Agronomy Research **21**(X), xxx–ccc, 2023
https://doi.org/10.15159/AR.23.090

Carbohydrate and protein metabolism of marandu grass affected by nitrogen fertilisation and number of cuts

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Received: June 18th, 2023; Accepted: September 30th, 2023; Published: October 23rd, 2023

Abstract. Understanding the metabolism of tropical grasses in response to management practises imposed in pastoral environments allows for improvements in the management and use of mineral fertilisers. This study aimed to quantify metabolite content in different plant parts of Marandu grass (*Urochloa brizantha*), with a specific focus on the influence of nitrogen fertilisation and its effects following successive cuts. The treatments corresponded to four nitrogen (N) rates (0, 75, 150, and 225 kg N ha^{-1}) and the number of cuts (one, two and three cuts). The plants were fractionated into leaves, stems, and roots to assess the content of water-soluble carbohydrates (WSC), starch, albumin, globulin, prolamin, and glutelin content. N fertilisation influenced the WSC and starch content in different parts of the plant, varying according to the cuts made. In the leaves and roots, fertilisation reduced the content of WSC and starch with one cut, as these were utilised as energy sources for assimilating the excess nitrogen in the soil. There was an increase in the concentration of all protein groups with nitrogen fertilisation in all parts of the plant with one cut. In plants cut two and three times, N fertilisation led to specific increases and decreases in different parts of the plants as an adaptive strategy for allocating resources as the number of cuts increased. Our results broaden our understanding of carbohydrate and protein metabolism in tropical grasses, thereby providing subsidies for the rational use of nitrogen fertilisers.

Key words: cuttings, grasses, nitrogen, regrowth, reserves.

INTRODUCTION

Pastures cover approximately 67% of the areas used for global farming activity, with an emphasis on tropical and subtropical regions (FAO, 2020). In Brazil, pastures occupy approximately 21% of its territory, with the *Urochloa* genus standing out, with

the marandu cultivar (*Urochloa brizantha*) currently being the most cultivated forage crop in the country (Lapig, 2020). Marandu grass is a forage grass of significant economic importance because of its remarkable adaptability to different soil and climate conditions, favoured its spread and application in animal production systems in the tropics (Jank et al., 2014). However, optimising the sustainable production of marandu grass requires a deep understanding of the complex interactions resulting from the management practises often employed in pastoral environments.

In these environments, forage plants are often subjected to leaf area loss and fertilisation strategies aimed at increasing productivity (Pereira et al., 2015). The primary studies related to Marandu grass have focused on the impacts of N fertilisation and defoliation practices on morphogenic, structural, productive, and nutritional characteristics (Alexandrino et al., 2004; Pereira et al., 2015; Pontes et al., 2017). These investigations reveal that the application of nitrogen, when combined with an appropriate approach to cutting intensity and frequency, plays a significant role in boosting growth rates (Alexandrino et al., 2004; Pereira et al., 2015), biomass production, and nutritional quality (Benett et al., 2008; Pontes et al., 2017).

Despite the scientific progress made, there are still substantial gaps in the understanding of these factors related to the metabolism of tropical grasses, especially concerning the synthesis, allocation, and remobilisation of carbohydrates and proteins in different parts of the plant and during different growth stages. Studies on the metabolism of grasses of this genus are scarce and use the content of non-structural carbohydrates and the content of total nitrogen and free amino acids as parameters to assess N and carbon metabolism (Fulkerson & Donaghy, 2001; Da Silva et al., 2014; Ferro et al., 2015; Garcez & Monteiro, 2022). However, these analyses do not delve into the necessary details and fail to consider specific types of carbohydrates, such as starch, which also plays a significant role in plant metabolism (Weise et al., 2011).

Soluble carbohydrates serve as an immediate source of energy produced during photosynthesis and are rapidly utilised to sustain vital metabolic processes like growth and stress response (Rosa et al., 2009). Conversely, starch functions as a storage carbohydrate that plants accumulate as an energy reserve, mobilised when needed-such as during the night or under unfavourable photosynthetic conditions (Volenec & Nelson, 2020). Furthermore, relying solely on the total N content or free amino acids to assess protein metabolism proves inadequate, as different proteins fulfil distinct functions in different plant parts (Taiz et al., 2018). Addressing these gaps could enhance our understanding of the metabolic adaptation of these grasses, facilitating improvements in pasture management and the efficient utilisation of mineral fertilisers.

Our study aims to quantify metabolite content in different segments of Marandu grass, with a specific focus on the influence of N fertilisation and its effects following successive cuts. Our hypothesis is that the application of N to the soil and its effects on successive cuts significantly influence the metabolism of carbohydrates and proteins in different parts of Marandu grass.

MATERIALS AND METHODS

The experiment was conducted in greenhouse belonging to the Forage and Pasture sector of the State University of Southwest Bahia in Itapetinga, Bahia, Brazil (15º14'S, 40º14'W) from February to July 2017. During the experimental period, the minimum,

maximum, and average temperatures recorded inside the greenhouse were 12.2 °C, 37.2 °C and 26.1 °C, respectively. The study was conducted in a 4×3 factorial design, with four nitrogen rates (0, 75, 150, and 225 kg N ha^{-1}) and three cutting regimes (one, two and three cuts) arranged in a completely randomised design, with four replications totalling 48 pots.

