<u>Original Research Article</u>

NIRS Estimation of the Nutritive Value of *Megathyrsus maximus* (Syn. *Panicum* sp.) Forages

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ABSTRACT

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Aimed to evaluate the estimation of the nutritive value of *Megathyrsus maximus* (Syn. *Panicum* sp.) forages by near-infrared reflectance spectroscopy (NIRS). The experimental design was completely randomized with four replications. The treatments were arranged in a split-plot arrangement, with nine cultivars of *M. maximus* in the main plot (Aries, Atlas, Massai, Mombasa, Paredao, Kenya, Tamani, Tanzania, and Zuri) and four harvesting ages in the subplot (30, 60, 90, and 120 days). The contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), indigestible neutral detergent fiber (iNDF), and ash were evaluated. A reduction in CP and ash contents is observed with the advancement of age in all evaluated cultivars. In contrast, an increase in DM, NDF, ADF, and iNDF contents is observed. The nutritive value of the forages from the evaluated cultivars deteriorates as age increases, recommending grazing periods of less than 60 days for cattle. The NIRS estimations are excellent (R^2_{cv}> 0.95) for CP, ash, NDF, and iNDF, demonstrating the potential of this technology for analyzing the nutritive value of *M. maximus* forages.

Keywords: age, cultivars, guinea grass, indigestible neutral detergent fiber, near-infrared spectroscopy

1. INTRODUCTION

The animal production system on pasture experiences a considerable increase in profitability due to its low investment cost and ease of implementation, requiring minimal labor. To achieve benefits in this practice, it is essential to make the right choice of forage plants, utilizing cultivars that best adapt to edaphoclimatic conditions.

For the production of cattle in tropical and subtropical regions, forages of the genus *Megathyrsus* (Syn. *Panicum*) are widely used due to their high potential for dry matter production per unit area, broad adaptability, good forage quality, and easy establishment. Therefore, several research institutions have developed cultivars of *Megathyrsusmaximus* adapted to deep and well-drained soils with good fertility. However, there are morphological and physiological differences that require further studies to determine the appropriate management strategies [1,2].

Among the factors related to the management of these forages, the interval between harvest is one of the factors that affects both production and grass quality. Reductions in harvesting intervals lead to a lower amount of forage mass but increase the leaf/stem ratio, providing higher nutritional value. As the plants age, there is increased lignification of the cell walls, making it more difficult for rumen microorganisms to act, resulting in reduced degradability of the fibrous fraction [3].

Forages of the genus *Megathyrsus* are less flexible in terms of management due to their high growth rate, large size, and generally robust stems, which impose limitations on continuous stocking, making rotational stocking preferable [4].

Stabile SS et al. [5], in their evaluation of guinea grass cultivars regarding the effect of maturity on bromatological composition and digestibility, observed a linear increase in dry matter, acid detergent fiber, and leaf lignin content, as well as a reduction in *in vitro* dry matter digestibility and neutral detergent fiber with advancing maturity.

Near-infrared spectroscopy (NIRS) has been successfully applied for determining bromatological composition, product quality evaluation, and production control. The spectrum provides a global signature of the bromatological composition (fingerprint), which, when combined with chemometric techniques, can be used to elucidate compositional characteristics that are not easily detected by traditional chemical analyses [6].

NIRS technology allows for the investigation of the nutritive value of a given sample without its destruction, physical separation, or chemical treatment [7]. *In vitro* and *in situ* procedures used to estimate indigestible neutral detergent fiber (iNDF) in forage or fecal samples are time-consuming, expensive, and limited by intrinsic factors [8]. On the other hand, NIRS technology enables rapid and accurate determination of the bromatological composition and digestibility of forages [9,10,11,12,13,14,15].

The conventional reference method requires approximately 200 g of chemical reagents per complete sample (dry matter, crude protein, neutral and acid detergent fiber, ether extract, and ash), with one-third of this weight consisting of strong acids that pollute the environment and pose risks to operators [16]. Furthermore, a well-equipped conventional laboratory can perform 50 complete analyses in five working days (40 hours). In NIRS technology, a technician spends two minutes per complete analysis, enabling them to perform 1200 analyses in 40 hours.

Thus, the aim was to evaluate the estimation of the nutritive value of *M. maximus* (Syn. *Panicum* sp.) forages using near-infrared spectroscopy (NIRS).

