Combining Fully Acidulated Phosphates with Phosphate-Solubilizing Microorganisms for *Urochloa brizantha* Fertilization

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aimed to evaluate the productive, morphological and nutritional characteristics, in addition to the accumulation of nutrients in the aerial part of *Urochloa brizantha* cv. Marandu subjected to phosphorus chemical sources and its interaction with phosphate-solubilizing inoculant. The experiment was developed in Colorado do Oeste, RO, Brazil, being adopted a randomized block design with three replications. The treatments consisted of different phosphate fertilizer doses associated or not with phosphate-solubilizing microorganisms (PSM): 0% P; 50% P; 100% P; 50% P + 250 mL ha⁻¹ PSM; 50% P + 500 mL ha⁻¹ PSM; 50% P + 750 mL ha⁻¹ PSM; and 50% P + 1000 mL ha⁻¹ PSM. Triple superphosphate was used as a chemical fertilizer, and fertilization with chemical fertilizer and PSM occurred on November 19th, 2021. The results for the morphological characteristics and yields of Marandu grass showed significant differences. The different arrangements and phosphate doses influenced the green matter yield and dry matter yield, both using the 50% P + 1000 mL ha⁻¹ PSM presented higher yields (3,642.75 kg ha⁻¹ and 1,049.10 kg ha⁻¹, respectively). Higher crop growth rate results in green matter were also found when employing a combination of 50% P + 500 mL ha⁻¹ PSM and 50% P + 1000 mL ha⁻¹ PSM. Dry matter had a significant effect on the bromatological composition, with higher results observed when using 50% P alone (34.55%). Regarding the accumulation of nutrients in the aerial part, the highest P accumulation in Marandu grass was observed when utilizing 100% P, resulting in an accumulation of 15.4 kg P ha⁻¹. Inoculation with PSM for fertilization of *U. brizantha* cv. Marandu grass does not have adverse effects on the increase in P availability to the plant, resulting in adequate yield and crop development associated with the productive potential of the area.

Keywords: Fertilizer doses; Marandu grass; nutrient transport; phosphorus; soil fertility.

1. INTRODUCTION

In the global scenario, Brazil has emerged as the second-largest cattle rancher and one of the top seven dairy producers [1], with an approximate herd size of 222 million [2] and a national dairy production of 33.8 billion liters, sourced from an estimated 16.35 million milked cattle [3].

In the context of livestock, grazing is the most important source of feed for a cattle herd, as it presents a lower cost compared to feeding concentrate [4]. However, in tropical regions where cattle are primarily fed with monocultures of certain grass species, this practice creates a dependence on external inputs [5], despite its low efficiency.

The *Urochloa* genus is one of the most widely cultivated grasses in Brazil, with *U. brizantha* [6,7] standing out for its fast and intense growth [8] and high adaptability to different soil and climate conditions [9,10]. Furthermore, considering that Brazilian livestock still faces challenges in terms of low productivity and income due to inadequate pasture management [11], *Urochloa* grass plays a crucial role in establishing the right path for managing this system. It is essential to understand the nutritional needs of *Urochloa* grass species within this context [12].

Therefore, the productivity of forage plants in a system characterized by extensive nutrient exploitation and the lack of nutrient replenishment requires fertilization and soil practices aimed at improving the nutritional use by the plants. In light of this, phosphorus (P) emerges as an essential element, playing a key role in the plant life cycle. It is involved in metabolic processes related to photosynthesis, cell division, and energy transfer [13,14].

From this perspective, P acts as a limiting factor for plant growth, particularly in tropical regions, where a large amount of P is required due to its high stability and consequently low solubility [15]. It is estimated that approximately 70% of the P used through chemical fertilizers or organic matter remains in the soil, but only a small portion is readily accessible to plants [16]. Thus, there is a need to apply high doses of phosphorus fertilizers in order to meet the plant's demand and achieve high yields [17].

Therefore, inoculating with phosphate-solubilizing microorganisms (PSM) represents an alternative for various crops, resulting in a significant increase in yields [18,19]. These microorganisms play a crucial role in facilitating the plants’ uptake of P from the soil. They achieve this by promoting lateral root growth, enhancing nutrient absorption, and increasing
the superficial area of the roots throughout the entire root system. In addition, they stimulate metabolic processes [20,21].

It is extremely important, therefore, to consider the role of PSM in relation to pasture growth. This understanding is crucial not only for comprehending the mechanisms involved in the soil-plant system but also for expanding the possibilities of research in this field, particularly concerning the frequently used soluble phosphate fertilizers.