Plastic pots with a capacity of 12 litres each were used and filled with 9 dm^3 of sandy loam soil collected from a depth of 0 to 20 cm. The soil underwent chemical analysis covering different parameters, such as pH (hydrogen potential in water), nutrient levels such as phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), as well as the presence of aluminium (Al) and hydrogen (H). Base sum (BS), cations exchange capacity at pH 7.0 (T) and effective (t), base saturation (V), and the quantity of organic matter (O.M.) were also assessed, as shown in Table 1.

Table 1. Chemical analysis of soil collected at a depth of 0–20 cm

pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	BS	T	t	V	O.M.
(H_2O)	mg dm^{-3}	----------			$cmol_c$ dm^{-3}	----------				%	g dm^{-3}
5.3	6	0.08	2.4	1.8	0.2	3.6	4.3	8.1	4.5	53	10

To determine the soil's water retention capacity, pots with dry soil were weighed and saturated with water for three days. After draining the excess water, the pots were weighed again, and the difference in weight between the wet and dry soil after draining was determined as the maximum water-holding capacity of the soil. This value was used to replenish the water in each pot each day. Based on the soil analysis, there was no need for liming. The levels of phosphorus (P) and potassium (K) were below those required by the species; therefore, 22 kg P ha^{-1} and 25 kg K ha^{-1} were applied. Four plants of *Urochloa brizantha* cv. Marandu were used per pot, produced from commercial seeds with 80% cultural value.

When the plants reached 30 cm in height, all pots were cut 10 cm from the soil and nitrogen rates 75, 150, and 225 kg N ha^{-1} were applied according to each treatment. After 28 days of fertilisation, 16 pots were divided into leaves, stems and roots. Two grammes of fresh mass were collected from each fraction, wrapped in aluminium foil, and frozen for protein fractionation analysis. The remaining material was placed in a forced-circulation (Solidsteel SSDCR 40L220V environment +5 °C to 200 °C with capacity of 40 litres) oven at 65 °C for 72 h. After pre-drying, the dried material was ground in a ball mill and then subjected to final drying in an oven at 105 °C for 24 h. The remaining pots were cut at a height of 10 cm, and the cut aerial part was discarded. The process was repeated twice more, as shown in Fig. 1.

The two grams of leaves, stem, and roots collected from the pots at each cutting were extracted and fractionated into globulins, prolamins, albumins, and basic glutelins according to their solubility in distilled water, sodium chloride (NaCl), ethanol, and sodium hydroxide (NaOH), respectively, as proposed by Osborne (1924) and shown in Fig. 2. The content of soluble proteins present in the different protein fractions was quantified by the method described by Bradford (1976) using Coomassie blue G-250 solution and read in a spectrophotometer (Broadband 4NM) at 595 nm absorbance. The protein content was calculated in milligrams per gram of dry matter as a function of the dry matter content of the samples.

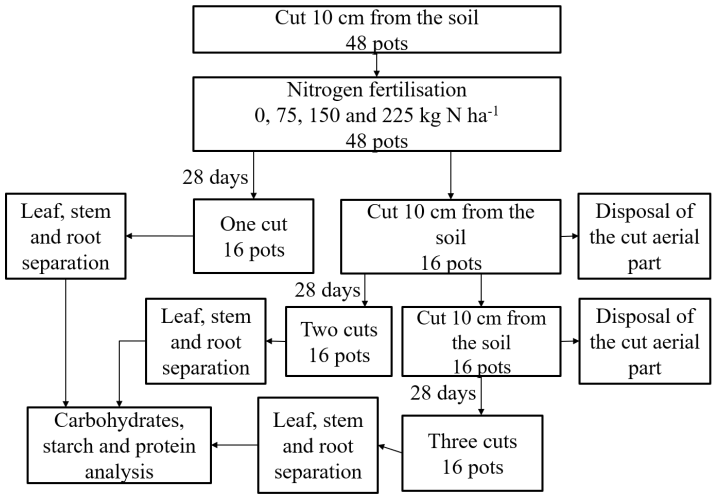

Figure 1. Schematic of cuts and sample collection in the experiment.

To quantify the carbohydrates, 0.3 g of the dry mass of leaf, stem, and root was subjected to the extraction of soluble sugar in distilled water by mixing the sample with water, followed by centrifugation. This process was repeated twice more and the supernatant was collected and used to quantify water-soluble carbohydrates (WSC). The resulting pellet was suspended in 5 mL of 200 mM potassium acetate buffer (pH 4.8) and placed in a water bath at 100 °C for 5 min to neutralise the enzymes. A solution containing amyloglucosidase enzyme was then added (0.08 mL), and the homogenate was incubated under the optimum conditions for 2 h under constant stirring. After incubation, centrifugation was performed at 9,000 g for 20 min, and the supernatant was collected for starch quantification. The content of WSC and starch was quantified using the Antrona method (Dische, 1962).

