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Response of *Brachiaria brizantha* cv. Piatã pastures to nitrogen fertilization

Resposta de pastagens de *Brachiaria brizantha* cv. Piatã à fertilização nitrogenada

Respuesta de pasturas de *Brachiaria brizantha* cv. Piatã a la fertilización nitrogenada

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Abstract

With the objective to evaluate the effects of nitrogen levels (0, 40, 80, 120 and 160 kg of N ha^{-1}) on green dry matter (GDM) yield and morphogenetic and structural characteristics and nitrogen nutrition index (NNI) of *Brachiaria brizantha* cv. Piatã, was installed an experiment under field conditions in Roraima´s savannas. Nitrogen fertilization increased significantly (P<0.05) GDM yields, number of tillers, number of leaves tiller^{-1}, average leaf size, leaf area index, leaf senescence rate, leaf appearance and elongation rates. Maximum GDM yields, leaf elongation rates, leaf length and number of leaves tiller^{-1} were obtain with the application of 145.9; 118.2; 108.9 and 133.6 kg of N ha^{-1}, respectively. Nitrogen nutrition index alone with 120 or 160 kg N application was higher than the grass N internal critical level. The NNI, efficiency of utilization and apparent N recovery were inversely proportional to the increased N levels.

Keywords: green dry matter; N-efficiency utilization; N-apparent recovery; senescence; tillering

Resumo

Com o objetivo de avaliar os efeitos da adubação nitrogenada (0, 40, 80, 120 e 160 kg de N ha^{-1}) sobre a produção de forragem, características morfogênicas e estruturais e índice de nutrição nitrogenada (INN) de *Brachiaria brizantha* cv. Piatã foi realizado um experimento em condições de campo nos cerrados de Roraima. A adubação nitrogenada afetou positiva e significativamente (P<0,05) a produção de matéria seca verde (MSV), número de perfilhos, número de folhas perfilho^{-1}, tamanho médio de folhas, índice de área foliar e taxas de aparecimento, expansão e senescência das folhas. Os maiores rendimentos de MSV, taxa de expansão foliar, tamanho médio de folhas e número de folhas perfilho^{-1} foram obtidas com a aplicação de 145,9; 118,2; 108,9 e 133,6 kg de N ha^{-1}, respectivamente. O nível crítico de N foi reduzido com o aumento da produtividade de forragem. O INN, para todos os níveis de N avaliados, foi insuficiente para suprir o nível crítico de N da gramínea. Os níveis críticos de N, a eficiência de utilização e sua recuperação aparente foram inversamente proporcionais às doses de N aplicadas.

Palavras–chave: eficiência de utilização de nitrogênio; matéria seca verde; perfilhamento; senescência; recuperação aparente de nitrogênio

Resumen

Con el objetivo de evaluar el efecto de la fertilización nitrogenada (0, 40, 80, 120 y 160 kg N ha^{-1}) sobre el rendimiento del forraje, las características morfogénicas y estructurales y el índice de nutrición de nitrógeno (INN) de *Brachiaria brizantha* cv. Piatã fue realizado un experimento en condiciones de campo em las sabanas de Roraima. La fertilización nitrogenada afectó positiva y significativamente ($P<0.05$) el rendimiento de la materia seca verde (MSV), el número de macollas, el número de hojas $macollas^{-1}$, el tamaño promedio de las hojas, el índice de área foliar, las tasas de aparición, expansión y senescencia de las hojas. Los rendimientos más altos de MSV, la tasa de expansión de la hoja, el tamaño promedio de la hoja y el número de hojas $macollas^{-1}$ se obtuvieron com la aplicación de 145,9; 118,2; 108,9 y 133,6 kg N ha^{-1}, respectivamente. El INN solo con la aplicación de 120 o 160 kg de N ha^{-1} fue mayor que el nivel crítico interno de N de gramínea. El nivel crítico de N se redujo al aumentar la productividad del forraje. Los niveles críticos de N, la eficiencia de utilización y la recuperación aparente de N fueron inversamente proporcionales a las tasas de N aplicadas.

Palabras-clave: macollamiento; materia seca verde; N-eficiencia de uso; N-recuperación aparente; senescência

1. Introduction

In the Western Amazon, about ten million hectares of forests are currently occupy with cultivated pasture. About 40% have pastures in different stages of degradation, which reflects the continuing need for sustainable management practices to reduce new deforestation and ensure adequate livestock feeding, avoiding the maintenance of itinerant and environmentally undesirable livestock (Costa et al., 2009).