Therefore, this study aimed to evaluate the productivity, morphological and nutritional characteristics, as well as the accumulation of nutrients in the aerial part of *U. brizantha* cv. Marandu subjected to different sources of phosphorus chemical fertilizers and their interaction with a phosphate-solubilizing inoculant.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out in a rural property located in Colorado do Oeste, RO, Brazil (13º 06’ 22.2” S and 60º 31’ 43.4” W). The total area of the property is 2.39 hectares, with 0.064 hectares allocated for the experiment.

Soil samples taken at a depth of 0 to 20 cm revealed the following chemical characteristics: pH H₂O = 5.2; P = 1.3 mg dm⁻³; K = 0.07 cmolₑ dm⁻³; OM = 15%; Al = 0.2 cmolₑ dm⁻³; Ca = 1.6 cmolₑ dm⁻³; Mg = 0.3 cmolₑ dm⁻³; cation exchange capacity = 5.4 cmolₑ dm⁻³; and base saturation = 36%.

According to Köppen-Geiger classification [22], the region’s climate is classified as tropical monsoon (Am) and exhibits two well-defined seasons. The average annual temperature in this region ranges between 24°C and 26°C, with maximum temperatures reaching 30°C to 35°C and minimum temperatures ranging from 16°C to 24°C. The average annual rainfall varies from 1,400 to 2,600 mm per year [23].

2.2 Experimental Design and Management

The experimental design employed was a randomized block with 7 treatments and 3 replications, resulting in a total of 21 experimental units. The treatments consisted of different doses of phosphate fertilizer, either applied alone or in combination with PSM: 1) 0% of P; 2) 50% of P; 3) 100% of P; 4) 50% of P + 250 mL ha⁻¹ of PSM; 5) 50% of P + 500 mL ha⁻¹ of PSM; 6) 50% of P + 750 mL ha⁻¹ of PSM; and 7) 50% of P + 1000 mL ha⁻¹ of PSM.

Triple superphosphate, containing 46% P₂O₅ and 36% soluble phosphorus, was used as a chemical fertilizer. It was manually applied to each experimental unit. The PSM inoculant was added using a calibrated knapsack sprayer, delivering the prescribed dose to each treatment on November 19th, 2021.

*U. brizantha* cv. Marandu was selected as the pasture for this study. The pasture had been established on the farm for 15 years without any prior phosphate fertilization in this particular season. Therefore, the fertilization process for this experiment was considered the initial one, including the supply of PSM. An area of 0.064 ha was designated for the experiment and isolated with a fence to prevent access by the animals on the property. Within this area, 21 experimental units were installed, each measuring 9.0 m² and spaced 1.0 m apart from each other.

2.3 Evaluation of Yield and Morphological Characteristics of Marandu Grass

The forage was harvested at 30, 60, 90, and 150 days after treatment implementation. Grass height was measured using a measuring tape attached to a wooden support surface. Two random spots were selected within each plot to obtain height measurements. Furthermore, the green matter yield was assessed by using a 1.0 m² sampling frame and a digital electronic scale. Afterward, all plots were trimmed to a height of 0.15 m using a petrol grass trimmer. This trimming aimed to preserve the apical bud and facilitate the proper reestablishment of the plants.

For statistical analysis, we calculated the average height and yield of Marandu grass for each treatment across the harvesting seasons. To determine the crop growth rate (CGR), we considered the total matter input during the last harvesting season, following the methodology described by Rajput A et al. [24].

Only at 60 days (second harvesting season) were the morphological components collected. To accomplish this, grass was harvested from the designated areas within the sampling frame (1.0 m²). The harvested material was then separated into morphological components (leaf, stem, and dead material), weighed using precision scales, and the values were later
converted to determine the proportions of each component.

2.4 Forage Chemical Analysis

At the time of the second grass harvesting season (at 60 days), forage samples were taken and placed in paper bags for chemical analysis. The forage samples were pre-dried and then milled in a Willey mill with a 1.0 mm screen to obtain dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents, following the method described by Silva JD and Queiroz AC [25].

2.5 Nutrients Accumulation in the Aerial Part of Marandu Grass

The concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were determined using the methodology recommended by Claessen MEC et al. [26]. With the dry mass and nutrient concentrations, we calculated the accumulation of nutrients in the aerial part of Marandu grass, in kilograms per hectare (kg ha\(^{-1}\)) for macronutrients and in grams per hectare (g ha\(^{-1}\)) for micronutrients.

2.6 Statistical Analysis

The data obtained from the experiment were subjected to a statistical analysis using analysis of variance followed by a post-hoc Tukey’s test for mean comparison. The significance level was set at \(P = .05\), with the assistance of Experimental Designs package of R Software, version 3.5.3 [27].

