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# Production and nutritive value of Tifton 85 bermudagrass pastures overseeded with annual ryegrass and inoculated with diazotrophic bacteria

**Animal Research Paper**

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**Abstract**

The inoculation with *Azospirillum brasilense* has the potential to reduce the use of mineral fertilizers with efficient capacity to promote plant growth and yield. Most studies on the *Azospirillum*–plant association have been conducted on cereals and annual grasses. More studies are needed in perennial pastures, such as bermudagrass (*Cynodon dactylon* (L.) Pers.) that require substantial nitrogen (N) fertilization to maximize their production potential. Therefore, pastures based on Tifton 85 bermudagrass in association with annual ryegrass (*Lolium multiflorum* Lam.), which were inoculated with *A. brasilense* and fertilized with increasing amounts of N fertilizer and grazed by lactating Holstein cows were evaluated. Three grazing systems were evaluated: (i) Tifton 85, inoculated + 180 kg N/ha per year; (ii) Tifton 85 + 230 kg N/ha per year; and (iii) Tifton 85 + 280 kg N/ha per year. Forage samples were collected before and after grazing to evaluate the responses of the plants and animals. The forage yields of the systems were 21.0, 20.8 and 22.1 t DM/ha per year and the stocking rates were 3.9, 3.8 and 4.0 animal unit/ha per day, respectively. Crude protein, total digestible nutrients and neutral detergent fibre concentrations were 162, 560 and 667 g/kg, respectively. Inoculation in pastures planted with Tifton 85 bermudagrass in combination with ryegrass (plus 180 kg N/ha per year) had a positive effect, providing forage yield and nutritional value equivalent to those with fertilization with 230 kg N/ha per year.

## Introduction

During livestock production, the establishment of grazing systems with perennial pastures is extremely important because they protect the natural resource base and are relatively more sustainable during periods of water scarcity or excess (Ojeda *et al.*, 2018). Among pasture species, grasses in the genus *Cynodon*, especially the cultivar Tifton 85, stand out for their high nutritional value and strong potential for forage production (Pedreira *et al.*, 2018), especially for pasture and hay (Mufatto *et al.*, 2016). Cultivation of these forages has increased in both tropical and subtropical regions. During the winter season in subtropical regions, the production potential of bermudagrass pastures is decreased. However, the introduction of winter forage species, such as ryegrass, supports the adequate use of these areas throughout the agricultural year (De Almeida *et al.*, 2019; Oliveira *et al.*, 2019). The productive potential and chemical composition of these bermudagrass pastures is associated with fertilization, especially nitrogen fertilization (Pereira *et al.*, 2011). Nitrogen is a tissue-forming element that interferes directly with the photosynthetic process. In the most diverse production systems, nitrogen influences plant development to a greater extent than other nutrients, being essential for forage production. Its availability is a limiting factor and has a high cost (Sollenberger, 2008; Dias *et al.*, 2019). Thus, the use of nitrogen fertilizer has intensified, notably due to the need to increase production per unit area. This practice leads to increases in productivity, but also has economic and environmental impacts (Krupa, 2003; Behera *et al.*, 2013).

In this context, the use of plant growth-promoting bacteria can contribute to increase plant productivity through the production of phytohormones, abscisic acid, gibberellic acid, indole 3-acetic acid and ethylene (Perrig *et al.*, 2007; Housh *et al.*, 2021) that act on the root system, thus increasing the water and nutrient absorption capacity (Hungria *et al.*, 2010; Leite *et al.*, 2019; Duarte *et al.*, 2020; Santos *et al.*, 2021). Studies on the use of these bacteria, especially

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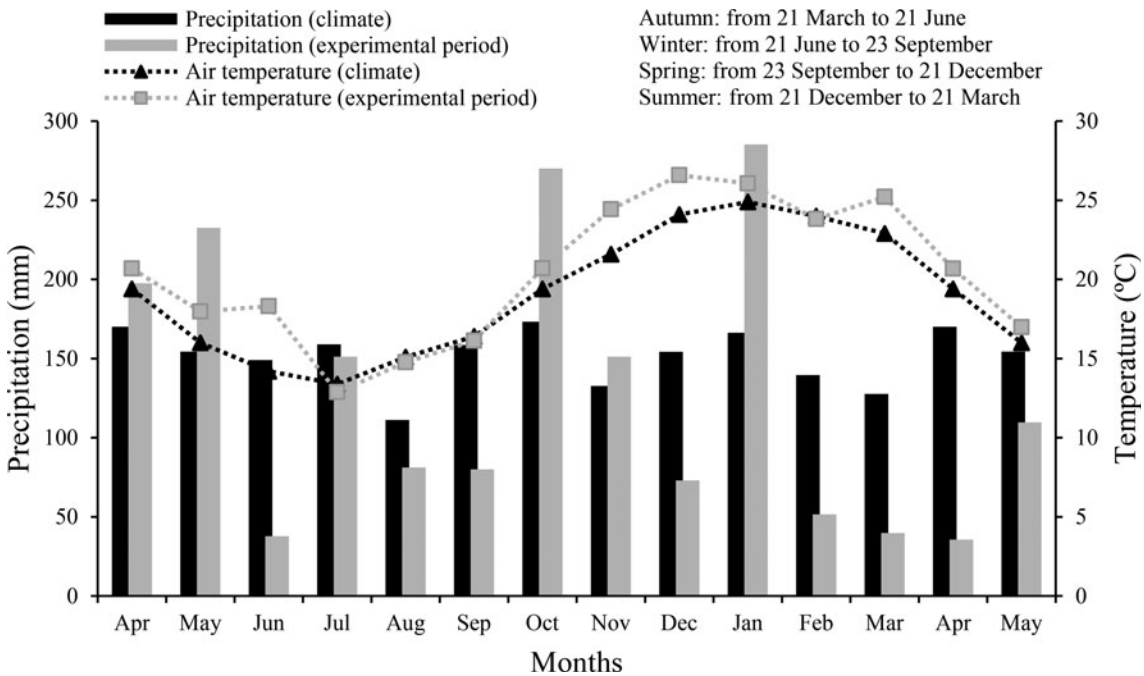


