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**Research Article**

# Growth promotion of Guinea grass by diazotrophic bacteria[1]

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## ABSTRACT

The *Panicum maximum* species (cv. Mombaça) is a forage plant well adapted to the different edaphoclimatic conditions found in Brazil; however, it requires high nitrogen fertilizer doses to ensure high yields. The present study aimed to assess the inoculation effect with associative diazotrophic bacteria strains on the yield and bromatological characteristics of the Guinea grass. A randomized block design was used, consisting of 25 treatments, being 23 of them inoculated with diazotrophic bacteria and two without inoculation (one with and the other without mineral nitrogen), with four repetitions. The analyzed variables were stem diameter; leaf width and length; leaf, stem and root dry mass yield; crude protein; acid and neutral detergent fibers. There were no differences among the treatments for root dry mass, stem diameter and acid detergent fiber. For the remaining variables, a positive effect of the inoculation with diazotrophic bacteria was observed, particularly for the following Unifenas strains: 100-06, 100-13, 100-26, 100-30, 10-35, 100-54, 100-69, 100-71 and 100-94. This reveals the potential of these bacterial strains for use at the sustainable production of the Guinea grass with a reduction in the use of nitrogen inputs.


KEYWORDS: *Panicum maximum*, biological $N_2$ fixation, forage grasses.


## RESUMO

Promoção de crescimento de capim Mombaça por bactérias diazotróficas

A espécie *Panicum maximum* cv. Mombaça é uma forrageira adaptada às diversas condições edafoclimáticas encontradas no Brasil; no entanto, necessita de altas doses de fertilizantes nitrogenados para garantir altas produtividades. Objetivou-se avaliar o efeito da inoculação com estirpes de bactérias diazotróficas associativas nas características produtivas e bromatológicas do capim Mombaça. Utilizou-se delineamento em blocos casualizados, constituído por 25 tratamentos, sendo 23 inoculados com bactérias diazotróficas e dois sem inoculação (um com e o outro sem nitrogênio mineral), com quatro repetições. As variáveis analisadas foram: diâmetro do colmo; largura e comprimento da folha; produtividade da matéria seca das folhas, colmos e raiz; proteína bruta; fibra em detergente ácido e neutro. Para as variáveis matéria seca da raiz, diâmetro do colmo e fibra em detergente ácido, não foram observadas diferenças entre os tratamentos. Para as demais, verificou-se contribuição positiva da inoculação com bactérias diazotróficas, destacando-se as seguintes estirpes Unifenas: 100-06, 100-13, 100-26, 100-30, 100-35, 100-54, 100-69, 100-71 e 100-94. Isto revela o potencial destas estirpes bacterianas para uso em produção sustentável do capim Mombaça com redução de insumos nitrogenados.


PALAVRAS-CHAVE: *Panicum maximum*, fixação biológica de $N_2$, gramíneas forrageiras.

## INTRODUCTION

Much like other macronutrients, nitrogen is vital to most agricultural crops, since it forms part of several biomolecules and participates in different cell metabolism processes (Nelson & Cox 2014). It is highly mobile in the soil, what reduces its agricultural efficiency and increases the potential for water and atmospheric pollution, due to greenhouse gas emissions such as nitrous oxide ($N_2O$) (Nogueira et al. 2015). In addition, Brazil is highly dependent on external suppliers to obtain nitrogen fertilizers and imports almost 70 % of the nitrogen used in agriculture (ANDA 2017), making it important to find alternative nitrogen sources, in order to reduce this dependency and ensure a sustainable agricultural production.

Biological nitrogen fixation (BNF) is an economically and environmentally viable substitute for nitrogen fertilizers. The process is carried out

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by a small group of prokaryotes responsible for $N_2$ reduction to $NH_3$, known as $N_2$ fixing or diazotrophic bacteria, which have been widely studied for potential agricultural applications.

However, most studies on BNF focus on the symbiosis between diazotrophic bacteria and leguminous plants such as soybean, which obtains all the nitrogen it needs for growth via inoculation with diazotrophs from the genus *Bradyrhizobium* (Zuffo et al. 2015). In addition to soybean, strains of $N_2$ fixing bacteria have been selected for peanut and cowpea, which can obtain 100 % of nitrogen from the association between hosts and diazotrophic bacteria (rhizobia) (Brasil 2011). In this respect, more research is needed to investigate the contribution of these bacteria to other agriculturally important plant families such as grasses, including maize, wheat, rice and forage crops.

