

## RESPONSE OF JIGGS GRASS TO INOCULATION WITH PLANT GROWTH-PROMOTING MICRORGANISMS

Sonia Purin da Cruz<sup>1\*</sup>, Kelen Cristina Basso<sup>1</sup>

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**ABSTRACT** - Brazil is the second world producer of bovine meat, which often relies on preserved forage to decrease seasonality of forage production. Increased forage accumulation may be favored by technologies such as inoculation. Research works on this theme have been conducted with *Azospirillum brasilense* associated to oats, ryegrass and guinea grass. However, response of jiggs, a widely cultivated perennial grass, to plant growth-promoting microorganisms is poorly understood. Hence this study aimed to assess effects of inoculation on development of *Cynodon dactylon* cv. Jiggs. Four treatments were tested: T1) control, T2) Inoculation with *A. brasilense*, T3) Inoculation with *Azospirillum*, *Rhizobium*, *Pseudomonas* and *Saccharomyces*, T4) Inoculation + Reinoculation with *Azospirillum*, *Rhizobium*, *Pseudomonas* and *Saccharomyces*. Forage mass, percentage of leaves, stems, dead mass, reproductive structures and leaf/stem ratio were measured. Data were submitted to analysis of variance, and when significant effects were detected, means were separated by LSD Test. Positive effects of inoculation were recorded at all evaluations, mostly concerning percentage of leaves and leaf/stem ratio. *A. brasilense* improved leaf/stem ratio up to 56%. Also, a 64% increment on this parameter was obtained with *Azospirillum*, *Rhizobium*, *Pseudomonas* and *Saccharomyces*. Modifications of plant morphology components are important since grasses with more percentage of leaves increase nutritional value of haylage. Morphology of *Cynodon dactylon* cv. Jiggs is affected by *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*, improving aspects related to palatability and preferred grazing of livestock animals.

**Keywords:** *Cynodon dactylon* (L.) Pers., rhizobacteria, haylage.

## RESPOSTA DO CAPIM JIGGS A INOCULAÇÃO COM MICRORGANISMOS PROMOTORES DE CRESCIMENTO DE PLANTAS

**RESUMO** - O Brasil é o segundo maior produtor mundial de carne bovina, que necessita muitas vezes de forragem conservada, para diminuir a estacionalidade de produção forrageira. O aumento do acúmulo de forragem pode ser favorecido por tecnologias como a inoculação. Trabalhos de pesquisa nesse tema vêm sendo conduzidos com *Azospirillum brasilense* associado a aveia, azevém e grama da Guiné. Entretanto, a resposta do jiggs, uma gramínea perene amplamente cultivada, a microrganismos promotores de crescimento vegetal, é pouco entendida. Esse estudo objetivou avaliar efeitos da inoculação no desenvolvimento de *Cynodon dactylon* cv. Jiggs. Quatro tratamentos foram testados: T1) controle, T2) Inoculação com *A. brasilense*, T3) Inoculação com *Azospirillum*, *Rhizobium*, *Pseudomonas* e *Saccharomyces*, T4) Inoculação + Reinoculação com *Azospirillum*, *Rhizobium*, *Pseudomonas* e *Saccharomyces*. Mensurou-se massa de forragem, porcentagem de folhas, colmos, material morto, estruturas reprodutivas e relação folha/colmo. Os dados foram submetidos a análise de variância, e quando efeitos significativos foram detectados, as médias foram separadas pelo Teste LSD. Efeitos positivos da inoculação foram registrados em todas as avaliações, principalmente sobre porcentagem de folhas e relação folha/colmo. *A. brasilense* aumentou a relação folha/colmo em até 56%. Além disso, um incremento de 64% nesse parâmetro ocorreu com *Azospirillum*, *Rhizobium*, *Pseudomonas* e *Saccharomyces*. Modificações nos componentes morfológicos vegetais são importantes, pois gramíneas com maior porcentagem de folhas aumentam o valor nutritivo do pré-secado. A morfologia de *Cynodon dactylon* cv. Jiggs é afetada por *Azospirillum*, *Rhizobium*, *Saccharomyces* e *Pseudomonas* melhorando aspectos relacionados a palatabilidade e pastejo preferencial pelo gado.