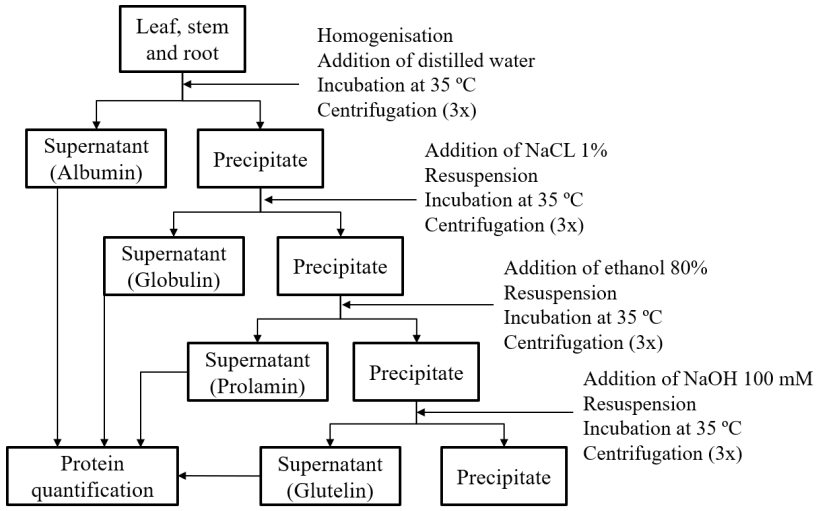

Figure 2. Schematic diagram of protein extraction.

The data were subjected to analysis of variance using the Statistical Analysis System (SAS) OnDemand for Academics programme (SAS Institute Inc., Cary, NC), considering nitrogen rates (N), cuttings (C) and the interaction of N and C as sources of variation. The effects of nitrogen fertilisation were assessed by simple regression analysis using an orthogonal decomposition of the nitrogen effect, the coefficients of which were assessed using the *F* test ($\alpha = 0.05$). Comparisons between cuts were made using the *Tukey's test*, with $\alpha = 0.05$.

RESULTS AND DISCUSSION

The interaction between N fertilisation and the number of cuts had a significant influence ($p < 0.0001$) on the content of WSC in the leaves, stems and roots (Table 2).

Table 2. Content of water-soluble carbohydrates (WSC) in leaves, stems and roots of marandu grass according to nitrogen rates and number of cuts

	Nitrogen rate (kg ha^{-1})				*P*-value					s.e.m.
	0	75	150	225	N*	C*	N×C*	L[1]	Q[1]	
WSC leaves (mg g^{-1} of DM)										
Cuts										
One	50^{a}	50^{b}	31^{c}	22^{c}	<.0001	<.0001	<.0001	<.0001	0.0009	
Two	18^{c}	96^{a}	86^{a}	85^{a}				<.0001	<.0001	1.21
Three	29^{b}	53^{b}	58^{b}	63^{b}				<.0001	<.0001	
WSC stem (mg g^{-1} of DM)										
Cuts										
One	20^{c}	37^{c}	42^{c}	36^{c}	<.0001	<.0001	<.0001	<.0001	<.0001	
Two	62^{a}	68^{b}	60^{b}	53^{b}				0.0016	0.0097	2.30
Three	48^{b}	97^{a}	105^{a}	92^{a}				<.0001	<.0001	
WSC roots (mg g^{-1} of DM)										
Cuts										
One	23^{b}	18^{c}	15^{c}	13^{c}	<.0001	<.0001	<.0001	<.0001	0.2238	
Two	14^{c}	26^{b}	31^{b}	23^{b}				<.0001	<.0001	1.40
Three	34^{a}	47^{a}	61^{a}	55^{a}				<.0001	<.0001	

Means followed by different letters, comparing the effect of cuts, are different by the *Tukey test* ($P < 0.05$). *Probability of significant for nitrogen fertilisation (N), number of cuts (C) and interaction (N×C). [1]Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; $P < 0.05$). s.e.m. = standard error of means.

In response to N fertilisation, a reduction of 57% in WSC content was observed in the leaves when fertilised with 225 kg N ha^{-1} in plants with one cut (Table 2). This decrease suggests that the plant reallocates its energy resources to maximise the assimilation of excess N (Bloom et al., 1992; Nunes-Nesi et al., 2010). According to Huppe & Turpin (1994), the capacity of the roots to absorb N and convert it into amino acids and N compounds in the leaves is directly conditioned by the amount of carbohydrates supplied through photosynthesis. The reallocation of energy resources to the synthesis of proteins represents a common adaptive mechanism in grasses (Conaghan et al., 2012; Olszewska, 2021). Conaghan et al. (2012) documented a 37% reduction in WSC content with 120 kg N ha^{-1}, whereas Olszewska (2021) observed a decrease of up to 32% with the

application of 240 kg N ha^{-1}. These results corroborate our findings, indicating a similar and even more pronounced response in grasses from tropical climates.

