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Ruminal degradation of *Panicum* grasses in three post-regrowth ages

Degradabilidade ruminal de gramíneas do gênero *Panicum* em três idades de pós-rebrota

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Abstract

The objective was to evaluate the chemical composition and *in situ* degradation of Maasai, Mombasa and Tanzania grasses belonging to the genus *Panicum*, at 22, 34 and 46 days after regrowth. The contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined. For the evaluation of ruminal degradation, 4 g sample were placed in nylon bags and incubated in the rumen of a fistulated animal, for 6, 24 and 72 h. The experiment had a completely randomized design for chemical composition analysis and a split-split plot completely randomized design for *in situ* degradation, with means compared by Tukey test at 5%. There was an increase in the contents of DM (+ 1.69%) and NDF (+ 5.06%) (P <0.05) of the grasses at the highest cutting ages with reduction in the CP fraction. The potential degradation (PD) of DM, NDF and CP decreased with advancing age of grasses, with an increase in colonization time (0.69 h) and NDF degradation rate (1.14%/h). The increase in the post-regrowth age of the Massai, Mombasa, and Tanzania grasses increases the acid detergent fiber content and reduces the crude protein content, with a negative effect on the degradation of DM, PB and NDF. The management of these cultivars is indicated at 22 and 34 days post-regrowth to obtain forage with better nutritional value.

Keywords: Massai grass; chemical composition; *in situ* degradation

Resumo

Neste trabalho, objetivou-se avaliar a composição química e a degradação *in situ* das gramíneas do gênero *Panicum* Massai, Mombaça e Tanzânia nas idades 22, 34 e 46 dias pós-rebrota. Quantificou-se os teores de

matéria seca (MS), proteína bruta (PB), fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA). Na avaliação da degradabilidade *in situ* foram pesadas 4 g de amostra em sacos de náilon incubados no rúmen de um bovino fistulado, nos tempos 6, 24 e 72 h. Adotou-se delineamento inteiramente casualizado para composição química e em parcela subdividida para degradação *in situ*, com médias comparadas pelo teste de Tukey a 5%. Houve aumento nos teores de MS (+1,69%) e FDN (+5,06%) ($P<0,05$) das gramíneas nas maiores idades de corte com redução da fração PB. A degradação potencial (DP) da MS, FDN e PB apresentou redução com o aumento da idade das gramíneas, com o aumento no tempo de colonização (0,69 h) e a taxa de degradação da FDN (1,14%/h). O aumento da idade pós-rebrota das gramíneas Massai, Mombaça, e Tanzânia eleva os teores de fibra em detergente ácido e reduz a proteína bruta, com efeito negativo na degradação da MS, PB e FDN, sendo indicado manejo desses cultivares aos 22 e 34 dias pós-rebrota para se obter forragem com melhor valor nutricional.
Palavras-Chave: Capim massai; Composição química; Degradação *in situ.*

Introduction

In the current feeding systems of ruminants on pastures, information is needed regarding the nutritional value and their rates of degradation and utilization in the digestive system, in order to balance the availability of energy and nitrogen in the rumen, maximizing microbial efficiency. However, in tropical regions, due to climatic conditions, forage plants have marked seasonality, with high availability and good nutritional value only in the rainy season, a phenomenon determined mainly by the limitations of light, water availability, and temperature in the dry season(1). Thus, the search for tropical grasses with high productivity in dry matter and adequate nutritional value is a challenge for the production of ruminants.

In Brazil, *Panicum maximum* grasses are one of the most important forage groups for ruminant production, as they have good nutritional value; tolerance to high temperatures and shading; adequate responses to more intensive cultural treatments; large production of dry matter; easy to establish, even in periods of water deficit, and possible use in intercropping systems(2, 3). However, there is little information about their chemical composition and nutritional value.

The cultivar Massai is a hybrid between *P. maximum* and *P. infestum*, and its characteristic is the growth forming clumps with an average height of 60 cm, intermediate tolerance to cold and high resistance to fire, with adaptations to the conditions of low soil fertility(2, 5). *Panicum maximum* CV Mombaça has a high dry matter production, is nutrient-demanding, with good responses when fertilized with nitrogen(5). Tanzania grass (*Panicum maximum* cv. Tanzania) is an alternative for soil areas with greater fertility, with good resistance to spittlebug and higher production of leaves with adequate nutritional value(15).