2. MATERIAL AND METHODS

2.1 Site and Experimental Design

The experiment was carried out at the Experimental Farm of the Federal University of Mato Grosso, located at coordinates 15° 47’ South Latitude, 56° 04’ West Longitude, and an altitude of 140 m. The climate, according to the Köppen classification, is Aw type (megathermal tropical climate), characterized by two well-defined seasons: dry season (May to September) and rainy season (October to April). The annual precipitation is 1500 mm, with the highest intensity in the months of December, January, and February.

The predominant soil type is Plintosol (Plintosol Tb albic moderate, medium texture, flat relief). It has a texture that facilitates water infiltration, soil aeration, root penetration, and root system development.

The experimental design was completely randomized with four replications. The treatments were arranged in a split-plot arragement, with nine cultivars of *M. maximus* in the main plot (Aries, Atlas, Massai, Mombasa, Paredao, Kenya, Tamani, Tanzania, Zuri) and four harvesting ages in the subplot (30, 60, 90, and 120 days).

The experiment was established in an already established pasture area with *M. maximus* cultivars, where soil samples were collected for chemical and particle size analysis in the 0-10 cm layer (Table 1).

Table 1. Chemical and particle size characteristics of the soil in the experimental area (Santo Antônio do Leverger, MT, Brazil)

pH	P	K	Ca	Mg	Al+H	OM	Sand	Silt	Clay	SB	CEC	V
$CaCl_2$	mg dm^{-3}		cmol$_c$ dm^{-3}			g dm^{-3}	g kg^{-1}			cmol$_c$ dm^{-3}		%
5.9	5.3	31.1	1.9	0.77	2.98	1.89	740	59	201	2.75	5.72	47.88

P: phosphorus; K: Potassium; Ca: calcium; Mg: magnesium; Al+H: potential acidity; OM: organic matter; SB: sum of bases; CEC: cation exchange capacity; V: sabe saturation

Considering that the cultivars have a minimum requirement of 50% base saturation, liming was not necessary. After the uniformization harvest, maintenance fertilization was applied as a topdressing, using 40 kg P_2O_5 ha^{-1}, 200 kg N ha^{-1}, and 200 kg K_2O ha^{-1} [17].

At the predetermined harvesting ages, the cultivars were harvested at residue heights of 25 cm (Tamani), 30 cm (Aries and Massai), 35 cm (Atlas, Kenya, and Tanzania), 40 cm (Zuri), and 45 cm (Mombasa and Paredao), following the recommendation by Costa JAA and Queiroz HP [18]. The forages were chopped, placed in paper bags, and dried in a convection drying oven at 55 °C until reaching a constant weight.

2.2 Chemical analyses

The pre-dried samples were weighed and milled using a stationary mill with a 1.0 mm sieve, and stored in polyethylene containers for the analysis of the definitive dry matter (DM) content in an oven at 105 °C for 4 hours, according to AOAC [19].

Forage samples were subjected to analysis of ash content, as described in Silva DJ and Queiroz AC [20]; crude protein (CP) by the micro Kjeldahl method [21]; neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest PJ et al. [22]. The indigestible neutral detergent fiber (iNDF) was determined according to Cochran RC et al. [23].

The obtained data were subjected to analysis of variance and regression, adopting a 1.0% probability of error.

2.3 Calibration and Validation Curves

Approximately 15 g of milled forage sample were transferred to a quartz bottom sample holder attached to an MPA FT-NIR device (BRUKER® OPTIK GmbH, Rudolf Plank Str. 27, D-76275 Ettlingen). The spectra were generated in triplicate with 64 different points scanned with a resolution of 16 cm^{-1} in the wavenumber range of 4,000 to 12,500 cm^{-1}.

Reference values for CP, NDF, ADF, iNDF, and ash (% of DM) were added to the forage sample spectra. Data preprocessing and chemometric model development, i.e., calibration curve construction, were performed using Opus 7.5 software employing the partial least squares (PLS) model [24].

The calibration model was selected based on the lowest root mean square error of cross-validation (RMSECV) and the highest coefficient of determination (R^2). Additionally, relative performance deviation (RPD) values above 3 and ratio of error range (RER) values above 10 were adopted as criteria [25].

Samples identified as outliers in the plots were detected and excluded from the models. A set of samples not included in the calibration step was used for external validation.

