Productivity and nutritive value of Tifton 85 bermudagrass inoculated with *Azospirillum brasilense* in association with nitrogen fertilization[1]

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ABSTRACT

Tifton 85 bermudagrass has been widely used in tropical and subtropical regions. Owing to its high forage yield, the fertilizer requirement of Tifton 85 is also high. Inoculation with *Azospirillum brasilense* is an alternative to reduce the use of nitrogen fertilizers and decrease their environmental impact. However, the effects of inoculation in tropical grasses remain poorly understood. This study aimed to evaluate the productivity, pasture characteristics, and nutritive value of Tifton 85 bermudagrass inoculated with *A. brasilense* in association with nitrogen fertilization. The study evaluated four nitrogen levels (0, 100, 200, and 300 kg ha^{-1}), inoculated and uninoculated, under cutting conditions. The experimental design was completely randomized, in a factorial arrangement, with two qualitative (inoculated with *A. brasilense* or uninoculated) and four quantitative levels (nitrogen doses) with three replications (24 plots). Botanical composition and morphological characteristics, leaf blade/stem+sheath ratio, nutritive value, and forage yield were evaluated. The inoculation increased the leaf blade/stem+sheath ratio and forage yield of Tifton 85, but did not affect crude protein concentration, neutral detergent fiber content, and total digestible nutrient content of the forage constituted by Tifton 85 leaf blades. The protein concentration and forage production corresponded linearly to the nitrogen dose increase.

Keywords: crude protein; *Cynodon* spp.; diazotrophic bacteria; forage yield; total digestible nutrients.

INTRODUCTION

The Tifton 85 bermudagrass (*Cynodon dactylon* (L.) Pers.) stands out for its high forage production, good nutritive value, response to fertilization, and better animal performance when compared to other cultivars of the same genus (Burton, 2001; Baseggio *et al.,* 2015; Olivo *et al.,* 2019). As a result, Tifton 85 is the most farmed grass of the *Cynodon* genus in Brazil (Silva *et al*., 2017). Tifton 85 has substantial requirements in terms of fertility, hence the application of fertilizers is crucial to enhance the production and the forage quality (Sohm *et al*., 2014).

In this context, nitrogen (N) is the nutrient in highest deficit and the one most required in quantitative terms (Alderman *et al*., 2011; Bourscheidt *et al*., 2019) to enhance the forage production of forage grasses and ensure a higher stocking rate and animal production per area (Oliveira *et al*., 2010). It is also important for improving the nutritive value of the forage (Anderson & Stewart, 2017; Olivo *et al*., 2019).

Currently, alternatives are being sought to reduce the use of fertilizers and make production systems less costly and more sustainable. Thus, the use of diazotrophic bacteria stands out. Diazotrophic bacteria may help reduce the application of fertilizers (Santos *et al*., 2021) and increase the efficiency of N use by reducing N leaching and vola-

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tilization through biological fixation (Cunha *et al.*, 2014).

Diazotrophic bacteria promote plant growth and fix atmospheric N. Its association with plant roots is capable of counteracting populations of phytopathogenic micro-organisms in the soil, fixing atmospheric N, and secreting phytohormones (Dobbelaere *et al.*, 2003). Studies have shown that the diazotrophic bacterium *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) promotes an increase in biomass and accumulated N in forage biomass (Hungria *et al.*, 2016).

A. brasilense (strains Ab-V5 and Ab-V6) research has been conducted previously, particularly in annual crops for grain production, such as wheat (Quatrin *et al.*, 2019) and maize (Mumbach *et al.*, 2017), but few studies have been conducted in pastures, especially with perennial species (Aguirre *et al.*, 2018a). In addition, results from research involving inoculation with *A. brasilense* and application of N fertilizers show variability (Galindo *et al.*, 2017), particularly in forage plants (Leite *et al.*, 2019). However, inoculation with *A. brasilense* and nitrogen fertilization in tropical perennial pastures can increase the productive characteristics and nutritive value of the pasture.

Thus, the objective of this study was to evaluate the forage production and nutritive value of Tifton 85 bermudagrass inoculated with the strains Ab-V5 and Ab-V6 of *A. brasilense* and subjected to different doses of N fertilizer.