In plants cut two and three times, the WSC content in the leaves increased with fertilisation, reaching maximum values with the application of 148 kg N ha^{-1} and 209 kg N ha^{-1}, respectively (Table 2). The content of WSC in the leaves decreased in the unfertilised plants with two and three cuts, unlike the fertilised plants, in which they increased at rates of 75, 150, and 225 kg N ha^{-1}. This response is associated with the internal remobilisation of N to recover the photosynthetic leaf area (Meuriot et al., 2018). Lu et al. (2017) observed a positive relationship between the content of soluble proteins and N compounds in the roots and the regeneration capacity of the aerial part of alfalfa (*Medicago sativa* L.). According to Gloser et al. (2007), plants fertilised with N have higher concentrations of proteins and N compounds, which are used as a N reserve, accelerating the recovery of photosynthetic leaf area and, consequently, WSC content in the plant.

The WSC content in the stem increased with fertilisation, reaching maximum values at rates of 143, 67, and 148 kg N ha^{-1} in plants that were cut one, two, and three times, respectively (Table 1). N fertilisation improves the photosynthetic capacity of the plant, increasing the synthesis of soluble carbohydrates (Rufty et al., 1989), even after three cuts. The plant directs part of the carbohydrates produced in the aerial part to supply the energy demand necessary for the assimilation of N by the roots, increasing the flow in that part of the plant (Bredemeier & Mundstock, 2000), or to store the surplus in permanent organs, such as the stem (Volenec & Nelson, 2020).

In contrast to the stem, fertilisation with 225 kg N ha^{-1} reduced the WSC content in the roots in plants with one cut by 51% (Table 1). The decrease in WSC content in the roots is due to the energy-intensive process of N assimilation (Masclaux-Daubresse et al., 2010). The proximity of the root to the nutrient and the greater availability of N in the soil allows the plant to redirect its energy resources towards the absorption and incorporation of N into amino acids (Huppe & Turpin, 1994; Bredemeier & Mundstock, 2000). De Faria et al. (2019) also found a reduction in WSC content in the roots of piatã grass (*Urochloa brizantha*) when fertilised with 100 mg N dm^{-3}.

However, in plants cut two and three times, there was an increase in WSC content in the roots, reaching maximum values with the application of 131 kg N ha^{-1} and 149 kg N ha^{-1}, respectively. Plants fertilised with N tend to accumulate greater N reserves at the base of the stem and in the roots (Gloser et al., 2007). The reserves act as a source of N and energy for the rapid restoration of the plant's photosynthetic capacity (Dierking et al., 2017). Restoring leaf area and carbohydrate synthesis allows carbohydrates to be directed to other parts of the plant, such as the roots (Vantini et al., 2005). This adaptive strategy helps plants cope with repeated cutting cycles more efficiently (Avice et al., 1996; Aranjuelo et al., 2014).

The interaction between N fertilisation and the number of cuts was significant ($p < 0.0001$) for starch content in the stem and roots (Table 3). In the stem, the starch content increased by 5.5 mg g^{-1} of DM when fertilised with 225 kg N ha^{-1} in plants with one cut. N potentiates the recovery of leaf area, increasing photosynthetic activity, and stimulating WSC synthesis in the plant (Bassi et al., 2018). These WSCs are then used for the synthesis of reserve carbohydrates, such as starch in the stem (Vantini et al., 2005). In leaves, starch is considered transitory, since all the starch produced during the day is decomposed during the night (Weise et al., 2011). In contrast, starch stored in

permanent organs, such as stems and roots of grasses, serves as a source of energy in the medium and long term (Volenec & Nelson, 2020).

In plants cut two and three times, there was an increase in stem starch content, reaching maximum estimated values with the application of 128 kg N ha^{-1} and 91 kg N ha^{-1}, respectively (Table 3). The increase of starch in the stems of fertilised plants is linked to the increase of WSC in plants, as previously observed. The internal redistribution of N in fertilised plants during subsequent cuts results in a more efficient recovery after cutting (Gloser et al., 2007; Dierking et al., 2017), resulting in an increase in the production of carbohydrates that can be stored in the form of starch in the stem (Slewinski, 2012).

Table 3. Starch content in stems and roots of marandu grass according to nitrogen rates and number of cuts

	Nitrogen rate (kg ha^{-1})				*P*-value					s.e.m.
	0	75	150	225	N*	C*	N×C*	L[1]	Q[1]	
Starch stem (mg g^{-1} of DM)										
Cuts										
One	2.5[c]	3.8[c]	5.8[c]	7.9[a]	<.0001	<.0001	<.0001	<.0001	0.0702	
Two	5.9[b]	6.2[b]	6.8[b]	5.9[b]				0.5177	0.0056	0.20
Three	7.9[a]	11.7[a]	11.5[a]	4.8[c]				<.0001	<.0001	
Starch root (mg g^{-1} of DM)										
Cuts										
One	3.8[b]	1.4[c]	1.4[c]	2.5[b]	<.0001	<.0001	<.0001	<.0001	<.0001	
Two	3.8[b]	3.5[b]	2.5[b]	2.4[b]				<.0001	0.2363	0.11
Three	5.4[a]	5.1[a]	3.9[a]	3.3[a]				<.0001	0.2891	

Means followed by different letters, comparing the effect of cuts, are different by the *Tukey test* ($P < 0.05$). * Probability of significant for nitrogen fertilisation (N), number of cuts (C) and interaction (N×C). [1]Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; $P < 0.05$). s.e.m. = standard error of means.

