

Short Communication

Chlorophyll concentration and production of *Urochloa decumbens* treated with diazotrophic bacteria and thiamine in the Brazilian Cerrado

Concentración de clorofila y producción del pasto Urochloa decumbens tratado con bacterias diazotróficas y tiamina en el Cerrado brasileño

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Abstract

The effects of application of *Azospirillum brasilense* and thiamine on chlorophyll concentration and forage mass of *Urochloa decumbens* were evaluated in a small plot experiment conducted in Goiânia, Goiás, Brazil. The treatments were applications of: *A. brasilense* (concentration of 10 mL/L); thiamine at 2 concentrations: 50 and 100 mg/L; combinations of *A. brasilense* and thiamine at the 2 concentrations; and a Control treatment (untreated grass). At the first harvest there was a trend for applying *A. brasilense*, either alone or in combination with thiamine, to increase the concentrations of chlorophyll, but differences were not always significant at $P < 0.05$. Dry mass of forage from applying *A. brasilense* plus thiamine at 100 mg/L was greater than that for Control and thiamine at both concentrations. At the second harvest, chlorophyll concentrations were not affected by treatment ($P > 0.05$), while dry matter production of forage from applying *A. brasilense* alone and thiamine at 100 mg/L was greater ($P < 0.05$) than that of Control and thiamine at 50 mg/L. Larger-scale and longer-term studies to validate these preliminary findings are needed.

Keywords: Biological fixation, forage, plant protection, tropical grasses, vitamin B1.

Resumen

En un experimento de campo conducido en Goiânia, Goiás, Brasil, se evaluó la producción de forraje y la concentración de clorofila en *Urochloa decumbens* en respuesta a la aplicación de *Azospirillum brasilense* y tiamina (vitamina B1). Los tratamientos consistieron en aplicaciones de: *A. brasilense* en concentración de 10 mL/L; tiamina en concentraciones de 50 y 100 mg/L; las combinaciones de *A. brasilense* con ambas concentraciones de tiamina; y un tratamiento testigo (gramínea no tratada). En el primer corte, la aplicación de *A. brasilense*, tanto sola como combinada con tiamina, incrementó la concentración de clorofila, pero en algunos casos las diferencias entre tratamientos no fueron significativas ($P < 0.05$). La producción de forraje seco fue más alta con la aplicación de *A. brasilense* más 100 mg/L de tiamina que la de los tratamientos testigo y tiamina en ambas concentraciones. En el segundo corte, las concentraciones de clorofila no presentaron diferencias ($P > 0.05$), mientras que la producción de materia seca fue más alta ($P < 0.05$) cuando se aplicó *A. brasilense* sola o tiamina en dosis de 100 mg/L, en comparación con el testigo y tiamina en dosis de 50 mg/L. Para validar estos resultados preliminares se necesitan estudios a escala mayor y plazo más largo.

Palabras clave: Fijación biológica, forraje, gramíneas tropicales, protección de plantas, vitamina B1.

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Introduction

Brazil has the largest cattle herd in the world, with about 218 million animals which represent approximately 15% of the world's total cattle population (FAO 2017). Most of these animals are raised on pasture, generating a product in great global demand (Jacinto et al. 2005). The production of cattle on pasture is dependent on both forage quantity and quality. As for other grasses, applying N fertilizer has a significant impact on the production of species of the genus *Urochloa* (Duarte et al. 2020). Economically, applying N fertilizer is a significant component of the costs of grass production (Costa et al. 2015).

Although N is commonly applied as chemical fertilizer, biological fixation through diazotrophic bacteria of the *Azospirillum* genus has been studied as a technique for the provision of atmospheric N to grasses (Hungria 2011), e.g. maize (Longhini et al. 2016). Nitrogen plays a major role in plant development, participating in the synthesis of several compounds, such as nucleic acids and proteins, as well as photosynthetic activity (Taiz et al. 2017). Chlorophyll concentration in a grass is generally accepted as a suitable indicator of a plant's N status and consequently crude protein (N \times 6.25) concentration (Rocha et al. 2010; Rincón Castillo et al. 2019).

In addition to N fertilizer, other compounds with bio-stimulating and protective effects, such as vitamins, can improve physiological and morphological characteristics of species of commercial interest. Among the vitamins studied, thiamine (vitamin B1) has the capacity to stimulate the production of secondary metabolites, which exert antioxidant activity and avoid the degradation of the photosynthetic system (Goyer 2010; Kaya et al. 2015). This vitamin is also related to the ability to stimulate the accumulation of energy reserves in plant tissues (Barakat 2003); its application has been shown to provide productive gains in beans (*Phaseolus vulgaris*) (Vendruscolo et al. 2018).

The present study aimed to evaluate the effect of inoculation with *Azospirillum brasilense* and treatment with thiamine, either alone or in combination, on forage mass production and chlorophyll concentration of *Urochloa decumbens*, an economically important grass in the Brazilian Cerrado.

