Research Article

Nitrogen fertilization time affects the root reserves of tropical grasses[1]

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ABSTRACT

Grass regrowth can reduce root mass, delaying reestablishment and grazing periods by reducing the nutrient absorption from the soil by plants. This study aimed to determine the optimal intervals for harvest and nitrogen fertilization in *Urochloa brizantha* cv. BRS Piatã and *Megathyrsus maximus* cv. BRS Quênia, as well as to investigate whether the flexibility of the fertilization time is related to the accumulation of water-soluble carbohydrates (WSC) and nitrogen in the roots. The experiment was conducted in a greenhouse, using a completely randomized design, with five treatments (nitrogen fertilization after harvest: 0, 2, 4, 6 and 8 days) and ten replicates. The nitrogen fertilization time linearly reduced the forage and root mass for the BRS Piatã, but had no effect on the BRS Quênia guinea grass. For the BRS Piatã, the WSC and starch concentrations in the roots showed a quadratic response, decreasing from harvest to day 2 and increasing until the day 8. However, the delayed fertilization led to a linear reduction in the root nitrogen content. The nitrogen fertilization time had a quadratic effect on the WSC concentration for the BRS Quênia roots. For the BRS Piatã, the nitrogen fertilization immediately after harvest improves the forage and root masses, WSC and nitrogen reserves. In contrast, the BRS Quênia exhibited a greater flexibility, concerning the nitrogen fertilization time. The interval between harvest and nitrogen fertilization is primarily depended on root mass, which influences the nitrogen accumulation in the roots.

KEYWORDS: *Megathyrsus maximus*, *Urochloa brizantha*, root starch, root nitrogen.

RESUMO

Momento de adubação nitrogenada afeta as reservas radiculares de gramíneas tropicais

A rebrota do capim pode reduzir a massa radicular, o que atrasa o restabelecimento e os períodos de pastejo, por reduzir a absorção de nutrientes do solo pelas plantas. Objetivou-se identificar o intervalo adequado de colheita e adubação nitrogenada para *Urochloa brizantha* cv. BRS Piatã e *Megathyrsus maximus* cv. BRS Quênia, e se a flexibilidade do momento de adubação está relacionada ao acúmulo de carboidratos solúveis em água (CS) e nitrogênio nas raízes. O experimento foi conduzido em casa-de-vegetação, em delineamento inteiramente casualizado, com cinco tratamentos [adubação nitrogenada após a colheita: 0, 2, 4, 6 e 8 dias] e dez repetições. O tempo da adubação reduziu linearmente a massa de forragem e de raízes do capim BRS Piatã, mas não afetou o BRS Quênia. Observou-se resposta quadrática sobre a concentração de CS e amido na raiz do capim BRS Piatã, diminuindo da colheita até o dia 2 e aumentando até o dia 8. Porém, com o atraso na adubação, o nitrogênio na raiz reduziu linearmente. A época de adubação nitrogenada afeta de forma quadrática a concentração de CS em raízes de capim BRS Quênia. A adubação nitrogenada do capim BRS Piatã logo após a colheita melhora a massa de forragem e de raízes, CS e reservas de nitrogênio, enquanto há maior flexibilidade quanto ao momento de realizar a adubação nitrogenada para o BRS Quênia. O intervalo entre a colheita e a adubação nitrogenada é mais dependente da massa radicular, que afeta o acúmulo de nitrogênio nas raízes.

PALAVRAS-CHAVE: *Megathyrsus maximus*, *Urochloa brizantha*, amido na raiz, nitrogênio na raiz.

INTRODUCTION

Nitrogen is a vital nutrient required in large quantities by plants and plays a crucial role in forage mass production (Rosado et al. 2017), as well as leaf elongation and appearance rate (Martuscello et al. 2019, Duarte et al. 2020), facilitating the faster reestablishment after defoliation.

In rotational grazing systems, nitrogen fertilization is typically conducted after cattle grazing. Following harvest, plants experience nutritional stress due to the nutrient requirements for regrowth. To fulfill these nutrient requirements, plants translocate organic reserves (carbohydrates and nitrogen) from basal stems and roots to leaves, resulting in a reduction in root mass (Gomide et al. 2019).

