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Productive and biochemical responses of Marandu grass under fertilization protocols

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Abstract. The aim of this study was to evaluate the effects of liming and chemical fertilization protocols, containing different combinations of nitrogen (N), phosphorus (P) and potassium (K), on the photosynthetic pigments, productive characteristics and carbohydrate concentration of *Urochloa brizantha* cv. Marandu. Five fertilization protocols were evaluated (PK fertilizer combination; NP fertilizer combination; NK fertilizer combination; NPK fertilizer combination; and without fertilizer - control), associated or not with soil correction via liming, in a completely randomized design, with four replicates. The experimental units consisted of plastic pots with a capacity of 12 liters, which were filled with 10 dm³ of sandy clay loam soil. Seeds of *Urochloa brizantha* cv. Marandu, with 80% cultural value were used. With the corrected soil, the NP protocol provided greater production of dry mass of residue (PMSRE), root volume, leaf area, leaf area index, total sugar content (AST) and starch in the root, compared to the other protocols. Without liming, the NP protocol provided greater production of aerial part dry mass (PMSPA), root volume, leaf aerial volume, leaf aerial index and AST content in leaves, roots and residue. The NPK protocol, with liming, was 73% higher in chlorophyll a content, 50% in carotenoid content, 90% in PMSPA, 78% in leaf area and 76.2% in leaf area index, compared to the soil without correction. The use of NP fertilization with liming is recommended as it provides positive responses on the photosynthetic pigments, productive characteristics and total sugar content of Marandu grass.

Key words: starch, *Brachiaria brizantha*, limestone, chlorophyll, phosphorus, nitrogen.

INTRODUCTION

In Brazil, approximately 80% of pastures are composed of the genus *Urochloa*, with emphasis on the species *Urochloa* brizantha cv. Marandu, widely spread and used throughout the country, representing approximately 50% of the pasture area (Cardoso et al., 2016). Its use in different production systems is justified by its characteristics, resistance to drought and pasture leafhoppers and high dry matter productivity, average of 20 ton ha year^{-1}, in addition to its high crude protein content (Valle et al., 2010).

However, the species has not expressed its maximum productive potential on many properties due to the degradation of cultivated areas. According to data from MapBiomas (2022), around 52% of the areas designated for pasture in Brazil face some level of degradation, which is associated with compaction and gradual decrease in soil fertility, especially due to NPK deficiency (Paciullo et al., 2007; Vendrame et al., 2010), which has resulted in low production and quality of grasses and consequently in lower animal performance and profitability.

The application of agricultural correctives, as liming and fertilization is crucial to avoid degradation and to be able to maintain the productivity of forage species, in addition, the stability of the system is ensured as the balance between the nutrients that are extracted from the soil and those that are supplied. Liming aims to raise the pH to values between 5.5 and 6.0, which is the range in which most nutrients are absorbed by plants (Rossetto et al., 2005). Furthermore, it increases the concentration of magnesium and calcium, promoting their binding with elements that are harmful to plants, such as aluminium, making the region more suitable for plant development.

Nitrogen, phosphate and potassium fertilizations are intended to maintain and promote the growth of forage grasses, being responsible for the constitution of vital organic compounds, plant development, in addition to influencing plant metabolism, efficient use of water, translocation of carbohydrates, growth and development of tillers, as well as the size and number of leaves and stems (De Morais et al., 2016).

Currently, research on grass development has focused on understanding biochemical parameters and their relationship with biomass production and root development (De Oliveira et al., 2018; Cruz et al., 2023; Seixas et al., 2023). Carbohydrates are synthesized in leaves through the photosynthetic process and serve as a source of energy for plant growth and development, in addition to being stored in the roots and base of the stem, functioning as a reserve carbohydrate that will be used according to the plant's needs (Taiz et al., 2017). Understanding the biochemical processes in forage grasses is essential, due to agronomic practices commonly carried out in the establishment of pastures, such as liming and fertilization, to propose pasture fertilization protocols that are appropriate to the possibility of adopting or not cultural treatments during planting.

