

Biomass, Efficiency of Nitrogen Conversion and Nitrogen Recovery in Millet Cultivars Under Nitrogen Fertilization

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Abstract

The objective of this study was to evaluate the production of green mass (GM), dry mass (DM), nitrogen contained (NC), bromatological composition, apparent nitrogen conversion efficiency (NCE), and apparent nitrogen recovery (ANR) of millet cultivars (ADR-500, ADR 7020 and LABH 70732) that received different doses of nitrogen (N) (0, 50, 100, 200 kg.ha⁻¹). The experimental design was randomized blocks in a 3x4 factorial scheme (three cultivars and four N rates) with four replications. About biomass, only GM showed an interaction between N and millet cultivars. The DM production showed difference only between N doses. The NCE and ANR differed between N doses with decreasing mean values, due to the increase of nitrogen fertilization. The best conversion efficiency was 54.06 kg DM/kg N applied. The best rate of recovery of N occurred with the application of 50 kg N.ha⁻¹. The values of NC varied in the fertilized plots. NCE and ANR differed between millet cultivars. The bromatological composition variables did not differ as a function of the N doses evaluated. In the conditions of the present study, nitrogen fertilization did not influence the production and bromatological composition of millet cultivars. The three cultivars present satisfactory characteristics for use in ruminant feed.

Keywords: fibers, dry mass, pennisetum glaucum, crude protein, urea, bromatological composition

1. Introduction

Initially, millet was used as cereal for human and animal feeding. Later, it was used as a forage crop because it can be grown in arid and semi-arid tropical areas, characterized by

high temperatures, low rainfall, and shallow or sandy soils. The crop is adapted to these conditions by a combination of important short periods and considerable developmental plasticity, maximizing the use of available soil moisture.

The successful adaptation of millet in Brazil is due to innumerable factors. In addition to adapting to the most diverse environments and climate types, millet cultivation stands out because it presents high resistance to water stress and adapts to the most different types of soil. According to Almeida et al. (2018), high efficiency of water use by millet allows it to produce the same amount of dry mass as maize while using up to 70% less water.

In addition to the high resistance to drought and production capacity, it presents good adaptability to low fertility soils, which are extremely limiting for maize and sorghum cultivation. In addition, it presents excellent forage potential (Martini et al., 2017), as it is an easily planted crop, has good acceptability by animals, great regrowth capacity, good nutritional value, and excellent biomass production capacity. Thus, millet can be used as forage for succession to the summer crop, the so-called safrinha, in the subtropical and tropical regions of Brazil (Silva, et al., 2012).

Despite these auspicious characteristics, there are few studies that contribute to and boost the cultivation of millet. It is known that nitrogen fertilization tends to improve the production and composition of grasses. It is also of great interest to evaluate the quality of the food available and how it can supply the nutritional requirements of the animals in a viable way. In this sense, the evaluation of the bromatological composition of this species will allow for a better characterization of the millet plant, as well as to evaluate the influence of the nitrogen fertilization on this crop. The objective of this work was to evaluate the production and quality of the cultivars of millet ADR-500, ADR 7020, and LABH 70732 submitted to different doses of nitrogen in the cerrado.

2. Materials and Methods

2.1 Experiment Location

The experiment was conducted from January to April 2017 in cerrado soil, located at latitude S 16° 35 '52' 'and longitude W 49° 17' 11 " with an altitude of 723 mpresenting a dry season that is well defined from the months of May to October. The annual mean temperature is 23.2°C. The climate in the region is type Aw, warm and semi-humid, with minimum average of 17.9°C, maximum average of 28.9°C, and annual precipitation of 1578 mm (Pereira, et al., 2010). The average annual air humidity is 71%, presenting the lowest index in August. The meteorological data related to the experimental period presented the following variation: insolation from 142.3h to 239.9h; rainfall of 178.0mm to 368.0mm, and average air temperature of 19.9°C to 31.3°C. Source: Firstclass evaporimeter station of the Sector for Biosystems Engineering of the School of Agronomy, UFG.