Materials and Methods

The study was conducted in Goiânia, Goiás, central Brazil (16°40' S, 49°15' W; 750 masl). The climate in the experimental area is tropical with a rainy season from October to April and a dry season from May to September. The monthly average temperatures range from 20.8 °C in June and July to 25.3 °C in October (Cardoso et al. 2014).

The soil of the experimental area was classified as Latossolo Vermelho (Oxisol) and had the following chemical characteristics: Organic matter = 12.0 g/kg; pH (CaCl₂) = 5.3; P (Mehlich) = 2.0 mg/dm³; K = 80.0 mg/dm³; Ca = 3.8 cmol/dm³; Mg = 0.8 cmol/dm³; H+Al = 2.6 cmol/dm³; Al = 0.0 cmol/dm³; cation exchange capacity = 7.4 cmol/dm³; and base saturation = 65.0%.

The experiment was conducted in a pasture of *U. decumbens* cv. Basilisk using a randomized complete block design with 5 replicates. The 6 treatments used in the study were as follows: 1 - Control; 2 - *Azospirillum brasilense* (NITRO 1000 Gramineae, NITRO 1000, Cascavél, PR, Brazil) at a concentration of 10 mL/L of water; 3 - Thiamine (Neon, Suzano, SP, Brazil) at a concentration of 50 mg/L; 4 - Thiamine at a concentration of 100 mg/L; 5 - Thiamine at a concentration of 50 mg/L + *A. brasilense*; 6 - Thiamine at a concentration of 100 mg/L + *A. brasilense*. The plots were established in an area of existing 5-year-old pasture, and each plot was 1 \times 1 m. Products were applied via a manual sprayer at a volume equivalent to 200 L/ha.

The pasture had previously been mown quarterly. Prior to the application of the treatments, on 29 November 2016, the area was mown, leaving a stubble height of approximately 10 cm. Treatments were applied 5 days after mowing. For treatments that combined *A. brasilense* and thiamine, the products were mixed before application.

The evaluations were carried out when plants started flowering, on 2 occasions, the first at 50 days after the initial mowing (first harvest, 18 January 2017) and the second after a further 45 days (second harvest, 4 March 2017). At this time, the concentrations of chlorophyll a, chlorophyll b and total chlorophyll were evaluated using a digital chlorophyllometer (CFL1030; Falker, Porto Alegre, RS, Brazil) on 5 flag leaves per plot. All forage on each plot was harvested at about 10 cm above ground level and weighed immediately. Samples (200 g) of green forage were selected, packed in brown paper bags and placed in a forced-ventilation oven at 65 °C until constant weight and weighed with a digital scale (ML 600, Marte, São Paulo, SP, Brazil) to determine dry matter yields. The data obtained for each variable were submitted to analysis of variance and compared by the Tukey test at $P < 0.05$. For data analysis the computer program System for Variance Analysis – SISVAR was used (Ferreira 2014).

Results

At the first harvest there was a trend for treatments that included *A. brasilense*, either alone or in combination with thiamine, to increase the relative concentrations of chlorophyll, but differences were not always significant at $P < 0.05$ (Table 1). Green mass of forage was greater

Table 1. Mean concentrations of chlorophyll a, chlorophyll b and total chlorophyll (CLA, CLB and CLT, respectively) and green and dry mass (GM and DM) production of *Urochloa decumbens* plants treated with *Azospirillum brasilense* (Azos) and thiamine.

Treatment	CLA	CLB	CLT	GM	DM
	(SPAD value)			(Mg/ha)	
	First harvest (50 days after mowing)				
Control	35.5b	11.4b	46.9b	36.9b	7.89b
Azos 10 mL/L	39.9ab	14.5a	54.4a	49.8ab	10.22ab
Thiamine 50 mg/L	38.5ab	13.0ab	51.5ab	39.9ab	7.84b
Thiamine 100 mg/L	39.4ab	13.6ab	53.0ab	38.3b	8.09b
Azos + Thiamine 50 mg/L	40.7a	14.6a	55.4a	43.4ab	8.44ab
Azos + Thiamine 100 mg/L	40.3a	14.3ab	54.5a	51.5a	10.80a
CV (%)	10.8	20.3	12.4	15.3	15.2
LSD	4.50	2.93	6.93	13.13	2.69
	Second harvest (45 days after first harvest)				
Control	30.1a	7.80a	37.9a	31.9c	6.26c
Azos 10 mL/L	30.8a	8.14a	39.0a	52.6a	10.76a
Thiamine 50 mg/L	29.0a	7.39a	36.4a	35.5bc	6.55bc
Thiamine 100 mg/L	29.4a	7.36a	36.8a	39.4bc	9.48a
Azos + Thiamine 50 mg/L	29.9a	8.09a	38.0a	41.6abc	8.68abc
Azos + Thiamine 100 mg/L	29.5a	7.56a	37.0a	45.4ab	8.93ab
CV (%)	7.88	17.14	9.66	14.65	14.94
LSD	3.11	1.76	4.81	11.96	2.51

Within columns means followed by the same letters do not differ significantly by the Tukey test ($P > 0.05$).