**Palavras-chave:** *Cynodon dactylon* (L.) Pers., rizobactérias, pré-secado.

### INTRODUCTION

Agriculture and Livestock activities were responsible for 5.2% of Brazilian Gross Domestic Product in 2019 (IBGE, 2020). The livestock sector, in particular, has great relevance once Brazil holds the second largest flock and is the top bovine meat exporter worldwide.

Livestock gross domestic product increased 8.3% from 2018 to 2019, and is expected to achieve 11.8% from 2019 to 2020 (CNA, 2020). Given the fact that bovine meat is the major component of livestock, research on intensive production systems that optimize pasture yield and quality is mandatory.

<sup>1</sup>Universidade Federal de Santa Catarina – UFSC, Campus Curitibanos, SC, Brasil. E-mail: [s.purin@ufsc.br](mailto:s.purin@ufsc.br). \*Corresponding Author.

Projections to the four next decades indicate that forage production in Brazil must be increased by 25% in order to sustain livestock production. This should be accomplished by higher yields in currently established pasture areas, instead of increasing extension of land used for this purpose (which would have negative environmental impacts). Grasslands with higher yield and improved nutrient contents can support larger flocks with no need to expand extension of livestock properties. In addition, many forage conservation strategies have been employed to minimize the effects of seasonal production of forage during the dry months of the year.

Inoculation with plant growth-promoting microorganisms, mostly with *Azospirillum brasilense*, is an economically feasible and environmentally friendly technique that improves yield and nutritional quality of pastures (HEINRICHS, 2000; OKUMURA et al., 2013). In Brazil, *A. brasilense* has been commercially recommended for the annual grass *Brachiaria* spp. (= *Urochloa* spp.), based on its effects on plant growth and nitrogen content. Increments on shoot biomass ranged from 13 to 25%, underlining the potential of microorganisms to be used when higher yields are aimed (HUNGRIA et al., 2016). Some researchers have explored other bacteria groups to improve pasture growth. Duarte et al. (2020a) inoculated *Urochloa* with *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Pantoea ananatis* and found positive results on leaf and stem elongation, as well as number of basal tillers, when bacteria were applied as single inoculants. However, different groups of microorganisms may be used in combination, a technique known as coinoculation (HUNGRIA et al., 2013). Despite its proved effects on yield and growth of some agricultural crops (HUNGRIA et al., 2013), coinoculation remains poorly understood in pasture species (SÁ et al., 2019a; SÁ et al., 2019b).

*Cynodon* is one of the most spread genera of perennial grass used as pasture worldwide, primarily in tropical and subtropical regions. Its response to rhizobacteria has been verified only through single inoculation with *A. brasilense*. Aguirre et al. (2018) confirmed that *A. brasilense* improved forage yield rate of *Cynodon dactylon* cv Coastcross-1 from 21.2 to 28.1 kg of dry mass per day. When reinoculation was performed, values as high as 33 kg were achieved, what represents a 56% increment compared to absence of inoculation. In a following experiment, *A. brasilense* improved dry matter from 3.9 to 7.4 Mg ha<sup>-1</sup> (AGUIRRE et al., 2020). Nitrogen accumulation increased from 81.8 to 156.6 kg ha<sup>-1</sup>. Both studies emphasize that more pronounced effects of bacteria were achieved without nitrogen fertilization.

Other *Cynodon* cultivars comprise jiggs (*Cynodon dactylon* cv. Jiggs), one the most recently groups introduced in Brazil (ATHAYDE et al., 2013). Preliminary data suggest that jiggs is preferred by animals over other cultivars such as Tifton 85, Tifton 68 and Russel due to its higher quantity of leaves, what makes plants more palatable (RANDÜZ, 2005). However, little is known

about cultivation practices, grass management and association with plant growth-promoting microorganisms.

Given the potential of inoculation in other species of grasses and pastures to improve yield and change morphological composition, this study aims to evaluate effects of plant growth-promoting microorganisms on jiggs (*Cynodon dactylon* cv. Jiggs) in field conditions.