Therefore, this study aimed to evaluate the productive characteristics and quantify photosynthetic pigments, Soluble and reserve carbohydrates of *U. brizantha* cv. Marandu as a function of liming and chemical fertilization protocols during two growth cycles. We hope to identify which combination of fertilizer protocol and soil correction can improve the biochemical and productive parameters evaluated in *U. brizantha* cv. Marandu.

MATERIALS AND METHODS

Experimental details and treatments

The experiment was conducted in a greenhouse located at the Southwest Bahia State University, Juvino Oliveira Campus, located at the following coordinates: 15°38’46’’ south latitude, 40°15’24’’ west latitude, average longitude of 280 m, in the municipality of Itapetinga-BA, from April to June 2019, with a maximum temperature of 41.4 °C and a minimum temperature of 20.8 °C throughout the experimental period. The research was conducted in a completely randomized design, in a 5×2 factorial design, five fertilization protocols (Control: absence of fertilizers; PK fertilizer combination; NP fertilizer combination; NK fertilizer combination; NPK fertilizer combination), associated or not with soil correction via liming, with four replicates. The experimental units consisted of plastic pots with a capacity of 12 liters, filled with 10 dm^3 of soil with a sandy-clay loam texture. The soil was collected at a depth of 0 to 20 cm and its chemical characteristics are described in Table 1.

Table 1. Soil chemical analysis

pH	mg dm^{-3}	------------------------- $Cmol_c$ dm^{-3} of soil -------------------------								%	
(H_2O)	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	S.B	t	T	V	m
4.7	1	0.1	0.6	0.4	1.3	5.9	1.1	2.4	8.3	13	54

S.B: Sum of bases; t: effective cation exchange capacity; T: pH cation exchange capacity; V: base saturation; m: Aluminium saturation.

The pots subjected to the soil correction factor had limestone applied 30 days before planting, with the application of 18.6 g of calcitic limestone with PRNT 82% per pot, determined by the exchangeable Al and Ca + mg method (Alvarez Vênegas & Ribeiro, 1999).

$$NC\ (t\ ha^{-1}) = Y * [Al^{3}+(mt.t/100)] + [X- (Ca^{2} + Mg^{2})] * 100/PRNT \qquad (1)$$

where NC = limestone requirement; Y = factor that varies with the soil's acidity buffering capacity and can be defined according to the texture; mt = maximum aluminum saturation tolerated by the crop; t = effective cation exchange capacity; X = calcium and magnesium requirement of the crop; PRNT = relative total neutralization power of the limestone to be applied

For the chemical fertilization factor, the P and K sources were applied at the time of planting, with 110 kg ha^{-1} of P_2O_5, (3.06 g of triple superphosphate per pot) and 60 kg ha^{-1} of K_2O (0.52 g of potassium chloride per pot), respectively. The N source (urea) occurred with the application of 150 kg ha^{-1} in a split form, in two applications, the first application being after the first standardization cut and the second application after the second standardization cut (0.84 g pot^{-1}).

Seeds of *Urochloa brizantha* cv. Marandu, with 80% cultural value were used. Twenty days after emergence (DAE), non-vigorous or non-homogeneous plants were removed, keeping only four plants in each experimental unit (repetitions). At 40 DAE, the first uniformity cut was made at a height of 10 cm at ground level, followed by the first application of N fertilization and at 68 DAE the second standardization cut was carried out with application of the second dose of N fertilization. After the distribution of the fertilization protocols, monitoring and evaluation of the plants began during two cycles growth period of 28 days each, when the grass reached an average height of 30 cm.

To determine the water holding capacity of soil, three pots containing dry soil were initially weighed and then saturated with water for three days. After draining excess water, the pots were weighed again and the difference in weight between the wet and dry soil afterwards was determined as the maximum water holding capacity of the soil, which was 80%. This value was then used as a reference to maintain the water levels of the experimental units close to the retention capacity, with the buckets being weighed daily and water replaced when necessary.