3. RESULTS

3.1 Yield and Morphological Characteristics of Marandu Grass

For the morphological characteristics and the yields of Marandu grass subjected to the chemical sources of phosphorus associated with PSM, the results showed that there were significant differences \((P = .05)\) for the variables green and dry matter yield, crop growth rate of green matter, height, leaf proportion, stem proportion, and dry matter content (Table 1).

As presented in Table 2, the different arrangements and phosphate doses (chemical and PSM) influenced the green matter yield \((P = .0165)\) and dry matter yield \((P = .039)\). On both, using the 50% phosphorus dose recommended associated with 1000 mL ha\(^{-1}\) of PSM presented higher yields than the other treatments.

In addition, the crop growth rate of green matter was also influenced \((P = .0212)\) by using chemical phosphorus sources and the PSM, and when using 50% of the recommended phosphorus dose associated with 500 mL ha\(^{-1}\) of PSM and 50% of the recommended phosphorus dose associated with 1000 mL ha\(^{-1}\) of PSM, a higher growth rate was obtained. The lowest growth rate was obtained when operating with 50% of the recommended phosphorus dose associated with 750 mL ha\(^{-1}\) of PSM.

In the morphological characteristics of Marandu grass subjected to the chemical sources of phosphorus associated or not with PSM, there was a significant effect on the proportions of leaf \((P = .008)\), stem \((P = .0047)\) and dead material \((P = .0086)\), leaf/stem ratio \((P = .0146)\) and plant height \((P = .0061)\) (Table 3).

The leaf proportion and leaf/stem ratio demonstrated superiority among the other treatments when it was not being subjected to phosphate fertilizer. On the other hand, making use of the 50% phosphorus dose recommended associated with 250 mL ha\(^{-1}\) PSM and 50% of the phosphorus dose recommended associated with 500 mL ha\(^{-1}\) PSM were the lowest on both variables.

In addition, the stem proportion showed higher mean when subjected to the 50% phosphorus dose recommended associated with 500 mL ha\(^{-1}\) PSM. The non-fertilization with phosphate provided a lower mean for this variable. As for the dead material proportion, higher results were found employing only 50% phosphorus dose recommended and 50% phosphorus dose recommended with 250 mL ha\(^{-1}\) of PSM. The inverse materialized when there was no phosphate fertilization and also when 50% phosphorus dose recommended associated to 750 mL ha\(^{-1}\) PSM were supplied.

3.2 Bromatological Composition of Marandu Grass Forage

There was a significant effect of chemical phosphorus sources associated with PSM only for dry matter content \((P = .0048)\) (Table 4).
Table 1. Summary of the analysis of variance for morphological characteristics, yields and dry matter content of Marandu grass (*Urochloa brizantha*) subjected to increasing doses of phosphate fertilization and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Mean</th>
<th>Treat.</th>
<th>Block</th>
<th>CV (%)</th>
<th>P (value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMY (kg ha⁻¹)</td>
<td></td>
<td>3,290.35</td>
<td>*</td>
<td>ns</td>
<td>7.69</td>
<td>0.0165</td>
</tr>
<tr>
<td>DMY (kg ha⁻¹)</td>
<td></td>
<td>958.33</td>
<td>*</td>
<td>ns</td>
<td>7.71</td>
<td>0.039</td>
</tr>
<tr>
<td>GMCGR (kg GM day⁻¹)</td>
<td></td>
<td>93.81</td>
<td>*</td>
<td>ns</td>
<td>8.52</td>
<td>0.0212</td>
</tr>
<tr>
<td>DMCGR (kg DM day⁻¹)</td>
<td></td>
<td>27.48</td>
<td>ns</td>
<td>ns</td>
<td>9.10</td>
<td>0.0663</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td>28.45</td>
<td>*</td>
<td>ns</td>
<td>4.61</td>
<td>0.0061</td>
</tr>
<tr>
<td>Leaf (%)</td>
<td></td>
<td>85.94</td>
<td>*</td>
<td>ns</td>
<td>2.24</td>
<td>0.008</td>
</tr>
<tr>
<td>Stem (%)</td>
<td></td>
<td>12.58</td>
<td>*</td>
<td>ns</td>
<td>14.43</td>
<td>0.0047</td>
</tr>
<tr>
<td>DMP (%)</td>
<td></td>
<td>1.07</td>
<td>*</td>
<td>ns</td>
<td>24.20</td>
<td>0.0086</td>
</tr>
<tr>
<td>LSR</td>
<td></td>
<td>7.36</td>
<td>*</td>
<td>ns</td>
<td>20.88</td>
<td>0.0146</td>
</tr>
</tbody>
</table>