Unlike the stem, the starch content in the roots decreased by 54% with N fertilisation, reaching minimum estimated value with the application of 101 kg N ha^{-1} in plants with one cut (Table 3). The trend of reducing starch content in the roots in response to N fertilisation was also observed in plants cut two and three times, with reductions of 41% and 42%, respectively, when fertilised with 225 kg N ha^{-1}. The removal in leaf area reduces the aerial part's capacity to generate energy for N assimilation (Gomide et al., 2002). As an adaptive response to take advantage of excess N, the plant uses its energy reserves in the roots to absorb and assimilate inorganic N (Morcuende et al., 2011; Soares Filho et al., 2013; Guo et al., 2017). This adaptive response was also confirmed by Soares Filho et al. (2013), who observed a 19% reduction in non-structural carbohydrates in the roots of Tanzânia grass (*Megathyrsus maximus*) treated with 150 kg N ha^{-1}. Similar results were reported by Vantini et al. (2005), demonstrating a reduction in root starch content of Tanzânia grass (*Megathyrsus maximus*) when exposed to 150 mg N dm^{-3}.

Even after three cuts, a higher starch content was observed in the roots and stem of all the plants (Table 3). When plants are cut frequently, they may allocate more resources to the root system as a survival strategy (Wiley et al., 2013). In fertilised plants, this

increase is related to the greater availability of WSC and root growth, contributing to the increase in starch in these parts of the plant (Lawlor, 2002; Kakabouki et al., 2020).

The interaction between N fertilisation and the number of cuts had a significant effect ($p < 0.0001$) on albumin content in the leaves, stems and roots (Table 4). Albumin concentration in leaves increased by 7, 16 and 5 mg g^{-1} of DM when fertilised with 225 kg N ha^{-1} in plants that were cut one, two and three times, respectively.

Table 4. Content of albumin in leaves, stems and roots of marandu grass according to nitrogen rates and number of cuts

	Nitrogen rate (kg ha^{-1})				*P*-value					s.e.m.
	0	75	150	225	N*	C*	N×C*	L[1]	Q[1]	
Albumin leaves (mg g^{-1} of DM)										
Cuts										
One	8.3[a]	10.7[a]	13.4[b]	15.2[b]				<.0001	0.3202	
Two	5.9[b]	9.9[a]	15.1[a]	21.5[a]	<.0001	<.0001	<.0001	<.0001	0.0001	0.28
Three	7.8[a]	8.5[b]	11.6[c]	12.3[c]				<.0001	0.9148	
Albumin stem (mg g^{-1} of DM)										
Cuts										
One	1.4[b]	3.7[a]	4.9[a]	7.8[a]				<.0001	0.1719	
Two	2.4[a]	3.2[a]	3.6[b]	3.7[b]	<.0001	<.0001	<.0001	<.0001	0.1345	0.22
Three	1.8[ab]	2.3[b]	2.6[c]	3.6[b]				<.0001	0.3138	
Albumin root (mg g^{-1} of DM)										
Cuts										
One	0.9[a]	3.4[a]	4.8[a]	3.7[a]				<.0001	<.0001	
Two	0.9[a]	1.5[b]	2.2[b]	2.4[b]	<.0001	<.0001	<.0001	<.0001	0.0931	0.12
Three	0.9[a]	1.6[b]	2.1[b]	2.5[b]				<.0001	0.2558	

Means followed by different letters, comparing the effect of cuts, are different by the *Tukey test* ($P < 0.05$). * Probability of significant for nitrogen fertilisation (N), number of cuts (C) and interaction (N×C). [1]Probability of significant effect due to nitrogen fertilisation (L = linear effect; Q = quadratic effect; $P < 0.05$). s.e.m. = standard error of means.

Hojilla-Evangelista et al. (2016) observed that when extracting proteins from alfalfa (*Medicago sativa*) leaves, albumins constituted the main fraction of the total protein. According to Rasheed et al. (2020), the greater participation of albumins in the leaves is due to the presence of enzymes such as Rubisco, ATP synthase, PEP-carboxylase, and glutamate synthase, all of which are soluble in water. When N is application to plants through fertilisation, the concentration of enzymes in the leaves increases because N stimulates enzyme synthesis and activity (Bassi et al., 2018; Yasuoka et al., 2018; Kocheva et al., 2020).

Despite the decrease in albumins in fertilised plants as the number of cuts increased, they remained highly concentrated in the leaves, particularly in plants with two cuts. This suggests that the plant prioritises the allocation of internal resources to maintain the concentration and activity of enzymes that act in primary metabolism as a way of maximising photosynthetic activity (Thornton & Millard, 1996; Masclaux-Daubresse et al., 2010).