Plant analysis

At the end of each growth cycle, two fully expanded leaves were collected on each experimental unit and packaged in envelopes made of aluminum foil and immediately stored on ice, which were taken to the laboratory to determine the levels of chlorophyll *a*, chlorophyll *b* and carotenoids, where 0.03 g of the fresh mass (MF) of the collected leaf was placed in a glass bottle containing 5 mL of dimethyl sulfoxide and wrapped in aluminum foil for 72 hours. Then, a reading was taken on the spectrophotometer for the quantification of photosynthetic pigments according to Wellburn (1994) and values expressed in mg g^{-1} MF.

Next, the aerial part of the plants was cut at 10 cm above the ground and separated into stem/pseudo-stem and leaf blade, then weighed in natural matter. All leaf blades were scanned using an HP G2710 photo scanner at 300 dpi resolution and subsequently analyzed with the aid of the ImageJ® program, with the leaf area (LA) calculated and its value expressed in cm^2 pot^{-1}. Based on the LA data, the leaf area index (LAI) was calculated according to the equation defined by Cairo et al. (2008).

The morphological fractions were grouped and dried in a forced circulation oven at 55 °C for 72 h, they were then weighed to obtain the dry mass production values of the aerial part (PMSPA). The residue, corresponding to 0–10 cm of soil, was also weighed to determine the dry mass production values of the residue (PMSRE). The pots were disassembled and then the root volume (RV) of each experimental unit was determined, using a graduated cylinder with a known volume of water, where the root was introduced and through the difference, the VR was obtained, then the roots were taken to the greenhouse forced circulation at 55 °C for 72 h to determine root dry biomass production (PMSR).

For quantification of total soluble sugars (AST), 0.3 g of leaf, residue and root samples were weighed and ground in a ball mill and subjected to extraction in distilled water, with maceration followed by centrifugation (9,000 g for 20 minutes). This process was repeated twice more and the supernatant was collected and used to quantify water-soluble carbohydrates by the Anthrone method (Dische, 1962). The pellet obtained after the extraction of carbohydrates from the residue and root fractions was again suspended in 5 mL of 200 mM potassium acetate buffer solution (pH 4.8) and heated in a water bath at 100 °C for 5 min under constant stirring. The mixture was then cooled to approximately 50 °C, at which point the enzyme solution containing 0.08 mL of the amyloglucosidase enzyme was added. The mixture was kept in a water bath at 50 °C for two hours, under stirring. After incubation, centrifugation was performed at 9,000 g for 20 minutes, the supernatant was collected and the volume completed to 5 mL with the same buffer, and then the starch content (AMD) was quantified by the Anthrone method (Dische, 1962).

Statistical analysis

Data were subjected to analysis of variance using the Statistical Analysis System (SAS) OnDemand for Academics program (SAS Institute Inc., Cary, NC), considering the fertilization, liming protocols and the interaction as sources of variation. Significant interactions were unfolded and the means compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

The interaction between liming and fertilization protocols was significant ($P < 0.05$) for the concentration of chlorophyll *a* and carotenoids (Table 2). For chlorophyll *b*, there was only an effect of the liming factor, in which, in its absence, the plants showed a reduction of 13.33% in relation to the plants subjected to liming, which presented an average of 1.5 mg g^{-1} MF.

This accessory pigment collaborates with chlorophyll *a* in absorbing light for photosynthesis, even in circumstances of low sunlight. This increase in photosynthetic efficiency allows plants to capture a greater diversity of wavelengths of sunlight and transform it into chemical energy. This highlights the importance of adequately correcting the soil to promote the development of plants with higher levels of photosynthetic pigments.

When liming was used, higher ($P < 0.05$) concentrations of chlorophyll *a* (mg g^{-1} MF) and carotenoids (mg g^{-1} MF) were recorded in the NK and NPK protocols. On the other hand, when the soil was not amended, a higher ($P < 0.05$) concentration of chlorophyll *a* (mg g^{-1} MF) was observed in the PK protocol compared to the NPK protocol. Without liming, the concentration of carotenoids (mg g^{-1} MF) was higher ($P < 0.05$) using the PK protocol (Table 2).