The experimental area soil is classified in Dystrophic Red Latosol. For the purpose of its chemical characterization, soil samples were collected at a depth of 0.20 m in order to evaluate the acidity profile and the natural fertility. The results revealed by the soil analysis were as follows: cmolc: Ca - 3.4; Mg – 1.1; K - 0.15; A l- 0.0; H - 2.8; mg.dm³: P (Mehlich)



3.8 and K - 69.0; pH (CaCl₂) - 5.9; V% - 62.0 e M.0. - 1.8g/k.

2.2 Treatment and Experimental Design

The experimental design was a randomized complete block design in a 3 x 4 factorial scheme, evaluating three cultivars of forage millet (ADR - 500, ADR – 7020, and LABH 70732) and four N doses (0.50, 100, and 200 kg.ha⁻¹) in the form of urea. The experimental plots consisted of five rows of five linear meters, spacing 0.30 m, and area of each experimental unit with 6.0 m², totaling 288 m² of area. The soil rotation of the experimental area was performed in a conventional way.

The sowing was carried out on January 6, 2017, adopting a sowing rate of 20 viable pure seeds (PVS) per linear meter. 60 kg of P_2O_5 /ha of phosphate fertilization (Fertilizer: Simple superphosphate), 30 kg of K₂O/ha of potassium (Fertilizer: Potassium chloride), and 50 kg/ha of micronutrients (FTE BR 16) were applied, following the recommendations of Marta-Júnior et al. (2007). Nitrogen and potassium cover fertilization were divided into two applications: 50% of the dose provided in 10 days and the rest 20 days after germination.

The three central rows of each experimental unit were used for evaluation, disregarding the two outer rows and 0.50 m from the extremities, thus leaving 12 linear meters of useful area per experimental unit. To determine biomass production and bromatological composition, manual cutting was performed after 87 days of vegetative growth, using steel scissors 0.15 m above the soil level. Under these conditions, the plants presented between 26 and 28% of the dry mass. Subsamples were collected and dried in a forced ventilation oven for 72 hours at a temperature of 55°C and, then, milled in a Willey mill with 1 mm mesh sieves for laboratory analysis.

2.3 Variables Analyzed

Dry mass (DM), nitrogen contained in the aerial part of the plant (NC), crude protein (CP), neutral detergent fiber, corrected for ash and protein (NDF_{cp}), acid detergent fiber, corrected for ash and protein (ADF_{cp}), hemicelluose (HEM), cellulose, lignin (LIG), mineral matter (MM) was determined according to Detmann et al. (2012).

The apparent nitrogen conversion efficiency (NCE) and apparent nitrogen recovery (ANR) were also considered. These were determined according to the formulas suggested by Carvalho and Saraiva (1987). The nitrogen contained in the roots and residue was not determined. The recovery of N absorbed from the total that was applied considers only the N absorbed by the aerial part of the plants.

The hemicellulose contents (HEM) were estimated using the following formula: HEM (% DM) = NDF (% DM) – ADF (% DM). However, the organic matter (OM) contents were obtained from the ash contents by means of the following formula: OM (% DM) = 100 - Ash (% DM). The non-fibrous carbohydrate (NFC) contents were obtained by the methodology described by Sniffen et al. (1992) in which:

$$NFC = 100 - (NFDcp + CP + EE + Ash)$$



2.4 Statistical Analysis

The variables were submitted to analysis of variance, and the means were compared by the tukey test at 5% probability using the software R (R Core Team, 2013). The polynomial regression analyses of the variables were performed with a level of significance of 95%. In order to evaluate the biomass biomatological composition parameters of the of different varieties of millet under different dosages of nitrogen fertilization (DM; CP NFDcp = neutral detergent fiber, corrected for ash and protein; ADFcp = acid detergent fiber, corrected for ashes and protein; NFC; LIG; MM), the principal components analysis (PCA) was performed with a covariance matrix, testing the percentage composition of each parameter with significance at 95%. Statistical analyses were undertaken using R 3.4.3 computer software (R Core Team, 2017).