Studies indicate that around 70 % of the cultivated pasture areas in Brazil are being degraded (Macedo et al. 2013, Hungria et al. 2016), what may explain the low natural fertility of tropical soils and the lack of nutrient replenishment, of which nitrogen is considered one of the most yield-limiting elements in these crops (Dias-Filho 2014).

Adopting a combined management and fertilization plan is important to obtain significant yield gains in forage crops and animal production. Hungria et al. (2016) found that the inoculation with *Azospirillum* spp. in *Brachiaria brizantha* and *B. ruziziensis* provides the equivalent of 40 kg of nitrogen fertilizer per hectare. Promising results have also been reported in Elephant grass (*Pennisetum purpureum*), which requires a high soil fertility, with research demonstrating that BNF-related N contributions account for about 36-132 kg $ha^{-1}$ of N (Morais et al. 2011), highlighting the potential of BNF in different forage grass species.

Diazotrophic bacteria also contribute to the plant development by producing growth-stimulating hormones such as indole-3-acetic acid (IAA). The inoculation with IAA-producing diazotrophs can increase the germination rate and initial development of lettuce seedlings (Florentino et al. 2017a), besides promoting growth in several economically important plants (Moreira et al. 2010).

The Guinea grass stands out among tropical grasses for its high nutritional value and good yield, making it an important plant in the animal production. In this respect, studies involving the selection of diazotrophic bacteria with a view to reducing mineral nitrogen use in the Guinea grass cultivation are highly relevant. As such, the present study aimed to assess the effect of inoculation with strains of associative diatrophic bacteria on the yield and bromatological characteristics of Guinea grass.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse of the Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais, in Bambuí, Minas Gerais state, Brazil (18º49'41.02"S, 41º58'52.07"W and altitude of 680 m), from April 2015 to March 2016. The climate in the region is humid subtropical, with an average annual temperature and rainfall of 22.5 ºC and 1,426.3 mm, respectively. The forage grass studied was *Panicum maximum* cv. Mombaça.

The soil used was classified as a Distroferric hematite-containing Red Latosol (Embrapa 2018) with a clay texture, collected in a first-crop area at 0-0.20 m. It was sieved through a 2 mm-mesh sieve and homogenized for chemical and particle size characterization (Embrapa 2017), with the following results: pH ($H_2O$) = 5.1; P = 1.3 mg $dm^{-3}$; $K^+$ = 48 mg $dm^{-3}$; $Ca^+$ = 1.42 $cmol_c$ $dm^{-3}$; $Mg^{2+}$ = 0.43 $cmol_c$ $dm^{-3}$; $Al^{3+}$ = 0.44 $cmol_c$ $dm^{-3}$; $H^+ + Al^{3+}$ = 2.64 $cmol_c$ $dm^{-3}$; sum of bases = 2 $cmol_c$ $dm^{-3}$; potential CEC = 4.6 $cmol_c$ $dm^{-3}$; base saturation (V%) = 42.7 %; organic matter = 26 g $kg^{-1}$; sand = 15.8 g $kg^{-1}$; clay = 65 g $kg^{-1}$; silt = 9.2 g $kg^{-1}$. The Guinea grass was grown in 23 dm³ pots and the soil sieved through a 4 mm-mesh sieve.

The soil analysis results indicated the need for liming, which was performed by elevating the base saturation to 60 % (Santos et al. 2014) and incorporating lime (PRNT = 80 %), followed by incubation for 90 days. The moisture content of the pots was maintained at 60 % of the water holding capacity (Matos et al. 2014). Following the incubation, topdressing was performed in all the experimental plots, with the recommended doses of 200 mg $dm^{-3}$ of phosphorus and 150 mg $dm^{-3}$ of potassium, using single superphosphate and potassium chloride, respectively.

Micronutrient fertilization was performed in all the plots, with boric acid, copper chloride, zinc chloride and sodium molybdate doses of 1.39 mg $dm^{-3}$, 2.61 mg $dm^{-3}$, 2.03 mg $dm^{-3}$ and 0.36 mg $dm^{-3}$, respectively (Rezende et al. 2016).

The bacterial strains used for the Guinea grass inoculation exhibited a rapid growth rate and were isolated from the rhizosphere of *Brachiaria brizantha* cv. Marandu plants previously characterized by Dias (2015). These strains have been studied for their ability to fix $N_2$ in *Brachiaria brizantha* cv. Xaraés (Dias et al. 2019), solubilize potassium (Florentino et al. 2017b) and produce IAA (Florentino et al. 2017a). A group of diazotrophic bacteria strains selected at the Universidade José do Rosário Vellano (Unifenas) was prioritized in this study.