MATERIAL AND METHODS

Study Site

This study was conducted at the Department of Animal and Dairy Sciences of the Federal University of Santa Maria (RS - Brazil) between August 2017 and May 2018, totaling 279 days. The soil is classified as Hapludult (Soil Survey Staff, 2014). The climate is Cfa (humid subtropical) according to the Köppen classification.

The daily average temperature and monthly precipitation were 21.2 °C and 140.3 mm month^{-1}, respectively, during the experimental period. The climatological means (1981–2010) of daily temperature and monthly precipitation for the respective period were 20.3 °C and 148.8 mm month^{-1}, respectively (INMET, 2018).

The results of the soil analysis (0–20 cm deep) were as follows: pH 5.9; organic matter 3.1; P 48.2 mg dm^{-3}; K 0.225 cmol dm^{-3}; Ca 7.6 cmol dm^{-3}; Mg 3.1 cmol dm^{-3}, and CEC 15.8 cmol dm^{-3}. Soil acidity was corrected with lime to 2.5 t ha^{-1}. Base fertilization was performed according to the soil analysis, as recommended by the Soil Chemistry and Fertility Commission, RS/SC (Tedesco *et al.*, 2004) for warm-season grasses. In total, 60 kg ha^{-1} year^{-1} of P_2O_5 and 60 kg ha^{-1} year^{-1} of K_2O were used.

Establishment of Pasture and Treatments

Tifton 85 was planted on September 14 using seedlings in furrows approximately 10 cm deep and spaced every 0.50 m. The experimental area was subdivided into 24 plots with an area of 3 × 4 m, separated from each other by 0.5 m wide borders. Eight treatments were arranged, based on the bermudagrass cultivar Tifton 85, with two qualitative levels (uninoculated or inoculated with *A. brasilense*) and four quantitative levels of N fertilizer (0, 100, 200, and 300 kg N ha^{-1}).

The N fertilizer (urea) was supplied according to each treatment and divided into five applications. In treatments involving the inoculation, the liquid inoculant Azototal® (*A. brasilense* bacterium, strains Ab-V5 and Ab-V6) was applied on the plant surface using a solution prepared according to the recommendations (500 mL of inoculant ha^{-1} for 200 L of water). The inoculant applications were fractioned, with half of the dose applied in January and the other half applied in February, and performed by spraying with a back spray.

Pasture Measurements

The first pasture cut was performed on December 7, 2017, using canopy height of 20–25 cm as the criterion. Pasture height was measured using a graduated ruler. The evaluations were performed by random cutting within each plot (0.5 × 0.5 m square). To determine the forage mass of the upper stratum, cuts were made at 7–9 cm above the ground. Subsequently, in the same place, the residue of the pasture was collected, making the cut close to the ground to determine the forage mass of the lower stratum. The sample collection site was demarcated with stakes to avoid sampling in the same location in later cuts. Subsequently, the grass of the entire plot was cut at 7–9 cm from the ground using a side mower. The forage was removed with the help of rakes.

The forage from the cut samples was homogenized and a subsample was taken per plot, which was then used to determine the botanical composition of the pasture and the morphological characteristics of the Tifton 85 bermudagrass. Subsequently, the samples were placed in a forced ventilation oven set at 55 °C for drying to constant weight, and the content of the partially dry matter hence obtained was determined.

Forage accumulation from the first cutting cycle was determined by adding the forage mass of the upper and lower strata. In the subsequent cutting cycles, the forage accumulation was calculated from the forage mass of the upper stratum alone (Zanine *et al.*, 2011). The forage accumulation rate was estimated from the relationship between forage accumulation and days between cutting cycles.

Nutritive Value

The samples used for the analysis of crude protein (CP) and neutral detergent fiber (NDF) consisted of the leaf blade fraction from the upper stratum of the Tifton 85 grass forage. These samples were ground in a "Willey" type mill and analyzed in the laboratory for CP concentration using the Kjeldahl method (AOAC, 1995) and NDF content (Van Soest *et al.*, 1991). The estimated values of total digestible nutrients (TDN) were obtained using the following equation: TDN = 83.79 − 0.4171 NDF; $r^2 = 0.82$ (Cappelle *et al.*, 2001).