Table 2. Green and dry matter yield and crop growth rate of Marandu grass (*Urochloa brizantha*) subjected to increasing doses of phosphate fertilization and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (kg ha⁻¹)</th>
<th>CGR (kg GM day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM</td>
<td>DM</td>
</tr>
<tr>
<td>Control</td>
<td>3,003.08 AB</td>
<td>887.37 AB</td>
</tr>
<tr>
<td>50% P</td>
<td>3,153.83 AB</td>
<td>950.36 AB</td>
</tr>
<tr>
<td>100% P</td>
<td>3,492.75 AB</td>
<td>1,020.89 AB</td>
</tr>
<tr>
<td>50% P + 250 mL ha⁻¹ PSM</td>
<td>3,380.83 AB</td>
<td>990.86 AB</td>
</tr>
<tr>
<td>50% P + 500 mL ha⁻¹ PSM</td>
<td>3,525.75 AB</td>
<td>980.52 AB</td>
</tr>
<tr>
<td>50% P + 750 mL ha⁻¹ PSM</td>
<td>2,833.42 B</td>
<td>829.22 B</td>
</tr>
<tr>
<td>50% P + 1000 mL ha⁻¹ PSM</td>
<td>3,642.75 A</td>
<td>1,049.10 A</td>
</tr>
</tbody>
</table>

CV (%)                  | 7.89 | 7.71 | 8.52 |

50% P: 50% of the recommended phosphorus dose. 100% P: 100% of the recommended phosphorus dose. 50% P + 250 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 250 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 500 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 500 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 750 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 750 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 1000 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 1000 mL ha⁻¹ of phosphate-solubilizing inoculant. CGR: crop growth rate. CV: coefficient of variation. GM: green matter. DM: dry matter. Means followed by the same letter in the column do not differ by the Tukey test (P = .05)

Table 3. Morphological characteristics of Marandu grass (*Urochloa brizantha*) subjected to increasing doses of phosphate fertilization and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm)</th>
<th>Leaf (%)</th>
<th>Stem (%)</th>
<th>DMP (%)</th>
<th>LSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.33 AB</td>
<td>90.15 A</td>
<td>9.10 C</td>
<td>0.43 B</td>
<td>10.52 A</td>
</tr>
<tr>
<td>50% P</td>
<td>30.00 A</td>
<td>86.89 AB</td>
<td>11.81 ABC</td>
<td>1.89 A</td>
<td>7.43 AB</td>
</tr>
<tr>
<td>100% P</td>
<td>25.55 B</td>
<td>85.36 AB</td>
<td>13.24 ABC</td>
<td>0.83 AB</td>
<td>6.47 AB</td>
</tr>
<tr>
<td>50% P + 250 mL ha⁻¹ PSM</td>
<td>27.70 AB</td>
<td>82.98 B</td>
<td>15.42 ABC</td>
<td>1.56 A</td>
<td>5.52 B</td>
</tr>
<tr>
<td>50% P + 500 mL ha⁻¹ PSM</td>
<td>30.43 A</td>
<td>83.13 B</td>
<td>16.17 A</td>
<td>1.02 AB</td>
<td>5.22 B</td>
</tr>
<tr>
<td>50% P + 750 mL ha⁻¹ PSM</td>
<td>28.22 AB</td>
<td>85.59 AB</td>
<td>10.76 BC</td>
<td>0.47 B</td>
<td>8.77 AB</td>
</tr>
<tr>
<td>50% P + 1000 mL ha⁻¹ PSM</td>
<td>29.93 A</td>
<td>87.44 AB</td>
<td>11.57 ABC</td>
<td>1.31 AB</td>
<td>7.61 AB</td>
</tr>
</tbody>
</table>

CV (%)                  | 4.61 | 2.24 | 14.43 | 24.20 | 20.88 |

50% P: 50% of the recommended phosphorus dose. 100% P: 100% of the recommended phosphorus dose. 50% P + 250 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 250 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 500 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 500 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 750 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 750 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 1000 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 1000 mL ha⁻¹ of phosphate-solubilizing inoculant. DMP: dead material proportion. LSR: leaf/stem ratio. CV: coefficient of variation. Means followed by the same letter in the column do not differ by the Tukey test (P = .05)
The higher dry matter content was obtained when only the 50% recommended phosphorus dose was used. Conversely, lower dry matter contents were observed when using 100% of the recommended phosphorus dose, 50% of the recommended phosphorus dose in association with 500 mL ha\(^{-1}\) of PSM, 50% of the recommended phosphorus dose in association with 750 mL ha\(^{-1}\) of PSM, and 50% of the recommended phosphorus dose in addition to 1000 mL ha\(^{-1}\) of PSM (Table 5).