The following Unifenas bacterial strains were assessed: 100-02, 100-06, 100-13, 100-16, 100-21, 100-26, 100-28, 100-30, 100-31, 100-34, 100-35, 100-40, 100-43, 100-50, 100-51, 100-54, 100-65, 100-69, 100-71, 100-78, 100-82 and 100-94. The strain Ab-V5 of the *Azospirillum brasilense* species, supplied by the Embrapa Soja, was also assessed. The bacteria were grown in an FAM medium (Magalhães & Döbereiner 1984) until isolated pure colonies were obtained. These were then transferred to a liquid FAM medium and cultivated for three days until the log phase, containing approximately $10^8$ cells $mL^{-1}$.

A randomized block design, with four repetitions, was used, consisting of 25 treatments, 23 of which involved inoculation with diazotrophic bacterial strains, plus two control treatments, one without inoculation and containing mineral nitrogen fertilization (positive control) and other without inoculation and nitrogen fertilization (negative control).

Twenty seeds were planted per pot, leaving five plants per pot after thinning. Following thinning, inoculation was performed in line with the respective treatments, when plants reached an average of 10 cm tall. In the treatment containing nitrogen fertilization, 25 mg $dm^{-3}$ of urea were applied as a nitrogen source. Inoculation was carried out by inserting a 2 mL aliquot of bacterial suspension into the soil close to the root of each plant.

The moisture content of the pots was maintained at the field capacity, determined based on the registered rainfall index for the study site and measured using a tensiometer.

Plants were assessed in three cutting cycles, with stem diameter and leaf width and length measured before each cutting. The variables analyzed after cutting were leaf and stem dry mass, crude protein, neutral and acid detergent fibers. The root dry mass was evaluated at the last cutting.

Cutting was performed when plants were 90 cm tall, leaving 30 cm. After each cutting, the nitrogen fertilizer was reapplied (25 mg $dm^{-3}$ of N) only for the nitrogen fertilization treatment, and potassium fertilization (100 mg $dm^{-3}$ of $K_2O$) for the remaining treatments, in the form of urea and potassium chloride, respectively. For the treatments involving inoculation, plants were also reinoculated after each cutting with 2 mL of bacterial suspension containing approximately $10^8$ cells $mL^{-1}$, to ensure a viable number of bacterial strains in the root/rhizosphere system.

At the third cutting, the plants were cut to a height of 30 cm and then down to the ground level, after which the roots were removed and washed to determine the root dry mass.

For the crude protein analysis, the nitrogen content was determined (AOAC 1996) and multiplied by 6.25. The methodology described by Van Soest et al. (1991) was applied for the biological nitrogen fixation and acid detergent fibers analyses. Nitrogen and shoot dry mass values (leaf dry mass + stem dry mass) were used to determine the accumulated N in the shoots.

The data for all variables were submitted to analysis of variance and treatment means were compared by the Scott-Knott test at 5 % of probability, using the Sisvar software (Ferreira 2014).

## RESULTS AND DISCUSSION

The effect of the treatments on the assessed variables at the three cuttings was very similar and the values were therefore presented as the means of three cuttings (Tables 1 and 2). Statistical differences were observed for leaf and stem dry mass, leaf width and length, crude protein and neutral detergent fibers, albeit not significant for stem diameter and acid detergent fibers. The root dry mass, assessed at the third cutting, also showed no statistical difference between the treatments. Table 1 shows the average results of three cuttings for stem diameter, leaf width, leaf length, leaf dry mass, stem dry mass and root dry mass, with the latter obtained after the third cutting.

There was no statistical difference between the treatments for stem diameter. In forage plants, the stem provides a support for leaves on the tiller. As observed by Castagnara et al. (2011), increasing nitrogen doses do not influence the stem or pseudostem diameter in Guinea grass. Bosa et

Table 1. Means[1] of three cuttings for stem diameter (SD), leaf width (LW) and length (LL), leaf (LDM), stem (SDM) and root (RDM) dry mass in Guinea grass submitted to inoculation with diazotrophic bacterial strains, or with and without nitrogen fertilization.