**Fig. 1.** Climate (1981–2010) and values recorded during the experimental period from April 2019 to May 2020, for average temperature and accumulated monthly precipitation. Santa Maria, RS, Brazil, 2019–2020.

for grain production, have been conducted (Quatrin *et al*., 2019; Schaefer *et al*., 2019; Hungria *et al*., 2021). However, few studies on forage plants, especially perennial species (Aguirre *et al*., 2018), are available. The use of microorganisms, especially those with facultative endophytic associations such as *Azospirillum brasilense*, may be an alternative that improves the nitrogen use efficiency of pastures (Duarte *et al*., 2020; Pedraza *et al*., 2020; Hungria *et al*., 2021). Therefore, a possible synergistic effect between *A. brasilense* and nitrogen fertilization can result in increased productivity and decreased production costs of Tifton 85 bermudagrass pasture (Aguirre *et al*., 2018). The objective of this study was to evaluate the effects of *A. brasilense* inoculation in grazing systems based on Tifton 85 bermudagrass and its association with annual ryegrass with different levels of nitrogen fertilizer. The focus was on the effects of inoculation on the resulting forage yield and nutritional value, grazing efficiency, roughage intake and stocking rate.

## Materials and methods

### Study site

This study was conducted in the Department of Animal and Dairy Sciences of the Federal University of Santa Maria, which is located in the Central Depression region of the state of Rio Grande do Sul, Brazil (95 m altitude, 29°43′S and 53°42′W). An area of 0.3 ha was divided into nine paddocks. The soil is classified as a sandy dystrophic Red Argisol belonging to the São Pedro mapping unit (Streck *et al*., 2008). The climate of the region is humid subtropical (Cfa) according to the Köppen classification (Alvares *et al*., 2014). During the experimental period from April 2019 to May 2020, the average daily temperature and monthly rainfall were 20.4°C and 128.3 mm, respectively (Fig. 1). The daily temperature for the same period and the 30-year average monthly rainfall (1981–2010) were 19.1°C and 151.5 mm, respectively. A previously established Tifton 85 bermudagrass pasture (approximately 6 years old) was used by transplanting whole seedlings into 10 cm-deep furrows, with 50 cm spacing between seedlings. During this period, the area was fertilized and grazed by lactating cows.

### Treatments and experimental design

The treatments consisted of three grazing systems, with Tifton 85 bermudagrass based during the spring, summer and early mid-autumn and bermudagrass in association with annual ryegrass based during the autumn, winter and early mid-spring as follows: (i) inoculation with *A. brasilense* + 180 kg N/ha per year (30 + 150); (ii) 230 kg N/ha per year (50 + 180) and (iii) 280 kg N/ha per year (70 kg in ryegrass + 210 kg/ha per year in Tifton 85 bermudagrass). The experimental design was completely randomized, with three treatments (grazing systems), three replicates (paddocks) and repeated measures over time (grazing cycles).

### Pasture and animal management

The timeline of the experiment is shown in Fig. 2. For soil amendment and fertilization, the guidelines of the CQFS – RS/SC (2016) were followed. The soil was amended with dolomitic limestone at a rate of 4.2 t/ha. In mid-April 2019, ryegrass (*Lolium multiflorum* Lam.) 'BRS Ponteio' was oversown at a rate of 50 kg/ha. For ryegrass, in all paddocks, fertilization was performed in May using $P_2O_5$ (50 kg/ha) and $K_2O$ (50 kg/ha), considering the historical production of 3 t DM/ha per year when associated with bermudagrass (Olivo *et al*., 2019). The nitrogen fertilization (urea 46% N) used during the autumn-winter was divided into two applications according to the dose proposed for each system. During the spring-summer, fertilization at Tifton 85 bermudagrass planting consisted of supplying $P_2O_5$ and $K_2O$ in October at 90 and 110