## MATERIAL AND METHODS

Inoculation experiments were established in two seasons: 2017-2018 and 2018-2019, in the city of Curitiba (SC), Brazil. Experimental sites were located in a property of Gemelli company, that produces milk and cheese. Most of the land in this property is cultivated with forage that is used for either cattle consumption *in situ* or haylage production. Species of grass are jiggs (*Cynodon dactylon*) in the summer and forage oat (*Avena sativa*) and ryegrass (*Lolium multiflorum*) in the winter. Gemeli company is located at 27°21' 08'' S and 50° 42' 32'' W, 987 m above sea level. Both experiments were conducted on areas with jiggs as perennial grass.

Soil is classified as Latosol, and analysis parameters were: 5.8 pH H<sub>2</sub>O; 5.2 pH CaCl<sub>2</sub>; 23 g dm<sup>-3</sup> organic carbon; 39.56 g dm<sup>-3</sup> organic matter; 7.26 cmol<sub>c</sub> dm<sup>-3</sup> Ca; 3.82 cmol<sub>c</sub> dm<sup>-3</sup> Mg; 0.27 cmol<sub>c</sub> dm<sup>-3</sup> K; 11.25 mg dm<sup>-3</sup> P; 16.70 cmol<sub>c</sub> dm<sup>-3</sup> CEC. Throughout the year, the company performed addition of 16 tons ha<sup>-1</sup> of chicken manure, 255 kg ha<sup>-1</sup> of urea and 150 kg ha<sup>-1</sup> of KCl.

The first experiment was carried on at the end of 2017-2018 season. It was established in March 2018, following a completely randomized block design. Three treatments were: T1- Control; T2- Inoculation with *Azospirillum brasilense* (1 L ha<sup>-1</sup>), T3- Inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas* (2 L ha<sup>-1</sup>). Four replicates were established for each treatment. Each replication (experimental unit) consisted of a 12 m<sup>2</sup> plot (4 m x 3 m). Both blocks and replication were spaced by 1 m. Inoculants were provided by Total Biotecnologia (Curitiba - PR, Brazil).

*Azospirillum brasilense* was used as liquid inoculant in its commercial version (AzoTotal®). This product was the first in Brazil to be recommended as growth-promoter of *Brachiaria decumbens*. Its composition consists of strains Ab-V5 (CNPSO 2083) and Ab-V6 (CNPSO 2084), at the concentration of 2 x 10<sup>8</sup> CFU mL<sup>-1</sup>. *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas* were also used as liquid inoculant in its commercial version (Accelerate Fertility). This product has been approved as currently recommended as a microbial additive in the composting process by the Brazilian Federal Government. Neither product has commercial recommendation as a plant growth-promoting inoculant to jiggs. Both inoculants were first diluted in water to a final volume of 150 L ha<sup>-1</sup>, and then spray-applied on soil surface.

In 2018-2019, studied treatments were: T1 - control, T2 - Inoculation with *Azospirillum brasilense* (2 L ha<sup>-1</sup>), T3 - Inoculation with *Azospirillum*, *Rhizobium*,

*Saccharomyces* and *Pseudomonas* (2 L ha<sup>-1</sup>) and T4 - Inoculation and reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas* (2L ha<sup>-1</sup>). Reinoculation procedure was performed in January 2019, month in which the highest temperatures are recorded and fastest growth of jiggs occurs. Experimental design, number and size of experimental units were the same as 2017-2018.

Cuttings and data collection were performed according to the company activities. Every time jiggs was cut from the remaining farm areas to produce grass haylage, sample collection was carried on in the experimental area. A single cutting was performed in the 2017-2018 season, and three cuttings were performed between 2018 and 2019. Analyzed variables were fresh shoot mass, dry shoot mass, percentage of leaves, percentage of stems, percentage of dead material and percentage of reproductive structures (if present). Based of mass of leaves and stems, the leaf/stem ratio was calculated.