Table 2. Chlorophyll *a* and carotenoid content of *Urochloa brizantha* cv. Marandu in five chemical fertilization protocols, with and without liming

Liming	Chemical fertilization protocols				
	Without	PK[1]	NP[2]	NK[3]	NPK[4]
	Chlorophyll *a* (mg g^{-1} MF)				
Without	2.1Aab	2.4Aa	2.1Aab	1.9Bbc	1.5Bc
With	1.6Bb	1.1Bc	1.7Ab	2.7Aa	2.6Aa
Without	3.2Aab	3.5Aab	3.7Aa	3.2Bab	2.8Bb
With	3.0Ab	2.5Bb	3.1Ab	4.2Aa	4.3Aa
	Carotenoids (mg g^{-1} MF)				
Without	0.3Ab	0.4Aa	0.3Ab	0.2Bc	0.2Bc
With	0.1Bb	0.1Bb	0.2Bb	0.4Aa	0.3Aa

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). [1]Phosphorus + potassium; [2]Nitrogen + phosphorus; [3]Nitrogen + potassium; [4]Nitrogen + phosphorus + potassium; MF: fresh matter.

When examining the effect of fertilization protocols on liming, the NK and NPK protocols with liming provided higher ($P < 0.05$) concentrations of chlorophyll *a* (mg g^{-1} MF) and carotenoids (mg g^{-1} MF), compared to unamended soil. The PK protocol, without liming, provided higher ($P < 0.05$) concentrations of chlorophyll *a* (mg g^{-1} MF) and carotenoids (mg g^{-1} MF) compared to the amended soil (Table 2).

Greater nutrient availability can enable better vegetative growth of plants, resulting in greater production of green biomass. According to Taiz et al. (2017), an increase in the synthesis of chlorophyll *a* enhances the production of ATP in chloroplasts, since this pigment increases the absorption of light, which is then converted into energy used during the photophosphorylation process. Therefore, carotenoids play a role as accessory pigments in the absorption and transfer of radiant energy, in addition to acting as protectors of chlorophyll against photodegradation caused by high irradiation (Taiz et al., 2017).

The interaction between liming and fertilization protocols was significant ($P < 0.05$) for production of dry mass of aerial part (PMSPA) and dry mass of residue (PMSRE) of *Urochloa brizantha* cv. Marandu (Table 3).

Table 3. Production of dry mass of aerial part (PMSPA) and production of dry mass of residue (PMSRE) of *Urochloa brizantha* cv. Marandu in five chemical fertilization protocols, with and without liming

Liming	Chemical fertilization protocols				
	Sem	PK[1]	NP[2]	NK[3]	NPK[4]
	PMSPA (g pot^{-1})				
Without	3.1Ad	4.0Acd	16.2Aa	5.3Bc	8.2Bb
With	4.2Ac	5.0Ac	17.3Aa	8.6Ab	15.6Aa
	PMSRE (g pot^{-1})				
Without	4.6Ab	5.1Ab	18.1Aa	6.7Bb	18.8Aa
With	5.1Ad	6.8Acd	18.6Aa	9.5Ab	8.6Bbc

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). [1]Phosphorus + potassium; [2]Nitrogen + phosphorus; [3]Nitrogen + potassium; [4]Nitrogen + phosphorus + potassium.

With the amended soil, higher ($P < 0.05$) PMSPA yields (g pot^{-1}) were observed with the NP and NPK protocols, while, in the absence of liming, the NP protocol provided higher ($P < 0.05$) PMSPA (g pot^{-1}). The use of the NK and NPK protocols, in the presence of liming, resulted in higher ($P < 0.05$) PMSPA (g pot^{-1}) (Table 3). This may have occurred due to the possibility that liming provided an increase in the pH, possibly within the recommended range (5.5–6.0), of the soil to a range where the availability of N and P were enhanced, thus contributing to greater plant growth and PMSPA. According to FERLIN et al. (1999), the presence of nitrogen is one of the main facilitators of the growth and development of grasses, since this nutrient participates directly in the plant's metabolism, contributing to the formation of nucleic acids, proteins, membranes and chlorophyll molecules.