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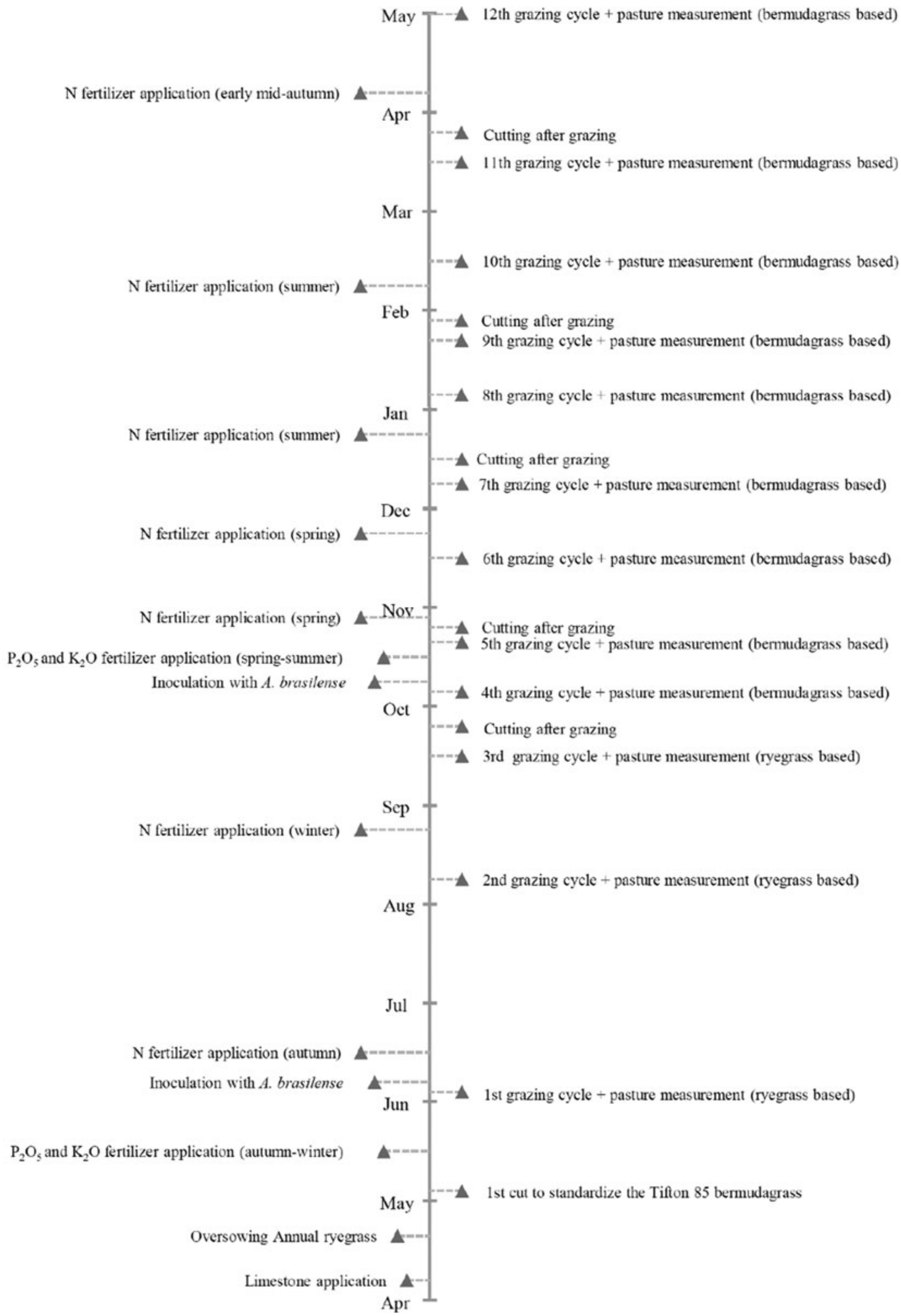


**Fig. 2.** Timeline of the experiment (from April 2019 to May 2020). Santa Maria, RS, Brazil.

kg/ha, respectively. Nitrogen fertilization proportional to the grazing systems was divided into five applications.

The criterion used to determine the beginning of grazing was the sward height, which was measured using a graduated ruler and a 600 $cm^2$ acetate sheet (Pedreira *et al*., 2018). Grazing started when the pastures reached a height of 20 cm for both the Tifton 85 bermudagrass and the ryegrass (Mezzalira *et al*., 2013). The pasture management method used here was rotational stocking, as calculated for one day of occupation, with free access to shade and water. The forage allowance ranged from 4 to 5.5 kg

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DM/100 kg body weight, and the mean value of the experimental period was 4.5 kg DM/100 kg body weight. With this management approach, we sought to maintain the post-grazing forage mass above sward height of 7 cm (Michelangeli *et al.*, 2010), estimating a range between 7 and 10 cm. Given that the forage mass was higher than the proposed range due to the increase in the grazing exclusion areas from the presence of faecal patches, the forage was cut to between 7 and 10 cm to standardize the pasture. During the experimental period, six cuttings were performed. The first cutting was conducted to provide cover to the ryegrass seeds and to standardize the Tifton 85 bermudagrass. The ryegrass was sown in April 2019. Grazing started in June 2019 and ended in May 2020.

In the inoculated grazing system, a commercial product (AzoTotal®) was used, which is a liquid inoculant composed of pure cultures of *A. brasilense* bacteria, Ab-V5 and Ab-V6 strains; a concentration of $2 \times 10^8$ CFU/ml was applied (via leaf-spray) to the pasture under good humidity and low light conditions at a rate of 0.5 litres/ha (Hungria *et al.*, 2021). The dilution was performed with water at a ratio of 0.5 litres of inoculant for each 199.5 litres of water. Two applications were carried out, with one (for ryegrass) in June and another (for Tifton 85 bermudagrass) in October, using a backpack sprayer.

The experimental animals were lactating Holstein cows, with an average weight of 582 kg and an average daily production of 22 litres. The cows were milked twice a day at 7:30 a.m. and 5:00 p.m. and weighed every 2 weeks. After each milking, they received food supplementation at rates accounting for their days in lactation and milk production. The mean value of food supplementation was 0.9 kg DM/100 kg body weight. When the animals were not grazing in the experimental area, they remained under similar management in the pasture, with free access to water and mineralized salt.