The beneficial effect of N fertilisation on albumin content was also observed in the stem, with an increase of 6, 1 and 2 mg g^{-1} DM when fertilised with 225 kg N ha^{-1} in plants that were cut one, two and three times, respectively (Table 4). This response demonstrates the significant impact of N on this specific protein group, particularly in

the plant stem under conditions of high N availability. This influence can be attributed to the positive effect of N, which amplifies the synthesis of enzymes responsible for nutrient transport and growth, contributing to the increase in albumins content in the stem (Bassi et al., 2018; Taiz et al., 2018). The decrease in albumin content in the stem of fertilised plants as the number of cuts increased may indicate that these proteins can be remobilised during regrowth for the synthesis of new enzymes in the leaves (Volenec et al., 1996; Masclaux-Daubresse et al., 2010; Dierking et al., 2017).

In the roots, the albumin content increased with N fertilisation, reaching maximum estimated value with the application of 123 kg N ha^{-1} in plants with one cut. In plants cut two and three times, the albumin content in the roots increased by 2 mg g^{-1} DM when fertilised with 225 kg N ha^{-1} (Table 4). The application of N tends to increase the synthesis of enzymes involved in nutrient assimilation and protein synthesis in the roots (Garcez & Monteiro, 2022). These proteins, in turn, contribute to increasing the albumin fraction in the roots of fertilised plants (Rasheed et al., 2020). Garcez & Monteiro (2022) observed an increase in glutamine synthetase (GS) activity and total free amino acid concentrations in the roots of grasses from the *Urochloa* and *Megathyrsus* genera in response to N fertilisation. It is important to note that, despite the reduction in albumins after three cuts, the plants fertilised with N maintained elevated albumin content (Table 4). The reduction in this protein group may be associated with the plant's adaptive mechanism, which, with greater internal N availability, prioritises the remobilisation of amino acids from other parts of the plant in order to maintain the levels and activity of the enzymes involved in primary metabolism in subsequent cuts (Masclaux-Daubresse et al., 2010).

The interaction between N fertilisation and the number of cuts had a significant effect ($p < 0.0001$) on the content of globulins in the leaves, stems and roots (Table 5). Globulins in leaves with one cut increased by 6 mg g^{-1} DM with the application of 225 kg N ha^{-1}. In plants cut twice, N fertilisation also increased the globulins content in the leaves, reaching maximum value with the application of 103 kg N ha^{-1}. However, in plants cut three times, the globulins content in the leaves decreased by 52% when fertilised with 225 kg N ha^{-1}.

Globulins are predominantly composed of hydrophobic proteins because of their association with the lipid layer of the membrane and their solubility in saline solution (Teng & Wang, 2012; Rasheed et al., 2020). In the leaves, there is a considerable increase in globulins with N fertilisation, possibly due to the effect of N in stimulating the synthesis of membrane proteins of the photosynthetic complex, which act together with chlorophylls and enzymes in carbon assimilation processes (Bassi et al., 2018; Taiz et al., 2018). This increase may have contributed to the increase in globulin content in this part of the plant.

In the stem, N fertilisation increased the globulin content by 2 and 0.7 mg g^{-1} DM with the application of 225 kg N ha^{-1} in plants cut one and three times. However, the globulin content in the stem decreased by 44% when fertilised with 225 kg N ha^{-1} in plants cut twice (Table 5). In the roots, N fertilisation also led to an increase of 2 mg g^{-1} DM in globulin content when fertilised with 225 kg N ha^{-1} in plants with one cut. There was no effect of fertilisation on subsequent cuts. It is important to emphasize the significant reduction in the content of this protein group in the leaves at a rate of 225 kg N ha^{-1} with three cuts. In both the stem and roots, there was a reduction in globulins in the evaluated N rates as the number of cuts increased.

Table 5. Content of globulin in leaves, stems and roots of marandu grass according to nitrogen rates and number of cuts

	Nitrogen rate (kg ha^{-1})				*P*-value					s.e.m.
	0	75	150	225	N*	C*	N×C*	L[1]	Q[1]	
Globulin leaves (mg g^{-1} of DM)										
Cuts										
One	4.8^{c}	6.1^{b}	8.5^{ab}	10.5^{a}	0.1112	<.0001	<.0001	<.0001	0.4002	
Two	6.9^{b}	8.9^{a}	9.7^{a}	8.1^{b}				0.0114	<.0001	0.37
Three	12.8^{a}	9.5^{a}	8.2^{b}	6.1^{c}				<.0001	0.0873	
Globulin stem (mg g^{-1} of DM)										
Cuts										
One	2.8^{a}	3.8^{a}	3.8^{a}	4.8^{a}	0.0001	<.0001	<.0001	<.0001	0.9689	
Two	1.6^{b}	1.5^{b}	1.1^{b}	0.9^{c}				0.0010	0.8945	0.16
Three	1.2^{b}	1.1^{b}	1.6^{b}	1.9^{b}				0.0006	0.1665	
Globulin root (mg g^{-1} of DM)										
Cuts										
One	2.7^{a}	3.2^{a}	4.6^{a}	4.5^{a}	0.0213	<.0001	<.0001	<.0001	0.1576	
Two	0.7^{b}	1.0^{b}	0.6^{b}	0.6^{b}				0.3735	0.3906	0.21
Three	1.2^{b}	1.0^{b}	0.9^{b}	0.9^{b}				0.2910	0.6011	

Means followed by different letters, comparing the effect of cuts, are different by the *Tukey test* ($P < 0.05$). * Probability of significant for nitrogen fertilisation (N), number of cuts (C) and interaction (N×C). [1]Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; $P < 0.05$). s.e.m. = standard error of means.