Experimental Design and Statistical Analysis

The experimental design was completely randomized, with a 2 × 4 factorial arrangement (Tifton 85 without and with inoculation × 4 levels of N fertilizer) and three replications (24 plots). Data were analyzed using analysis of variance at a 5% probability of error; when significant, Tukey's test for comparison of means was used to further analyze the treatment effect using the Statistical Analysis System (SAS, 2001). The statistical additive model used was as follows: $Y_{ijk} = m + T_i + D_j + T_iD_j + \varepsilon_{ijk}$, where Y_{ijk} represents the dependent variables; i, the treatment index a, qualitative (inoculation); j, the treatment index b, quantitative (N levels); k, the repetition index; m, the mean of all observations; T_i, the effect of the inoculation (i = 2); D_j, the effect of the N dosage (j = 4); T_iD_j, the correlation between the inoculation and the N dosage; and ε_{ijk}, the residual or experimental error. Regression analysis was used when at least one significant effect was found. The criteria for choosing the model were the coefficient of determination (R^2) and a p-value of ≤ 0.05.

RESULTS

Upper Stratum Forage Mass

In the 234 days of the experimental period, 5, 6, 6, and 7 cutting cycles were carried out, with an average of 25, 20, 20, and 21 days between the cycles, considering the beginning of pasture use, for the treatment combinations of 0, 100, 200, and 300 kg N ha^{-1}, respectively. The average height of the pasture before cutting was 24 cm.

There was no correlation between the inoculation and N doses for forage mass, both in the available form and in the upper stratum (Table 1). For the same variables, no inoculation effect was observed either. The highest value ($p \leq 0.05$) of forage mass was obtained in the pasture in which 300 kg N ha^{-1} was applied. The forage masses of the upper stratum differed depending on N doses ($p \leq 0.05$), with an ascending linear effect (y = 0.0013*N + 1.03; $r^2 = 0.985$; $p \leq 0.001$) associated with increased N doses.

There was also no effect of the inoculation in the botanical composition of the forage mass of the upper stratum (Table 1). In the botanical composition, Tifton 85 was higher ($p \leq 0.05$) in pastures where N fertilizer was applied, with a linear upward trend (y = 0.0387*N + 19.077; $r^2 = 0.953$; $p \leq 0.001$) with increasing N dose. In contrast, the other species showed an opposite trend (y = −0.0366*N + 78.2; $r^2 = 0.955$; $p \leq 0.001$), with greater botanical composition ($p \leq 0.05$) in the pasture without N fertilizer. These species were composed of, in particular, *Urochloa plantaginea* (Link) Hitch., *Cynodon* spp., and *Ipomoea acuminata* (Vahl.) Roemer & Schultes. There was no effect of the inoculation and N application on dead material in the forage mass of the upper stratum. As for the morphological characteristics of Tifton 85 (Table 1), there was a difference ($p \leq 0.05$) in the samples of the plot applied 300 kg N ha^{-1}, with a higher percentage of leaf blades in the inoculated pasture. Further, inoculation provided the highest values ($p \leq 0.05$) of leaf blade/stem+sheath ratio in samples of the plots applied 100 and 300 kg N ha^{-1}.

Lower Stratum Forage Mass

There was no correlation between inoculation and N application in the forage mass of the lower stratum (Table 1). For this variable, a linear ascending trend with increasing N doses was observed (y = 1.051*N + 932.1; $r^2 = 0.938$; $p \leq 0.001$). The botanical composition of Tifton 85 in the forage mass was lower ($p \leq 0.05$) in the uninoculated pasture without N fertilizer application. Among the four N levels, no difference was noted in the botanical composition of Tifton 85. The N dosage had a significant effect ($p \leq 0.05$) on the fraction of other species, with a lower botanical composition of these species in the pasture with the highest

Table 1: Effect of N levels and inoculation with *Azospirillum brasilense* on pastures consisting of Tifton 85 bermudagrass and warm season spontaneous growth species