### Pasture parameters

To estimate the forage mass, four cuts were made close to the ground in each paddock before and after grazing using scissors and a $0.5 \times 0.5$ m square. The forage from the samples was weighed and homogenized, and two subsamples were collected. The first subsample of pre-grazing forage mass was used to estimate the percentage of dry matter using a microwave (Lacerda *et al.*, 2009). The values were used to calculate the stocking density. With the material from the second subsample, the botanical components of the pasture and morphological components of Tifton 85 bermudagrass (leaf blade, stem + sheath and senescent material) were quantified. Subsequently, these samples were pre-dried in a forced-air oven at 55°C to a constant weight to determine forage mass. Forage samples were collected using the grazing simulation method, by hand-plucked samples, observing the feeding behaviour of the animals (Euclides *et al.*, 1992) at the beginning and end of each grazing time. The sampled material was pre-dried in a forced-air oven at 55°C to a constant weight and ground in a Wiley mill (Wiley-type mill, model TE-680 Tecnal Laboratory Equipment LTDA, Piracicaba, Brazil). These samples were used to determine the crude protein (CP) (AOAC, 1995) and neutral detergent fibre (NDF) contents (Van Soest *et al.*, 1991). To estimate the total digestible nutrients (TDN) content, the following equation was used: TDN = 83.79–0.4171 NDF (Cappelle *et al.*, 2001).

The forage accumulation rate for the first grazing period was calculated from the forage availability divided by the number of days since ryegrass sowing. The accumulation rate of the cycles to follow was calculated by taking the difference between the pre- and post-grazing forage mass of the previous evaluation and dividing this result by the number of days between grazing intervals (Alava *et al.*, 2015).

The total forage production (in kg DM/ha) was determined by summing the forage accumulation of each grazing cycle (Olivo *et al.*, 2010). The leaf blade:stem + sheath ratio was calculated by determining the ratio of the leaf blade weight to the stem + sheath weight (Kirchner *et al.*, 2010). Protein production was obtained by multiplying the forage production by the CP concentration (g/kg). The same procedure was performed for TDN content.

### Parameters of dairy cows

The grazing efficiency (% of pre-grazing forage mass) was estimated using the equation $\{[(\text{pre-grazing}_{\text{cycle } n} - \text{post-grazing}_{\text{cycle } n}) / \text{pre-grazing}_{\text{cycle } n}] \times 100\}$ (Hodgson, 1979). The apparent forage dry matter intake (DMI) (kg DM/100 kg body weight) was estimated by the agronomic difference method as follows: DMI = $\{[(\text{pre-grazing forage mass}_{\text{cycle } n} - \text{post-grazing forage mass}_{\text{cycle } n}) / \text{stocking density}] \times 100\}$ (Burns *et al.*, 1994).

The stocking density was determined by adding the weight of the animals/ha. To calculate the stocking rate (AU/ha per day), the stocking density was divided by the number of days of the grazing cycle and by 450 (the weight equivalent to one animal unit (AU)).

### Statistical analysis

The data on the grazing cycles were grouped by nearest season and subjected to analysis of variance, and the means were compared by Tukey's test at a 5% probability of error. All the variables were subjected to Pearson's correlation analysis. The variables were analysed individually, by treatment and season, using the MIXED procedure statistical software of SAS (SAS Institute, 2016) considering the random effect of paddocks and the fixed effects of the grazing systems. The statistical model for the variables was as follows:

$$Y_{ijk} = m + T_i + R_j(T_i) + S_k + (TS)_{ik} + \varepsilon_{ijk}$$

where $Y_{ijk}$ represents the dependent variables; $m$ is the mean of all the observations; $T_i$ is the effect of the treatments (grazing systems); $R_j(T_i)$ is the replicate (paddocks) effect within treatments (error *a*); $S_k$ is the effect of the seasons; $(TS)_{ik}$ represents the interaction between treatments and seasons; and $\varepsilon_{ijk}$ is the residual effect (error *b*).

## Results

### Pre and post-grazing forage mass

Over the experimental period lasting 412 days, 12 grazing cycles were performed, four in the autumn and winter and eight in the spring and summer, with a mean interval between cycles of 44 and 24 days, respectively. The mean sward heights of the pastures before and after grazing were 20.5 and 8.5 cm, respectively.

A difference ($P \leq 0.05$) was found in autumn for the pre-grazing forage mass (Table 1). Among the seasons, lower values were observed in the winter in all the grazing systems. For the leaf blade:stem + sheath ratio of Tifton 85 bermudagrass,

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**Table 1.** Forage mass of three pasture systems (PS), Santa Maria, RS, Brazil, 2019–2020