In each experimental unit, a sampler device (0.5 m<sup>2</sup>) was placed in two randomly chosen places. All plants within the sampler were cut at 5 cm above soil level. This criterion was chosen to simulate the remaining height of jiggs after harvesting machines have cut all grass in the farm. Plants were placed inside plastic bags and weighed for fresh shoot mass. A subsample was transferred to paper

bags and kept at 65°C for 72 h, in order to estimate moisture and calculate dry shoot mass. The remaining fresh material sampled was then classified as leaves, stems, dead material and reproductive structures. Each fraction was weighted separately. Moisture was deducted from the obtained values and percentage of leaves, stems, dead material and reproductive structures (if present) were calculated as fractions of shoot dry mass. All values were estimated and standardized based on an area of one hectare (10,000 m<sup>2</sup>).

Data were submitted to analysis of variance (ANOVA). When significant differences were found among treatments, means were ranked with the LSD Test (p<0.10), using the software SISVAR (FERREIRA, 2011).

## RESULTS AND DISCUSSION

The first experiment revealed promising effects of inoculation with the consortium of plant growth-promoting microorganisms on some evaluated variables. Combined use of *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas* affected both percentage of leaves and leaf/stem ratio (Table 1). Control treatment promoted and average of 32% leaves, while inoculation changed this characteristic to 44.75%. Also, leaf/to stem ratio was improved in 64% by the combined use of these four bacteria. Other evaluated parameters did not respond to treatments tested.

**TABLE 1** - Inoculation treatments on growth and yield parameters of jiggs (*Cynodon dactylon*).

Treatments	Fresh shoot mass (kg ha <sup>-1</sup> )	Dry shoot mass (kg ha <sup>-1</sup> )	% of leaves	% of stems	% of dead mass	Leaf/stem ratio
T1	13,580.7 <sup>ns</sup>	3,076.7 <sup>ns</sup>	32.33a1*	46.39 <sup>ns</sup>	21.28 <sup>ns</sup>	0.70 b
T2	12,768.7	3,061.5	38.58a1a	39.94	21.48	1.02ab
T3	13,781.2	3,235.5	44.76a	39.22	16.02	1.15a
Average	13,376.9	3,124.5	38.56	41.85	19.59	0.95
CV(%)	14.32	12.54	19.17	12.18	27.90	28.45

ns = no significant differences according to the analysis of variance, \* = means followed by the same letter and number are not different (LSD Test, p<0.1). CV = coefficient of variation, T1 = control, T2 = Inoculation with *Azospirillum brasilense*, T3 = inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*.

On January 2019, significant effects of *Azospirillum brasilense* were observed on leaf/stem ratio. This bacterium, when inoculated on jiggs, increased this parameter from 0.34 to 0.53, a 56% improvement. The remaining characteristics were not affected by any studied treatment (Table 2). The highest percentages of leaves were observed with treatments 3 and 4 (Table 3). No significant response was recorded in any other measured variable.

In the last sampling procedure performed, reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas* produced higher percentage of leaves and leaf/stem ratio (Table 4). Mean percentage of leaves changed from 36.58 (without inoculation) to 41.07 (T4). Leaf/stem ratio was 25% improved with treatment 4 compared to the absence of inoculation. No statistical difference among treatments was reported in any other growth or yield parameter.

**TABLE 2** - Inoculation treatments on growth and yield parameters of jiggs (*Cynodon dactylon*).

Treatments	Fresh shoot mass (kg ha <sup>-1</sup> )	Dry shoot mass (kg ha <sup>-1</sup> )	% of leaves	% of stems	% of dead mass	% of reproductive structures	Leaf/stem ratio
T1	15,237.9 <sup>ns</sup>	4,248.4 <sup>ns</sup>	19.43 <sup>ns</sup>	57.81 <sup>ns</sup>	11.56 <sup>ns</sup>	11.20 <sup>ns</sup>	0.34b*
T2	17,773.7	4,376.6	27.43	54.05	10.20	8.32	0.53a
T3	15,789.6	4,119.1	22.20	58.63	9.49	9.68	0.39b
T4	14,902.4	4,018.0	23.08	54.67	12.34	9.91	0.42ab
Average	15,925.90	4,190.5	23.04	56.29	10.89	9.78	0.42
CV(%)	21.89	30.96	20.50	12.52	58.84	51.32	25.83

ns = no significant differences according to the analysis of variance, \* = means followed by the same letter and number are not different (LSD Test, p<0.1), CV = coefficient of variation, T1 = control, T2 = inoculation with *Azospirillum brasilense*, T3 = inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*, T4 = inoculation + reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*.