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The decrease in root mass during grass regrowth can affect nutrient absorption from the soil, thereby delaying shoot reestablishment and the grazing period. This is of particular importance considering that urea, the main source of nitrogen fertilizer, exhibits high nitrogen losses through volatilization (Wang et al. 2023). Therefore, a fast root recovery can enhance nitrogen uptake and reduce volatilization losses, thereby increasing nitrogen availability for new leaf growth and chlorophyll synthesis, ultimately leading to a higher photosynthetic rate (Jin et al. 2022) and promoting greater carbohydrate and nitrogen reserves in the roots (Gomide et al. 2019).

Carbohydrate variations in grass forages typically occur in roots after defoliation (Alderman et al. 2011), particularly under conditions of high grazing intensity (Silva et al. 2015) or shading (Coura et al. 2020). Plants with greater nutrient reserves may exhibit increased tolerance to a longer delay in nitrogen replacement in the soil, initiating the regrowth process. Therefore, it is crucial to determine the optimal number of days between grass harvest and fertilization that yields the most favorable response, in terms of grass regrowth and accumulation of carbohydrates and nitrogen in the roots.

Thus, this study aimed to identify the optimal interval between harvest and nitrogen fertilization for two grass species, as well as whether the flexibility in the fertilization time is associated with the accumulation of water-soluble carbohydrates and nitrogen in the roots.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse located in Rondonópolis, Mato Grosso state, Brazil (16º28'15"S, 54º38'08"W and about 227 m of altitude), from July to December 2018, in a region classified as *Aw* (tropical climate with a dry season), according to the Köppen-Geiger climate classification.

Two separate experiments were performed, each one focusing on a grass species: *Urochloa brizantha* (Hochst. ex A. Rich.) cv. BRS Piatã and *Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. BRS Quênia. The experimental design was completely randomized, with five treatments and ten replications. The treatments comprised nitrogen fertilization times after harvest: day 0, which involved nitrogen fertilization on the same day of harvest, and 2, 4, 6 and 8 days after harvest. Each experimental plot consisted of a pot containing 5 dm³ of soil and three plants.

The soil used in the experiment was an Oxisol (Santos et al. 2018), equivalent to Ferralsols (FAO 1998), collected from the 0-20 cm depth layer and characterized by its clayey composition. The soil chemical and grain size analyses (Table 1) were performed according to Teixeira et al. (2017). The soil was sieved through a 4-mm sieve and then transferred to 5.5-dm³ pots. The soil's base saturation was adjusted to 60 % using dolomitic limestone (PRNT = 86 %; Vilela et al. 2007). Irrigation was conducted daily using the gravimetric method (Cabral et al. 2018), to ensure that the applied water mass elevated the soil moisture to its maximum water retention capacity.

At 30 days after lime application, each pot was sown with 20 seeds. At the time of sowing, a dose of 300 mg dm^{-3} of phosphorus (P_2O_5) was applied. At 10 days after fertilization, plant stand thinning was conducted to maintain four plants per plot. After thinning, all treatments received 100 mg dm^{-3} of nitrogen in the form of urea and 70 mg dm^{-3} of potassium as potassium chloride.

A standardized harvest was conducted at 30 days after sowing, with a residue height of 20 cm for BRS Piatã palisade grass and 30 cm for BRS Quênia guinea grass. Subsequently, 200 mg dm^{-3} of nitrogen were immediately applied after harvest, corresponding to treatment 0. The same nitrogen dose was applied after 2, 4, 6 and 8 days of regrowth, corresponding to each treatment.

The first evaluation was performed at 20 days after the standardized harvest. The grass height was measured using a graduated ruler, and the number of tillers per pot was counted. The cut was made at

Table 1. Chemical and grain size characteristics of the experimental soil.

pH	P	K	Ca + Mg	Al + H	CEC	BS	m	OM	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}		cmol dm^{-3}			%		g kg^{-1}			
4.9	4.6	108	2.4	3.4	6.1	44	0.0	19.2	290	150	560

CEC: cation exchange capacity; OM: organic matter; BS: base saturation; m: aluminum saturation.

20 cm for BRS Piatã and 30 cm for BRS Quênia. The material above the cutting height was collected and separated into leaf blades and stem + sheath. No dead material was observed. The number of leaves was counted, and the separated components were dried in a forced air circulation oven at 55 ± 5 ºC, for 72 hours, before being weighed. Four evaluations were conducted at intervals of 20 days. In the last harvest, the material below the cutting height was collected and subjected to the same procedures. This material was considered the residual mass.