($P < 0.05$) for *A. brasilense* plus thiamine at 100 mg/L than for Control and thiamine at 100 mg/L. Dry mass of forage for *A. brasilense* plus thiamine at 100 mg/L was greater than that for Control and thiamine at both concentrations.

At the second harvest, chlorophyll concentrations were totally independent of treatment. Application of *A. brasilense* alone produced more green mass than the Control and thiamine at 100 mg/L (Table 1). However, dry matter production of forage was greater ($P < 0.05$) for *A. brasilense* alone, and for thiamine at 100 mg/L was greater than that of Control and thiamine at 50 mg/L.

Discussion

The positive response to the inoculation with *Azospirillum* is due to the bacteria's capacity to biologically fix atmospheric N through the enzyme nitrogenase (Hungria 2011), leading to increased synthesis of nucleic acids, proteins and hormones, which are essential to plant development, as well as to increased photosynthetic activity (Taiz et al. 2017). This is shown by the increase in the chlorophyll concentrations at the first harvest, since these concentrations are directly related to the N concentrations in the leaves (Rocha et al. 2010).

The superior results obtained at the first harvest for the combined application of *A. brasilense* and thiamine are also related to the ability of the vitamin to improve the conditions of the photosystem by protecting it, avoiding its degradation by oxidation (Goyer 2010; Kaya et al. 2015). Consequently,

with the improvement of physiological conditions, there is an increase in the accumulation of energy reserves of tissues (Barakat 2003), which can be used for plant development and tissue maintenance during periods of stress (Taiz et al. 2017).

Although treatments had no effects on chlorophyll concentration at the second harvest, applying *A. brasilense* and thiamine at 100 mg/L either alone or in combination significantly increased dry mass yields over that of Control. This response may be related to the priming effect of both the bacteria and the vitamin. Both are recognized for their ability to mitigate stress caused by water restriction (Leite et al. 2019; Vendruscolo et al. 2020) and improve the nutritional condition of plants (Kaya et al. 2015; Galindo et al. 2020). However, under adequate growth conditions, including e.g. good availability of water, their effect is reduced (Goyer 2010; Naoe et al. 2020). In the present study, this became evident, since the second harvest occurred at the end of the rainy season, while the first harvest was on plant regrowth that occurred under conditions of erratic rainfall.

Inoculation with *A. brasilense* and application of thiamine, alone or in combination, seemed to have some potential for the maintenance and improvement of physiological and productive characteristics of *U. decumbens*. Larger-scale studies to test the repeatability of these findings seem warranted as well as longer-term studies to endeavor to clarify the medium- and long-term effectiveness of these treatments.

References

(Note of the editors: All hyperlinks were verified 11 January 2020.)