**TABLE 3** - Inoculation treatments on growth and yield parameters of jiggs (*Cynodon dactylon*).

Treatments	Fresh shoot mass (kg ha <sup>-1</sup> )	Dry shoot mass (kg ha <sup>-1</sup> )	% of leaves	% of stems	% of dead mass	Leaf/stem ratio
T1	8,226.4 <sup>ns</sup>	2,824.0 <sup>ns</sup>	37.78ab*	43.28 <sup>ns</sup>	18.94 <sup>ns</sup>	0.87 <sup>ns</sup>
T2	8,483.6	2,849.1	34.85b	41.45	23.70	0.84
T3	7,935.3	2,489.0	41.33a	43.36	15.31	0.97
T4	7,575.7	2,499.1	43.64a	42.68	13.68	1.03
Average	8,055.2	2,665.3	39.40	42.70	17.90	0.93
CV(%)	24.71	24.99	12.41	11.16	35.74	17.51

Ns = no significant differences according to the analysis of variance, \* = means followed by the same letter and number are not different (LSD Test, p<0.1), CV = coefficient of variation, T1 = control, T2 = inoculation with *Azospirillum brasilense*, T3 = inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*, T4 = inoculation + reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*.

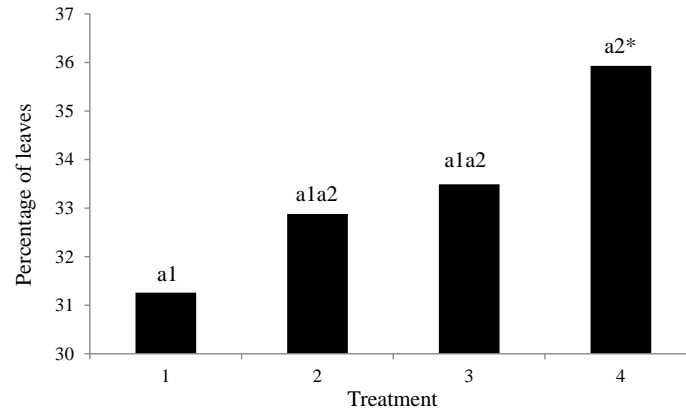
**TABLE 4** - Inoculation treatments on growth and yield parameters of jiggs (*Cynodon dactylon*).

Treatments	Fresh shoot mass (kg ha <sup>-1</sup> )	Dry shoot mass (kg ha <sup>-1</sup> )	% of leaves	% of stems	% of dead mass	% of reproductive structures	Leaf/stem ratio
T1	6,067.5	2,277.7	36.59b	50.19	8.04	5.18	0.73b
T2	5,888.4	2,284.5	36.39b	49.84	6.66	7.11	0.73b
T3	5,911.4	2,343.2	36.94b	50.45	8.28	4.33	0.73b
T4	6,100.7	2,401.2	41.07a	45.59	6.93	6.41	0.91a
Average	5,992.0	2,326.68	37.74	49.02	7.48	5.76	0.77
CV(%)	22.00	18.17	7.93	7.68	61.90	53.55	12.38

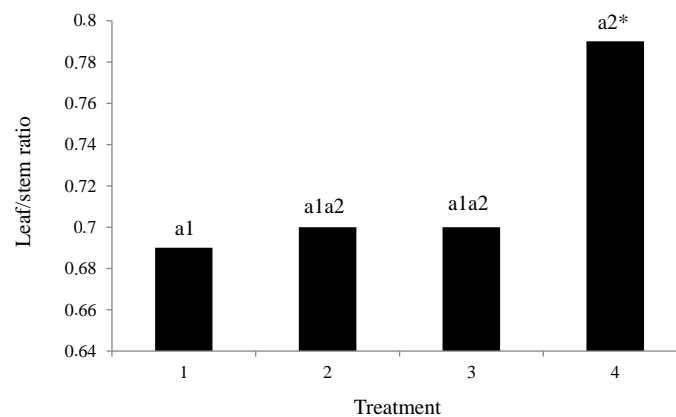
ns = no significant differences according to the analysis of variance, \* = means followed by the same letter and number are not different (LSD Test, p<0.1), CV = coefficient of variation, T1 = control, T2 = inoculation with *Azospirillum brasilense*, T3 = inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*, T4 = inoculation + reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*.