To date, there are no studies in the literature that offer a better understanding of globulins in different plant tissues. Since these proteins are associated with the membrane, they can perform multiple functions in plants, including photosynthesis, transport, nutrient absorption, signalling, and storage in plant tissues (Taiz et al., 2018; Shewry et al., 1995). In our study, we observed that globulins are highly responsive to N fertilisation. Their reduction in stems and roots as the number of cuts increased may be linked to the internal remobilisation of this protein group as an adaptive process to the loss of leaf area, varying according to its concentration in each part of the plant (Volenec et al., 1996).

The interaction between N fertilisation and the number of cuts was significant ($p < 0.0001$) for the content of prolamins in the leaves, stems and roots (Table 6). N fertilisation increased the prolamins content in the leaves, reaching maximum estimated value with the application of 230 kg N ha^{-1} and 100 kg N ha^{-1} in plants cut one and two times, respectively. With three cuts, the prolamins content in the leaves decreased by 58% when fertilised with 225 kg N ha^{-1}.

The unfertilised plants increased prolamins in the leaves after the second and third cuts, unlike the fertilised plants, which showed a reduction with the third cut. Prolamins are considered to be storage proteins unique to plants and play a fundamental role as the main source of amino acids during germination and the initial phase of plant growth (Shewry & Tatham, 1990; Shewry et al., 1995). Abbaraju et al. (2022) observed that N fertilisation increased the concentration of vegetative storage proteins in maize (*Zea mays*) leaves. According to Abbaraju et al. (2022), the concentration of storage proteins is influenced by the availability of N in the soil, which represents a strong drain on surplus assimilated N.

Vegetative storage proteins in grasses are produced by plants to store N during periods of growth, acting as a source of N and energy to survive in unfavourable conditions (Avice et al., 1996; Meuriot et al., 2018). The presence of prolamins in leaves may represent an adaptive strategy to optimise the use of nutrients and resources due to the greater availability of N.

Table 6. Content of prolamin in leaves, stems and roots of marandu grass according to nitrogen rates and number of cuts

	Nitrogen rate (kg ha^{-1})				*P*-value					s.e.m.
	0	75	150	225	N*	C*	N×C*	L[1]	Q[1]	
Prolamin leaves (mg g^{-1} of DM)										
Cuts										
One	2.4^{b}	3.7^{b}	5.2^{a}	5.7^{a}	<.0001	<.0001	<.0001	<.0001	0.0079	
Two	3.6^{a}	5.3^{a}	5.8^{a}	3.6^{b}				0.7343	<.0001	0.15
Three	3.8^{a}	2.7^{c}	2.1^{b}	1.6^{c}				<.0001	0.0910	
Prolamin stem (mg g^{-1} of DM)										
Cuts										
One	2.1^{a}	3.1^{a}	3.0^{a}	4.0^{a}	<.0001	<.0001	0.0091	<.0001	0.9337	
Two	1.8^{a}	2.3^{b}	2.2^{b}	2.6^{b}				0.0017	0.7280	0.16
Three	0.8^{b}	1.1^{c}	1.2^{c}	1.4^{c}				0.0144	0.6722	
Prolamin root (mg g^{-1} of DM)										
Cuts										
One	5.0^{a}	7.5^{a}	7.7^{a}	6.4^{a}	0.0011	<.0001	<.0001	0.0041	<.0001	
Two	3.6^{b}	3.1^{b}	2.8^{b}	1.6^{b}				<.0001	0.2896	0.31
Three	2.2^{c}	1.5^{c}	1.5^{c}	1.1^{b}				0.0152	0.6452	

Means followed by different letters, comparing the effect of cuts, are different by the *Tukey test* ($P < 0.05$). * Probability of significant for nitrogen fertilisation (N), number of cuts (C) and interaction (N×C). [1]Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; $P < 0.05$). s.e.m. = standard error of means.

In the stem, N fertilisation resulted in a linear increase in the prolamins content when fertilised with 225 kg N ha^{-1} in plants cut one, two and three times (Table 6). In the roots, the prolamins content in plants with one cut increased with N fertilised, reaching maximum estimated value with the application of 109 kg N ha^{-1}. In plants cut two and three times, N fertilisation led to a significant reduction in prolamins content in the roots, with reductions of 55% and 53% observed at application rates of 225 kg N ha^{-1}, respectively. The positive response of N fertilisation on prolamin synthesis with one cut may indicate a greater investment of the excess N assimilated in the accumulation of reserve proteins in the stem and roots (Dierkung et al., 2017). These proteins were used in subsequent cuts as a source of N to restore the plant's photosynthetic capacity (Gloser et al., 2007). According to Lehmeier et al. (2013), organic reserves made up of N provide almost half of the N used during grass regrowth, indicating the importance of reserve proteins in plant regrowth.