3. RESULTS

3.1 Chemical composition of forage

In the evaluated cultivars, there was a linear increase in the contents of DM, NDF, and ADF with advancing age (Table 2), except for Massai, which had NDF and ADF contents of 74.52% and 45.74%, respectively.

Aries and Atlas cultivars had the highest daily increments in DM content (0.43% and 0.32%), NDF (0.33% and 0.19%), and ADF (0.32% and 0.18%), respectively. In contrast, lower increments in NDF (0.11% per day) and ADF (0.11% per day) were obtained by Tamani and Tanzania cultivars, respectively.

With advancing age, there was a linear reduction in CP and ash contents in all evaluated cultivars (Table 2). The cultivars that had the highest decreases in CP content for each additional day of age were Aries (0.14%); Atlas, Tamani, and Zuri (0.12%). In contrast, the smallest reduction in CP content was observed in the Massai cultivar (0.05% per day).

The largest reductions in ash contents were observed in Aries cultivar (0.05% per day); Tamani and Zuri (0.02% per day). On the other hand, the Massai cultivar showed the smallest daily reduction in ash content (0.0096%).

There was a linear increase in iNDF content with advancing age in all evaluated cultivars (Table 2). The largest daily increases were observed in Atlas (0.11%); Kenya (0.10%); Aries and Zuri (0.09%) cultivars. Conversely, the smallest daily increment was found in the Massai cultivar (0.04%).

Table 2. Means of dry matter (DM, %), crude protein (CP, % DM), neutral detergent fiber (NDF, % DM), acid detergent fiber (ADF, % DM), ash (% DM), and indigestible neutral detergent fiber (iNDF, % DM) contents in forages of *Megathyrsus maximus* cultivars (Syn. *Panicum* sp.) at different ages (days)

	Ages (days)						
Contents	30	60	90	120	Regression equation[1]	R^2	CV (%)
		Aries					
DM	-	22.86	35.71	48.68	ŷ= -2.9695 + 0.4302x**	0.99	3.61
CP	15.22	8.84	3.91	2.47	ŷ= 18.4037 - 0.1438x**	0.94	5.66
NDF	55.77	62.27	76.86	84.45	ŷ= 44.6862 + 0.3320x**	0.98	4.15

ADF	29.73	35.04	48.68	57.68	ŷ= 18.4175 + 0.3249x**	0.97	5.79
Ash	11.33	9.65	8.31	6.73	ŷ= 12.7975 - 0.0505x**	0.99	5.39
iNDF	20.15	23.46	27.11	27.92	ŷ= 17.9225 + 0.0898x**	0.94	2.72
Atlas							
DM	22.38	23.94	35.41	51.4	ŷ= 8.6500 + 0.3284x**	0.90	5.05
CP	15.99	9.48	5.81	4.67	ŷ= 18.3925 - 0.1253x**	0.91	7.99
NDF	63.36	72.87	77.82	80.85	ŷ= 59.3775 + 0.1913x**	0.94	2.76
ADF	36.85	44.70	50.97	53.73	ŷ= 32.3450 + 0.1896x**	0.96	4.76
Ash	9.71	8.78	8.10	8.09	ŷ= 10.0562 - 0.0184x**	0.87	2.13
iNDF	17.67	22.85	25.69	27.7	ŷ= 15.2487 + 0.1097x**	0.95	2.32
Massai							
DM	32.37	32.6	42.54	47.86	ŷ=24.7412 + 0.1880x**	0.90	5.28
CP	8.94	6.20	5.00	4.31	ŷ= 9.8925 - 0.0503x**	0.91	11.17
NDF	75.12	71.8	75.72	75.42	ȳ= 74.52	-	3.28
ADF	47.04	41.71	46.64	47.56	ȳ= 45.74	-	3.98
Ash	8.84	8.15	7.81	7.99	ŷ= 8.9275 - 0.0096x**	0.68	3.87
iNDF	25.18	25.65	28.12	28.82	ŷ= 23.6012 + 0.0446x**	0.92	3.57
Mombasa							
DM	25.65	27.34	34.85	45.06	ŷ= 16.7949 + 0.2191x**	0.92	6.09
CP	11.36	6.71	4.95	3.05	ŷ= 13.1887 - 0.0889x**	0.93	10.66
NDF	71.32	76.06	79.19	83.22	ŷ= 67.7400 + 0.1294x**	0.99	1.63
ADF	43.64	51.31	55.2	56.8	ŷ= 40.9000 + 0.1445x**	0.91	6.68
Ash	9.01	8.36	8.13	7.71	ŷ= 9.3325 - 0.0137x**	0.96	1.51
iNDF	22.09	25.28	26.1	28.16	ŷ= 20.6525 + 0.0634x**	0.94	2.74
Paredao							
DM	24.22	26.51	34.31	49.1	ŷ= 12.9337 + 0.0889x**	0.89	7.76
CP	12.07	7.40	5.70	3.23	ŷ= 14.1612 - 0.0940x**	0.95	13.89
NDF	70.47	77.45	79.18	82.72	ŷ= 67.8337 + 0.1283x**	0.93	2.60
ADF	43.44	49.22	51.91	56.03	ŷ= 40.0350 + 0.1349x**	0.98	4.20
Ash	8.95	8.33	8.24	7.67	ŷ= 9.2812 - 0.0130x**	0.94	2.16
iNDF	21.40	24.5	26.12	27.3	ŷ= 20.0500 + 0.0644x**	0.95	3.00
Kenya							
DM	24.13	27.92	38.70	40.50	ŷ= 17.8387 + 0.1997x**	0.93	8.16
CP	12.44	7.81	3.85	4.57	ŷ= 14.0587 - 0.0918x**	0.82	8.10
NDF	68.74	76.79	81.20	81.54	ŷ= 66.3675 + 0.1427x**	0.86	2.88
ADF	40.50	48.99	52.77	54.73	ŷ= 37.6312 + 0.1549x**	0.91	8.06
Ash	9.60	8.35	7.87	8.13	ŷ= 9.7125 - 0.0163x**	0.68	2.39
iNDF	20.62	23.98	26.88	29.52	ŷ= 17.8525 + 0.0986x**	0.99	2.81
Tamani							