| | Autumn | | | Winter | | | Spring | | | Summer | | | Mean | | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | SEM | *T* | *S* | *T* × *S* |
| Pre-grazing forage mass (t DM/ha) | 2.80 | 2.50 | 2.80 | 1.70 | 1.70 | 1.60 | 2.80 | 2.70 | 2.90 | 2.80 | 2.70 | 2.90 | 2.50 | 2.40 | 2.60 | 0.22 | 0.049 | <0.001 | 0.193 |
| *Botanical composition – pre-grazing forage mass (g/kg DM)* | | | | | | | | | | | | | | | | | | | |
| Tifton 85 bermudagrass | 860 | 833 | 869 | 84 | 97 | 118 | 503 | 490 | 489 | 905 | 943 | 937 | 588 | 591 | 603 | 26.4 | <0.001 | <0.001 | <0.001 |
| Annual ryegrass | 57 | 82 | 53 | 754 | 764 | 713 | 393 | 418 | 423 | – | – | – | 401 | 421 | 396 | 24.8 | <0.001 | <0.001 | <0.001 |
| Other species | 8.4 | 9.2 | 8.1 | 4.9 | 4.6 | 4.7 | 4.0 | 4.6 | 3.9 | 8.2 | 7.8 | 7.2 | 6.4 | 7.6 | 6.0 | 0.52 | 0.030 | <0.001 | 0.169 |
| Dead material | 75 | 76 | 70 | 156 | 134 | 165 | 100 | 87 | 83 | 87 | 52 | 55 | 105 | 87 | 93 | 15.3 | <0.001 | <0.001 | <0.001 |
| LRS – Tifton 85 bermudagrass | 1.50 | 1.70 | 1.50 | 0.70 | 0.60 | 0.40 | 1.10 | 1.00 | 0.90 | 1.70 | 1.70 | 1.50 | 1.20 | 1.30 | 1.10 | 0.03 | <0.001 | <0.001 | 0.361 |
| Post-grazing forage mass (t DM/ha) | 1.30 | 1.20 | 1.30 | 0.80 | 0.80 | 0.80 | 1.50 | 1.50 | 1.60 | 1.60 | 1.50 | 1.50 | 1.30 | 1.30 | 1.30 | 0.21 | 0.149 | <0.001 | 0.561 |
| *Botanical composition – post-grazing forage mass (g/kg DM)* | | | | | | | | | | | | | | | | | | | |
| Tifton 85 bermudagrass | 830 | 832 | 844 | 235 | 277 | 239 | 590 | 561 | 564 | 925 | 906 | 931 | 645 | 644 | 645 | 45.3 | 0.943 | <0.001 | <0.001 |
| Annual ryegrass | 24 | 31 | 8 | 504 | 487 | 511 | 236 | 284 | 280 | – | – | – | 191 | 200 | 200 | 21.6 | 0.008 | <0.001 | <0.001 |
| Other species | 28.3 | 14.7 | 27.0 | 26.3 | 22.7 | 26.9 | 7.4 | 6.0 | 6.5 | 10.3 | 10.6 | 11.1 | 18.1 | 13.5 | 17.9 | 3.50 | <0.001 | <0.001 | <0.001 |
| Dead material | 117 | 123 | 121 | 235 | 214 | 223 | 167 | 147 | 150 | 65 | 84 | 59 | 146 | 142 | 138 | 17.7 | 0.012 | <0.001 | <0.001 |
| LRS – Tifton 85 bermudagrass | 0.60 | 0.60 | 0.60 | 0.50 | 0.50 | 0.80 | 0.70 | 0.60 | 0.50 | 0.80 | 0.90 | 0.70 | 0.60 | 0.70 | 0.70 | 0.01 | <0.001 | <0.001 | <0.001 |

PS = I, 180 kg N/ha + *Azospirillum brasilense*; II, 230 kg N/ha; III, 280 kg N/ha; DM, dry matter; LSR, leaf blade/stem + sheath relation; SEM, standard error of the mean; *P* value, significance level; T, treatment; S, season.

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higher values were observed in the pasture that received an intermediate level of nitrogen fertilizer in all seasons; and higher values were observed in inoculated pasture in winter, spring and summer. For the post-grazing forage mass, no difference ($P > 0.05$) was observed between the grazing systems. For the leaf blade: stem + sheath ratio of Tifton 85 bermudagrass, the value remained close to 0.7.

For the pre-grazing botanical composition, an interaction between the grazing systems and season was observed ($P <$ 0.001), except for the other species fraction. With the highest dose and the lowest dose of nitrogen combined with *A. brasilense* inoculation, a higher percentage of Tifton 85 bermudagrass was obtained in the autumn; during the winter and summer, Tifton 85 bermudagrass participated less in the pasture under inoculation, with no difference in the spring. For ryegrass, at the beginning of its use in autumn, no difference was found; in the winter, greater participation was noted in pastures with lower N doses; and in the spring, ryegrass had a greater contribution in pastures with higher levels of nitrogen fertilization. The presence of other species remained below 1 g/kg DM during all seasons. For the dead material fraction, greater participation was observed in the winter and spring, with a greater contribution in the pasture with a lower N level.

In the post-grazing botanical composition, no difference in Tifton 85 bermudagrass participation was found between the pastures. With respect to the seasons, even in the winter, significant participation of this forage was observed. A higher value of ryegrass was observed in the winter while a lower value was observed in the spring in the inoculated pasture. For the other species fraction, the contribution remained below 2 g/kg DM. For dead material, higher values were obtained in the winter.

## Forage accumulation and forage yield

In the autumn, winter and spring, no differences were observed between the grazing systems for the forage accumulation rate (Table 2). In the summer, the highest ($P \leq 0.05$) rate was observed in the pasture in which the highest dose of nitrogen was applied. Between the seasons, lower values were observed for autumn and winter, and higher values were observed for spring and summer, with averages of 35.7 and 69.1 kg DM/ha per day, respectively. The Tifton 85 bermudagrass was influenced by the grazing systems, with higher rates ($P \leq 0.05$) at the highest dose of nitrogen fertilizer in the summer than those in the other grazing systems. The Tifton 85 bermudagrass accumulation rates were higher for all grazing systems in the summer. For ryegrass, no difference was observed between the grazing systems in the autumn and winter; in the spring, pastures receiving 230 and 280 kg of N showed higher forage accumulation rates.