| Treatments | SD[2] (mm) | LW (cm) | LL (cm) | LDM (g) | SDM (g) | RDM[2] (g) |
|---|---|---|---|---|---|---|
| Without mineral N | 3.57 | 2.21 c | 57.67 b | 59.75 d | 44.25 b | 84.50 |
| With mineral N | 5.70 | 3.47 a | 68.00 a | 94.75 a | 51.00 a | 86.50 |
| Unifenas 100-02 | 4.47 | 2.30 c | 65.42 a | 67.75 d | 43.00 b | 89.25 |
| Unifenas 100-06 | 4.18 | 2.76 c | 64.58 a | 65.25 d | 52.00 a | 82.25 |
| Unifenas 100-13 | 4.69 | 2.44 c | 62.67 b | 58.75 d | 51.25 a | 78.00 |
| Unifenas 100-16 | 3.72 | 2.43 c | 60.25 b | 56.75 e | 39.00 b | 76.50 |
| Unifenas 100-21 | 4.51 | 2.55 c | 67.58 a | 47.50 e | 43.00 b | 67.50 |
| Unifenas 100-26 | 4.25 | 2.49 c | 66.50 a | 75.50 c | 53.25 a | 73.00 |
| Unifenas 100-28 | 3.72 | 2.64 c | 61.17 b | 62.50 d | 47.50 b | 97.25 |
| Unifenas 100-30 | 3.95 | 2.66 c | 64.17 a | 65.50 d | 53.50 a | 75.50 |
| Unifenas 100-31 | 4.09 | 2.33 c | 56.50 b | 52.75 e | 38.75 b | 81.00 |
| Unifenas 100-34 | 4.36 | 2.42 c | 62.83 b | 56.50 e | 44.50 b | 75.00 |
| Unifenas 100-35 | 4.71 | 2.35 c | 65.17 a | 63.25 d | 46.25 b | 73.50 |
| Unifenas 100-40 | 4.21 | 2.64 c | 62.25 b | 51.25 e | 39.00 b | 72.75 |
| Unifenas 100-43 | 3.88 | 2.28 c | 62.25 b | 52.75 e | 41.50 b | 66.75 |
| Unifenas 100-50 | 4.12 | 2.48 c | 60.50 b | 70.50 c | 54.50 a | 67.00 |
| Unifenas 100-51 | 4.83 | 2.27 c | 60.42 b | 53.75 e | 46.75 b | 73.00 |
| Unifenas 100-54 | 3.95 | 2.47 c | 68.50 a | 83.00 b | 44.00 b | 65.75 |
| Unifenas 100-65 | 4.57 | 2.38 c | 58.67 b | 55.25 e | 41.25 b | 71.75 |
| Unifenas 100-69 | 4.52 | 2.60 c | 68.25 a | 81.75 b | 52.75 a | 87.50 |
| Unifenas 100-71 | 4.50 | 2.69 c | 62.25 b | 54.50 e | 48.75 a | 84.50 |
| Unifenas 100-78 | 4.37 | 2.57 c | 68.00 a | 49.75 e | 46.25 b | 64.50 |
| Unifenas 100-82 | 4.14 | 2.32 c | 62.92 b | 64.50 d | 42.25 b | 74.25 |
| Unifenas 100-94 | 3.80 | 2.08 c | 64.00 a | 62.75 d | 46.25 b | 78.50 |
| Ab-V5 | 4.48 | 2.90 b | 67.67 a | 64.25 d | 44.75 b | 74.75 |
| CV (%) | 17.45 | 12.71 | 7.32 | 9.63 | 9.35 | 25.92 |

[1] Means followed by the same letter in the column do not differ according to the Scott-Knott test at 5 % of probability. [2] Variable with no significant ($p > 0.05$) treatment effects.

Table 2. Means[1] (%) for acid and neutral detergent fibers (ADF and NDF, respectively), crude protein (CP) and crude protein increase in relation to the control without inoculation and mineral nitrogen (ICP), in Guinea grass submitted to inoculation with diazotrophic bacterial strains, or with and without nitrogen fertilization.