DM	31.61	32.07	41.17	-	$\hat{y}$= 25.3950 + 0.1593x**	0.79	14.8
CP	11.93	6.42	4.42	-	$\hat{y}$= 15.0975 - 0.1250x**	0.93	12.17
NDF	74.64	79.22	81.69	-	$\hat{y}$= 71.4675 + 0.1175x**	0.97	2.09
ADF	45.50	51.13	53.99	-	$\hat{y}$= 41.7233 + 0.1414x**	0.96	3.45
Ash	9.39	8.34	8.00	-	$\hat{y}$= 9.9700 - 0.0231x**	0.92	2.35
iNDF	22.82	25.39	26.82	-	$\hat{y}$= 21.0100 + 0.0667x**	0.97	2.29
Tanzania							
DM	22.89	27.53	36.90	44.96	$\hat{y}$=14.1808+0.2519x**	0.98	5.87
CP	12.56	6.78	4.91	3.06	$\hat{y}$=14.4162-0.1011x**	0.91	10.66
NDF	72.64	72.74	81.27	82.72	$\hat{y}$=67.6525+0.1292x**	0.86	2.97
ADF	42.78	49.47	53.17	53.22	$\hat{y}$=40.9075+0.1167x**	0.84	4.00
Ash	9.34	8.24	8.00	7.68	$\hat{y}$=9.6187-0.0173x**	0.87	2.90
iNDF	20.87	24.09	26.99	27.75	$\hat{y}$=19.0462+0.0784x**	0.94	2.19
Zuri							
DM	20.48	26.97	39.48	47.37	$\hat{y}$=10.2775+0.3106x**	0.98	10.03
CP	14.75	7.47	4.65	2.89	$\hat{y}$=17.0400-0.1279x**	0.90	18.21
NDF	75.14	74.03	81.60	82.81	$\hat{y}$=70.7525+0.1019x**	0.79	2.14
ADF	35.83	47.83	52.57	54.18	$\hat{y}$=32.6600+0.1992x**	0.86	6.93
Ash	9.76	8.54	8.03	7.80	$\hat{y}$=10.1362-0.0213x**	0.89	2.32
iNDF	19.69	24.63	26.55	27.96	$\hat{y}$=18.0312+0.0890x**	0.91	2.76

*CV: coefficient of variation; R^2: coefficient of determination; [1]**: Significant at a 1.0% level of probability, according to the F-test*

3.2 NIRS Estimations

A total of 148 samples were used for the calibration set, while the external validation set consisted of 27 samples. In the calibration phase, a maximum limit of 10% outliers was adopted, with iNDF content showing the lowest percentage of outliers (3.86%) (Table 3).