Variables	Inoculation	N levels (kg ha^{-1})				Means	CV (%)
		0	100	200	300		
Forage mass (t DM ha^{-1})	Ns	2.0^{C}	2.1^{BC}	2.4^{B}	2.7^{A}		13,9
Upper stratum[1]							
Forage mass (t DM ha^{-1})	Ns	1.0^{C}	1.1^{BC}	1.3^{AB}	1.4^{A}	1.2	12.5
Botanical composition (%)							
Tifton 85	Ns	18.2^{C}	23.8^{B}	27.9^{A}	29.7^{A}	24.9	9.5
Other species[3]	Ns	79.1^{A}	73.4^{B}	70.2^{BC}	68.0^{C}	72.7	7.8
Dead material	Ns	2.7^{A}	2.8^{A}	1.9^{A}	2.3^{A}	2.4	18.5
Morphological composition Tifton 85 bermudagrass (%)							
Leaf blead	No	50.6^{Aa}	51.1^{Aa}	50.3^{Aa}	50.5^{Ab}	50.6	8.0
	Yes	52.5^{Aa}	54.7^{Aa}	51.8^{Aa}	55.3^{Aa}	53.6	
Stem+sheath	Ns	44.0^{A}	43.5^{A}	44.0^{A}	44.0^{A}	43.9	9.2
Senescent material	Ns	4.5^{A}	3.4^{A}	5.0^{A}	3.2^{A}	4.1	15.4
Leaf blade/stem + sheath ratio Tifton 85 bermudagrass							
	No	1.2^{Aa}	1.1^{Ab}	1.1^{Aa}	1.1^{Ab}	1.1	9.4
	Yes	1.2^{Aa}	1.4^{Aa}	1.2^{Aa}	1.4^{Aa}	1.3	
Lower stratum[2]							
Forage mass (t DM ha^{-1})	Ns	0.9^{C}	1.0^{BC}	$1,1^{B}$	1.3^{A}	1.1	13.4
Botanical composition (%)							
Tifton 85	No	11.1^{Bb}	16.5^{Aa}	20.0^{Aa}	18.0^{Aa}	16.4	12.3
	Yes	18.2^{Aa}	19.9^{Aa}	16.9^{Aa}	16.2^{Aa}	17.8	
Other species[3]	Ns	76.3^{A}	71.8^{AB}	72.1^{AB}	69.9^{B}	72.5	10.3
Dead material	Ns	9.0^{B}	9.9^{B}	9.4^{B}	13.0^{A}	10.3	13.8
Morphological composition Tifton 85 bermudagrass (%)							
Leaf blead	Ns	34.1^{A}	32.2^{A}	35.3^{A}	34.0^{A}	33.9	9.6
Stem+sheath	Ns	50.5^{B}	55.4^{A}	51.5^{AB}	53.1^{AB}	52.6	10.1
Senescent material	Ns	15.3^{A}	12.3^{A}	13.2^{A}	14.0^{A}	13.6	14.2
Leaf blade/stem + sheath ratio Tifton 85 bermudagrass							
	Ns	0.7	0.6	0.7	0.7	0.7	11.6

Means followed by different letters, upper case in the same row and lower case in the same column, are significantly different by Tukey's test at 5% probability. DM= dry matter. Ns= not significant to inoculation. Cv= coefficient of variation. [1]Cutting at 7 and 9 cm above the soil; [2]Cutting close at the soil level. [3]Spontaneous growth species. 5, 6, 6 e 7 cuts were made to 0, 100, 200 and 300 kg N ha^{-1}, from December to May.

dose of N compared to the unfertilized one. The fraction of spontaneously growing species showed a downward linear trend with increasing N doses ($y = -0.019*N + 75.357$; $r^2 = 0.822$; $p \leq 0.001$). The highest value ($p \leq 0.05$) of dead material was found in the pasture applied 300 kg N ha^{-1}.

The inoculation did not affect the morphological characteristics and leaf blade/stem+sheath ratio of Tifton 85 (Table 1). There was a difference in terms of stem+sheath ($p \leq 0.05$) among the samples from the plots applied different N doses, with a lower value in the unfertilized pasture compared to the one that was applied 100 kg N ha^{-1}. Among the N-fertilized pastures, no difference was noted in terms of stem+sheath.

