Abstract

M.N. Lopes, M.J.D. Cândido, R.C.F.F. Pompeu, R.G. da Silva, C.F. de Lacerda, and T.D. Maranhão. 2020. Yield and chemical composition of massai grass fertilized with nitrogen. Int. J. Agric. Nat. Resour. 69-78. Nitrogen fertilization, in addition to increasing biomass production, can also positively influence the chemical composition of a forage plant. The aim of this research was to evaluate the yield and chemical composition of *Megathyrsus maximus* cv. Massai grass under five nitrogen fertilization levels (0, 150, 300, 450 and 600 mg N dm⁻³ soil, equivalent to 0, 300, 600, 900 and 1,200 kg ha⁻¹ year⁻¹, respectively) and during growth cycles (establishment and regrowth cycles) in a greenhouse. A completely randomized design with split-plot arrangement was adopted in which the five nitrogen levels were the plots and the cycles were the subplots. The total forage biomass increased with the nitrogen fertilization levels in the three growth cycles (28.4 g pot⁻¹, 32.0 g pot⁻¹ and 29.8 g pot⁻¹ for establishment, regrowth 1 and regrowth 2, respectively, at the level of 600 mg N dm⁻³), and it was reduced from establishment to regrowth at lower N levels. The dry matter content was not affected by the N levels. However, this content presented higher values at the regrowth cycle in comparison to the establishment cycle. The crude protein (CP) content increased (7.0% CP at the level of 600 mg N dm⁻³), while the neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (HEM) contents all decreased at higher N levels, with the highest CP content being observed at the establishment, and the opposite occurring for NDF, ADF and HEM. Nitrogen fertilization provides positive responses on yield and chemical composition of massai grass.

Keywords: Crude protein, forage biomass, *Megathyrsus maximus*, neutral detergent fiber, nitrogen fertilization.

Abbreviations used: N (nitrogen); TFB (total forage biomass); DM (dry matter); CP (crude protein); NDF (neutral detergent fiber); ADF (acid detergent fiber); HEM (hemicelluloses).
Introduction

The need to intensify grazing production systems has increased the demand for studies on forage cultivars and their responses to intensive management. Massai grass (*Megathyrsus maximus*) has been shown to be promising and well adapted to intensive management because of its great capacity to emit leaves (Lopes *et al.*, 2013a) and tillers (Lopes *et al.*, 2016) and its quick regrowth after cutting or grazing (Lopes *et al.*, 2011).

The production potential of a forage plant is determined genetically, but for this potential to be reached, abiotic conditions and management should be observed (Fagundes *et al.*, 2005). The perennialism and productivity of forage plants demand adequate replacement of nutrients, which can be achieved through maintenance fertilization. Among the nutrients, nitrogen stands out in forage production (Fagundes *et al.*, 2006) as it provides an increase in tillering (Alexandrino *et al.*, 2004) and consequently in forage biomass production (Magalhães *et al.*, 2006).

Nitrogen fertilization, besides enhancing the growth rate, also influences the chemical composition of forage plants (Benett *et al.*, 2008; Mesquita & Neres, 2008). The positive effect of nitrogen on the crude protein (CP) content of forage plants can be verified in the studies conducted by Andrade *et al.* (2003) and Rodrigues *et al.* (2005) in which the CP content of the forages increased with the increase of the nitrogen fertilization. Nitrogen fertilization may also lead to a decrease in the neutral detergent fiber (NDF) content (Vitor *et al.*, 2009; Mota *et al.*, 2010), although contradictory responses, such as positive responses of the NDF content to the nitrogen application, have also been verified in the literature (Rocha *et al.*, 2002).

The chemical composition is one of the parameters used in the evaluation of the nutritive quality of forage plants. This approach shows the importance of knowing the chemical composition of forage plants in response to factors, such as environment and management, especially regarding nitrogen fertilization, as this nutrient has a great influence on the biomass production of forage plants. In this context, the objective of this research was to evaluate the yield and chemical composition of massai grass during the establishment and regrowth phases under different levels of nitrogen fertilization.

Materials and Methods

Description, treatments, and experimental design

This research was performed in a greenhouse at the Department of Plant Science of the Federal University of Ceará (UFC), in Fortaleza, Ceará (Brazil). Fortaleza represents climate type Aw’, rainy, tropical, according to the Köppen climate classification (Köppen, 1948). Minimum and maximum temperatures were recorded daily (inside the greenhouse), with mean values of 25.8 and 40.5 °C (establishment), 25.6 and 40.6 °C (regrowth 1), and 25.9 and 40.9 °C (regrowth 2), respectively.