Among the factors related to the management of these forages, the interval between cuts affects both production and quality. Shortening the period between cuts result in lower forage mass, however, increasing the leaf/stem ratio, raising the nutritional value. With advancing age, there is a greater lignification of the cell wall, hindering the action of ruminal microorganisms and resulting in lower degradability of the fiber fraction[26].

In this sense, the assessment of the potentially degradable fractions in the rumen can determine the best time for harvesting the plant, associated with adequate nutritional value. The in-situ degradability technique is adequate to this end and allows quantifying the disappearance of nutrients over time, considering the real conditions of the rumen environment[19, 21]. The objective of this study was to determine the chemical composition and in situ degradability of grasses of the genus *Panicum* at three post-regrowth ages.

Material and methods

The research was carried out at the Animal Sciences Department, Agricultural Sciences Center, Federal University of Piauí, Teresina Campus, at 05°05'21"S, 42°48'07"W and 74.4 m altitude, with Aw local climate - according to the Köppen classification, tropical and rainy (megathermal), with dry winter and rainy summer. Chemical analyses were performed at the Animal Nutrition Laboratory (LANA/CCA/UFPI).

The grasses Massai (*Panicum maximum* Jacq. Cv. Massai), Tanzania (Pa*nicum maximum* Jacq. Cv. Tanzania) and Mombasa (*Panicum maximum* Jacq. Cv. Mombasa) were evaluated, divided into plots, subjected to a standardization cut at 30 cm from the ground, and NPK fertilization (100-80-50 kg/ha). The plots were irrigated by conventional sprinkler, with sprinklers spaced at 12 meters, with a flow rate of 7.3 mm/h and a four-day watering shift, adjusting the water depth to replace the average monthly evapotranspiration of the experimental period (138.6 mm)[23].

The grasses were harvested at 30 cm from the ground 22, 34 and 46 days after regrowth, identified, weighed and oven dried at 55 ºC for 72 hours for subsequent grinding in a Wiley mill to 1 mm particles. Samples were analyzed for dry matter (DM), crude protein (CP), mineral matter (MM)[6], neutral detergent fiber (NDF) and acid detergent fiber (ADF)[7].

For analysis of ruminal degradation, 4-g samples were weighed in 12x8 cm nylon bags with 50 µm porosity[8]. The bags were incubated for, 6, 24 and 72 h[9] in the rumen of two adult cattle, with an average weight of 450 kg, fistulated, previously fed a diet composed of 80% forage (chopped wilted elephant grass) and 20 % concentrate (corn, and soybean meal) twice a day (8 and 16 hours), with mineral salt and water at will. Four bags were used per treatment in each incubation time, totaling 288 bags, distributed in two animals. The soluble fraction was obtained by immersing bags not incubated in a water bath at 39°C for one hour. After removing from the rumen, the bags were immersed in ice water, washed and taken to a forced air oven with at 55°C for 72 h to quantify the contents of DM, CP and NDF.

The in situ degradation parameters (a, b and c) and the potential degradability of DM and CP were estimated by the model: $PD = A – B^{e\text{-}c \times t}$ and effective degradability estimated by the formula $ED = a + [(b\ c)/(c + k)]$ in the rates of passage of 2.5 and 8%[10], in order to determine the one that best fits the grasses. The NDF degradation parameters were determined according to Mertens & Loften[11]: Rt = B.e-ct + I, with standardization of fractions, according to Waldo, Smith & Cox[12].

The study had a 3x3 factorial randomized complete block design (three cutting ages x three forages) for chemical composition, and a split plot randomized complete block design for the in situ degradability of DM, CP and NDF, with the treatments representing plots and incubation times, the split plots. PROC MEANS and PROC NLIN of SAS (Statistical Analysis System, version 8) were adopted to obtain means and standard deviation, and degradation parameters, respectively. Tukey's test was applied at 5% probability to compare the mean values of chemical composition.