Forage Accumulation

There was no correlation between the inoculation and N application for the production variables (Table 2). The inoculation affected the accumulation rate and forage production in the unfertilized pasture and the pasture in which 200 kg N ha^{-1} was applied ($p \leq 0.05$). Further, the inoculation affected the forage production of Tifton 85 ($p \leq 0.05$) only in the unfertilized pasture, with a value 26% higher than that in the uninoculated one. Forage production showed a linear ascending trend with increasing N application, both in the uninoculated and inoculated pastures (Figure 1). As for the forage production of the spontaneously growing species, there was a difference ($p \leq 0.05$) in the unfertilized pasture in comparison to that applied 300 kg N ha^{-1}, with lower values in the inoculated pasture. Forage production of the spontaneously growing species showed a linear increasing trend with increasing N dosage, both in uninoculated and inoculated pastures (Figure 1).

The nutritive value of the evaluated forage, leaf blades of Tifton 85 from the upper stratum, was not affected by the inoculation (Table 3). However, CP concentration differed significantly among the different N doses ($p \leq 0.05$), with higher concentrations in the fertilized pastures. CP concentration showed a linear ascending trend as a function of increasing N dosage ($y = 0.0173*N + 14.2$; $r^2 = 0.983$; $p \leq 0.001$).

DISCUSSION

Forage Mass

The average of 24 days between the cutting cycles is suitable for pastures of Tifton 85 grass. Research evaluating different cutting intervals has shown that intervals of up to 27 days are associated with high forage production and nutritive value (Michelangeli *et al.*, 2010).

The increase in forage masses of the upper and lower strata are linearly associated with the application of increasing N doses, which is related to the utilization of N by

Table 2: Pasture productivity, inoculated with *Azospirillum brasilense* and N fertilized, consisting of Tifton 85 bermudagrass and spontaneous growth species of warm season

Variables	Inoculation	N levels (kg ha^{-1}) 0	100	200	300	Means	CV (%)
Forage accumulation ratio (kg ha^{-1} day^{-1})	No	27,6^{Db}	35,5^{Ca}	40,0^{Bb}	46,1^{Aa}	37,3	2,5
	Yes	30,8^{Da}	38,2^{Ca}	43,7^{Ba}	47,5^{Aa}	40,0	
Herbage yield (t DM ha^{-1})	No	5,7^{Db}	7,3^{Ca}	8,2^{Bb}	10,8^{Aa}	8,0	2,5
	Yes	6,3^{Da}	7,8^{Ca}	9,0^{Ba}	11,1^{Aa}	8,6	
Tifton 85 (t DM ha^{-1})	No	1,4^{Db}	2,6^{Ca}	3,2^{Ba}	4,3^{Aa}	2,9	4,6
	Yes	1,9^{Da}	2,8^{Ca}	3,2^{Ba}	4,4^{Aa}	3,1	
Others species (t DM ha^{-1})	No	3,9^{Ca}	4,6^{Ba}	4,8^{Ba}	6,6^{Aa}	5,0	3,8
	Yes	3,3^{Cb}	4,5^{Ba}	4,7^{Ba}	5,9^{Ab}	4,6	

Means followed by different letters, upper case in the same row and lower case in the same column, are significantly different by Tukey's test at 5% probability. DM= dry matter. Cv= coefficient of variation.