When average values from all three cuts were combined, the effect of inoculation + reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas* was highly pronounced on percentage of

leaves and leaf/stem ratio. Non inoculated plants had an average 31.26% of leaves, while this number changed to 35.93 with T4 (Figure 1). Leaf/stem ratio increased by 14.5% from T1 to T4 (Figure 2).



**FIGURE 1** - Inoculation treatments on percentage of leaves of jiggs (*Cynodon dactylon*). T1 = control, T2 = inoculation with *Azospirillum brasilense*, T3 = inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*. T4 = inoculation + reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*. Average data combined from three cuttings = January, February and April 2019, \* = means followed by the same letter and number are not different (LSD Test,  $p < 0.1$ ).



**FIGURE 2** - Inoculation treatments on leaf/stem ratio of jiggs (*Cynodon dactylon*). T1 = control, T2 = inoculation with *Azospirillum brasilense*, T3 = inoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*. T4 = inoculation + reinoculation with *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*. Average data combined from three cuttings = January, February and April 2019, \* = means followed by the same letter and number are not different (LSD Test,  $p < 0.1$ ).

Inoculation of grasses such as *Urochloa* has been demonstrated as an environmental-friendly and feasible technique to improve plant growth and yield not only in Brazil, but also in several other countries (HUNGRIA et al., 2016; DUARTE et al., 2020a; DUARTE et al., 2020b). However, studies about plant growth-promoting microorganisms and *Cynodon* species, mainly Jiggs cultivar, remained to be performed despite its need and potential of application. Our results highlight the effects of inoculation on plant morphology and suggest positive outcomes related to forage consumption and animal nutrition. Inoculation, therefore, may be a tool to be considered in sustainable pasture systems and more productive livestock systems.

Jiggs yield was not affected by any treatment evaluated in this study. Although inoculation is known to improve yield of other grass species (HEINRICHS et al., 2020; HUNGRIA et al., 2016; AGUIRRE et al., 2018), this was not observed in the current experiment. According to Guimarães et al. (2011), for example, the use of *Azospirillum* shows an increase of 10% over the values of

number of leaves in pasture of *Brachiaria decumbens* when compared to the absence of the inoculant. It must be emphasized that this is a preliminary study and other inoculant formulations, concentrations and methods of application should be further tested. These factors have shown to alter growth and yield components of other crops - mainly soybean and have just begun to be explored on grass species.

For instance, it has been reported that some growth components of soybean were altered by inoculant formulation. Zilli et al. (2010) showed that number of nodules produced when plants were inoculated with liquid inoculant was 270. When peat-based inoculant was applied, this value significantly changed to 369 (a 37% increase).

Concentration of bacteria has also to be considered. For decades, it is known that variations in bacteria concentrations may respond for up to 75% variation in plant-growth responses (WEAVER; FREDERICK, 1974). Inoculation of wheat with *Azospirillum brasilense* under greenhouse conditions was

evaluated by Fukami et al. (2016). When bacteria were inoculated in planting furrow, associated to 75% N, tillers were different between inoculum concentrations. At 1 dose ( $1.74 \times 10^4$  cells seed<sup>-1</sup>), number of tillers was of 6.2 per plant. On the other hand, when this dose was of 2.5x, an average of only 4.4 tillers was documented in each plant.