Results and discussion

There was an average increase of 1.69% in the DM content ($P < 0.05$) of forages when the cut was performed 46 days after regrowth, which is related to the incorporation of fiber constituents in the plant cell structure, associated with DM, with thickening of the wall and increased lignification of the tissues, which is evidenced by the higher content of NDF and ADF in this post-regrowth age (Table 1). The increase in the percentage of NDF and ADF, with advancing cutting ages, indicates a reduction in the proportion of nutrients that can be digested by the animal and directly reflects the intake of DM, and rumen microbial metabolism[26].

Table 1. Chemical composition of *Panicum* grasses harvested at three post-regrowth ages

Grass	Constituents[1]	Cutting age (days)[2]		
		22	34	46
Massai	DM	91.73^{bA}	92.25^{bA}	93.56^{aB}
	MM	9.10^{aC}	8.57^{aA}	8.17^{aB}
	CP	13.18^{aB}	9.02^{bB}	7.41^{bB}
	ADF	54.04^{bB}	55.09^{bB}	58.63^{aB}
	NDF	87.30^{bA}	87.51^{bA}	89.79^{aA}
Mombasa	DM	91.99^{bA}	92.06^{bA}	93.33^{aB}
	MM	11.01^{aA}	9.13^{bA}	7.83^{bB}
	CP	12.55^{aB}	11.57^{abA}	9.23^{bAB}
	ADF	53.36^{bA}	51.88^{bC}	58.54^{bB}
	NDF	81.75^{bB}	82.80^{bA}	85.24^{aB}
Tanzania	DM	91.27^{bB}	92.60^{bA}	93.75^{aA}
	MM	9.87^{aB}	9.28^{aA}	11.17^{aA}
	CP	12.70^{aB}	9.36^{bB}	8.19^{bAB}
	ADF	46.21^{bB}	46.26^{bD}	48.94^{aB}
	NDF	84.96^{bA}	85.16^{bA}	88.17^{Ab}

[1]DM: Dry matter; MM: Mineral matter; CP: Crude protein; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

[2]Mean values followed by different lowercase letters, in the same row, or different uppercase letters, in the same column, are significantly different by Tukey's test at 5%.

The increased proportion of fiber constituents is associated with the rapid thickening and lignification of the cell wall with advancing plant age, mainly due to the increase in the proportion and thickness of stem in relation to the leaves. This trend is in accordance with the results obtained for grasses of the genus *Panicum* by Pereira *et al*.[13], Carvalho *et al*. [14] and Garcez *et al*.[26], corroborating the increase in the concentrations of structural carbohydrates with advancing age of the plant for greater support at the vegetative stage. However, variations in the chemical composition of tropical grasses also depend on the type of cultivar, the presence of side tillers, and the light intensity, which does not make the leaf: stem ratio an exclusive determinant of the nutritional value of these species[15].

There was a decrease of 43.78, 26.45 and 31.51% in CP content at the highest cutting age for the Massai, Mombasa and Tanzania grasses, respectively. The reduction in the contents of this constituent with advancing forage age is associated with the higher complexation of nitrogen compounds with the lignocellulosic fraction of the cell wall. However, even at the highest cutting age, the CP content is above the minimum of 6-8% in DM, recommended for the maintenance of 8 mg NH_3-N.dL^{-1} in the rumen fluid and for the efficient ruminal degradation, with an increase in the production of microbial protein[24]; these values are equivalent to those obtained for tropical grasses[1, 5].

The lower proportion of CP in forages becomes the first limiting factor to intake, as a result of the lower activity of microorganisms in the rumen because of the low availability of nitrogen substrate for synthesis of microbial protein, especially cellulolytic microorganisms , which reduce their growth rate leading to less degradation of fiber fractions, with a consequent reduction in the rate of passage of rumen digesta and negative effects on the digestibility of the other components of the diet[7].

The PD and the potentially degradable fraction of dry matter decreased with advancing cutting age, associated with an increase in the ADF fraction, with low degradability in forages (Table 2). The lower rates of degradation are justified by the thickening of the cell wall and the increase in the proportion of lignin in the leaves. The stem has, in most of its composition, lignified tissues that hinder microbial access to the degradable fraction of the fiber, promoting lower rates of ruminal degradation and limiting the production of energy from glucose present in these polysaccharides[16].