It is important to highlight that the expressive results of the NP protocol, in PMSPA and PMSRE indicate that, in the possibility of not being able to carry out soil correction, in the short term, in sandy clay loam soils, with 1 mg dm^{-3} of P, pH in H_2O of 4.7 and base saturation of 13% (Table 1), Marandu grass planting can be carried out using NP fertilization, as it responds positively to the production of aerial part mass and residue. For PMSRE, higher ($P < 0.05$) yields were observed in the absence of liming when NP and NPK were applied and, in the presence of liming, when only NP was used (Table 3). When analyzing the effects of different chemical fertilization protocols on PMSRE, it is highlighted that the combination of NP, regardless of liming, and the use of NPK in the absence of liming resulted in higher averages for this variable (Table 3).

These results highlight the importance of the positive contributions of the protocols, NP, even in the absence of liming and the NPK protocol, not only in PMSPA and PMSRE, as well as in the concentrations of chlorophyll *a* (mg g^{-1} MF), carotenoids, respectively (Table 2). The results obtained demonstrate the positive influence of the nutrients N and P on the productive processes of Marandu grass, highlighting especially the importance of P when associated with liming, with crucial participation in the development of the root system and in the increase of photosynthetic pigments of

Marandu grass. Even in unamended soils, the presence of adequate nutrients can result in greater residue production, which can benefit the tillering process.

In the presence of liming, higher ($P < 0.05$) values of root dry mass production (PMSRA) were recorded in the NP and NK protocols and root volume in the NP fertilization protocol, while, without liming, greater ($P < 0.05$) PMSRA was observed in the NP and NPK protocols and root volume in the NP protocol (Table 4).

When examining the effect of fertilization protocols, in liming, on PMSRA and root volume, the NP and NPK protocols, without liming, provided better responses when compared to the amended soil (Table 4), indicating that even without the possibility of liming, the combination of NP and NPK can contribute positively to stimulating root production, since the soil used in the experiment had a low P content (Table 1). The increase in availability, mainly of P and N, regardless of the correction, enabled satisfactory performance of the plants in these protocols, reflecting in the increase of their photosynthetic rate and, consequently, in the increase in the production of photoassimilates, thus promoting greater development of both the aerial part and the root system of the plants (Table 4).

Table 4. Root dry mass production (PMSRA) and root volume of *Urochloa brizantha* cv. Marandu in five chemical fertilization protocols, with and without liming

Liming	Chemical fertilization protocols				
	Sem	PK[1]	NP[2]	NK[3]	NPK[4]
	PMSRA (g pot^{-1})				
Without	11.6Ab	19.4Ab	35.8Aa	14.5Ab	31.9Aa
With	10.8Ac	14.6Abc	26.1Ba	22.4Aab	16.5Bbc
	Volume de raiz (mL)				
Without	0.08Ad	0.11Ac	0.24Aa	0.09Bcd	0.20Ab
With	0.07Ac	0.11Abc	0.18Ba	0.13Ab	0.11Bb

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). [1]Phosphorus + potassium; [2]Nitrogen + phosphorus; [3]Nitrogen + potassium; [4]Nitrogen + phosphorus + potassium.

For the variables, leaf area and leaf area index, a significant interaction ($P < 0.05$) was observed between liming and fertilization protocols (Table 5). Regardless of liming, the NP fertilization protocol provided greater ($P < 0.05$) leaf area and leaf area index. When examining the effect of fertilization protocols on liming, the NK and NPK protocols with liming provided greater ($P < 0.05$) leaf area and leaf area index compared to unamended soil (Table 5). The importance of the leaf area index in a crop is well recognized as an indicator of productivity, since the photosynthetic process depends on the interception of light energy and its conversion into chemical energy by the leaves.

When examining the impact of liming on total soluble sugars (TSS) present in leaves, a higher content of this sugar was found in the absence of liming when the fertilizer contained NP, and in the presence of liming when NPK was used. Regarding fertilization, a higher AST content in the leaves was observed in the absence of fertilization, with PK and NP without the application of liming, and with NPK associated with liming.