- Barakat H. 2003. Interactive effects of salinity and certain vitamins on gene expression and cell division. *International Journal of Agriculture & Biology* 5:219–225. [bit.ly/3sDJPLe](https://doi.org/10.1007/s11738-014-1741-3)
- Cardoso MRD; Marcuzzo FFN; Barros JR. 2014. Climatic classification of Köppen-Geiger for the state of Goiás and the Federal District. *ACTA Geográfica* 8(16):40–55. (In Portuguese). [bit.ly/369AaCP](https://doi.org/10.1071/CP18105)
- Costa NR; Andreotti M; Bergamaschine AF; Lopes KSM; Lima AES. 2015. Cost of the silage production crop-livestock integrated under no-tillage system. *Revista Ceres* 62:9–19. (In Portuguese). doi: [10.1590/0034-737X201562010002](https://doi.org/10.1590/0034-737X201562010002)
- Duarte CFD; Cecato U; Hungria M; Fernandes HJ; Biserra TT; Galbeiro S; Toniato AKB; Silva DR da. 2020. Morphogenetic and structural characteristics of *Urochloa* species under inoculation with plant-growth-promoting bacteria and nitrogen fertilisation. *Crop and Pasture Science* 71:82–89. doi: [10.1071/CP18455](https://doi.org/10.1071/CP18455)
- FAO. 2017. Faostat: Agricultural Statistics Database 2017. Live animals. [fao.org/faostat](https://www.fao.org/faostat)
- Ferreira DF. 2014. Sisvar: A guide for its bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia* 38:109–112. doi: [10.1590/S1413-70542014000200001](https://doi.org/10.1590/S1413-70542014000200001)
- Galindo FS; Teixeira Filho MCM; Buzetti S; Santini JMK; Boleta EHM; Rodrigues WL. 2020. Macronutrient accumulation in wheat crop (*Triticum aestivum* L.) with *Azospirillum brasilense* associated with nitrogen doses and sources. *Journal of Plant Nutrition* 43:1057–1069. doi: [10.1080/01904167.2020.1727511](https://doi.org/10.1080/01904167.2020.1727511)
- Goyer A. 2010. Thiamine in plants: Aspects of its metabolism and functions. *Phytochemistry* 71:1615–1624. doi: [10.1016/j.phytochem.2010.06.022](https://doi.org/10.1016/j.phytochem.2010.06.022)
- Hungria M. 2011. Inoculação com *Azospirillum brasilense*: Inovação em rendimento a baixo custo. Embrapa Soja, Londrina, PR, Brazil. bit.ly/3p0DpDN
- Jacinto EJ; Martins Filho R; Malhado CHM; Azevedo DMMR; Carneiro PLS; Lôbo RNB; Facó O; Machado CHC; Souza JC de. 2005. Appropriate models to estimate genetic parameters for weight at 205 days of age of the Tabapuã Zebu cattle in Northeast region of Brazil. *Revista Ciência Agronômica* 36:221–226. (In Portuguese). bit.ly/39NArwd
- Kaya C; Ashraf M; Sonmez O; Tuna AL; Polat T; Aydemir S. 2015. Exogenous application of thiamine promotes growth and antioxidative defense system at initial phases of development in salt-stressed plants of two maize cultivars differing in salinity tolerance. *Acta Physiologiae Plantarum* 37:1741–1753. doi: [10.1007/s11738-014-1741-3](https://doi.org/10.1007/s11738-014-1741-3)
- Leite RC; Santos JG dos; Silva EL; Alves CR; Hungria M; Leite RC; Santos AC dos. 2019. Productivity increase, reduction of nitrogen fertiliser use and drought-stress mitigation by inoculation of Marandu grass (*Urochloa brizantha*) with *Azospirillum brasilense*. *Crop and Pasture Science* 70:61–67. doi: [10.1071/CP18105](https://doi.org/10.1071/CP18105)
- Longhini VZ; Souza WCR de; Andreotti M; Soares NA; Costa NR. 2016. Inoculation of diazotrophic bacteria and nitrogen fertilization in topdressing in irrigated corn. *Revista Caatinga* 29:338–347. doi: [10.1590/1983-21252016v29n210rc](https://doi.org/10.1590/1983-21252016v29n210rc)
- Naoe AML; Peluzio JM; Campos LJM; Naoe LK; Silva RA. 2020. Co-inoculation with *Azospirillum brasilense* in soybean cultivars subjected to water deficit. *Revista Brasileira de Engenharia Agrícola e Ambiental* 24:89–94. doi: [10.1590/1807-1929/agriambi.v24n2p89-94](https://doi.org/10.1590/1807-1929/agriambi.v24n2p89-94)
- Rincón Castillo A; Álvarez de León M; Pardo Barbosa O; Amaya MA; Díaz Giraldo RA. 2019. Estimating chlorophyll concentration and its relationship with crude protein concentration in three species of *Urochloa* in the Piedemonte Llanero, Colombia. *Tropical Grasslands-Forrajes Tropicales* 7:533–537. (In Spanish). doi: [10.17138/tgft\(7\)533-537](https://doi.org/10.17138/tgft(7)533-537)
- Rocha RNC; Galvão JCC; Teixeira PC; Miranda GV; Agnes EL; Pereira PRG; Leite UT. 2010. Relations between the SPAD index determined by chlorophyll meter with nitrogen content in leaves and grain yield in maize genotypes. *Revista Brasileira de Milho e Sorgo* 4:161–171. (In Portuguese). doi: [10.18512/1980-6477/rbms.v4n2p161-171](https://doi.org/10.18512/1980-6477/rbms.v4n2p161-171)
- Taiz L; Zeiger E; Moller IM; Murphy A. 2017. Plant physiology and development. 6th Edn. Sinauer Associates, Sunderland, MA, USA.
- Vendruscolo EP; Rodrigues AHA; Martins APB; Campos LFC; Seleguini A. 2018. Seed treatment with niacin or thiamine provides bean plant development and productivity. *Revista de Ciências Agroveterinárias* 17:83–90. (In Portuguese). doi: [10.5965/223811711712018083](https://doi.org/10.5965/223811711712018083)
- Vendruscolo EP; Rodrigues AHA; Oliveira PR; Leitão RA; Campos LFC; Seleguini A; Lima SF de. 2020. Exogenous application of thiamine on upland rice submitted to water deficit. *Revista de Ciências Agroveterinárias* 19:48–53. (In Portuguese). doi: [10.5965/223811711912020048](https://doi.org/10.5965/223811711912020048)

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