3.3 Accumulation of Nutrients in the Aerial Part of Marandu Grass

There was a significant effect of chemical sources of phosphorus associated with PSM on all macronutrients and micronutrients (Table 6). Significant evidence regarding P accumulation in the aerial part of Marandu grass was observed, demonstrating the impact of the different treatments in this study. Among the treatments, the highest P accumulation was found when applying 100% of the recommended phosphorus dose. In contrast, the control treatment exhibited low P accumulation due to the absence of phosphorus supply. These findings, indicating the importance of phosphorus management, are summarized in Table 7.

The treatments that combined phosphorus sources with varying doses of PSM demonstrated intermediate levels of P accumulation, contrasting with the highest accumulation observed in the treatment utilizing 100% of the recommended phosphorus dose.

Regarding the accumulation of K and S in the aerial part of Marandu grass, higher levels were observed when using 100% of the recommended phosphorus dose, 50% of the recommended phosphorus dose with 500 mL ha\(^{-1}\) of PSM, and 50% of the recommended phosphorus dose with 1000 mL ha\(^{-1}\) of PSM.

### Table 4. Summary of the analysis of variance for the bromatological composition of Marandu grass (Urochloa brizantha) subjected to increasing doses of phosphate fertilization and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Effect</th>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Treat.</th>
<th>Blocks</th>
<th>P (value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM (%)</td>
<td>31.97</td>
<td>*</td>
<td>ns</td>
<td>0.0048</td>
</tr>
<tr>
<td></td>
<td>CP (%)</td>
<td>8.86</td>
<td>ns</td>
<td>ns</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>NDF (%)</td>
<td>62.97</td>
<td>ns</td>
<td>ns</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>ADF (%)</td>
<td>29.84</td>
<td>ns</td>
<td>ns</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**DM**: dry matter. **CP**: crude protein. **NDF**: neutral detergent fiber. **ADF**: acid detergent fiber. *Significant at 5% probability of error by the F test. ns: not significant

### Table 5. Dry matter content of Marandu grass (Urochloa brizantha) subjected to increasing doses of phosphate fertilization and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.74 AB</td>
</tr>
<tr>
<td>50% P</td>
<td>34.55 A</td>
</tr>
<tr>
<td>100% P</td>
<td>30.53 B</td>
</tr>
<tr>
<td>50% P + 250 mL ha(^{-1}) PSM</td>
<td>32.40 AB</td>
</tr>
<tr>
<td>50% P + 500 mL ha(^{-1}) PSM</td>
<td>30.92 B</td>
</tr>
<tr>
<td>50% P + 750 mL ha(^{-1}) PSM</td>
<td>30.32 B</td>
</tr>
<tr>
<td>50% P + 1000 mL ha(^{-1}) PSM</td>
<td>32.31 B</td>
</tr>
</tbody>
</table>

**CV**: coefficient of variation. Means followed by the same letter in the column do not differ by the Tukey test (P = .05)

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50% P: 50% of the recommended phosphorus dose. 100% P: 100% of the recommended phosphorus dose. 50% P + 250 mL ha\(^{-1}\) PSM: 50% of the recommended phosphorus dose + 250 mL ha\(^{-1}\) of phosphate-solubilizing inoculant. 50% P + 500 mL ha\(^{-1}\) PSM: 50% of the recommended phosphorus dose + 500 mL ha\(^{-1}\) of phosphate-solubilizing inoculant. 50% P + 750 mL ha\(^{-1}\) PSM: 50% of the recommended phosphorus dose + 750 mL ha\(^{-1}\) of phosphate-solubilizing inoculant. 50% P + 1000 mL ha\(^{-1}\) PSM: 50% of the recommended phosphorus dose + 1000 mL ha\(^{-1}\) of phosphate-solubilizing inoculant. DM: dry matter. CV: coefficient of variation.
Table 6. Summary of the analysis of variance for the accumulation of nutrients in the aerial part of Marandu grass (Urochloa brizantha) subjected to increasing doses of phosphate fertilization and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Treat.</th>
<th>Blocks</th>
<th>P (value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (kg ha⁻¹)</td>
<td>11.30</td>
<td>*</td>
<td>ns</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>K (kg ha⁻¹)</td>
<td>96.10</td>
<td>*</td>
<td>ns</td>
<td>0.0004</td>
</tr>
<tr>
<td>Ca (kg ha⁻¹)</td>
<td>11.90</td>
<td>*</td>
<td>ns</td>
<td>0.000058</td>
</tr>
<tr>
<td>Mg (kg ha⁻¹)</td>
<td>8.40</td>
<td>*</td>
<td>ns</td>
<td>0.0004</td>
</tr>
<tr>
<td>S (kg ha⁻¹)</td>
<td>4.20</td>
<td>*</td>
<td>ns</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>B (g ha⁻¹)</td>
<td>30.30</td>
<td>*</td>
<td>ns</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cu (g ha⁻¹)</td>
<td>16.70</td>
<td>*</td>
<td>ns</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Fe (g ha⁻¹)</td>
<td>386.30</td>
<td>*</td>
<td>ns</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Mn (g ha⁻¹)</td>
<td>557.90</td>
<td>*</td>
<td>ns</td>
<td>0.0121</td>
</tr>
<tr>
<td>Zn (g ha⁻¹)</td>
<td>96.10</td>
<td>*</td>
<td>ns</td>
<td>0.021</td>
</tr>
</tbody>
</table>