As the grass approaches its physiological maturity and the flowering stage, changes occur in the proportions of its structural components[17], with an increase in cell wall components, associated with the dilution of their content components (organic acids and soluble starch) that have high digestibility[27], negatively impacting the degradation of DM by rumen microorganisms.

There was a high potentially degradable fraction for CP, with a reduction according to advancing cutting age (Table 3), which influences the degradation of the other DM components by making less N available to the rumen environment. Even with advanced ages, the PD values of this research for forages with up to 34 days after regrowth were higher than those obtained by Guadayo *et al*. (2019)[18] for cultivars of *Panicum maximum* (61.47%) and *Brachiaria mutica* (64.39%) at 28 days after regrowth, and by Pinheiro *et al*. (2019)[19] for Tanzania grass with 24 days of age (63.3%).

Table 2. Ruminal degradability of dry matter of *Panicum* grasses harvested at three post-regrowth ages

Grass	Cutting age (days)	Parameters¹							R^2
		A	B	c (%/h¹)	PD	ED			
						2%	5%	8%	
Massai	22	20.37	71.42	4.32	86.78	56.21	44.12	38.14	97.12
	34	18.86	52.93	4.37	67.15	51.76	41.14	36.70	92.25
	46	20.15	43.29	5.17	56.81	48.21	40.43	36.19	96.55
Mombasa	22	19.98	50.40	3.67	70.38	52.60	41.31	35.83	95.41
	34	21.95	36.03	4.34	67.98	50.26	43.38	39.19	94.69
	46	19.64	31.15	4.33	50.79	45.29	39.92	36.41	82.95
Tanzania	22	20.52	52.65	3.62	73.23	54.49	42.69	36.98	98.52
	34	21.10	46.22	3.20	67.32	49.54	39.14	34.31	99.07
	46	19.76	39.33	4.67	59.09	47.30	38.75	34.26	97.74

¹Soluble fraction (*a*), potentially degradable (*b*), degradation rate of fraction b (*c*), potential degradation (PD) and effective degradation (ED) determined by the models
$PD = A - B.e^{-c.t}$ and $ED = a + [(b \times c)/(c + k)]$[10].

Table 3. Ruminal degradability of crude protein of *Panicum* grasses harvested at three post-regrowth ages

Grass	Cutting age (days)	Parameters¹							R^2
		A	B	c (%/h¹)	PD	ED			
						2%	5%	8%	
Massai	22	38.25	48.72	2.56	86.97	59.6	49.84	46.2	99.88
	34	35.21	36.18	2.34	75.12	51.34	47.75	41.98	96.21
	46	35.10	22.12	2.92	57.22	52.26	47.94	45.36	97.12
Mombasa	22	30.25	59.76	2.31	90.01	62.28	49.13	43.64	96.92
	34	32.47	44.06	2.23	76.53	55.70	46.06	42.07	94.89
	46	50.15	32.56	3.83	62.71	51.54	44.27	40.69	95.22
Tanzania	22	29.85	65.76	2.00	95.61	62.73	48.64	43.0	95.72
	34	26.68	59.98	1.53	89.83	53.71	41.02	36.31	98.24
	46	26.81	52.48	2.49	79.81	49.76	39.40	35.59	89.73

¹Soluble fraction (*a*), potentially degradable (*b*), degradation rate of fraction b (*c*), potential degradation (PD) and effective degradation (ED) determined by the models
$PD = A - B.e^{-c.t}$ and $ED = a + [(b \times c)/(c + k)]$[10].

Reductions in the degradation rate of nitrogen fractions from 34 days can reduce the amount of ammonia nitrogen available to rumen microorganisms, which use it for the synthesis of amino acids, cell structures, and growth[25], which negatively impacts the

DM degradability (Table 2). In tropical grasses, a reduction in the protein degradation rate is expected with advancing age, associated with factors such as climate, water availability, and temperature, with the incorporation of this fraction in the less soluble components of the forage[1, 13].