During the calibration step, it was observed that the values of the cross-validation coefficient of determination (R^2_{cv}), the RPD, and the RER were greater than 0.95, 3.0, and 10.0, respectively, indicating an excellent estimation of the CP, ash, and iNDF content in *M. maximus* forages (Table 4 and Figure 1). Additionally, low values of RMSECV were also observed. No preprocessing was required for the ash and iNDF content.

In the external validation phase, it was found that the values of the correlation coefficient (r) and the RPD were greater than 0.95 and 3.0, respectively, indicating an excellent estimation of the CP, ash, and iNDF content in *M. maximus* forages (Table 5 and Figure 2). Similarly, in the external validation, the values of the RMSEP were low.

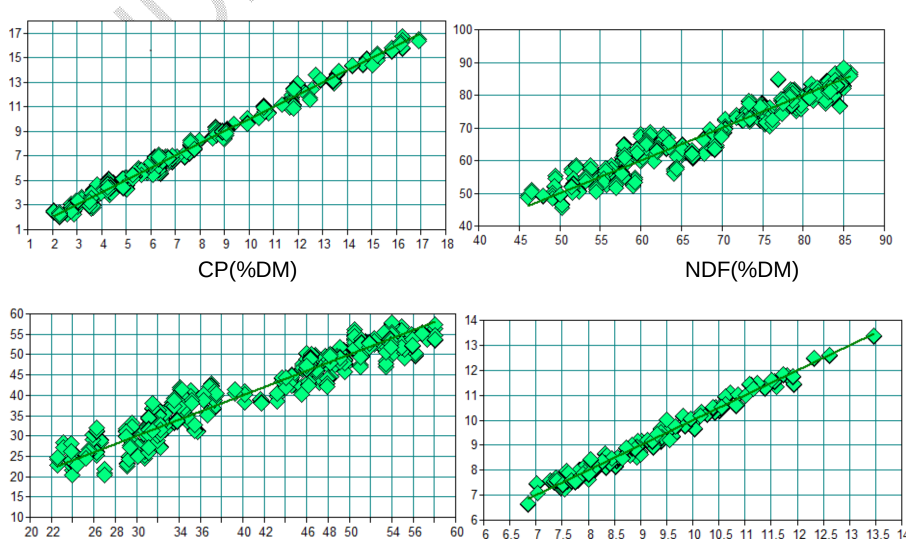
Table 3. Minimum and maximum contents, and percentage of outliers, for crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), ash, and indigestible neutral detergent fiber (iNDF) in forages of *Megathyrsus maximus* (Syn. *Panicum* sp.) cultivars for the calibration and external validation sets

Variables	Calibration			External Validation	
	Minimum	Maximum	Outliers (%)	Minimum	Maximum
CP (% DM)	2.00	16.74	6.52	2.27	15.95
NDF (% DM)	45.60	88.35	4.50	51.40	85.02
ADF (% DM)	20.27	57.87	4.72	27.26	55.87
Ash (% DM)	6.63	13.37	9.42	7.28	11.46
iNDF (% DM)	16.88	30.46	3.86	3.86	30.42

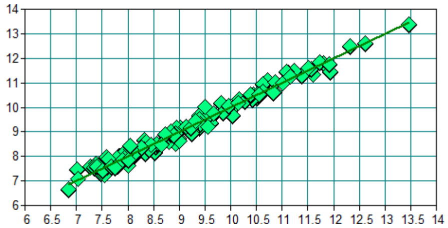
Table 4. Parameters and preprocessing models used for the prediction of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), ash (ASH), and indigestible neutral detergent fiber (INDF) contents in forages of *Megathyrsus maximus* (Syn. *Panicum* sp.) cultivars for the calibration set

Variables	RMSECV (% DM)	R^2_{CV}	RPD	RER	Preprocessing
CP (% DM)	0.37	0.99	10.3	39.83	MMN
NDF (% DM)	3.19	0.92	3.46	13.40	FDNV
ADF (% DM)	3.04	0.91	3.36	12.36	SLS
Ash (% DM)	0.20	0.98	6.86	33.70	NSDP
iNDF (% DM)	0.28	0.99	10.50	48.50	NSDP

RMSECV: root mean square error of cross-validation; R^2_{cv}: coefficient of determination of cross-validation; RPD: relative performance deviation; RER: relative error ratio; MMN: Min-Max Normalization; FDNV: First Derivative + Vector Normalization; SLS: Straight Line Subtraction;NSDP:No Spectral Data Preprocessing