For the accumulation of Ca, Mg, B, and Cu in the aerial part of Marandu grass subjected to the use of chemical sources of phosphorus and PSM, higher levels were observed when providing 100% of the recommended phosphorus dose and when supplying 50% of the recommended phosphorus dose with 1000 mL ha⁻¹ of PSM.

Regarding Fe accumulation, statistical superiority was observed only when 50% of the recommended phosphorus dose was supplied in association with 500 mL ha⁻¹ of PSM. On the other hand, for Mn accumulation, higher levels were observed when providing 100% of the recommended phosphorus dose, 50% of the recommended phosphorus dose with 500 mL ha⁻¹ of PSM, and 50% of the recommended phosphorus dose with 1000 mL ha⁻¹ of PSM.

Finally, considering Zn accumulation, the treatments that exhibited the highest accumulations were the supply of 100% of the recommended phosphorus dose, 50% of the recommended phosphorus dose with 250 mL ha⁻¹ of PSM, 50% of the recommended phosphorus dose with 500 mL ha⁻¹ of PSM, and 50% of the recommended phosphorus dose with 1000 mL ha⁻¹ of PSM.

Table 7. Accumulation of nutrients in the aerial part of Marandu grass (Urochloa brizantha) subjected to increasing doses of phosphate fertilizers and inoculation with phosphate-solubilizing microorganisms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>50% P</th>
<th>100% P</th>
<th>50% P + 250 mL ha⁻¹ PSM</th>
<th>50% P + 500 mL ha⁻¹ PSM</th>
<th>50% P + 750 mL ha⁻¹ PSM</th>
<th>50% P + 1000 mL ha⁻¹ PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (kg ha⁻¹)</td>
<td>7.1 D</td>
<td>10.4 C</td>
<td>15.4 A</td>
<td>11.00 C</td>
<td>12.1 B</td>
<td>10.2 C</td>
<td>12.6 B</td>
</tr>
<tr>
<td>K (kg ha⁻¹)</td>
<td>83.3 B</td>
<td>87.0 B</td>
<td>106.1 A</td>
<td>90.20 B</td>
<td>107.3 A</td>
<td>81.3 B</td>
<td>117.5 A</td>
</tr>
<tr>
<td>Ca (kg ha⁻¹)</td>
<td>9.8 B</td>
<td>12.1 B</td>
<td>13.2 A</td>
<td>11.80 B</td>
<td>11.7 B</td>
<td>10.9 B</td>
<td>13.5 A</td>
</tr>
<tr>
<td>Mg (kg ha⁻¹)</td>
<td>6.7 C</td>
<td>8.3 B</td>
<td>9.7 A</td>
<td>8.50 B</td>
<td>8.4 B</td>
<td>7.0 C</td>
<td>9.8 A</td>
</tr>
<tr>
<td>S (kg ha⁻¹)</td>
<td>3.4 B</td>
<td>3.1 B</td>
<td>5.3 A</td>
<td>2.9 B</td>
<td>5.2 A</td>
<td>3.8 B</td>
<td>5.6 A</td>
</tr>
<tr>
<td>B (g ha⁻¹)</td>
<td>30.6 B</td>
<td>27.7 B</td>
<td>35.2 A</td>
<td>29.4 B</td>
<td>24.1 B</td>
<td>28.0 B</td>
<td>37.3 A</td>
</tr>
<tr>
<td>Cu (g ha⁻¹)</td>
<td>16.9 A</td>
<td>13.9 B</td>
<td>17.6 A</td>
<td>14.7 B</td>
<td>16.1 B</td>
<td>14.0 B</td>
<td>23.3 A</td>
</tr>
<tr>
<td>Fe (g ha⁻¹)</td>
<td>421.4 B</td>
<td>447.1 B</td>
<td>48.4 D</td>
<td>353.4 C</td>
<td>603.0 A</td>
<td>364.4 C</td>
<td>466.3 B</td>
</tr>
<tr>
<td>Mn (g ha⁻¹)</td>
<td>570.9 A</td>
<td>516.5 B</td>
<td>638.1 A</td>
<td>541.2 B</td>
<td>558.8 A</td>
<td>469.5 B</td>
<td>610.8 A</td>
</tr>
<tr>
<td>Zn (g ha⁻¹)</td>
<td>81.6 B</td>
<td>90.1 B</td>
<td>114.4 A</td>
<td>99.4 A</td>
<td>100.5 A</td>
<td>84.1 B</td>
<td>102.6 A</td>
</tr>
</tbody>
</table>