The forage mass was determined by summing the mass of leaves and stem + sheath; the individual leaf mass by dividing the dry mass of leaves by the number of leaves; the individual tiller mass by dividing the dry mass of tillers by the tiller population density; the number of leaves per tiller by dividing the number of leaves by the tiller population density in each pot; the leaf appearance rate by the relationship between the number of leaves per tiller and the cutoff interval; and phyllochron was estimated as the inverse of the leaf appearance rate.

At the final harvest, the residue below the cutting height was collected along with the roots, which were washed using a 4-mm sieve to remove soil particles. Both the roots and the residue were dried following the aforementioned procedure. The roots were then ground in a Willey mill using a 1-mm sieve for analysis of water-soluble carbohydrates (WSC) and starch (Passos 1996). The nitrogen analysis was performed using the AOAC method (2001.11; AOAC 2002) and the accumulation calculated by multiplying the root mass by the nitrogen and WSC contents quantified in the roots.

The data were analyzed using the general linear mixed model method through the PROC MIXED procedure (SAS® Institute Inc., Cary, NC). The time of N fertilizer application was treated as a fixed effect, while each regrowth cycle and pot replication were considered as random effects. The N fertilizer application time effects were evaluated using orthogonal polynomial contrasts, with significance set at $p < 0.05$. This allowed the assessment of linear or quadratic effects resulting from the N fertilizer application time.

RESULTS AND DISCUSSION

The fertilization time had a significant effect on all the variables examined for the BRS Piatã, except for leaf blade and stem + sheath mass, height and phyllochron (Table 2). The interval between fertilization and harvest quadratically reduced the forage mass, reaching a 39.82 % decrease at 6 days after harvest. The highest forage mass was observed when fertilization was performed on the day of the harvest (Figure 1). On average, the leaf blade and stem + sheath masses were 754.23 and 245.77 g kg^{-1}, respectively. The fertilization conducted at 8 days after harvest resulted in decreases of 29.20 % for individual leaf mass and 30.79 % for individual tiller mass, when compared to fertilization on the day of defoliation.

The development of the BRS Piatã was affected by the increased interval between harvest and nitrogen fertilization. Linear decreases of 34.70 and 26.68 % were observed for residual and root mass, respectively (Table 2). This reduction can be attributed to the nutritional stress experienced by BRS Piatã, due to delayed nitrogen fertilization. When nitrogen is not promptly supplied, the grass uses its reserves in the roots to minimize the damage to shoot productivity. The use of nitrogen reserves stored in the stem base and roots under stressful conditions has been reported in the literature (Volenec et al. 1996, Silva et al. 2014).

The results highlight the importance of nitrogen as a critical nutrient for grass regrowth. Even a delay of just 8 days in the nitrogen supply can negatively impact forage, residual mass and roots. This is because nitrogen is essential for various plant processes, including chlorophyll synthesis,

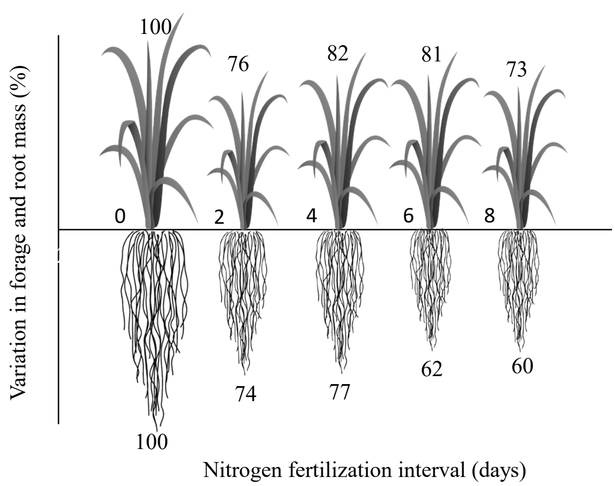

Figure 1. Variation in forage and root masses of BRS Piatã palisade grass at nitrogen fertilization intervals.

Table 2. Productive, morphological and structural characteristics of BRS Piatã palisade grass at nitrogen fertilization intervals after defoliation.