| Treatments | ADF[2] | NDF | CP | ICP |
|---|---|---|---|---|
| Without mineral N | 43.91 | 67.98 b | 7.26 b | 0.00 |
| With mineral N | 40.40 | 61.70 a | 11.27 a | 4.01 |
| Unifenas 100-02 | 44.43 | 67.26 b | 7.70 b | 0.44 |
| Unifenas 100-06 | 44.03 | 68.86 b | 7.91 b | 0.65 |
| Unifenas 100-13 | 42.35 | 67.40 b | 8.40 b | 1.14 |
| Unifenas 100-16 | 43.95 | 69.92 b | 8.70 b | 1.44 |
| Unifenas 100-21 | 42.91 | 68.36 b | 8.72 b | 1.46 |
| Unifenas 100-26 | 47.30 | 67.49 b | 8.35 b | 1.09 |
| Unifenas 100-28 | 43.50 | 68.93 b | 8.43 b | 1.17 |
| Unifenas 100-30 | 44.09 | 67.94 b | 8.20 b | 0.94 |
| Unifenas 100-31 | 41.28 | 68.85 b | 7.68 b | 0.42 |
| Unifenas 100-34 | 44.37 | 69.54 b | 9.01 b | 1.75 |
| Unifenas 100-35 | 43.87 | 68.88 b | 9.30 b | 2.04 |
| Unifenas 100-40 | 43.36 | 69.72 b | 7.20 b | -0.06 |
| Unifenas 100-43 | 41.85 | 67.45 b | 8.25 b | 0.99 |
| Unifenas 100-50 | 42.50 | 67.71 b | 8.26 b | 1.00 |
| Unifenas 100-51 | 42.78 | 68.22 b | 8.63 b | 1.37 |
| Unifenas 100-54 | 44.37 | 62.24 a | 7.45 b | 0.19 |
| Unifenas 100-65 | 47.06 | 68.14 b | 7.97 b | 0.71 |
| Unifenas 100-69 | 43.73 | 68.07 b | 8.43 b | 1.17 |
| Unifenas 100-71 | 43.10 | 68.07 b | 9.57 b | 2.31 |
| Unifenas 100-78 | 43.32 | 70.16 b | 7.68 b | 0.42 |
| Unifenas 100-82 | 44.64 | 67.65 b | 7.45 b | 0.19 |
| Unifenas 100-94 | 42.37 | 65.73 b | 9.23 b | 1.97 |
| Ab-V5 | 43.89 | 69.14 b | 8.43 b | 1.17 |
| CV (%) | 4.83 | 3.64 | 16.67 | - |

[1] Means followed by the same letter in the column do not differ according to the Scott-Knott test at 5 % of probability. [2] Variable with no significant ($p > 0.05$) treatment effects.

al. (2016) studied the effect of mineral nitrogen or inoculation on different bacterial strains in *Brachiaria brizantha* cv. Xaraés (*Brachiaria brizantha*) and observed a positive response for the stem diameter values in the treatment containing mineral N, when compared to those involving inoculation.

With respect to the leaf width, the mineral N treatment produced the best results, followed by the inoculation with *Azospirillum brasilense* (Ab-V5). The best leaf length results were also obtained in plants treated with mineral N or inoculated with the Ab-V5 strain, as well as with the following Unifenas strains: 100-02, 100-06, 100-21, 100-26, 100-30, 100-35, 100-54, 100-69, 100-78 and 100-82.

In general, the leaf development is directly related to a greater photosynthetic activity and, consequently, higher yields (Santos et al. 2012), which may also directly influence a greater nitrogen availability in the plant tissue, contributing to longer and wider Guinea grass leaves, as observed here.

Regarding inoculation, the *A. brasilense* strain Ab-V5 contributed to both the leaf width and length. In addition to its $N_2$ fixing capacity, this strain has a proven ability to produce indole-3-acetic acid (IAA), a plant growth hormone that contributes to increasing the leaf area (Almeida et al. 2015). Hungria et al. (2016) found that the inoculation with *A. brasilense* contributes to the *B. brizantha* and *B. ruziziensis* production and considered the use of this biotechnology a key component in pasture recovery. Since there are no studies in the literature that test *A. brasilense* in Guinea grass, the data obtained here provide an important information on expanding its application in the forage grass inoculation.

The Unifenas strains 100-21, 100-26 and 100-54 promoted a longer leaf length, due to their ability of producing IAA (Florentino et al. 2017a), what may be an important mechanism on selecting inoculant strains for forage grasses.

The leaf dry mass was influenced by the different treatments, with the highest value recorded for the mineral nitrogen treatment, followed by the inoculation with the Unifenas strains 100-54 and 100-69. The Guinea grass requires a highly fertile soil and, therefore, responds well to the nitrogen fertilization (Castro et al. 2016), justifying the high values recorded in the mineral nitrogen treatment.

On the other hand, the Unifenas strains 100-54 and 100-69 positively influenced the leaf dry mass, particularly when compared to the treatment without inoculation or mineral nitrogen, with both the strains also increasing the leaf length (Table 1).