3. Results

Nitrogen conversion efficiency (NCE) and apparent N recovery (ANR) differed between doses (P> 0.05). The ANR showed a greater recovery at the 50 kg.ha⁻¹ N dose (81.42%) and lower recovery at a dose of 200 kg.ha⁻¹ N (22.26%) (Table 2). The NCE presented a mean of 54.07 kg of DM/ha⁻¹ at a dose of 50 kg.ha⁻¹ N and 17.59 50 kg of DM/ha⁻¹ N for a dose of 200 kg.ha⁻¹ of N. The interaction between N doses and different millet cultivars was observed for green mass production (GM). On the other hand, dry mass (DM) production, nitrogen contained in the aerial part of the plant (NC), apparent N conversion efficiency (NCE), and apparent N recovery (ANR) differed only as a function of N doses evaluated (P> 0.05), as shown in table 1.

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Table 1. Average values for green mass (GM) production, dry mass (DM), nitrogen contained in the aerial part of the plant (NC) of different cultivars of millet submitted to different doses of nitrogen

		N Doses (kg.ha ⁻¹)				
Variables	Cultivar	0	50	100	200	
GM(t.ha ⁻¹)	ADR500	$31.86 \pm 12.42c$	$46.98 \pm 8.01 Aab$	$43.20\pm5.73b$	$50.96 \pm 6.21 \text{Aa}$	
	ADR7020	$30.54\pm3.15c$	$37.70 \pm 14.59 Bb$	$44.79\pm3.98a$	$48.82 \pm 8.32 \text{Aba}$	
	LABH70732	32.89 ±7.89c	$41.15\pm 6.31ABb$	43.63 ±12.15ab	44.99 ±4.87Ba	
DM(t.ha ⁻¹)	ADR500	$7.66 \pm 3.22 b$	$11.48\pm2.20a$	$11.17\pm2.19a$	$11.86 \pm 1.66a$	
	ADR7020	$8.16 \pm 1.06b$	$10.88 \pm 3.08 ab$	$11.34\pm2.19a$	$12.46\pm2.77a$	
	LABH70732	$8.62\pm2.09b$	$10.19 \pm 1.41 ab$	$10.68\pm3.59a$	$11.02 \pm 1.61a$	
NC(kg ha ¹)	ADR500	$103.6\pm12.03b$	$180.9\pm8.72a$	$154.1\pm7.40ab$	$166.0 \pm 9.79a$	
	ADR7020	$121.1\pm5.55c$	$159.6 \pm 8.48 ab$	$143.4\pm11.29b$	$178.8 \pm 11.02a$	
	LABH70732	$135.6\pm10.40b$	$149.7\pm9.02a$	$168.3\pm9.59a$	$147.5\pm9.09ab$	

GM- Green mass; DM- dry mass; NC- nitrogen contained in the aerial part of the plant. Means followed by lower case letters in the rows and upper case in the columns differ by the tukey test at the 5% probability level.

Table 2. Average values of apparent nitrogen conversion efficiency (NCE) and apparent nitrogen recovery (ANR) of different millet cultivars submitted to different dosages of nitrogen

		N doses (kg.ha ⁻¹)				
Variables	Cultivar	50	100	200		
	ADR500	76.42±16.67Aa	35.10±11.13Ab	18.54±8.69Ac		
NCE	ADR7020	54.30±24.73Aab	31.79±16.51Abc	20.39±11.55Ac		
	LABH70732	31.48±15.48Ba	20.62±12.22Ba	13.85±4.32Bb		
	ADR500	127.02±62.03Aa	49.47±19.34Ab	26.61±15.51Ac		
ANR	ADR7020	92.44±41.80Ba	44.27±25.00Ab	27.09±12.79Ab		
	LABH70732	56.23±23.32Ca	27.19±22.63Bb	13.07±8.44Bc		

NCE- apparent nitrogen conversion efficiency; apparent nitrogen recovery (ANR). Means followed by lower case letters in the rows and upper case in the columns differ by the tukey test at the 5% probability level.