Characteristics	Nitrogen fertilization interval (days)					p-value[1]		SEM
	0	2	4	6	8	L	Q	
Productive characteristic								
FM (g pot^{-1})	25.460	18.750	19.690	15.690	15.320	< 0.001	0.020	3.444
LP (g kg^{-1})	724.400	781.900	737.100	775.300	752.200	0.415	0.270	18.880
SP (g kg^{-1})	275.600	218.100	262.900	224.700	247.800	0.415	0.270	18.880
ILM (g leaf^{-1})	0.113	0.090	0.090	0.080	0.080	< 0.001	0.006	0.016
ITM (g tiller^{-1})	0.591	0.445	0.448	0.399	0.409	< 0.001	0.003	0.022
RESM (g pot^{-1})	30.720	24.520	24.470	22.350	20.060	< 0.001	0.102	0.918
RM (g pot^{-1})	16.750	12.760	13.800	13.560	12.280	0.003	0.174	0.794
Morphological and structural characteristic								
Height (cm)	46.000	45.000	46.000	44.000	45.000	0.242	0.348	0.860
NL (leaves pot^{-1})	165.000	157.000	165.000	148.000	134.000	0.001	0.110	6.400
TPD (tillers pot^{-1})	52.000	51.000	51.000	48.000	47.000	0.035	0.531	2.000
NLT	3.170	3.070	3.230	3.080	2.850	0.003	0.997	0.700
LAR (leaf tiller^{-1} day^{-1})	0.123	0.115	0.116	0.111	0.102	0.008	0.727	0.021
PHY (days leaf^{-1})	9.970	9.810	9.640	9.960	10.810	0.178	0.121	1.678

FM: forage mass; LP: leaf proportion; SP: stem proportion; ILM: individual leaf mass; ITM: individual tiller mass; RESM: residual mass; RM: root mass; NL: number of leaves; TPD: tiller population density; NLT: number of leaves per tiller; LAR: leaf appearance rate; PHY: phyllochron; SEM: standard error of the mean. [1] Orthogonal contrast (L: linear; Q: quadratic; $p < 0.05$).

protein formation and enzymatic activities involved in photosynthesis, which is the primary energy-producing metabolic pathway in plants (Bassi et al. 2018). An adequate nitrogen supply in the soil is necessary for the synthesis of new cells and proteins after defoliation, to support the plant reestablishment. Thus, any delay in nitrogen availability can compromise the regrowth of the grass (Cabral et al. 2021).

An average height of 45.2 cm was observed for BRS Piatã (Table 2). The number of leaves and tillers, leaves per tiller and leaf appearance rate exhibited a linear decrease as the interval between defoliation and nitrogen fertilization increased, resulting in reductions of 18.78, 9.61, 10.09 and 17.07 %, respectively (Table 2). The mean interval between the emergence of two successive leaves (phyllochron) was 10.04 days. The reduction in forage mass for BRS Piatã can be primarily attributed to the decline in the number of leaves and individual leaf mass. When comparing the treatment where grass was fertilized on the day of harvest with delayed fertilization, there was a reduction of 34.70 % in the number of leaves and 29.20 % in the individual leaf mass.

These findings indicate that the reserve content in roots alone was insufficient to meet nitrogen demands for a sustained leaf emergence, thus requiring a closer time between fertilization and harvest. The reduction in the number of leaves and individual leaf mass is detrimental to the productivity of the system, as leaf blades have a higher nutritional value when compared to stems and are preferred by grazing animals (Pedreira et al. 2017). In addition to the impact on leaves, there was a decrease in the tiller population density and individual tiller mass for BRS Piatã, with the fertilization at 8 days after harvest resulting in a reduction of 26.68 % in the tiller population density, if compared to the fertilization on the day of defoliation.

Proportionally, the fertilization time affected the leaf emergence more than the tiller emergence for BRS Piatã, as evidenced by the decrease in the number of leaves per tiller. This effect is further supported by the decline in the leaf appearance rate, which decreased from 0.123 leaf tiller^{-1} day^{-1} on the day of defoliation to 0.102 leaf tiller^{-1} day^{-1} after 8 days. Nitrogen plays a crucial role in promoting the leaf appearance rate, which is essential for canopy structure and can influence the leaf size, number of tillers and number of leaves per tiller (Pereira et al. 2015). Pereira et al. (2012) observed a reduction in the number of leaves per tiller for Marandu and Xaraés palisade grasses and recommended applying nitrogen on the day of harvest for these grasses.