The forage plant used was *Megathyrsus maximus* cv. Massai (massai grass). Five nitrogen levels were assessed (0, 150, 300, 450 and 600 mg N dm⁻³ soil), which are equivalent to 0, 300, 600, 900 and 1,200 kg ha⁻¹ year⁻¹, respectively, and growth cycles (establishment, regrowth 1 and regrowth 2) were used for the evaluation of total forage biomass, establishment and regrowth, and for chemical composition, considering the average of the regrowth cycles. The treatments (nitrogen levels x growth cycles) were evaluated following the principles of a completely randomized design in split plot in time, with five replications, considering the five nitrogen levels as plots and the growth cycles (establishment, regrowth 1 and regrowth 2) as subplots.

The soil used in the experiment was Yellow Argisol. After collected, the soil was sieved for better
homogenization and retention of coarse materials. Soil samples from the 0 – 20 cm-deep layer presented the following chemical composition: 4 mg dm$^{-3}$ of P; 76 mg dm$^{-3}$ of K; 2.0 cmol$_c$ dm$^{-3}$ of Ca$^{2+}$; 1.9 cmol$_c$ dm$^{-3}$ of Mg$^{2+}$; 0.0 cmol$_c$ dm$^{-3}$ of Al$^{3+}$; 11 mg dm$^{-3}$ of Na$^+$; 9.10 g kg$^{-1}$ of organic matter; sum of bases (SB) = 4.14 cmol$_c$ dm$^{-3}$; total cation exchange capacity (tCEC) = 4.14 cmol$_c$ dm$^{-3}$; pH in water 5.7; 19.0 mg dm$^{-3}$ of Fe$^{2+}$; 0.14 mg dm$^{-3}$ of Cu$^{2+}$; 3.91 mg dm$^{-3}$ of Zn$^{2+}$ and 12.18 mg dm$^{-3}$ of Mn. For the fertilization, the recommendation of Ribeiro et al. (1999) was followed for fertility levels suggested for grasses with high yield potential and high technological level.

The pots were randomly distributed inside the greenhouse. When filling the pots, which had a capacity of 10 dm$^3$ each, the soil correction was performed with the application of 6.169 g pot$^{-1}$ of limestone, according to the fertility analysis, aiming to increase calcium content and pH value. The samples received daily irrigation for ten days to speed up the limestone reaction.

The control of the daily irrigation to be applied was done by installing mercury tensiometers (Hg) in the pots (two tensiometers per treatment), with water replacement when the mercury column (h) reached approximately 10 cm (approximate $\psi_m$ = –7.98 kPa) in the treatments supplied with higher levels of nitrogen (600 mg N dm$^{-3}$ soil). The treatment that received the 600 mg N dm$^{-3}$ of soil was the reference for the water replacement applied in the other treatments, according to the equation $\psi_m = -12.6 \ h + h_1 + z$, where $\psi_m$ = matric potential in cm of water column; $h$ = height of mercury column (Hg) in the gauge in cm; $h_1$ = height of the Hg level in the gauge in cm, in relation to the soil surface, and $z$ = depth of the porous cup in cm (10 cm) (Amaro Filho et al., 2008). At the end of the irrigation, the Hg column height in the gauge (cm) was registered, which presented approximately 5.0 cm (approximate $\psi_m$ = –1.80 kPa) for all treatments. Thus, the soil moisture conditions were kept near field capacity in all experimental units.

Sowing was performed using an average of 50 seeds per pot. Prethinning was done eight days after emergence maintaining 12 plants per pot, which were reduced to three after the final thinning, 13 days after emergence.

Three cuts were performed; the first cut was carried out for uniformity (at the end of the establishment cycle), which used the horizontal length of the pseudo stem as a reference. Based on that, all plants in the pot were cut, with only a residue of 10 cm of stem length remaining, 43 days after seedling emergence. This criterion was adopted with the aim to standardize the cut plants, since they presented growth with different inclination angles of the tiller, impairing the cutting uniformity in case the cut was done at a fixed vertical height. The second and third cuts (at the end of the regrowth 1 and regrowth 2 cycles, respectively) followed the same criterion and were performed after a rest period of 28 days.