ADF(%DM) Ash(%DM)



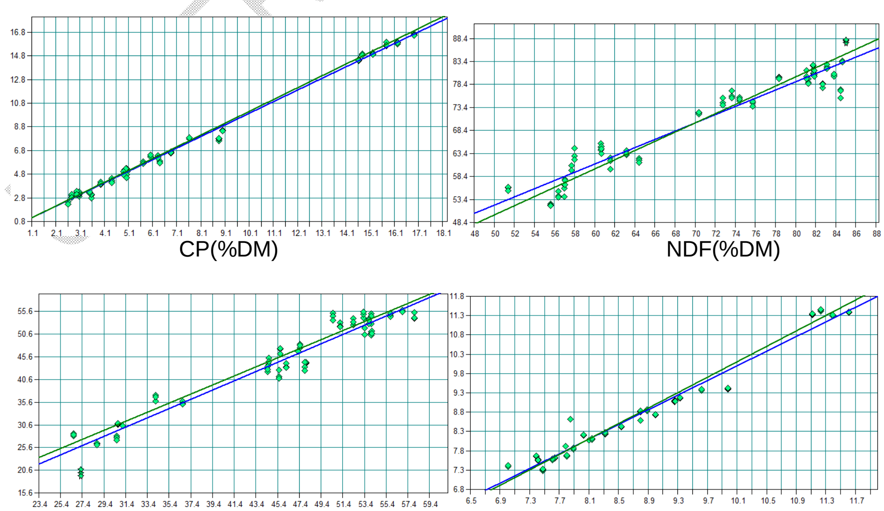
iNDF(%DM)

Fig. 1.Curve of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, and indigestible neutral detergent fiber (iNDF) contents of *Megathyrsus maximus* (Syn. *Panicum* sp.) forage in the calibration set

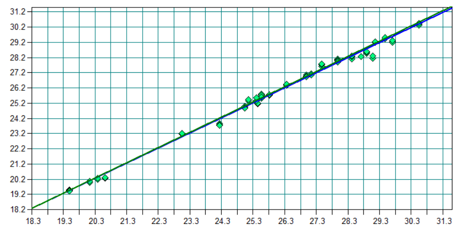
Table5. Adjusted parameters for estimating crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, and indigestible neutral detergent fiber (iNDF) contents in *Megathyrsus maximus* (Syn. *Panicum* sp.) forage for the external validation set

Variables	RMSEP (% DM)	r	RPD	Slope
CP (% DM)	0.34	0.99	14.30	0.98
NDF (% DM)	2.90	0.96	3.86	0.89
ADF (% DM)	2.64	0.97	4.01	1.02
Ash (% DM)	0.22	0.98	5.97	0.95
iNDF (% DM)	0.25	0.99	13.10	0.99

RMSEP: root mean square error of prediction; r: correlation coefficient; RPD: relative performance deviation



ADF(%DM) Ash(%DM)



iNDF(%DM)

Fig 2. Reference values versus predicted values for the contents of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, and indigestible neutral detergent fiber (iNDF) in *Megathyrsus maximus* (Syn. *Panicum* sp.) forage samples in the calibration sets

4. DISCUSSION

4.1 Chemical composition of forages

The nutritive value of forage from different cultivars of *M. maximus* was influenced by harvesting age. There was a linear increase in the contents of DM, NDF, ADF, and iNDF, and a linear decrease in the contents of CP and ash with advancing age. According to Stabile SS et al. [5], the most significant changes that occur in the chemical composition of forage plants are those that accompany their maturation. As the plant ages, the proportion of potentially digestible components tends to decrease, while the proportion of fibers increases.

Regarding the DM content, the increment ranged from 0.08% to 0.43% per day for Paredao and Aries grasses, respectively (Table 2). The estimated DM contents ranged from 15.60% to 48.65% at ages 30 and 120 days, respectively. These results were similar to those found by Araujo LMB et al. [26], who evaluated Mombasa grass at different harvesting ages and also found that the DM content increased linearly with age, ranging from 22.62% to 32.99% over a period of 49 days, with a more pronounced effect as the age advanced.