The accumulated root nitrogen (p = 0.045; SEM = 0.0042) showed a linear decrease of 0.014 g day^{-1} after harvest (Figure 2). The content of water-soluble carbohydrates (WSC) and starch

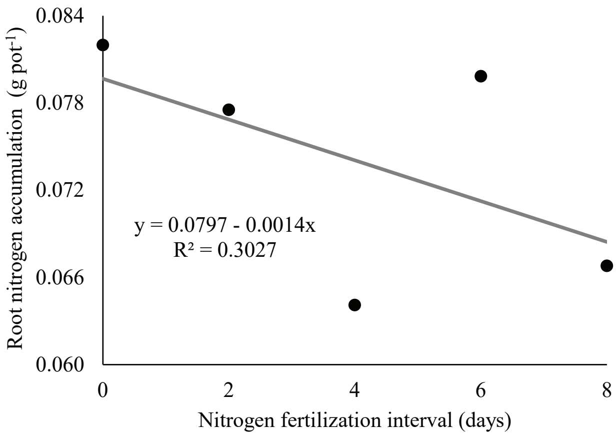

Figure 2. Root nitrogen accumulation for BRS Piatã palisade grass at nitrogen fertilization intervals.

in the roots exhibited a quadratic effect, decreasing when fertilization was performed at 2 days after harvest and increasing with subsequent fertilization times (Table 3). For the BRS Piatã, the lowest accumulation of WSC and starch occurred with a 2-day interval between fertilization and harvest. From the 4-day interval onwards, there was an increase in the WSC and starch accumulations, although the starch accumulation showed a greater increase when compared to WSC (Figure 2). The simultaneous increase in starch and WSC accumulations suggests that the increase in these carbohydrates did not occur due to starch breakdown.

According to Varma et al. (2018), plants increase the mobilization of root reserves when certain nutrients in the soil are limited. When nitrogen is adequately supplied, the accumulated WSC is used for grass regrowth (Schnyder & Visser 1999). This can be observed when nitrogen was supplied closer to harvest, as there was a reduction in the WSC content and an increase in the forage mass. Therefore, it is hypothesized that the plants mobilized the carbohydrates synthesized during photosynthesis to the roots to recover their root mass and enhance the uptake of inorganic nitrogen from the soil.

The regrowth stress observed for BRS Piatã can be attributed to the deficiency of nitrogen reserves, as indicated by the lower nitrogen accumulation in the roots. This deficiency is primarily caused by the reduction in root mass, which compromises the absorption of nitrogen from the soil (Silva et al. 2015, Gomide et al. 2019). The decrease in root nitrogen content can be attributed to the use of reserves by the plant for the emergence of new leaves and tillers (Silva et al. 2015). Therefore, when the nitrogen replacement in the soil is delayed, the rate of nitrogen utilization is reduced to a greater extent than its absorption, leading to a negative balance and a depletion of reserves in the roots. Consequently, grasses with low nitrogen accumulation in the roots do not possess sufficient nitrogen reserves, and, in the case of delayed fertilization, this results in a lower forage mass due to nutritional deficiencies.

In contrast, none of the productive characteristics of the BRS Quênia guinea grass were affected by the nitrogen fertilization time after harvest, except for residue mass, which exhibited a quadratic response (Table 4). Since the harvest was performed at 30 cm above the ground, only leaves were collected, and no stem mass was observed. Therefore, the forage mass is solely represented by the mass of leaves, with an average value of 15.09 g pot^{-1} (Table 4).

A linear increase in the height of the BRS Quênia was observed when fertilization was delayed by 8 days, if compared to the fertilization applied on the day of the harvest (Table 4). The content of WSC in the roots (g kg^{-1}) exhibited a quadratic effect in response to the fertilization time (Table 5). The average WSC content per pot was 0.764 g, while the accumulation of starch in the roots were 80.81 g kg^{-1} and 3.682 g pot^{-1}. The nitrogen content in the roots

Table 3. Root carbohydrate and starch accumulations for BRS Piatã palisade grass at nitrogen fertilization intervals after defoliation.

Characteristics	Nitrogen fertilization interval (days)					p-value[1]		SEM
	0	2	4	6	8	L	Q	
Root WSC (g kg^{-1})	43.830	24.610	27.040	34.890	41.140	0.517	< 0.001	2.338
Root starch (g kg^{-1})	34.590	24.890	43.940	71.340	84.000	< 0.001	< 0.001	1.609
Root WSC (g pot^{-1})	0.734	0.314	0.373	0.473	0.505	< 0.001	0.001	0.030
Root starch (g pot^{-1})	0.579	0.317	0.606	0.968	1.030	< 0.001	< 0.001	0.040

WSC: water-soluble carbohydrate; SEM: standard error of the mean. [1] Orthogonal contrast (L: linear; Q: quadratic; $p < 0.05$).