Table 5. Leaf area and leaf area index of *Urochloa brizantha* cv. Marandu with and without liming and subjected to different fertilizations

Liming	Chemical fertilization protocols				
	Sem	PK[1]	NP[2]	NK[3]	NPK[4]
	Leaf area (cm^2 pot^{-1})				
Without	453.0Ad	559.0Ad	2,869.1Aa	942.9Bc	1,461.9Bb
With	627.9Ad	732.5Ad	2,991.4Aa	1,472.4Ac	2,607.6Ab
	Leaf area index				
Without	0.6Ad	0.8Ad	4.1Aa	1.3Bc	2.1Bb
With	0.9Ad	1.0Ad	4.2Aa	2.1Ac	3.7Ab

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). [1]Phosphorus + potassium; [2]Nitrogen + phosphorus; [3]Nitrogen + potassium; [4]Nitrogen + phosphorus + potassium.

With the corrected soil, the NPK protocol contributed to higher ($P < 0.05$) total soluble sugar (AST) levels, in the leaves and residue. The NP protocol contributed to higher ($P < 0.05$) AST contents in the root and starch in the root, while in the absence of liming, the NP protocol provided higher ($P < 0.05$) AST contents in the leaves, residue and root, and the PK protocol provided higher ($P < .05$) starch contents in the residue (Table 6).

Table 6. Total soluble sugar (TSS) content in the leaf, residue and root and starch content in the residue and root of *Urochloa brizantha* cv. Marandu with and without liming and subjected to different fertilizations

Liming	Chemical fertilization protocols				
	Sem	PK	NP	NK	NPK
	AST Leaf (mg g^{-1} DM)				
Without	66.4Ac	72.4Ab	93.4Aa	55.2Ad	69.8Bbc
With	54.9Bc	53.1Bc	84.6Bb	56.3Ac	104.2Aa
	AST residue (mg g^{-1} DM)				
Without	59.3Bb	60.4Ab	68.9Ba	43.7Ac	46.0Bc
With	66.5Ac	58.2Ad	82.4Ab	46.5Ae	87.8Aa
Without	33.8Ab	25.1Bc	38.8Ba	16.9Bd	23.2Bc
With	28.3Bd	34.6Ac	66.5Aa	25.9Ad	52.8Ab
	Starch residue (mg g^{-1} DM)				
Without	67.6Bb	72.0Aa	55.4Bc	44.4Ad	63.7Ac
With	73.3Aa	69.4Aa	60.6Ab	42.2Ac	53.7Bb
Without	93.7Aa	81.4Ab	81.4Bb	73.4Ac	71.5Bc
With	88.1Bb	72.9Bc	96.2Aa	71.1Ac	86.2Ab

Means followed by the same capital letter in the row and lowercase letter in the column do not differ from each other according to the Tukey test ($P > 0.05$). [1]Phosphorus + potassium; [2]Nitrogen + phosphorus; [3]Nitrogen + potassium; [4]Nitrogen + phosphorus + potassium.

When examining the effect of fertilization protocols on liming, the NPK protocol, with liming provided higher ($P < 0.05$) AST content in the leaves, in the residue and starch content in the root, compared to the unamended soil. The NP protocol provided a higher ($P < 0.05$) AST content in the root and residue, and starch content in the residue and root with the corrected soil (Table 6).

Carbohydrates play a fundamental role in the respiration required for the formation of new tissues, and are therefore crucial during the regrowth process (Avice et al., 1996). The expressive results of the NP and NPK protocols with liming indicate the positive contribution in the allocation of photoassimilates, mainly in the residue and in the root, with the purpose of accumulating essential energy reserves for the initial regrowth process. When there is a greater supply of N to plants, their photosynthetic capacity is maximized due to the potentiation of photoassimilates, resulting in greater production of sugars, which are end products of photosynthesis. This explains the results of this study, where higher AST levels were observed in the presence of N, especially when associated with phosphate fertilization.

CONCLUSIONS

The combination of NP fertilization and liming favors the increase in plant mass of *Urochloa brizantha* cv. Marandu grass, in addition to promoting positive effects on pigments related to photosynthesis, production characteristics and total sugar levels.Promoting plant growth and development after cutting, facilitating the understanding of resource allocation and adaptation strategies of forage plants, especially with regard to carbohydrate metabolism, highlighting significant sustainable plant management practices and the rational use of fertilizers.

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