Fertilizations of phosphate (simple superphosphate), potassium (potassium chloride) and micronutrients (FTE BR-12, with 0.1% Mo, 0.8% Cu, 1.8% B, 2.0% Mn, 3.0% Fe, and 9.0% Zn) were performed according to the results of the soil analysis as well as the application of limestone. The applications of nitrogen (urea) and potassium were split. At the establishment cut, the nitrogen dose for each treatment was divided into two; the first half was applied soon after final thinning, and the second half was applied after 14 days. In all nitrogen applications, the urea was diluted in the irrigation water for a more uniform fertilizer application. The nitrogen levels assessed (0, 150, 300, 450 and 600 mg N dm$^{-3}$ soil) were calculated for pots that had the capacity of 10 dm$^3$ each.

The potassium was supplied in two applications; the first (120 mg dm$^{-3}$ of K$_2$O) was applied at the moment of sowing, and the second (120 mg dm$^{-3}$ of K$_2$O) was applied via water solution, which was performed soon after the uniformity cut along with the first nitrogen dose at regrowth 1. Phosphorus
supply (125 mg dm\(^{-3}\) of P\(_2\)O\(_5\)) was performed once at the time of sowing. At that moment, micronutrients (25 mg dm\(^{-3}\) of FTE BR-12) were also provided. The second half of the nitrogen level for each treatment applied at regrowth 1 was applied in the middle of the rest period (28-day cycles). At regrowth 2, the same management was followed.

Response variables

At the end of each cycle, following the rest period adopted for the forage plant, all plants of each experimental unit were harvested and taken to the laboratory. The samples were placed in a forced ventilation oven (55 °C until reaching constant weight) for later calculation of the total forage biomass yield (TFB) in a dry matter (DM) basis (g DM pot\(^{-1}\)).

For the chemical determinations, the growth of establishment and the average of the two regrowth cycles (representing the growth in regrowth 1 and 2, separately, due to the little amount of dry biomass of the treatment control) were evaluated. After predrying, the contents of DM (\%, method ID 930.15), crude protein (CP, \% in the DM, method ID 984.13) (Helrich, 1990), neutral detergent fiber (NDF, \% in the DM), acid detergent fiber (ADF, \% in the DM) and hemicellulose (HEM, \% in the DM) were analyzed (Van Soest & Robertson, 1985; Van Soest \textit{et al.}, 1991).

Data analysis

The statistical model used in the experiment was as follows:

\[
y_{ijk} = \mu + \alpha_i + \varepsilon_{i(k)} + \tau_j + (\alpha\tau)_{ij} + \varepsilon_{ijk},
\]

where \(y_{ijk}\) = the value observed in the experimental plot that received level \(i\) of factor \(\alpha\) (nitrogen fertilization) and level \(j\) of factor \(\tau\) (growth cycles) in replication \(k\);

\(\mu = \) the general constant;

\(\alpha_i = \) the effect of level \(i\) of factor \(\alpha\) \((i = 1, 2, 3, 4,\) and 5);

\(\varepsilon_{i(k)} = \) the effect of level \(i\) of factor \(\alpha\) in replication \(k\) (error a);

\(\tau_j = \) the effect of level \(j\) of factor \(\tau\) \((j = 1, 2,\) and 3 for the evaluation of total forage biomass and \(j = 1\) and 2 for chemical composition);

\((\alpha\tau)_{ij} = \) the effect of the interaction between level \(i\) of factor \(\alpha\) and level \(j\) of factor \(\tau\); and

\(\varepsilon_{ijk} = \) the experimental error (error b).

The data were subjected to analysis of variance, means comparison test and regression analysis. The interaction of fertilization levels and growth cycles was analyzed when significant at P<0.05 through the F test. The effect of the nitrogen fertilization levels was assessed by regression analysis. The growth cycles were compared by Tukey’s test. The models were chosen based on the significance of the linear and quadratic coefficients through Student’s t-test (P<0.05) and on the coefficient of determination. SAS (Statistical Analysis System, version 9.0, SAS Institute, Inc., Cary, NC) procedures MIXED and GLM were employed.