50% P: 50% of the recommended phosphorus dose. 100% P: 100% of the recommended phosphorus dose. 50% P + 250 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 250 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 500 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 500 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 750 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 750 mL ha⁻¹ of phosphate-solubilizing inoculant. 50% P + 1000 mL ha⁻¹ PSM: 50% of the recommended phosphorus dose + 1000 mL ha⁻¹ of phosphate-solubilizing inoculant. P: phosphorus. K: potassium. Ca: calcium. Mg: magnesium. S: sulfur. B: boron. Cu: copper. Fe: iron. Mn: manganese. Zn: zinc. Means followed by the same letter in the column do not differ by the Tukey test (P = .05)
4. DISCUSSION

4.1 Yield and Morphological Characteristics of Marandu Grass

From the evaluation of the productive and morphological characteristics of Marandu grass subjected to chemical sources of phosphorus and the interaction with phosphate-solubilizing inoculant, this study reveals that the aspects of production and plant configuration are closely related to the maintenance of supplied nutrients. This finding reinforces the benefits of using inoculants for forage grasses, leveraging microorganisms already utilized in commercial inoculants [28-30].

When evaluating the yield of Marandu grass subjected to increasing doses of phosphorus, Porto EMV et al. [31] also observed increases in dry matter production with the increment of P doses, reaffirming the significance of this nutrient in forage yield in phosphorus-deficient soils [32,33].

Although most forage grasses, including those belonging to the Urochloa genus, possess inherent morphological characteristics for extensive soil exploitation, studies have highlighted the significance of utilizing microorganisms to enhance phosphorus availability to the plants [34-36], thereby directly contributing to their establishment, growth, and regrowth in low-fertility soils.

A study investigating the combined effects of chemical fertilizer doses and co-inoculated microorganisms on promoting growth and nutrient efficiency in Marandu grass demonstrated that inoculation with microorganisms, along with 50% of the recommended phosphorus dose, resulted in increased grass growth [37], which is consistent with the findings of the present study. This approach could offer a valuable solution to address the issue of low productivity and profitability in Brazilian livestock systems that are often associated with inadequate pasture management practices [11].

Highlighting the potential for utilizing pasture recovery methods in this context, Souza R et al. [38] emphasize that the growth of forage grasses requires improvements in soil fertility to sustain adequate pasture growth. This requirement, in conjunction with the morphological characteristics of Marandu grass, is further supported by the higher proportions of plant parts observed when using chemical phosphorus sources associated with PSM, except for the leaf portion. The limitation in leaf proportion could be attributed to other nutrient requirements, such as nitrogen, which plays a crucial role in promoting new cell generation and facilitating leaf elongation rate [39].

4.2 Bromatological Composition of Marandu Grass Forage

Regarding Marandu grass subjected to chemical phosphorus sources and phosphate-solubilizing inoculant, only the dry matter content showed significant variation among treatments during chemical analysis. This highlights the importance of compositional chemical analysis for evaluating forage grasses, aiding in precise quantification of nutritional constituents [38]. Determining dry matter content is crucial for formulating diets, assessing feed intake, and evaluating animal performance.

It is important to note that achieving nutritional balance in animal feed relies on both organic and chemical nutrients [40], both of which are present in the dry matter [41]. Therefore, the observed differences in the dry matter content of the aerial part of Marandu grass indicate variations in nutrient intake, despite the absence of differences among the treatments in terms of the bromatological composition of the evaluated material.

Although the contents of CP, NDF, and ADF did not differ significantly among the treatments, they exhibited appropriate contents according to the Brazilian Cattle Feed Composition Table [42], with values of 8.86%, 62.97%, and 29.84%, respectively, indicating satisfactory bromatological composition of the grass.

The mean CP content exceeded the desirable minimum of 7.0% for proper functioning of the rumen microbiota. According to Sampaio FAR et al. [43], tropical grasses with CP content below 7.0% in the dry matter exhibit reduced digestibility due to inadequate nutrient levels in the plant.

Although the contents of CP, NDF, and ADF did not differ significantly among the treatments, they exhibited appropriate contents according to the Brazilian Cattle Feed Composition Table [42], with values of 8.86%, 62.97%, and 29.84%, respectively, indicating satisfactory bromatological composition of the grass.