For forage yield, a difference ($P \leq 0.05$) was noted between the pastures only in the summer, with a higher value in the pasture with a higher N level. The same result was observed for the total forage yield. An interaction with the season was identified for Tifton 85 bermudagrass forage production, with a higher value in the autumn for the lowest N level compared to the intermediate dose. The highest total production of Tifton 85 bermudagrass was obtained under the highest dose of N. For ryegrass forage production, no differences were observed between the grazing systems; between seasons, higher ryegrass production was observed between winter and spring.

Differences in CP/ha and TDN/ha production were observed only in the summer, with higher values at the highest N dose.

**Table 2.** Forage productivity of three pasture systems (PS), Santa Maria, RS, Brazil, 2019–2020

| Variables | Autumn | | | Winter | | | Spring | | | Summer | | | Mean | | | Total | | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | SEM | *T* | *S* | *T* × *S* |
| Forage accumulation rate (kg DM/ha per day) | 40.1 | 35.2 | 38.9 | 33.4 | 32.9 | 33.4 | 94.6 | 71.4 | 69.6 | 67.8 | 65.8 | 75.5 | 51.5 | 51.3 | 54.4 | NA | NA | NA | 9.20 | 0.043 | <0.001 | 0.218 |
| Forage accumulation rate of Tifton 85 bermudagrass (kg DM/ha per day) | 34.5 | 30.2 | 34.1 | 2.8 | 3.2 | 3.9 | 32.1 | 33.2 | 33.1 | 61.3 | 64.2 | 71.5 | 32.6 | 32.7 | 35.6 | NA | NA | NA | 9.56 | 0.045 | <0.001 | 0.100 |
| Forage accumulation rate of annual ryegrass (kg DM/ha per day) | 1.7 | 2.6 | 1.7 | 25.0 | 25.0 | 23.5 | 24.9 | 28.4 | 28.6 | – | – | – | 12.9 | 14.0 | 17.3 | NA | NA | NA | 3.21 | 0.046 | <0.001 | 0.199 |
| Forage yield (t DM/ha) | 5.80 | 5.10 | 5.60 | 2.50 | 2.50 | 2.50 | 5.90 | 6.60 | 6.40 | 6.80 | 6.60 | 7.60 | 5.25 | 5.20 | 5.53 | 21.00 | 20.80 | 22.10 | 0.75 | 0.042 | <0.001 | 0.160 |
| Forage yield of Tifton 85 bermudagrass (t DM/ha) | 5.00 | 4.30 | 4.90 | 0.20 | 0.20 | 0.30 | 3.00 | 3.10 | 3.00 | 6.10 | 6.40 | 7.20 | 3.57 | 3.50 | 3.85 | 14.30 | 14.00 | 15.40 | 0.60 | 0.015 | <0.001 | 0.024 |
| Forage yield of annual ryegrass (t DM/ha) | 0.20 | 0.40 | 0.20 | 1.90 | 1.90 | 1.80 | 2.30 | 2.60 | 2.60 | – | – | – | 1.47 | 1.63 | 1.53 | 4.40 | 4.90 | 4.60 | 0.56 | 0.258 | <0.001 | 0.184 |
| CP (t DM/ha) | 0.90 | 0.80 | 0.90 | 0.50 | 0.50 | 0.50 | 0.80 | 0.90 | 0.90 | 0.90 | 0.90 | 1.10 | 0.78 | 0.78 | 0.85 | 3.10 | 3.10 | 3.40 | 0.15 | 0.042 | <0.001 | 0.177 |
| TDN (t DM/ha) | 3.10 | 2.70 | 3.00 | 1.50 | 1.50 | 1.60 | 3.20 | 3.50 | 3.50 | 3.50 | 3.40 | 4.00 | 2.82 | 2.78 | 3.03 | 11.30 | 11.10 | 12.10 | 0.55 | 0.047 | <0.001 | 0.315 |

PS = I, 180 kg N/ha + *Azospirillum brasilense*; II, 230 kg N/ha; III, 280 kg N/ha; DM, dry matter; CP, crude protein; TDN, total digestive nutrients; Total = t DM/ha per year; SEM, standard error of the mean; *P* value, significance level; T, treatment; S, season; NA, not applicable.

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The same result was obtained for total production for these two variables.

### Nutritional value

No difference in CP concentrations was found between pastures (Table 3); between the seasons, higher values were obtained in the winter, which was related to the presence of ryegrass ($r = 0.73$; $P < 0.001$). A correlation was found between CP and NDF ($r = -0.81$; $P < 0.001$). For the NDF concentrations, a difference was observed between the grazing systems ($P \leq 0.05$) only in the spring, with a lower value in the pasture under intermediate nitrogen fertilization compared to the pasture subjected to inoculation. Variability between seasons was noted, with higher NDF concentrations in the summer, followed by autumn, due to the greater presence of Tifton 85 bermudagrass ($r = 0.96$; $P < 0.001$). For the TDN concentration, associations with ryegrass ($r = 0.73$; $P < 0.001$) and Tifton 85 bermudagrass ($r = -0.74$; $P < 0.001$) were identified.

### Grazing efficiency, forage intake and the stocking rate

No effect of the grazing systems on grazing efficiency was observed, with values close to 500 g/kg DM (Table 4). Higher values ($P \leq 0.001$) for grazing efficiency were noted in the autumn-winter, and an association was found with the NDF concentration of the forage ($r = -0.76$; $P = 0.004$). In addition, no effect of the grazing systems on the apparent forage intake was observed, with an average of 23 and 17 g/kg body weight for the autumn-winter and spring-summer, respectively. Forage intake was associated with the concentrations of CP ($r = 0.60$; $P < 0.001$) and NDF in the pasture ($r = -0.75$; $P < 0.001$). Additionally, for the stocking rate, no effect of the grazing system was identified. Among the seasons, the highest stocking rates were observed in the spring and summer.