In contrast to the DM content, the CP contents decreased with advancing age, with reductions of 0.05% to 0.14% per day for Paredao and Aries grasses, respectively (Table 2). The estimated CP contents ranged from 14.63% to 1.15% at ages 30 and 120 days, respectively. Garcez BS et al. [27], evaluating *P. maximum* cv. Colonião (Guinea grass), observed that CP contents also decreased with advancing age, with a 5.73% reduction at 46 days compared to 22 days. This decrease was associated with a greater complexation of nitrogenous compounds with the ADF fraction as the age advanced, reducing the availability of nitrogen and consequently the CP contents.

The decrease in CP in forage becomes the primary limiting factor for intake, as a result of reduced rumen microbial activity due to low availability of nitrogenous substrate, causing negative effects on digestibility [3]. Therefore, it is recommended that harvesting or grazing be done up to 57 days (Massai), 64 days (Tamani), 69 days (Mombasa), 73 days (Tanzania), 76 days (Kenya and Paredao), 78 days (Zuri), 79 days (Aries), and 90 days (Atlas). Above these ages, if there is no supplementation of the animals' diet to replenish the deficient nutrients in the forage, there will be a reduction in weight gain or even negative performance [28].

With advancing age, there was an increment in the NDF content ranging from 0.10% to 0.33% per day for Zuri and Aries grasses, respectively. The estimated NDF contents ranged from 54.65% to 84.53% at ages 30 and 120 days, respectively (Table 2). Similarly, there was an increase in the ADF content ranging from 0.11% to 0.32% per day for Tanzania and Aries grasses, respectively. The estimated ADF contents ranged from 28.18% to 58.24% at ages 30 and 120 days, respectively (Table 2). Castro GHF et al. [29] also observed an elevation in the NDF and ADF contents in Tanzania grass with increasing age, ranging from 69.98% to 76.64% for NDF and from 36.10% to 43.60% for ADF at ages 42 to 126 days, respectively.

In experiments with hybrids and cultivars of *M. maximus* under harvesting intervals of 35 days during the rainy season and 60 to 90 days during the dry season, in response to fertilization for two consecutive years, Braga GJ et al. [30] also observed CP, NDF, and ADF ranging from 9.4% to 11.5%, 35.2% to 38.5%, and 64.8% to 69.1%, respectively.

With the age advancement, the ash contents decreased, with a reduction ranging from 0.0096% to 0.05% per day for the Massai and Aries grasses, respectively (Table 2). The estimated ash contents varied from 11.28% to 6.74% at the ages of 30 and 120 days, respectively. These results differ from those obtained by Garcez BS et al. [27], who found ash contents ranging from 9.82% at 22 days to 11.08% at 46 days. The ash content showed little variation throughout the harvesting ages, indicating that the aging of the plant has little influence on this characteristic.

The iFDN is employed to estimate the effective digestion of fibrous components, with its isolated investigation being paramount to the understanding of physical repletion within the ruminal environment [31]. As the age advances, there was an increase in iFDN contents ranging from 0.04% to 0.11% per day for the Massai and Atlas grasses, respectively. The estimated iFDN contents varied from 18.54% to 29.68% at the ages of 30 and 120 days, respectively (Table 2).

In contrast, Detmann E et al. [31] observed iFDN contents ranging from 10.84% to 39.89%, evaluating samples of *in natura* forages including sugarcane (*Saccharum* sp.), Elephant grass (*Pennisetumpurpureum*), and *Cynodon* sp.; samples of tropical grasses used under grazing conditions (*Urochloadecumbens*, *U. brizantha*, *U. arrecta*, and *P. maximum* cv. Tobiatã); silage samples (*Cynodon* sp., corn, and sorghum); and hay samples (*Andropogongayanus*, *Cynodon* sp., and *Melinisminutiflora*).

4.2 NIRS Estimations

The RPD and RER values for CP, ash, NDF, ADF, and iNDF are above 3.0 and 10.0, respectively, indicating that they are considered suitable for the estimation of the nutritional value of *M. maximus* forages. In contrast, Parrini S et al. [32], evaluating the NIRS estimations of DM, CP, ash, ether extract, NDF, and ADF in forages of *Avenafatua* L., *Capsella bursa-pastoris* L., *Dactylis glomerata* L., *Festucaovina* L., *F. pratensis* L., *Holcuslanatus* L., *Loliumperenne* L., *Poa pratensis* L., *P. annua* L., *Trifolium pratense* L., *T. repens* L., *Ranunculus bulbos*us L., and *Taraxacum officinale* GH Weber ex Wiggers in Italy, found that only DM and CP achieved RPD > 3.0.