As shown in the polynomial regression equations (Figure 1), the value of maximum dry mass production was observed at the dose of 154 kg of N.ha⁻¹. The maximum value of nitrogen contained in the plant was observed with the dose of 146 kg of N.ha⁻¹.



Figure 1. Regression curves of the variables: (A) dry mass (DM) and (B) contained nitrogen in the aerial part of the plant (NC), determined in millet cultivars submitted to nitrogen fertilization

No interactions were observed between millet cultivars and N doses evaluated for bromatological composition (Table 3). The DM contents of the different cultivars varied from 24.52 to 26.66%, and CP levels ranged from 9.30% to 9.92%. For NDFcp, mean values of 62.57% were obtained; ADFcp averaged at 34.13%; LIG averaged at 6.68%; CEL averaged at 27.45%; HEM average content was 28.35%; and MM average content was 9.22% of the dry matter.



Table 3.	Variable	averages for dr	y matter	r bromato	logical	composi	tion betw	een tre	atments of	of
nitrogen	n doses in	millet cultivar	5							

Variablas	Cultivars	N Doses (kg.ha ⁻¹)				CV(0)	EDM	מ
variables		0	50	100	200	- CV (%)	EPM	r
	ADR 500	24.0	24.5	26.0	23.6		1.182	0.458
DM%	ADR 7020	26.7	29.2	25.3	25.4	9.31		
	LABH 70732	26.3	24.8	23.8	25.3	-		
	ADR 500	8.91	10.38	9.15	9.19			
CP%	ADR 7020	9.91	9.70	8.36	9.23	16.59	0.792	0.573
	LABH 70732	10.4	9.73	10.76	8.81			
	ADR 500	63.7	62.3	63.4	63.2	_		0.641
NDFcp%	ADR 7020	61.9	62.1	61.9	62.7	3.07	0.960	
	LABH 70732	62.9	61.4	63.0	62.5	-		
	ADR 500	33.7	34.1	33.9	33.4	-	0.793	0.270
ADFcp%	ADR 7020	32.4	34.6	35.0	35.0	4.65		
	LABH 70732	34.1	34.4	35.1	34.0	-		
	ADR 500	30.0	28.10	29.50	29.80			
HEM	ADR 7020	29.5	27.50	26.90	27.70	9.34	0.300	0.290
	LABH 70732	28.8	27.00	27.90	28.50			
	ADR 500	26.8	27.0	27.8	27.3			
CEL	ADR 7020	25.7	28.2	29.4	27.7	27.45	0.256	0.100
	LABH 70732	26.8	27.9	28.0	26.9			
	ADR 500	16.6	17.6	16.8	16.5	_		
NFC%	ADR 7020	18.2	17.8	19.8	18.0	15.77	1.366	0.721
	LABH 70732	14.9	17.7	15.7	18.5			
LIG%	ADR 500	6.91	7.11	6.11	6.09	15.15	0.507	0.394
	ADR 7020	6.70	6.41	5.62	7.29			
	LABH 70732	7.29	6.46	7.12	7.15			
MM%	ADR 500	9.06	9.27	8.76	9.31	_		0.270
	ADR 7020	8.74	9.38	8.95	9.01	6.54	0.3018	
	LABH 70732	9.63	9.95	9.54	9.18	_		

DM = dry mass; CP= crude protein; NDFcp = neutral detergent fiber, corrected for ash and protein; ADF = acid detergent fiber, corrected for ash and protein; NFC= non-fibrous carbohydrates; LIG = lignin; MM = mineral matter. Averages do not differ statistically by the tukey test at the 5% probability level

In the bromatological characteristics, the principal components analysis shows that millet varieties do not present significant response differences for the nitrogen fertilization (Figure 2). On the other hand, the bromatological parameters presented relations between them. As demonstrated in the regression analysis, the parameters of CP, N, NFC, and DM were relevant in this analysis. Among the variables, GM, DM, and CEL are positively related, and they are also related to nitrogen fertilization. CP and N were positively related, but they were



inversely proportional. LIG and nitrogen fertilization showed that higher fertilization resulted in lower CP.