The highest stem dry mass means were observed for inoculation with the Unifenas strains 100-06, 100-13, 100-26, 100-30, 100-50, 100-69 and 100-71, and with the control containing mineral nitrogen. The dry mass yield of leaves and sheaths are a vital component in the forage production, because both parts are responsible for storing organic substances, what may interfere in rebudding and production (Castagnara et al. 2011). According to Costa et al. (2016), the nitrogen fertilization has a positive effect on dry mass yield, leaf length and tiller density.

For the root dry mass, there was no statistical difference between the inoculated and non-inoculated treatments. Unlike in the present study, Brasil et al. (2005) found that the inoculation with *Azospirillum* strains promotes a better root development in *B. humidicola*, what was attributed to the production of plant hormones by the bacteria (Brasil et al. 2005). The authors also discuss the increased water and nutrient absorption provided by the increased root development.

The highest crude protein content was recorded for the mineral nitrogen treatment (Table 2). Crude protein values in treatments without inoculation containing or not mineral N were similar to those found by Vogel et al. (2014), who also observed a correlation between greater nitrogen supply and high crude protein values in Guinea grass.

There was no statistical difference for the crude protein values in the inoculated treatments, with statistical similarity to controls without mineral nitrogen or inoculation (Table 2). Nevertheless, it is important to underscore that the Unifenas strains 100-35, 100-71 and 100-94 promoted a 2 % increase in the crude protein content, when compared to the treatment without mineral nitrogen or inoculation. This is relevant because proteins are involved in different metabolic functions, including tissue growth and repair, enzymatic catalysis, transport and storage, coordinated movement, mechanical support, immune protection, generation and transmission of nerve impulses, and control of plant metabolism, growth and cellular differentiation, making them important in maintaining the animal homeostasis (Valadares Filho et al. 2009). Nitrogen is also used in the production of RuBisCO (ribulose bisphosphate carboxylase), responsible for the carbon fixation in photosynthesis (Taiz & Zeiger 2013).

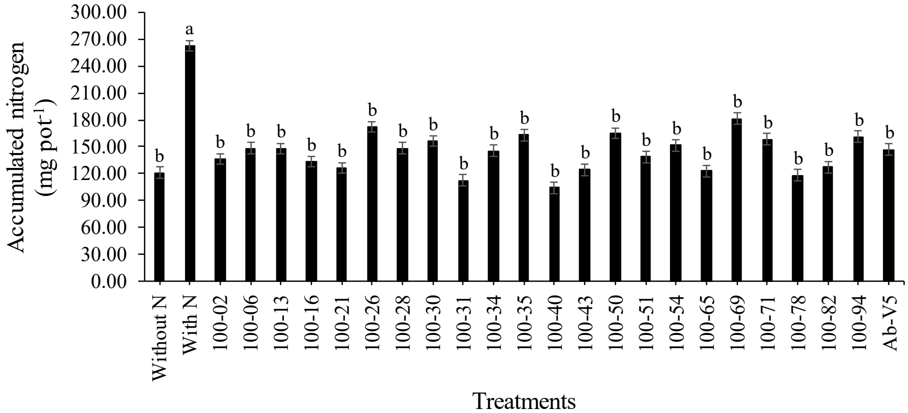


Figure 1. Accumulated nitrogen (mg pot$^{-1}$) in Guinea grass shoots submitted to inoculation with diazotrophic bacteria strains, or with and without nitrogen fertilization (the treatments identified with number 100-02 to 100-94 are Unifenas bacterial strains).

No differences were also found among the treatments for acid detergent fibers (Table 2), with similar values to those reported by Mari (2013). As observed for crude protein, the accumulated N content was higher for mineral nitrogen fertilization, when compared to the remaining treatments (Figure 1).

The analysis of the effects of inoculation with diazotrophic bacterial strains on the yield and bromatological traits of Guinea grass showed to be justified its use, and with significant influence of the following Unifenas strains: 100-06, 100-13, 100-26, 100-30, 10-35, 100-54, 100-69, 100-71 and 100-94. Furthermore, additional tests should be conducted to investigate the effect of bacteria with growth-promoting properties, such as biological nitrogen fixation and indolic compounds.

## CONCLUSIONS

Inoculations with associative diazotrophic bacteria positively affect the Guinea grass development and production, thereby contributing to a more sustainable crop and reducing the use of nitrogen inputs. The most promising results come from inoculation with the following Unifenas strains: 100-06, 100-13, 100-26, 100-30, 10-35, 100-54, 100-69, 100-71 and 100-94.

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