In tillage and burning, non-volatile nutrients from forest biomass are incorporate into the soil in the form of ashes, which implies increased soil pH and fertility. However, high fertility is only temporary. Nitrogen (N) can be lost by leaching, volatilization (transformation into gas) or immobilization, a process where the nutrient becomes unusable by the plant, its deficiency being pointed as one of the main causes of pasture degradation (Costa et al., 2009; Silva, 2018; Freitas et al., 2019).

Proteins, amino acid sequence, represent the major form of organic N present in plants. Its molecules contain only 16% of this nutrient, representing about 1 to 5% of plant biomass. Its positive effect on grass forage primary productivity will be maximize when there is synchronization with C fixation, biosynthesis, by improving photosynthetic efficiency and prioritize carbon allocation to shoots, resulting in increased photosynthetic area (Liu et al., 2017; Taiz et al., 2017; Silva, 2018). Much of the N involved in photosynthesis is presented as a soluble protein, notably in the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisco), one of the main components of the Benson-Calvin Cycle regulation process in chloroplasts and the respiratory enzymes of peroxisomes and mitochondria in carbonic anhydrase and ribosomes. N integrates structures such as the chloroplast tilak membranes and participates in protein, pigment and electron transporter complexes (Taiz & Zeiger, 2013), as well as composing DNA, the nucleic acid that defines protein synthesis (Silva, 2018; Liu et al., 2019).

In this work were evaluated the effects of nitrogen fertilization on forage yield and morphogenic and structural characteristics and nitrogen nutrition index of *Brachiaria brizantha* cv. Piatã.

2. Methodology

The experiment was carried out at Embrapa Roraima Experimental Field, located in Boa Vista, from May to September 2013. The soil of the experimental area is a Yellow Latosol, medium texture, with the following chemical characteristics, at a depth of 0-20 cm: pH (H_2O) = 4.7; P = 1.8 mg kg^{-1}; Ca + Mg = 0.98 $cmol_c.dm^{-3}$; K = 0.03 $cmol_c.dm^{-3}$; Al = 0.58 $cmol_c.dm^{-3}$; H + Al = 2.64 $cmol_c.dm^{-3}$. The experimental design was completely random with three replications. The treatments consisted of five nitrogen levels (0, 40, 80, 120 and 160 kg of N ha^{-1}), applied as urea. The size of the plots was 2.0 x 2.0 m, with a usable area of 1.0 m^2. Nitrogen application was splitted twice, half when pasture was clear at the beginning of the experiment and half after 45 days. During the experimental period three cuts were performed at 45-day intervals.

The parameters evaluated were green dry matter (GDM) yield, nitrogen utilization efficiency (NUE), number of tillers m^{-2} (NT), number leaves $tiller^{-1}$ (NLT), leaf appearance rate (LAR), leaf expansion rate (LER), leaf senescence rate (LSR), average leaf size (ALS) and leaf area index (LAI). The LER and LAR were calculate by dividing the accumulated leaf length and the total number of leaves in the tillers, respectively, by the regrowth period. The ALS was determined by dividing the total leaf elongation of the tillers by the number of leaves.

To calculate the leaf area, samples of completely expanded green leaves was collect, trying to obtain an area between 200 and 300 cm^2. The samples were digitalize and the leaf area estimated with the aid of an electronic optical planimeter (Li-Cor 3100C). Subsequently, the samples was take to the greenhouse with forced air at 65ºC until they reached constant weight, obtaining the leaf GDM. Specific leaf area (EFA) was determinate by the relationship between green leaf area and its GDM (m^2/g leaf GDM). The leaf area index (LAI) was determinate from the product of the total green leaf GDM (g GDM/m^2) by AFE (m^2/g leaf GDM). The TSF was obtain by dividing the length of the leaf that was yellowish or necrotic by the regrowth age.

NUE was estimate by relating the GDM yield to the applied N dose. N contents were measure by micro-Kjeldahl method. Apparent nitrogen recovery (ANRec.) was obtain by the formula: ANRec. = 100 x N extracted from fertilized plants - N extracted from unfertilized plants ÷ applied N dose. The critical nitrogen level (CNL) was determined by the methodology described by Lemaire et al. (1984a), Lemaire et al. (1984b), for C_4 grasses, by the formula: CNL = 3.6.GDM $^{(-0.34)}$, which estimates the N dilution curve as a function of GDM accumulation. Nitrogen nutrition index (NNI) was obtained by the ratio between the N content in GDM and the NCN, being considered satisfactory when the result is equal to or higher than 1.0 (Lemaire & Gastal, 1997; Lemaire & Maynard, 1997).