The interaction between N fertilisation and the number of cuts was significant ($p < 0.0001$) for the glutelin content in the leaves, stem and roots of the Marandu grass (Table 7). The glutelin content in the leaves increased by 60% when fertilised with 225 kg N ha^{-1} in plants with one cut.

Table 7. Content of glutelin in leaves, stems and roots of marandu grass according to nitrogen rates and number of cuts

	Nitrogen rate (kg ha^{-1})				*P*-value					s.e.m.
	0	75	150	225	N*	C*	N×C*	L[1]	Q[1]	
Glutelin leaves (mg g^{-1} of DM)										
Cuts										
One	11.1^{a}	12.3^{a}	16.6^{a}	17.7^{a}	0.0168	<.0001	<.0001	<.0001	0.8788	
Two	12.1^{a}	10.8^{a}	9.8^{b}	10.7^{b}				0.0676	0.0694	0.61
Three	11.1^{a}	7.6^{b}	6.3^{c}	7.2^{c}				<.0001	0.0013	
Glutelin stem (mg g^{-1} of DM)										
Cuts										
One	3.7^{b}	5.5^{a}	7.2^{a}	9.6^{a}	<.0001	<.0001	<.0001	<.0001	0.4274	
Two	7.0^{a}	4.7^{a}	4.4^{b}	5.2^{b}				0.0025	0.0003	0.38
Three	3.2^{b}	3.5^{b}	3.9^{b}	4.8^{b}				0.0047	0.3734	
Glutelin root (mg g^{-1} of DM)										
Cuts										
One	8.2^{b}	11.8^{a}	14.6^{a}	9.4^{a}	0.0036	<.0001	<.0001	0.0088	<.0001	
Two	10.4^{a}	9.8^{b}	8.5^{b}	8.4^{a}				0.0051	0.6399	0.52
Three	9.3^{ab}	6.5^{c}	6.6^{c}	6.9^{b}				0.0055	0.0068	

Means followed by different letters, comparing the effect of cuts, are different by the *Tukey test* ($P < 0.05$). * Probability of significant for nitrogen fertilisation (N), number of cuts (C) and interaction (N×C). [1]Probability of significant effect due to nitrogen rate (L = linear effect; Q = quadratic effect; $P < 0.05$). s.e.m. = standard error of means.

However, in plants cut twice, there was no significant difference. In plants cut three times, there was a reduction of glutelin in the leaves with N fertilised, reaching maximum estimated value with the application of 150 kg N ha^{-1}. In the stem, glutelin content increased by 159% and 50% when fertilised with 225 kg N ha^{-1} in plans cut one and three times, respectively. Unlike the others, in plants cut twice, there was a reduction in glutelin content in the stem of fertilised plants, reaching minimum estimated value with the application of 190 kg N ha^{-1}. The glutelin content increased in the roots of the plants fertilised with one cut, reaching maximum estimated value with the application of 121 kg N ha^{-1}. However, for the other cuts, there was a reduction in glutelin content in the roots, reaching minimum estimated value with the application of 200 kg N ha^{-1} for plants cut two and three times.

The function of glutelin in different parts of plants is not yet fully understood, and more studies are needed to gain a deeper understanding of their role in different plant parts, as well as their involvement in the metabolism of tropical grasses. In the literature, glutelins are identified as proteins with a storage function exclusive to plants, but they possess larger and more complex structures than prolamins, and play a specific role as amino acid reservoirs in seeds during plant germination (Osborne, 1924; D'ovidio & Masci, 2004). However, due to their extraction using sodium hydroxide, they may include proteins with structural functions in plant tissues (Rasheed et al., 2020). In our study, we found that glutelins represent the predominant group of proteins in different parts of Marandu grass and respond significantly to N fertilisation. We observed a marked reduction in glutelin content in the stems and roots of fertilised plants, as well as in the leaves at rates of 150 and 225 kg N ha^{-1}, as the number of cuts increased. This indicates that, at high content, this group of proteins can also act as a N reserve during regrowth.

Although our results offer valuable insights into how N fertilisation and the number of cuts impact carbohydrate and protein metabolism in Marandu grass, further research is necessary to enhance our understanding. This necessity is justified by the fact that the growth conditions within a greenhouse environment might not entirely replicate the natural habitat of the plants, potentially influencing the outcomes. To mitigate these potential effects, conducting experiments that closely simulate field conditions is recommended. These findings can serve as a foundation for future investigations, including studies on specific fertilisation and management strategies, interactions with other nutrients, effects on different soil types and climates, and contributing to the reduction of the dependency on N fertilisers.

CONCLUSIONS

The Marandu grass demonstrates a remarkable ability to adapt to leaf area loss through the strategic allocation of resources. In situations of energy imbalance due to leaf area loss, the plant directs its carbon reserves towards the assimilation of excess nitrogen in the soil, increasing the synthesis of different protein groups in the plant. These proteins play a fundamental role in the growth and recovery of the plant after subsequent cuts. These findings contribute to a better understanding of resource allocation and adaptive strategies of tropical forage plants, especially concerning carbohydrate and protein metabolism, with significant implications for proper plant management and the use of nitrogen fertilisers.

ACKNOWLEDGEMENTS. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, and by Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB).

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