It effectively compares the ADF mean content in the current study with the findings of Magalhães AF et al. [44] in U. decumbens, highlighting the difference between the two studies. It also mentions that the mean ADF content in Marandu grass is considered adequate (29.84%) according to Nussio LG et al. [45], providing...
additional context on the implications of ADF content on forage intake and digestibility.

4.3 Accumulation of Nutrients in the Aerial Part of Marandu Grass

The evaluation of nutrient accumulation in the aerial part of Marandu grass under the influence of chemical sources of phosphorus, in combination with a phosphate-solubilizing inoculant, yielded crucial findings in this study.

The accumulation of macro and micronutrients in the leaves of Marandu grass varied among the treatments, indicating significant changes in the plant's nutritional status. This effect aligns with a study conducted by Andrade RA et al. [46], which observed appropriate macronutrient accumulation in the leaves of Tamani grass (Megathyrsus maximus) when fertilized with natural phosphate and inoculated with Azospirillum brasilense.

This effect, observed across different plant species, can be attributed to biochemical changes and the production of phytohormones. These mechanisms promote an increase in the superficial root area, enhancing nutrient extraction from the soil, nitrogen fixation, and the solubilization of poorly soluble compounds. Furthermore, fertilization and the presence of inorganic phosphorus in the soil contribute to improved phosphorus utilization [47-49].

Forage grasses typically have a low cation exchange capacity in their root systems, leading to more effective adsorption of elements with higher valence, such as calcium and magnesium, by the soil. As a result, forage plants that exhibit greater efficiency in extracting monovalent cations, such as potassium, may experience limitations in the uptake of other nutrients, including calcium [46].

This fact becomes evident when examining the relationships among the minerals present in the aerial part of Marandu grass. It is possible to observe that as the dose and source of phosphorus for the plant increase, the other nutrients are influenced either positively or negatively. Nevertheless, no deficiency levels were observed for any of the evaluated nutrients, as their contents fall within the ranges considered adequate for forage plants of the Urochloa genus [50].

Andrade RA et al. [46] demonstrated that the interaction between phosphorus sources and rhizobacteria enhanced the efficiency of phosphate fertilization, regardless of the source used. This interaction resulted in increased phosphorus, potassium, magnesium, and sulfur contents in leaf tissues, positively influencing plant growth parameters. Additionally, Somavilla A et al. [51] reported that reactive natural phosphates require more time to become available in the soil but have a positive residual effect, particularly for perennial crops such as forage plants [52,53].

Therefore, it is evident why there has been a significant increase in the commercialization of microbial inoculants in recent years. However, their adoption in pasture systems remains relatively low. Nonetheless, this research highlights the positive contribution of microorganisms to the soil-plant environment in pasture-based animal production systems. This finding represents a step forward in addressing the challenges of low productivity and financial performance that persist in Brazilian cattle farming, primarily due to inadequate pasture management practices.

Supplying either 100% of the recommended phosphorus dose alone, or combining 50% of the recommended phosphorus dose with 500 mL ha\(^{-1}\) of phosphate-solubilizing microorganisms, or alternatively, 50% of the recommended phosphorus dose with 1000 mL ha\(^{-1}\) of phosphate-solubilizing microorganisms, appears to be associated with enhanced and balanced availability of other nutrients to the plant. This synergistic interaction potentially contributes to improved growth and development of Marandu grass.

Considering the favorable plant responses observed in relation to the utilization of phosphate-solubilizing microorganisms as a partial substitute for chemical phosphorus sources, it is of utmost importance to undertake investigations concerning the economic viability and environmental impact assessment associated with this practice. Such comprehensive studies will equip livestock farmers with the necessary knowledge and insights to make informed decisions in this regard.

It is also recommended that further studies be conducted to analyze the response of other tropical forage grass species associated with the
identification and characterization of specific microorganisms involved in phosphorus solubilization in tropical forage grasses, in addition to the definition of optimal conditions for the activity of the inoculants.

5. CONCLUSION

The inoculation of phosphate-solubilizing microorganisms for *U. brizantha* cv. Marandu grass fertilization exhibits no adverse effects on phosphorus availability, leading to satisfactory yield and crop development in line with the productive capacity of the area.

The utilization of 50% of the recommended phosphorus dose in conjunction with 500 mL ha\(^{-1}\) of phosphate-solubilizing microorganisms, as well as 50% of the recommended phosphorus dose in combination with 1000 mL ha\(^{-1}\) of phosphate-solubilizing microorganisms, demonstrates an association with increased yields and enhanced growth rates of Marandu grass.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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