Data were subject to analysis of variance and regression considering the significance level of 5%, using the statistical software Sisvar (Ferreira, 2011). To estimate the response of the evaluated parameters, as a function of the nitrogen fertilization levels, the choice of regression models was based on the significance of the linear and quadratic coefficients by Student's t-test.

3. Results and Discussion

Nitrogen fertilization affected ($P < 0.05$) the yields of GDM, being the quadratic relationship described by the equation: $Y = 1{,}843 + 26.304\,X - 0.0901\,X^2$ ($R^2 = 0.95$) and the maximum value estimated with application of 145.9 kg of N ha^{-1} (Table 1). For *B. brizantha* cv. Piatâ, Menezes et al. (2019) did not detect significant effect of nitrogen fertilization (100 or 200 kg N ha^{-1}) on total biomass, leaves and dead material, regardless of defoliation frequency (28 or 35 days), whereas Avelino et al . (2019) reported higher forage yields with the application of 200 or 300 kg of N ha^{-1} at both evaluated defoliation intensities (8, 16 24 and 32 cm above ground). Similarly, Nascimento et al. (2019) found a linear effect of nitrogen fertilization on the green biomass yield of *B. brizantha* cv. Paiaguás with the application of up to 250 kg of N ha^{-1}. However, the highest rates of efficiency of N utilization were estimated with the application of 50 (56.94 kg of GDM/kg of N) or 100 kg of N ha^{-1} (32.86 kg of GDM/kg of N). For *Brachiaria hibrida* cv. Mulato II, Teixeira et al. (2018) observed that the application of 100 kg of N ha^{-1} $year^{-1}$ was necessary to maximize the effect of phosphate fertilization (0; 45; 90; 135 and 180 kg of P_2O_5 ha^{-1}).

Table 1. Green dry matter (GDM - kg ha^{-1}) yields, number of tillers m^{-2} (NT), number of leaves $tiller^{-1}$ (NLT), leaf appearance rate (LAR - leave day^{-1} $tiller^{-1}$), leaf expansion rate (LER - cm day^{-1} $tiller^{-1}$), average leaf size (ALS - cm), leaf area index (LAI) and leaf senescence rate (LSR) of *Brachiaria brizantha* cv. Piatã, as affected by nitrogen fertilization.

Nitrogen (kg ha^{-1})	GDM[1]	NP	NLT	LAR	LER	ALS	IAF	LSR
0	1,876 d	517 d	3.98 c	0.088 d	1.73 d	19.6 c	2.74 d	0.119 d
40	2,742 c	685 c	4.55 b	0.101 c	2.51 c	24.8 b	3.11 c	0.143 c
80	3,201 b	779 b	4.81 b	0.107 b	2.75 b	25.7 b	3.48 b	0.157 b
120	3,987 a	818 ab	5.17 a	0.114 a	3.11 a	27.1 a	3.95 a	0.172 b
160	3,655 a	861 a	5.02 a	0.111 ab	2.82 b	25.3 b	3.52 b	0.196 a

- Means followed by the same letter do not differ (P>.05) by Tukey test.
- SOURCE: Research Data
[1] Means of three cuts.

The NT was positively and linearly affected by nitrogen fertilization (Y = 567.8 + 2.0525 X - r^2 = 0.91) (Table 1). The correlation between NT and GDM yield was positive and significant (r = 0.94; P <0.01), which explained in 88.4% the increases in grass GDM yields as a function of nitrogen fertilization (Table 1). N strongly interferes with activation of meristematic tissues (axillary buds), as its deficit increases the number of dormant buds, while its adequate supply allows maximum grass tillering (Nabinger & Carvalho, 2009). Individual tillers have a limited and variable life span, due to biotic and abiotic factors, and their population can be maintain by a continuous replacement of dead tillers, this behavior being the key point for grass perennity (Lemaire et al., 2011; Santos et al, 2012).