Methods of application may also interfere in plant response to inoculation, as observed by Zilli et al. (2008). Seed inoculation produced an average of 3.8 g of shoot dry mass per plant, while post-emergence inoculation reduced shoot mass by 53%. Grain yield also decreased from 3,680 to 2,946 kg ha<sup>-1</sup> with seed and post-emergence inoculation, correspondingly. In a greenhouse study with maize and *Azospirillum brasilense* with several inoculant doses and N concentrations, plants responded to inoculation methods only when bacteria were used at 2.5x recommended dose and 75% N (FUKAMI et al., 2016). In these conditions, when inoculant was sprayed on leaves, shoot dry weight per plant was 7.22 g, but when it was sprayed on soil surface, this value changed to 8.5 g.

Overall, the combination of four genera of plant-growth promoting microorganisms (*Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*), applied two times in the 2018-2019 cycle, produced the best overall results regarding percentage of leaves and leaf/stem ratio. The three bacteria genera are commonly found in the rizosphere of grasses and legume pastures, such as clover, red clover, maize and alfafa (AFZAL et al., 2019). Inoculation of plants with distinct microorganisms as a mixed inoculum is a potential synergistic tool, given that different growth-promoting mechanisms are combined. *Azospirillum* is far known for biological nitrogen fixation and production of phytohormones that promote proliferation of roots (BASHAN; DE-BASHAN, 2010; FUKAMI et al., 2018). *Bacillus* and *Pseudomonas* action of mode relies on the ability to form biofilms, which improve water retention in the rizosphere and act against pathogens through antibiotic production (RAAIJMAKERS et al., 2010; ALTAF, 2017; TORRES et al., 2020). *Pseudomonas* and *Rhizobium* are also phosphate solubilizers and have been report to promote growth of grass species such as maize, Zuri guinea grass and mavuno grass (CHABOT et al., 1996; SÁ et al., 2019a). Processes performed by *Saccharomyces* consist of phosphate solubilization, induction of resistance and secretion of hydrolitic enzymes with parasitism function (HESHAM; MOHAMED, 2011; FREIMOSER et al., 2019).

Positive effects of reinoculation were also reported by Sá et al. (2019b), working with Zuri guinea grass [*Megathyrsus* (syn. *Panicum*) *maximus*]. All single, coinoculation and reinoculatoin treatments tested with *Azospirillum*, *Pseudomonas* and *Rhizobium* were able to improve shoot and root dry weight, as well as tiller number, compared to the negative control treatment (without N or inoculation). However, no effect of treatments was detected on leaf blade percentage, the opposite to the observed in the current study.

Even with no increment on jiggs yield, there was a very positive change in percentage of leaves and

leaf/stem ratio, and this is a crucial aspect to forage quality. Jiggs is described to have higher percentage of leaves compared to other pasture species (RANDÜZ, 2005; INAM-UR-RAHIM et al., 2008), and plant growth-promoting microorganisms may even improve this characteristic, what is strongly related to acceptability and palatability of plants by animals. Indeed, O'Reagain and Mentis (1989) have researched the acceptability of nine indigenous grass species to cattle, and leaf percentage was one of the nine characteristics related to preferred acceptability. Waldron et al. (2010) also observed that percentage of leaves has a strong correlation with *Kochia prostrata* biomass consumption by cattle ( $r = 0.70$ ,  $p < 0.01$ ).

Increased leaf/stem ratios are also an important aspect once stemminess is reduced in the entire forage composition. In fact, stemmy grass species are frequently avoided for grazing by animals (O'REAGAIN; MENTIS, 1989). Oliveira et al. (2018) evaluated preference of equines for different alfafa hay types, classified as A, B and C based distinct morphological and bromatological properties. Horses showed preference to hay type A, with the highest leaf/stem ratio (0.77). Hence, higher leaf/stem ratios, as found in the current study to be promoted by reinoculation with different microorganisms, are interesting to improve forage consumption by animals because of higher palatability. However, other characteristics of jiggs that are related to grazing preference must be analyzed, such as nutrient concentration, crude protein, water-soluble carbohydrates, neutral detergent fiber and acid detergent fiber.

## CONCLUSION

Morphology of *Cynodon dactylon* cv. Jiggs is affected by *Azospirillum*, *Rhizobium*, *Saccharomyces* and *Pseudomonas*, improving aspects related to palatability and preferred grazing of livestock animals.

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