Table 4. Productive, morphological and structural characteristics of the BRS Quênia guinea grass at nitrogen fertilization intervals after defoliation.

Characteristics	Nitrogen fertilization interval (days)					p-value[1]		SEM
	0	2	4	6	8	L	Q	
Productive characteristic								
FM (g pot^{-1})	15.540	14.780	14.810	15.160	15.180	0.730	0.119	0.930
ILM (g leaf^{-1})	0.102	0.101	0.102	0.101	0.103	0.856	0.716	0.002
ITM (g tiller^{-1})	0.234	0.222	0.217	0.227	0.226	0.549	0.061	0.005
RESM (g pot^{-1})	70.920	73.560	72.520	69.550	67.180	0.003	0.009	1.192
RM (g pot^{-1})	41.300	47.100	44.450	56.680	43.550	0.539	0.397	5.650
Morphological and structural characteristics								
Height (cm)	59.000	58.000	60.000	62.000	64.000	< 0.001	0.058	0.652
NL (leaves pot^{-1})	154.000	149.000	147.000	155.000	154.000	0.723	0.228	3.141
TPD (tillers pot^{-1})	66.720	66.760	68.300	66.980	67.820	0.550	0.070	1.066
NLT	2.307	2.220	2.132	2.285	2.232	0.531	0.053	0.042
LAR (leaf tiller^{-1} day^{-1})	0.109	0.105	0.101	0.108	0.106	0.531	0.053	0.002
PHY (days leaf^{-1})	9.384	9.715	10.260	9.618	9.823	0.243	0.068	0.210

FM: forage mass; ILM: individual leaf mass; ITM: individual tiller mass; RESM: residual mass; RM: root mass; NL: number of leaves; TPD: tiller population density; NLT: number of leaves per tiller; LAR: leaf appearance rate; PHY: phyllochron; SEM: standard error of the mean. [1] Orthogonal contrast (L: linear; Q: quadratic; $p < 0.05$).

Table 5. Root carbohydrate and starch accumulations of BRS Quênia guinea grass at nitrogen fertilization intervals after defoliation.

Characteristics	Nitrogen fertilization interval (days)					p-value[1]		SEM
	0	2	4	6	8	L	Q	
Root WSC (g kg^{-1})	21.870	12.570	14.570	12.940	15.920	0.055	0.006	1.751
Root starch (g kg^{-1})	81.980	73.420	80.840	76.550	88.240	0.307	0.121	4.680
Root WSC (g pot^{-1})	0.965	0.588	0.746	0.789	0.732	0.453	0.257	0.104
Root starch (g pot^{-1})	3.364	3.413	3.587	4.290	3.755	0.346	0.759	0.438

WSC: water-soluble carbohydrate; SEM: standard error of the mean. [1] Orthogonal contrast (L: linear; Q: quadratic; $p < 0.05$).

($p > 0.05$; SEM = 1.0037) was not significantly affected by the fertilization time. BRS Quênia showed no significant response to the interval between harvest and fertilization, except for the WSC content, with no changes observed in the content and accumulation of starch in the roots.

The higher tolerance for the BRS Quênia guinea grass may be attributed to a higher proportion of accumulated starch, when compared to WSC. By producing more root mass, the grass can increase its starch storage capacity, what enhances its tolerance to variations in time of nutrient supply, particularly during periods of plant stress following harvest. This hypothesis is supported by the lower tolerance observed for the BRS Piatã, which stored a higher proportion of WSC, when compared to starch. Changes in the starch and WSC contents can occur under stress conditions, such as after harvest, competition with other plants or during dry periods (Priyadarshini et al. 2016).

CONCLUSIONS

1. The immediate nitrogen fertilization of BRS Piatã palisade grass after harvest enhances forage mass, root mass, water-soluble carbohydrate and nitrogen reserves. On the other hand, BRS Quênia guinea grass exhibits a greater flexibility, in terms of nitrogen fertilization time;
2. The interval between harvest and nitrogen fertilization is more influenced by root mass for BRS Piatã than for BRS Quênia, what directly affects the nitrogen accumulation in the roots.

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