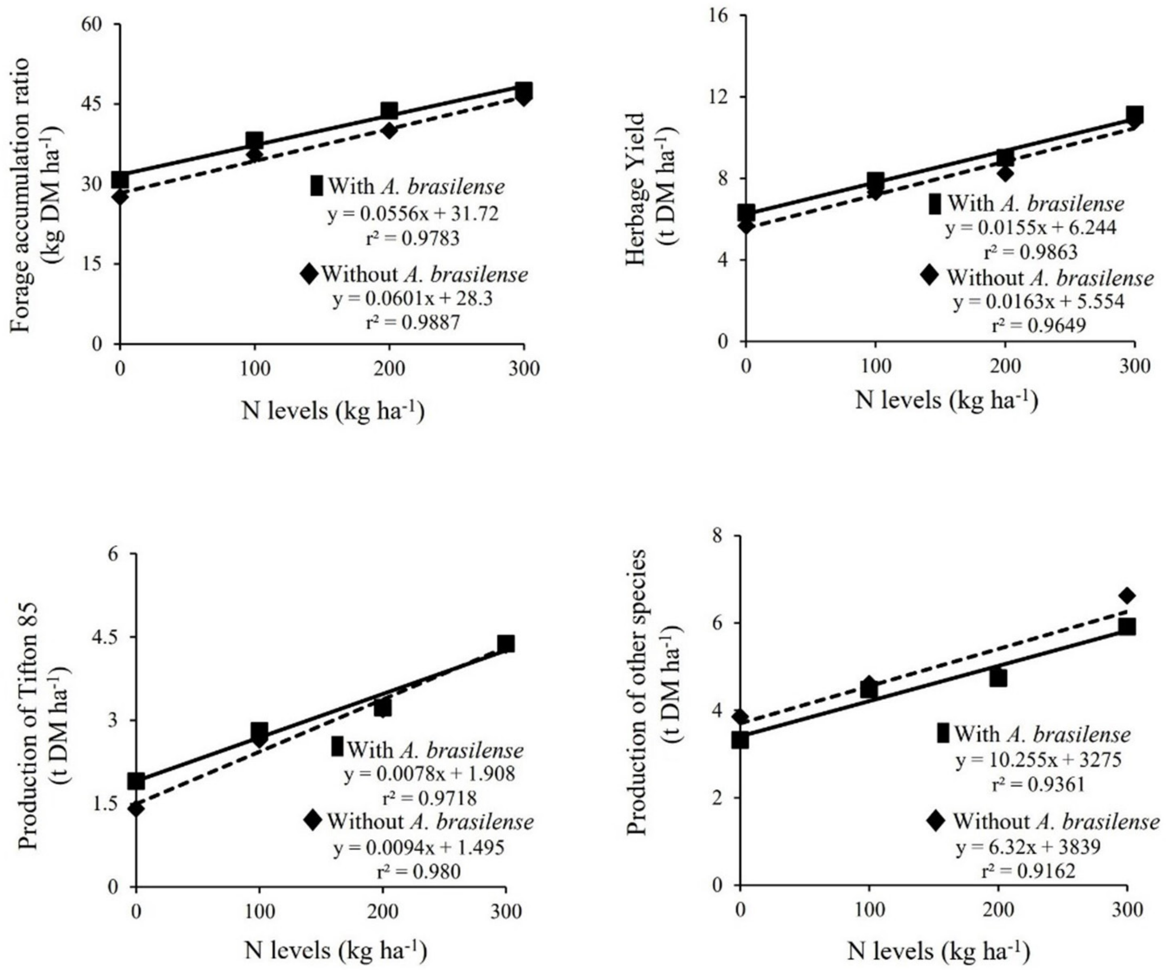

Figure 1: Effect of N levels and inoculation with *Azospirillum brasilense* in pastures of Tifton 85 and spontaneous growth species of warm season. Santa Maria, RS, 2017-2018.

Table 3: Nutritive value of leaf blades of the upper stratum of Tifton 85 bermudagrass, submitted to different levels of nitrogen fertilizer and inoculated with *Azospirillum brasilense*

Variables	Inoculation	N levels (kg ha^{-1})				Means	CV (%)
		0	100	200	300		
CP (g/kg^{-1} DM)	Ns	140^{C}	161^{B}	179^{AB}	191^{A}	168	3,1
NDF (g/kg^{-1} DM)	Ns	697^{A}	680^{A}	684^{A}	673^{A}	684	3,3
TDN (g/kg^{-1} DM)	Ns	547^{A}	552^{A}	554^{A}	557^{A}	552	1,69

Means followed by different letters, upper case in the same row and lower case in the same column, are significantly different by Tukey's test at 5% probability. CP= crude protein, NDF= neutral detergent fiber, TDN= total digestible nutrients. Ns= not significant to inoculation. Cv= coefficient of variation.

plants. Greater availability of N in the soil implies shorter harvest time and higher forage production (Pereira *et al.*, 2012). N fertilizers also affected the botanical composition of the pasture, as shown in the present study. Thus, higher levels of fertilizers implied greater botanical composition of Tifton 85 as it responds well to N fertilization (Borges *et al.*, 2017). As for the other spontaneously growing species, the opposite trend was observed, revealing a linear descending trend as a function of N dosage. These species are usually less productive (Anjos *et al.*, 2016) and less consumed by the animals (Olivo *et al.*, 2014).