Figure 2. Principal component analysis for the biomass biomatological composition of different varieties of millet under different dosages of nitrogen fertilization

(DM = % dry mass; DMt= dry mass (t.ha⁻¹) CP = crude protein; NDFcp = neutral detergent fiber corrected for ash and protein; ADFcp = fiber in acid detergent corrected for ashes and protein; NFC = non-fibrous carbohydrates; LIG = lignin; MM = mineral matter; Treat = Treatment: Triangle - ADR500, White Circle - ADR7020, and Black Circle - LABH70732; Trat - 22.36%, DM- 15.79%, CP-13.83%, N-11.76%, GM_t - 8.53%)

4. Discussion

The GM and DM contents found in this study are similar to those found in the literature (Table 1). In another study, Oliveira et al. (2002) found average yield of green mass of 45.76 t.ha⁻¹ for common millet. This average value was similar to that found in the present study. Distinct results can also be found. When working with cultivars ADR300 and ADR500, Pires et al. (2007) observed GM production close to 62 t/ha, being values higher than those obtained in this study. The authors also report dry matter production on the order of 14.18 t/ha, which also surpasses the average yields reported here. The justifications for the greater production reported by these authors are given according to the methodology adopted by them. A higher sowing rate (40 to 50 seeds per meter), spacing of 0.25 m, and cutting at ground level after 100 days of vegetative growth yielded DM content on average 34.4%. It is interesting to note that the sowing rate adopted in this study was 20 seeds per linear meter, spacing of 0.30 m, and cutting performed to leave a residue of 0.15 m (post-cut) at 87 days of vegetative growth. During this period, the plants had an average DM content of 25.4%, which is the most adequate parameter when the non-standard forage is destined to the silage process.



For the NC values, the control treatment (without nitrogen fertilization) was sufficient (Table 1), since under these conditions, the increase of the dose of N (urea) had no influence on this variable (P > 0.05) in cultivars ADR7020 and LABH70731. This shows that each cultivar has its own physiological capacity to absorb the available N in the soil. In quantitative terms, the NC values for the control treatment of all cultivars can be considered quite satisfactory.

The ANR was higher in ADR500 (78.76%), followed by ADR 7020 (42.68%) and LABH 70732 (21.18%) (Table 2). This reinforces the physiological ability of this forage to absorb the available nitrogen in the soil solution as long as fertility is corrected, according to studies conducted by Silva et al. (2012) and Buso et al. (2015). On the other hand, the cultivars showed a difference in NCE. The cultivar ADR 500 presented the best conversion efficiency with an average value of 43.34 kg DM/kg N applied, followed by ADR 70732 with 35.49 kg DM/kg N applied and LABH 70732 with 21.97 kg DM/kg N applied. For Martha Júnior et al. (2007) in the case of tropical grasses, N-fertilizer conversion efficiency can reach values of up to 83 kg DM/kg N applied, presenting an average efficiency of 26 kg of DM/kg N applied. According to these authors, the best efficiencies are determined with doses up to 150 kg of N.ha⁻¹.

The ANR differed between doses of N ranging from 21.98 to 86.54%, and there was a mean value of 47.88% (Table 2). The highest recovery rate of N (86.54%) was determined at the lowest applied N dose (50 kg.ha⁻¹), while the lowest N recovery (21.98%) occurred at the highest dose (200 kg.ha⁻¹). This confirms the statements of Martha Júnior et al. (2004), concluding that lower doses of N result in higher ANR rates. It is important to emphasize that factors such as nitrogen source and application management can influence this variable.

According to the polynomial regression equations (Figure 1), the values of maximum dry matter and nitrogen content in the plant were observed close to the dose of 150 kg of N.ha⁻¹, corroborating with results found by Guimarães Júnior et al . (2009) and Silva et al. (2012).