The NIR region provided excellent estimations for CP, ash, and iNDF with R^2_{cv} values > 0.95 and r > 0.95. Similar to the findings of this study, Anderson WF et al. [32], in a study of 30 accessions of *P. purpureum* Schum. in the United States, also reported excellent NIRS estimations for CP and ash with R^2_{cv} values of 0.99 and 0.98, respectively. The authors concluded that these findings allowed for a faster evaluation of Elephant grass biomass for use by the industry or geneticists.

Garcia J and Cozzolino D [34], evaluating 650 samples of grasses and legume forages at different growth stages from March to December in Uruguay, found R^2_{cv} values of 0.95, 0.98, and 0.95 for DM, CP, and ADF contents, respectively. The results demonstrated the potential of NIR to predict the chemical composition of forages; however, it is suggested that the technique can be used as a routine procedure in breeding programs only if calibration is performed for each species, season, and specific conditions.

The result obtained for CP content was similar to that observed by Lobos I et al. [35]. These authors assessed the nutritional quality of 295 samples of forage grasses in southern Chile (*L. perenne* L., *Agrostis* sp., *Holcuslanatus* L., *Bromusvaldivianus* Phil., *D. glomerata* L., *Medicago sativa* L., *T. pratense* L., and *T. repens* L.) using NIRS, and the R^2_{cv} and r values were excellent: 0.99 and 0.99, respectively.

The calibration indexes obtained in the present study were not satisfactory for estimating NDF and ADF contents using NIRS, with R^2_{cv} values of 0.92 and 0.91, respectively. Similarly, Serafim CC et al. [13], evaluating 105 samples of forages and hay from *C. dactylon* cv. Tifton 85, observed low R^2_{cv} values for ADF (0.80) and ADF (0.80). Furthermore, Guerra GL [36], when assessing 360 samples of forages from *U. brizantha* cultivars (Marandu and Piatã), also did not find adequate R^2_{cv} values for NDF (0.88) and ADF (0.86).

On the other hand, Massignani C et al. [12], evaluating 200 samples of forages from 26 species of grasses and 6 species of legume forages, found high R^2_{cv}, RPD, and r values for CP (0.98, 7.23, and 0.98), NDF (0.95, 4.34, and 0.94), and ADF (0.96, 4.75, and 0.96), respectively. The authors concluded that the calibration curves were suitable for evaluating CP, NDF, and ADF contents of different grasses and legume forages and for routine use in estimating nutritional value.

Fontanelli RS et al. [37], evaluating 129 samples of forages from *C. dactylon* cultivars (Tifton 68, Tifton 85, Florakirk, and Coastcross), observed excellent R^2_{cv} values for the contents of DM (0.99), CP (0.98), NDF (0.97), ADF (0.99), phosphorus (0.94), and potassium (0.97). They concluded that despite the literature not presenting good results for mineral prediction using NIRS, the authors obtained satisfactory calibration indexes, which could be attributed to appropriate associations between spectral bands and the mineral fraction complexed with the organic matrix or to the high correlation between mineral content and fluctuations of other organic compounds present in the samples.

On the other hand, Brogna N et al. [8], evaluating 1281 fecal samples for iNDF estimation using NIRS, obtained low R^2_{cv} (0.86) and RPD (2.57) values. Peters JF et al. [15] also developed NIRS calibrations to predict the nutrient composition of fecal matter in 12 different forage-based diets for beef cattle. They concluded that it was possible to obtain calibrations for fecal composition and digestibility in cattle fed high-forage diets. High R^2_{cv} values were found for nitrogen content (0.94), ADF (0.94), acid detergent lignin (0.95), and calcium (0.97), while low values were obtained for iNDF (0.89) and phosphorus (0.86).

5. CONCLUSION

There is a decrease in crude protein and ash content with advancing age in all evaluated cultivars. Conversely, an increase is observed in dry matter, neutral detergent fiber, acid detergent fiber, and indigestible neutral detergent fiber content.

The nutritive value of the forages from the evaluated cultivars worsens as age increases, recommending grazing of cattle at an age younger than 60 days.

The estimations using NIRS are excellent (R^2_{cv}> 0.95) for crude protein, ash, and indigestible neutral detergent fiber, demonstrating the potential of this technology for analyzing the nutritive value of *M. maximus* (Syn. *Panicum* sp.) forages.

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