As for the nutritive value of Tifton 85 grass leaf blades from the upper stratum, the increase in CP concentration, due to the increase in the applied N dose, indicates the dependence of this grass on N (Nascimento *et al.*, 2017). A similar result was obtained by Almeida *et al.* (2019) in a study on Tifton 85 for hay production fertilized with urea in increasing doses, and by Olivo *et al.* (2019) in pastures of Tifton 85 for dairy production. These differences in CP concentration, in contrast with the unfertilized pasture, may increase in the following year if there is no adequate N replacement in the soil. On the contrary, the absence of a difference in the nutritive value variable does not corroborate with the findings in Coastcross grass (*Cynodon* spp.) inoculated with *A. brasilense* in the same region, which showed an increase in the concentrations of CP and NDT in the pasture (Aguirre *et al.*, 2018b).

The effect of the inoculation on the botanical composition of the forage mass of the lower stratum was observed only in the unfertilized pasture, with greater botanical composition of Tifton 85. This may be attributed to the high competitive capacity of *A. brasilense* under conditions of low N availability in the soil (Bourscheidt *et al.*, 2019).

The increase in the leaf blade/stem+sheath ratio of Tifton 85 from the upper stratum in two of the three N levels of the inoculated pastures suggests an association between *A. brasilense* and fertilization. A similar effect, with a larger contribution of leaf blades, was observed in Coastcross-1 pasture (Aguirre *et al.*, 2018a) and in maize plants (Morais *et al.*, 2015) while validating an associative effect between N levels and the *A. brasilense* inoculant. This effect, with a greater contribution of leaf blades, was also found in inoculated winter cycle cultures (Díaz-Zorita & Fernandez-Canigia, 2009).

Forage Yield

The forage accumulation rate increase in two of the three quantitative levels of N in the inoculated pastures suggests a synergistic relationship between inoculation and N fertilization. This is an important finding as the predominant result in related research is the increase in forage production with inoculation when there is no N application (Vogel *et al.*, 2013; Aguirre *et al.*, 2020) or when low amounts of N fertilizer are used (Hungria *et al.*, 2010; Lana *et al.*, 2012).

The effect of inoculation in pastures applied different doses of N is attributed to the contribution of the *A. brasilense* bacterium, capable of inducing greater development of the root system, thereby resulting in substantial increases in nutrient and water absorption by the host plant (Bashan & Bashan, 2010; Licea-Herrera *et al.*, 2020) and usually involving greater tillering and contribution of leaf blades (Guimarães *et al.*, 2011). N synthesis also occurs on a smaller scale (Hungria *et al.*, 2016; Santos *et al.*, 2021). There is also an increase in plant resistance to stress conditions (Moreira *et al.*, 2010). A comparable result was observed by Leite *et al.* (2019), with increased plant height and forage production in Marandu - palisade grass (*Urochloa brizantha*), and by Andrade *et al.* (2019), with an increase in the leaf elongation rate of Tamani grass (*Panicum maximum* cultivar BRS Tamani) inoculated with *A. brasilense* and fertilized with different doses of N.

The lower forage production of spontaneously growing species in the inoculated pasture is possibly related to the better response of Tifton 85 to inoculation with *A. brasilense*. A similar finding was noted by Sabundjian *et al.* (2013), who found a lower inoculation response of *Urochloa ruziziensis* compared to maize.

CONCLUSIONS

Inoculation with *A. brasilense*, strains Ab-V5 and Ab-V6, resulted in greater participation in botanical composition of Tifton 85 in the pasture and a larger leaf blade/stem+sheath ratio. Forage production was higher in inoculated pastures, responding linearly with increasing N doses.

The inoculation did not affect the nutritive value of Tifton 85 bermudagrass leaf blades. CP concentration of Tifton 85 bermudagrass leaf blades responded linearly to the increase in N fertilization.

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