The crude protein (CP) content of millet cultivars evaluated in the present study (mean of 9.54%) also resemble those found by Guimarães Júnior et al. (2010), who evaluated pearl millet genotypes for the ensiling process and found an average of 10.95% CP in the dry matter. Higher concentrations of CP in the millet crop (close to 20%) have already been reported by Silva (2012) and Buso et al. (2015), but this was in a cut-off regime. Thus, the CP concentrations decrease with biomass accumulation, as a function of the maturity process of the forage crop, but this does not limit its use in animal feed. According to Sampaio et al. (2009), CP levels higher than 7% are considered for the maintenance of rumen microorganism activity.

The NDF and ADF levels were not influenced by N rates. These variables were more influenced by the physiological stage of the plant. As reported by Faria Júnior et al. (2013), NDF levels were higher in plants with advanced physiological stage, ranging from 54.81% at 31 days to 71.99% NDF at 65 days. The cut at 87 days of vegetative growth leads to a decrease in CP levels, and this causes the thickening of the cell wall and the lignification of its tissues as maturity occurs, which may imply higher NDF and ADFFDA values. The NDF represented by cell wall hemicellulose, cellulose, and lignin negatively correlates with



voluntary consumption, while acid detergent fiber with respect to cellulose and lignin negatively correlates with digestibility. According to Van Soest (1965), the DM consumption becomes negative when the cell wall content of the forage crop is above 55%, which is the lower limit of the forage and cultivars evaluated in the present study.

The average cellulose value of is within the ranges reported in the literature, being 27.45% (Table 3). According to Van Soest (1994), this polysaccharide of greater abundance in the nature and main constituent of the cell wall presents variation of 20 to 40% in the DM of higher plants. For hemicellulose, the mean value found in this study was 28.35%. However, this cannot be considered detrimental to forage crop, since hemicellulose is a homogeneous mixture of amorphous polysaccharides with polymerization degree much lower than that of cellulose (Van Soest, 1994).

The mean LIG values reported in the present study (8.92%) are above those reported by Guimarães Júnior et al. (2005) (4.16% to 4.44%). Lignin is a polymer that associates with structural carbohydrates, cellulose, and hemicellulose during the process of cell wall formation, negatively influencing the digestibility of forage crops (Van Soest and Wine, 1968). This suggests that cuts of this grass at earlier stages of growth guarantee better digestibility of this grass.

The mean levels of MM (9.22%) are within those described in the literature. However, this variable can be altered as a function of plant maturity and soil fertility. Although they are required in small quantities by animals, the mineral elements are essential to all other nutrients (carbohydrates, lipids, and proteins), being very important in energy metabolism (Wood et al., 1983).

The mean values of NFC found in this work are similar to those found by Possenti et al. (2005) for ensilage maize plants (*Zea mays*), considered a standard silage crop. Non-structural carbohydrates include the carbohydrates found in the cell contents, such as glucose, fructose, plant carbohydrates (starch, sucrose, and fructose), and they are the main substrate for the production of lactic acid, which is of fundamental importance for the reduction of pH in the ensiling process. Thus, the presence of high NFC contents in the dry mass of forage millet increases the possibilities of using this crop, which can also be used in silage production.

Although NFC presented a positive relation with the fertilization treatment, it was also inversely proportional to CP and N in the bromatological characteristics. Thus, in general, the multivariate analysis corroborates with the analysis of variance, in which they emphasize that nitrogen fertilization increases the biomass production of the different varieties of millet; however, they alter the bromatological composition of this same biomass, as the percentages of proteins are reduced in this study. In a study with millet, Pacheco et al. (2014) also observed a reduction in percentages of CP with the increase of nitrogen fertilization, and this is related to the higher growth rates of biomass, causing lower percentages of protein in the forage biomass.



5. Conclusions

Under the conditions of the present study, nitrogen fertilization did not influence the dry mass production and did not improve the bromatological composition of ADR-500, ADR 7020, and LABH 70732 forage millet cultivars. However, both cultivars present high biomass production potential and satisfactory bromatological composition for use in ruminant feed.

As expected, nitrogen conversion efficiency and apparent nitrogen recovery decreased with increasing doses. Compared with cultivar LABH70731, cultivars ADR 500 and ADR 7020 showed higher efficiency in the conversion and apparent nitrogen recovery.

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