Results

Total forage biomass

An interaction effect (P<0.05) between nitrogen levels and growth cycles was observed on TFB, which increased (P<0.01) as the nitrogen levels increased in the three growth cycles (Figure 1) but decreased (P<0.05) from the establishment to the regrowth cycles in the absence and at lower levels of nitrogen (Figure 1). The interaction effect reflects the increase of TFB that is due to the N levels being more pronounced in regrowth
2 in comparison to the increments observed in the regrowth 1 cycles and establishment (Figure 1). The total forage biomass ranged from 16.7 to 28.4 g pot\(^{-1}\) (establishment), from 9.4 to 32.0 g pot\(^{-1}\) (regrowth 1) and from 3.5 to 29.8 g pot\(^{-1}\) (regrowth 2) for the levels 0 to 600 mg N dm\(^{-3}\). Increases of 71% (establishment), 240% (regrowth 1) and 751% (regrowth 2) were observed at the level 600 mg N dm\(^{-3}\) in comparison to the absence of nitrogen fertilization.

**Chemical composition**

For DM content, no effect of the interaction between growth cycles and nitrogen levels was observed (P>0.05). An increase (P<0.05) in DM content was observed when comparing the establishment (29.3%) to the average of the two regrowth cycles (32.3%) (Figure 2), and the DM content was not affected (P>0.05) by the nitrogen fertilization.

The CP and NDF contents were not affected (P>0.05) by the interaction between nitrogen levels and growth cycles, with significance (P<0.05) being limited to the main factors. A positive linear response (P<0.01) was observed for the CP content (Figure 3A), while for the NDF content, a linear decrease was observed (P<0.01) as the nitrogen levels increased (Figure 3B). Estimated values of 6.0 to 7.0% for CP and 77.4 to 70.3% for NDF were observed for the levels of 0 and 600 mg N dm\(^{-3}\), respectively, corresponding to an increase of 17.4% in the CP content and a reduction of 10.1% in the NDF content, at the level 600 mg N dm\(^{-3}\) in comparison to the control treatment (absence of N). The CP content reduced (P<0.05) when comparing the establishment (7.1% CP) to the mean of the two regrowth cycles (5.9% CP) while the NDF responded inversely and was higher (P<0.05) in the regrowth cycles (Figure 2).

For ADF and HEM contents, no interaction was observed between growth cycles and nitrogen levels (P>0.05), but both variables increased (P<0.05) from the establishment (31.9% of ADF and 32.9% of HEM) to the average of the two regrowth cycles (34.0% of ADF and 37.0% of HEM) (Figure 2). For the contents of ADF (Figure 4A) and HEM (Figure 4B), a decreasing linear response (P<0.01) was observed with the increase in the nitrogen levels, revealing estimated values of 33.9 and 32.0% of ADF and 36.9 and 32.9% of HEM for 0 and 600 mg of N dm\(^{-3}\) of soil, respectively.
Discussion

Total forage biomass

The plants that had higher nitrogen input presented higher TFB production (Figure 1), thus proving the beneficial effect of the nitrogen fertilization in intensively managed pastures (Fagundes et al., 2006). The influence of nitrogen on TFB in massai grass can be attributed to the increase in the leaf appearance rate (Martuscello et al., 2005) and the effect of nitrogen on the leaf elongation rate (Martuscello et al., 2006; Lopes et al., 2013), which can be especially attributed to the increase in cell production (Volence & Nelson, 1984) and to the influence of nitrogen on physiological processes of the plant (Fagundes et al., 2005), which contributes to the growth and development of the forage plant. It is also worth mentioning the positive effect of nitrogen on the tiller population density (TPD), which contributes to the increase in total forage biomass of massai grass (Lopes et al., 2011). With successive cuts, a more pronounced reduction in TFB at lower levels was observed, which denotes greater nitrogen depletion and is due to the lower dose applied (Figure 1).

Chemical composition

The DM content was not influenced by the nitrogen fertilization, and this may be a relevant indicator of the adequate adjustment of the irrigation management in which the water demand of the different treatments under nitrogen levels was treated differently according to the daily quantity of water for each level of nitrogen. Thus, treatments of higher nitrogen levels presented higher water demand and were supplied with larger daily water amounts. Such management in the water...
supply possibly provided equilibrium on water balance in the plant for the different nitrogen levels, thus neutralizing some indirect effect of the nitrogen levels on the dry matter content of massai grass. An increase in the DM content was observed when comparing the establishment to the average of the two regrowth cycles (Figure 2), reflecting the slower growth of the grass at the establishment, resulting in lower physiological age, despite the higher chronological age (43 vs. 28 days).