The relationship between nitrogen fertilization and NLT was adjusted to the quadratic regression model and described by the equation $Y = 3.97 + 0.01632 X - 0.000061 X^2$ (R^2 = 0.92) and the maximum value obtained with the application of 133.6 kg of N ha^{-1} (Table 1). The values obtained were higher than those reported by Luna et al. (2012) for *Brachiaria brizantha* cvs. Xaraés and Piatã, which estimated 3.91 and 4.33 green leaves $tiller^{-1}$, respectively. The main effect of N on NLT would be the increase in leaf life by maintaining higher photosynthetic capacity for longer periods without significant internal remobilization of N from older leaves (Nabinger & Carvalho, 2009). The correlation between GDM yield and LER was positive and significant (r = 0.94; P <0.01), whereas with LAR the correlation was positive but not significant (r = 0.69; P> 0 .05).

According to the data presented in the Table 1, LAR was positively and linearly affected by nitrogen fertilization: Y = 0.0926 + 0.00021 X (r^2 = 0.85), while for LER, ALS and IAF the effects were quadratic and described, respectively, by equations: Y = 1.742 + 0.02127 X - 0.000091 X^2 (R^2 = 0.92); Y = 19.83 + 0.1307 X - 0.00061 X^2 (R^2 = 0.92) and Y = 2.66 + 0.0167 X - 0.0000669 X^2 (R^2 = 0.89) and the maximum values obtained with the application of 118.2; 108.9 and 124.8 kg of N ha^{-1}. Alexandrino et al. (2010) found a quadratic effect of nitrogen fertilization (0, 45, 90, 180 and 360 mg N kg $soil^{-1}$) on *B. brizantha* cv. Marandu and estimating the maximum values with the application of 335; 325 and 347 mg N kg $soil^{-1}$, respectively. For Lemaire et al. (2011), LER, in responding to the supply of N, would be LAR's main modifying agent. Successive leaves appearing at very close insertion levels, but under high elongation rates, supported by the additional N supply, would establish higher LAR. The LER and LAR are negatively correlated, indicating that the higher the TAF, the shorter the time available for leaf elongation (Santos et al., 2012). In this study, the correlation between these two variables was positive and significant (r = 0.91; P <0.05), possibly as consequence of favorable environmental conditions, which allowed the plants to express their maximum growth potential.

The LSR was directly proportional to the N rates applied, being the relation adjusted to the linear regression model (Y = 0.1208 + 0.00052 X - r^2 = 0.96) (Table 1). Similar results were reported by Costa et al. (2009) for *B. brizantha* cv. Xaraés, who found higher LSR with the application of 120 (0.177 cm $tiller^{-1}$) and 180 kg of N ha^{-1} year-1 (0.198 cm $tiller^{-1}$). LSR is one of the most important factors when evaluating forage production dynamics, as it determines the net accumulation of forage biomass by area available for animal consumption (Pereira, 2013). The senescence process represents the last stage of leaf development, started after its complete expansion and progressively accentuated with the increase of the leaf area, due to the shading of the leaves inserted in the inferior portion and the low supply of photosynthetically active radiation, besides strong competition for light, nutrients and water among the various plant strata (Nabinger, 2002). When the tillers reach a certain NLT, there is a balance between the LAR and the senescence of the leaves that exceeded its life period, so that for the emergence of a new leaf it implies the senescence of the preceding leaf, keeping the NLT relatively constant (Lemaire et al., 2011). Senescence negatively affects forage quality, however, it represents an important physiological process in grass tissue flow, since about 35.1; 68.3; 86.1 and 42.8% of nitrogen, phosphorus, calcium and magnesium, respectively, can be remobilize from senescent leaves and use for the production of new leaf tissues (Sarmiento et al., 2006).

According to the data presented in the Table 2, the efficiency of N utilization was inversely proportional to the N rates applied, being the linear relation and defined by the equation Y = 77.134 - 0.3608 X (r^2 = 0.92). Similar tendencies was reported by Costa et al. (2009) who found maximum forage yields of *B. brizantha* cv. Xaraés and *B. ruziziensis* with the application of 181.2 and 211.3 kg of N ha^{-1}, respectively, however the highest rates of N utilization efficiency were registered at doses between 80 and 120 kg of N ha^{-1}.

Table 2. Nitrogen content (g kg^{-1}), nitrogen utilization efficiency (NUE - kg GDM/kg of N), apparent nitrogen recovery (ANR - %), critical nitrogen level (CNL - g kg^{-1})[1] and nitrogen nutrition index (NNI - content of N/CNL) of *Brachiaria brizantha* cv. Piatã, as affected by nitrogen fertilization.