The level of rumen degradability of CP can be variable between different forage species, which determines the availability of ammonia nitrogen in the rumen, and the proportion of amino acids from the diet and microbial protein, that reach the intestine, which are directed to tissue protein synthesis[25]. The concentration of crude protein is higher at the vegetative stages of forages and decline at the flowering stage, which may vary depending on differences between species, initial level of protein in the plant, stem and leaf proportions, content of fiber components in the cell wall and ambient temperature[15, 21].

There was an increase in the undegradable NDF fraction (Ip) with advancing cutting age of the grasses, which may justify the negative variations in their potential and effective DM degradation, since the NDF accounts for more than 70% of its composition (Table 4). Lower NDF degradation in tropical grasses with advanced regrowth ages is common in tropical regions, since they quickly reach phenological maturity, incorporating support structures represented by lignified fiber[20].

Table 4. Ruminal degradability of neutral detergent fiber of *Panicum* grasses harvested at three post-regrowth ages.

Grass	Cutting age (days)	Parameters¹				R^2
		Bp	Ip	Lag	k (%/h)	
	22	72.75	27.25	4.06	4.50	99.16
Massai	34	66.09	33.91	4.89	3.60	98.96
	46	64.66	35.34	4.27	1.90	99.24
	22	70.02	29.98	4.65	3.02	99.14
Mombasa	34	62.14	37.86	4.81	2.70	96.19
	46	51.07	48.93	4.85	2.49	95.16
	22	74.80	25.20	4.42	4.10	89.94
Tanzania	34	67.91	32.09	4.94	4.04	88.91
	46	66.83	33.17	4.76	2.24	94.96

¹Standardized potentially degradable fraction (Bp), standardized undegradable fraction (Ip), colonization time (lag), degradation rate (k) obtained by the models
$Rt = B^{e-cxt} + I$[11], $Bp = B/(B+I) \times 100$[12] and $Ip = I/(B+I) \times 100$[12].

The results obtained for the degradable NDF fraction at the lowest cutting ages were, on average, 12% lower than reported by Costa et al.[4] for Mombasa grass (80.41%) and Tifton-85 (80.06%) with 21 days after regrowth. The composition of the cell wall varies according to the forage species, the soil, the ambient temperature, the resilience of the plant, and its phenological characteristics, the latter is important in tropical grasses that reach the maturity earlier, and may present reductions in degradation of fiber fractions in shorter intervals.

The NDF colonization time (lag) (0.69 h) increased, leading to a decline in the rate of passage (1.14%/h) at the highest cutting ages. The colonization time of the fiber fraction represents the time between the start of incubation and ruminal degradation and is associated with the ability of rumen microorganisms to attach to the particles[21], increasing when there is a greater proportion of lignified cell wall in the forage, makes it difficult for microorganisms to adhere. Associated with this effect, there is a reduction in ruminal degradation of the fiber fraction, as observed in this study, which can affect the ruminal passage rate and negatively imply the intake of DM.

As for the colonization time (lag), the average values were high and higher than those found for grasses, with an increase with advancing age of forages. This effect may be associated with the dissolution of soluble carbohydrates and protein components that, although with a lower proportion in species, showed greater availability for microbial degradation (Tables 1 and 2) at earlier ages, consequently, with greater competition for these substrates, affecting the lag time of cell wall by fibrolytic bacteria[22].

NDF degradability and the rate of passage of the digesta are directly associated with forage intake, which also depends on the rate of degradation of the nutritional components in the diet. Leaves and stems have different rumen retention times, due to the greater resistance to degradation of stems, which result in changes in food intake, with a decrease in consumption with increasing plant maturity, depending on the chemical characteristics of the cell wall that becomes thicker and lignified; a determining factor in the digestive process of ruminants[20].

Conclusions

The increase in the post-regrowth age in Massai, Mombasa and Tanzania grasses increases the acid detergent fiber content and reduces the crude protein content, with a negative effect on the degradation of DM, PB and NDF. The management of these cultivars is indicated at 22 and 34 days post-regrowth to obtain forage with better nutritional value.

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