The increase in CP content with increasing nitrogen levels (Figure 3A) occurred due to the relevant role of this nutrient. After absorption, nitrogen is reduced to the ammoniacal form, and when it is combined in the organic chains, it forms glutamic acid, which is a precursor of different amino acids, from which approximately 20 are used in protein formation (Raij, 1991). Thus, the increase observed for CP content reflects the greater presence of free amino acids, which maintain the nitrogen in its structure, and in the form of small peptides in the plant tissue, in response to the higher nitrogen input in the soil (Freitas et al., 2007). Despite the increase in the CP content, it is worth noting that only at the highest level (600 mg N dm⁻³) did this variable reach the critical value (7.0%) proposed by Van Soest (1965). According to this author, with CP contents lower than 7.0%, a reduction in intake and digestion of forage occurs because of inadequate levels of nitrogen for rumen microorganisms. To meet the protein requirements of ruminant animals, it is necessary to formulate a diet with CP content higher than 7.0% as reported by Bennett et al. (2008). In this study, the conditions of elevated temperature (greenhouse), the fixed rest period for all nitrogen levels and probably the effect of dilution of nitrogen on forage biomass, contributed to the small increments on CP content, even at high levels of nitrogen. Therefore, in order to overcome a low CP content in a pasture, managers should design management strategies based on the morphophysiological characteristics of massai grass. This includes, for example, defining the moment of defoliation based on variables related to the physiology of the pasture, such as interception of photosynthetically active radiation, number of leaves per tiller, and beginning of leaf senescence, to balance quantity and quality without compromising the perennialism and productivity of the pasture.

The reduction observed for the NDF content (Figure 3B) probably occurred because of the nitrogen stimulating effect, causing the rise of new axillary tillers, which present smaller leaves that also have a smaller midrib (Andrade et al., 2003), resulting in lower proportion of fibrous tissue. The response pattern observed for the NDF content in the present study shows the beneficial effect of nitrogen fertilization on this variable, corroborating the results found by Vitor et al. (2009) and Mota et al. (2010). These authors observed a decrease in NDF content along with nitrogen fertilization, which may be related to the fact that nitrogen stimulates the growth of new plant tissues that have lower contents of structural carbohydrates, leading to a reduction in the percentage of NDF.

The response observed for both variables (higher CP content and lower NDF content in the establishment) (Figure 2) occurred because of the higher proportion of young tillers in the establishment, resulting in a higher proportion of biomass components with physiologically younger tissues in the establishment cycle, as verified during the biomass flow assessments (Lopes et al., 2013b). With the greater presence of physiologically younger tissues, the participation of cellular content is higher, in detriment to the constituents of the cell wall, explaining the 1.2 and 6.1 percentage points of difference for the CP and NDF contents, respectively, from the establishment to the regrowth.

The effect of nitrogen in both constituents of the cell wall (ADF and HEM) possibly reflects the favoring of the morphogenic process, with the more intense flow in massai grass (Lopes et
of the regrowth cycles is explained by the effect of the growth cycle factor on the NDF and ADF variables, which increased from the establishment to the regrowth, since the variable in consideration (HEM) is the result of the difference between the concentrations of NDF and ADF (HEM = NDF – ADF).

Nitrogen fertilization promotes positive responses on forage biomass production and the chemical composition of massai grass up to the nitrogen level of 600 mg dm⁻³ of soil. The forage biomass yield and the chemical composition of massai grass are modified by the growth cycles, with more pronounced differences in productivity found between cycles at the lowest nitrogen levels and with better quality forage biomass found in the establishment growth.

The observed response for the ADF content (increasing from the establishment to the average of the regrowth cycles) occurred because of the faster growth of the grass during regrowth, leading to a higher physiological age, despite the lower chronological age, as previously mentioned. The superior hemicellulose content in the average of the regrowth cycles is explained by the effect of the growth cycle factor on the NDF and ADF variables, which increased from the establishment to the regrowth, since the variable in consideration (HEM) is the result of the difference between the concentrations of NDF and ADF (HEM = NDF – ADF).

Nitrogen fertilization promotes positive responses on forage biomass production and the chemical composition of massai grass up to the nitrogen level of 600 mg dm⁻³ of soil. The forage biomass yield and the chemical composition of massai grass are modified by the growth cycles, with more pronounced differences in productivity found between cycles at the lowest nitrogen levels and with better quality forage biomass found in the establishment growth.
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