## Discussion

### Pasture responses

The mean pre-grazing and post-grazing sward height results showed the adequacy of the strategy for better pasture use and maintenance of perennial crops (Michelangeli *et al.*, 2010). With the proposed approach, grazing cycles of less than 30 days can be conducted, a condition usually associated with forage production and better nutritional value (Gonçalves *et al.*, 2002; Pedreira *et al.*, 2018).

The available pre-grazing forage mass data show that in the pasture subjected to inoculation that received less nitrogen fertilizer, the values were equivalent to the highest fertilization level. For the leaf blade:stem + sheath ratio of Tifton 85 bermudagrass, the highest values are associated with lower doses of N, which restrict plant growth, consequently resulting in lower participation by the stem (Alderman *et al.*, 2011).

For the post-grazing forage mass values, the balance between the pastures indicates correct management when using a forage supply close to 4.5 kg DM/100 kg body weight; for the post-grazing leaf blade: stem + sheath ratio of Tifton 85 bermudagrass, the values are similar, both between pastures and between seasons due to the management approach, resulting in remaining leaf blade amounts of approximately 250 g/kg DM. Under this condition, in addition to a lack of intake limitation, the leaves are

**Table 3.** Nutritional value of the forage in the three pasture systems (PS), Santa Maria, RS, Brazil, 2019–2020

| | Autumn | | | Winter | | | Spring | | | Summer | | | Mean | | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | SEM | *T* | *S* | *T* × *S* |
| Crude protein (g/kg DM) | 154 | 157 | 166 | 202 | 209 | 209 | 132 | 148 | 148 | 133 | 146 | 138 | 155 | 165 | 165 | 29.6 | 0.123 | <0.001 | 0.797 |
| Total digestive nutrients (g/kg DM) | 544 | 542 | 544 | 601 | 605 | 610 | 557 | 571 | 565 | 527 | 520 | 528 | 557 | 560 | 562 | 13.2 | 0.042 | <0.001 | 0.216 |
| Neutral detergent fibre (g/kg DM) | 704 | 709 | 703 | 568 | 558 | 546 | 673 | 640 | 654 | 745 | 761 | 744 | 672 | 667 | 662 | 31.5 | 0.045 | <0.001 | 0.217 |

PS = I, 180 kg N/ha + *Azospirillum brasilense*; II, 230 kg N/ha; III, 280 kg N/ha; SEM, standard error of the mean; *P* value, significance level; T, treatment; S, season.

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**Table 4.** Animal responses in the three pasture systems (PS), Santa Maria, RS, Brazil, 2019–2020

| Variables | Autumn | | | Winter | | | Spring | | | Summer | | | Mean | | | SEM | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III | | *T* | *S* | *T* × *S* |
| Grazing efficiency (g/kg DM) | 549 | 517 | 516 | 501 | 494 | 478 | 456 | 462 | 460 | 434 | 432 | 469 | 487 | 476 | 481 | 75.4 | 0.592 | <0.001 | 0.281 |
| Apparent forage intake (g/kg BW) | 23 | 21 | 21 | 25 | 25 | 24 | 18 | 19 | 18 | 14 | 15 | 16 | 20 | 20 | 20 | 3.99 | 0.642 | <0.001 | 0.295 |
| Stocking rate (AU/ha) | 1.86 | 1.68 | 1.89 | 1.73 | 1.70 | 1.61 | 5.80 | 6.00 | 6.50 | 6.22 | 6.40 | 6.47 | 3.09 | 3.85 | 4.12 | 0.80 | 0.086 | <0.001 | 0.572 |

PS = I, 180 kg N/ha + *Azospirillum brasilense*; II, 230 kg N/ha; III, 280 kg N/ha; DM, dry matter; BW, body weight; AU, animal unit; SEM, standard error of the mean; *P* value, significance level; T, treatment; S, season.

rapidly restored, providing new grazing in less than 30 days (Vieira and Michel Filho, 2010).

In terms of the botanical composition, Tifton 85 bermudagrass predominated in the pre-grazing forage mass in the spring, summer and early-mid autumn and ryegrass predominated during winter and early-mid spring. For the other species fraction consisting of *Sida* spp., *Cynodon* spp. and *Cyperaceae* spp., low participation in the pasture composition was observed, which is a reflection of the management approach adopted here, with residual pasture heights between 7 and 10 cm (Michelangeli *et al.*, 2010). The contribution of dead material was high in the winter due to the senescence of Tifton 85 bermudagrass, resulting from the action of the cold and frosts, and due to ryegrass maturation in the spring (Aguirre *et al.*, 2018).

With respect to the post-grazing composition of pastures, less participation of ryegrass is evident due to the cows' greater preference for winter species than for summer species (Vieira *et al.*, 2019). For the dead material fraction, an increase occurred due to the effect of trampling and self-shading (Alderman *et al.*, 2011). The lower values for dead material are related to the higher doses of nitrogen fertilizer, which prolong the useful life of the leaf and reduce the leaf senescence rate (Farias Filho *et al.*, 2018). Regarding the presence of other plants, an increase in pasture composition was noted because these plants are less frequently consumed by cows (Simonetti *et al.*, 2019).