Nitrogen (kg ha^{-1})	Nitrogen content	NUE	ANR	CNL	NNI
0	15.89 c	---	---	27.75 a	0.573
40	17.32 c	68.55 a	44.2 a	24.39 b	0.710
80	19.67 b	40.01 b	41.4 b	23.14 bc	0.850
120	23.48 a	32.80 b	42.3 b	21.57 cd	1.088
160	25.11 a	22.84 c	38.7 c	22.12 c	1.135

- Means followed by the same letter do not differ (P>.05) by Tukey test.
[1] CNL = 3.6.GDM $^{(-0.34)}$
- SOURCE: Research Data

According to the data presented in the Table 2, NUE (Y = 77.13 - 0.3609 X; r^2 = 0.85) and ANR (Y = 45.55 - 0.0398 X; r^2 = 0.87) were inversely proportional to the N doses evaluated. Costa et al. (2007) reported similar trends for *Brachiaria humidicola* pastures (29.1; 20.8 and 15.5 kg of DM kg^{-1} of N and 49.1; 44.7 and 31.1%, respectively for 100, 200 and 300 kg N ha^{-1}). Townsend (2008) evaluating the effects of nitrogen fertilization (0, 60, 180 and 360 kg of N ha-1 $year^{-1}$) in *Paspalum notatum* cv. André da Rocha estimated maximum forage yield with the application of 239 kg N ha-1, however, the highest NUE were reached under fertilization levels between 80 and 160 kg N ha^{-1} $year^{-1}$. Primavesi et al. (2004) found that *Cynodon* dactylon cv. Coastcross was inversely proportional to N doses, however the highest values were obtained with the use of ammonium nitrate (75; 68 and 45%)

compared to urea (52; 46 and 37%), respectively for 50, 100 and 200. kg N ha^{-1}. NUE and ANR are affect by forage species, plant development stage, applied doses and their fractionation, frequency of pasture use, environmental factors and soil fertility. Decreases in NUE and ANR may be associated with N losses from leaching, NH3 volatilization and denitrification, notably with the use of high N rates and under high soil moisture conditions (Magalhães et al., 2012; Paiva et al., 2019).

The relationship between GDM and N content was adjusted to the linear regression model: Y = 15.37 + 0.0615 X (r^2 = 0.92), not showing the characteristic effect of dilution of its contents, due to the higher accumulation of GDM with increasing levels of nitrogen fertilization. This behavior reflects the higher proportion of structural material and reserves with plant growth, which contain low N concentrations, as well as the non-uniform distribution of N between leaves as a function of the level of solar radiation intercepted within the canopy (Gastal & Lemaire, 2002; Paiva et al., 2019).

The N levels recorded with the application of 120 and 160 kg of N ha^{-1} were above the N critical level proposed by Lemaire et al. (1984a) and Lemaire et al. (1984b), whereas with the application of 40 or 80 kg of N ha^{-1}, the estimated NNI was insufficient to provided the requirement of grass per N, since the ratio of N content in GDM to CNL was less than 1.0. The N uptake can be reduce by low phosphorus availability, which limits the energy supply to the photochemical phase of photosynthesis and subsequently to the carboxylation processes of CO^2 in sheath cells (Gastal & Lemaire, 2002). Barro et al. (2012), in *Paspalum regnellii* pastures, fertilized with 100 kg N ha^{-1} year and subjected to three shading levels, the grass NNI was only reached at 80% shading level (1.01), compared to 50% (0.79) or full sun (0.75), which is attributed to the lower GDM accumulation of grass with the increase of shading level.

4. Final Considerations

The evaluation of *Brachiaria brizantha* cv. Piatã pastures under different levels of nitrogen fertilization allows the recommendation of the most suitable for their efficient management.

Nitrogen fertilization positively affects the production of GDM, N concentrations and morphogenic and structural characteristics of the grass.

Utilization efficiency and apparent N recovery are inversely proportional to the applied doses, and the opposite was observe for leaf senescence rate.

The maximum technical efficiency dose for GDM production was estimate at 145.9 kg N ha^{-1} and the internal critical level of N was reduce with increasing forage yield.

Nitrogen nutrition index alone with 120 or 160 kg N application was higher than the grass N internal critical level.

Experiments are suggested and preferably with the use of animals in order to endorse the recommended nitrogen levels for the grass.

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