For the forage accumulation rate, the introduction of ryegrass helped reduce the seasonality of the Tifton 85 bermudagrass forage yield. The lack of difference between the grazing systems observed during the three seasons demonstrates an effect from *A. brasilense*. This assumption is confirmed by the total forage yield, which did not differ between the pasture inoculated and fertilized with 180 kg N/ha and the pasture fertilized with 230 kg N/ha corroborating studies showing that *A. brasilense* inoculation enables a reduction between 20 and 30% in the use of nitrogen fertilizer (Pindi and Satyanarayana, 2012; Leite *et al.*, 2019; Duarte *et al.*, 2020; Hungria *et al.*, 2021). A superior value was obtained in pastures of the Coastcross-1 bermudagrass when it was inoculated and fertilized with 100 kg N/ha, and the forage production was equivalent to that in the pasture fertilized with 200 kg N/ha per year (Aguirre *et al.*, 2018).

The lack of difference in forage yield between the pasture inoculated and fertilized with 180 kg N/ha and the pasture that received 230 kg N/ha confirms the action of *A. brasilense*, enabling reductions in the use of nitrogen fertilizer of 50 kg N/ha. This increased forage yield response may be associated with the action of *A. brasilense* in solubilizing phosphates and other soil minerals (Hungria *et al.*, 2010) and in increasing hormone synthesis, providing greater root development (Pedraza *et al.*, 2020; Housh *et al.*, 2021; Hungria *et al.*, 2021) and with nitrogen synthesis to a smaller extent (Hungria *et al.*, 2016). These actions enable greater nutrients absorption from the soil and water (Bashan and Bashan, 2010; Duarte *et al.*, 2020; Santos *et al.*, 2021), which may have contributed to the higher performance of ryegrass and Tifton 85 bermudagrass subjected to inoculation.

The production of 21 t DM/ha of forage in the inoculated pasture was higher than that observed in the same region with Tifton 85 bermudagrass plus ryegrass fertilized with 200 kg N/ha (Olivo *et al.*, 2019). Ryegrass production (4.6 t DM/ha) was more significant than that found in other studies with overseeding of Tifton 85 bermudagrass of 2.3 t DM/ha (Olivo *et al.*, 2019) and 3.2 t DM/ha (Ribeiro *et al.*, 2009). However, the higher forage production observed in the pasture with a higher N dose confirms

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that Tifton 85 bermudagrass responds well to nitrogen fertilization (Taffarel *et al*., 2016). This response was observed in the summer (Table 2), when the production of this forage was highest.

For nutritional value, most variables showed no difference between the grazing systems. The values implying better nutritional value for the forage, including higher CP and TDN concentrations and lower NDF as observed in the winter, are associated with the presence of ryegrass during the vegetative period. The mean CP, TDN and NDF values were 160, 560 and 680 g/kg DM, respectively, which are similar to values obtained in the same region, with ryegrass overseeding on Coastcross-1 bermudagrass (Diehl *et al*., 2014). For the CP of Tifton 85 bermudagrass in the summer, the season in which this pasture was predominant, the mean value between grazing systems, 139 g/kg DM, was lower than that observed in a study in which this grass was mixed with forage legumes (Olivo *et al*., 2019).

The equivalence in TDN and CP production between the grazing systems with lower nitrogen fertilization (and inoculated with *A. brasilense*) and intermediate fertilization is due to the lack of difference between these pastures, both in the concentration of these components and in forage yield. In the pasture receiving the highest dose of nitrogen fertilizer, higher TDN and CP production was associated with higher forage yield.

### Animal responses

The lack of difference in the inoculated pasture compared to the others with higher doses of nitrogen fertilizer indicates that using *A. brasilense* affected the performance of the cows. The grazing efficiency values during the autumn and winter are adequate because they are close to the range that does not limit intake and are associated with the best nutritional value for the forage. In the spring and summer, the values are below 500 g/kg DM, resulting in limited intake (Delagarde *et al*., 2001). This behaviour is associated with a higher NDF concentration in the forage, as observed in the summer. Additionally, environmental aspects (Fig. 1), such as air temperature above the comfort level of the animals (Rhoads *et al*., 2009), should be considered.

For the stocking rate, the results show that inoculation resulted in a performance equivalent to that of the other pastures, which can be attributed to the forage yield (which did not differ during three of the four seasons) and the higher leaf blade: stem + sheath ratio of Tifton 85 bermudagrass from the pasture inoculated with *A. brasilense*. A higher stocking rate, with an annual average of 6.1 AU/ha, was observed in an experiment conducted with Tifton 85 bermudagrass fertilized with 200 kg N/ha (Olivo *et al*., 2019).

## Conclusions

*Azospirillum brasilense* inoculation in pastures planted with Tifton 85 bermudagrass-based in combination with ryegrass (plus 180 kg N/ha per year) had a positive effect, providing forage yield and nutritive value equivalent to those with fertilization with 230 kg N/ha per year. Fertilization with 280 kg N/ha per year results in a higher forage yield. The pastures performed similarly in terms of forage nutritional value. The grazing systems used here do not affect parameters related to animals, grazing efficiency or forage intake.

**Author contributions.** L. G. Casagrande and C. J. Olivo: conceived and designed the study, performed statistical analyses and wrote the article. J. F. Aires: contribution to the statistical analyses. A. C. Vieira, A. A. Charão, M. P. Quatrin, F. A. Grzelak, M. D. Frantz, M. E. C. Salvador, B. M. de Andrade, P. B. dos Santos and T. J. Tonin: conducted data gathering.

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**Conflict of interest.** None.

**Ethical standards.** The techniques and procedures used in this study were approved by the Ethics Committee on Animal Experimentation – UFSM (protocol n. 6130 221 019; 04/28/2020).

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