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PASTURE AND FORAGE UTILIZATION

Effects of glyphosate plus foliar manganese application on the production and quality of marandu grass

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ABSTRACT. In this study, the stimulatory effects of application of glyphosate herbicide coupled with manganese sulfate (8%) foliar fertilizer on the production and bromatological characteristics of *Brachiaria brizantha* 'Marandu' were evaluated. The experiments were performed using randomized complete block design with a 5×4 factorial scheme in plots subdivided over time (across four evaluations) with four repetitions, totaling 100 observations. The effects of sublethal doses of acid equivalent (a.e.) of glyphosate (5.40, 21.60, 64.80, and 108.00 g·a.e.·ha^{-1}) and control plus manganese sulfate foliar fertilizer (1,000 g ha^{-1}) were assessed in four successive evaluations at a defoliation frequency of 21 days. Foliage at 20 cm height (to evaluate forage production); leaf/stem ratio (LSR); and contents of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG) were measured in experimental plots with a usable area of 7.5 m^2. Application of sublethal doses of glyphosate plus manganese sulfate did not increase forage production, increased LSR, reduced LIG content, and did not affect CP content in all four evaluations. NFD and ADF indicated satisfactory qualitative indices for animal feed following the application of sublethal doses of glyphosate plus manganese sulfate in all four evaluations.

Keywords: acid equivalent; sublethal doses; hormone; *Brachiaria brizantha*.

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Introduction

Glyphosate is a systemic broad-spectrum herbicide and a crop desiccant, which has proved to be a revolution for agricultural production since 1974 (Galli & Montezuma, 2005). Following the withdrawal of its patent, glyphosate became the most used phytosanitary product in Brazil (Vasconcelos, 2018), especially in no-till farming, which is considered an advancement from the edaphic and environmental viewpoints.

Glyphosate has no effects on genetically modified crops that are tolerant to chemical herbicides. However, its drift may produce beneficial effects on non-target plants, such as *Brachiaria decumbens* (Meschede, Velini, Carbonari, Trindade, & Gomes, 2011), *Oryza sativa* 'Primavera' (Gitti et al., 2011), and *Phaseolus vulgaris* 'IPR-139' (Silva, Gerlach, Rodrigues, & Arf, 2016). Application of sublethal doses of chemical herbicides alters the morphophysiological and biochemical characteristics of plants by affecting hormones (Nascentes, Fagan, Soares, Oliveira, & Brunelli, 2015).

Glyphosate affects plant physiology; for instance, application of sublethal doses of glyphosate can degrade chlorophyll, leading to temporary yellowing of newly emerged leaves (Yamada, & Castro, 2007). These symptoms are similar to those of manganese deficiency, indicating that glyphosate interferes with the balance of elements in plants. Manganese is a vital micronutrient involved in various physiological and metabolic processes of plants, such photosynthesis, photolysis of water, and nitrogen assimilation, and it is an important component of enzyme precursors, such as aromatic amino acids, hormones, phenols, and lignin (Campbell & Nable, 1988).

Previous studies verifying the effects of glyphosate on plants were conducted over short periods, which did not allow for specific evaluations and limited the time of herbicide exposure; therefore, these studies did not produce substantial evidence of the effects of glyphosate on yield (Brito, Tripaldi, Carbonari, & Velini, 2018). We hypothesized that foliar manganese supplementation would alter the metabolism of plants treated with glyphosate. To test this hypothesis, we assessed the effects of sublethal doses of glyphosate in the presence of manganese on the yield and nutritional value of *B. brizantha* 'Marandu' (Marandu grass) applied in four cycles at a defoliation frequency of 21 days.

Material and methods

The experiments were conducted at the Federal Institute of Education, Science and Technology of Rondônia, Câmpus Ariquemes, from January to June 2019. Climatic data during the experimental period were obtained from the automatic station of the National Institute of Meteorology, located at 400 m from the experimental site (Figure 1). The experiments were conducted using randomized complete block design with a 5×4 factorial scheme on plots subdivided over time and with four repetitions. The main treatments were organized into five levels: control and four sublethal glyphosate doses. Data were collected from subplots through four sequential evaluations at a defoliation frequency of 21 days.

At the experimental site, cassava was grown for 2 consecutive years (2017 and 2018). Soil properties were as follows: pH in H_2O = 6.71; P (Mehlich^{-1}) = 2.70 mg·dm^{-3}; K = 90 mg dm^{-3}; Ca^{2+} (KCl mol·L^{-1}) = 2.74 cmol$_c$ dm^{-3}; Mg^{2+} = 1.62 cmol$_c$·dm^{-3}; Al^{3+} = 0; H^+ Al^{3+} = 1.17 cmol$_c$·dm^{-3}; and base saturation (v%) = 79.7%. The soil texture is clayey (720 g·kg^{-1} clay). Liming was not required, and mechanical soil preparation followed conventional practices, including plowing (disc plow) and harrowing (leveling harrow) for soil mobilization, removal, and leveling.

On January 11, 2019, seeds of Marandu grass, obtained from a commercial lot sample [2017/2018 crop; cultural value (VC), 80%], were sown at a depth of 2.5 cm and line spacing of 0.50 m. At the time of sowing, 270 kg·ha^{-1} of the formulated fertilizer 04-30-16 was supplied (Cantarutti et al., 1999). The experimental plots comprised seven 4-m-long lines and a usable area of 7.5 m^2.

At 52 days after sowing (DAS), the first cut was made to stimulate tillering. At 71 DAS, the grass was cut uniformly at a height of 20 cm from soil surface (Dias-Filho, 2012). After cutting at 71 DAS, a nitrogen fertilizer (urea) was supplied at the first (92 DAS), second (113 DAS), and third (134 DAS) evaluation, totaling 50 kg·ha^{-1} of N (Cantarutti et al., 1999).

On seventh day after cutting for uniformization followed by the first three evaluations, acid equivalent (a.e.) of glyphosate was applied at sublethal doses of 5.40, 21.60, 64.80, and 108.00 g·a.e. ha^{-1}; control treatment included no glyphosate application. Glyphosate used was a commercial (a.e.) product, Shadow (356 g·L^{-1}), with a prescribed re-entry period of 2 days. The glyphosate and control syrups were supplemented with a manganese sulfate foliar fertilizer (1,000 g·ha^{-1}) and chelating agent (56%). Mineral composition of the foliar fertilizer was as follows: 8 manganese, 6.5 sulfur, 3 zinc, 1 copper and zinc, 0.5 boron, and 0.1% molybdenum.

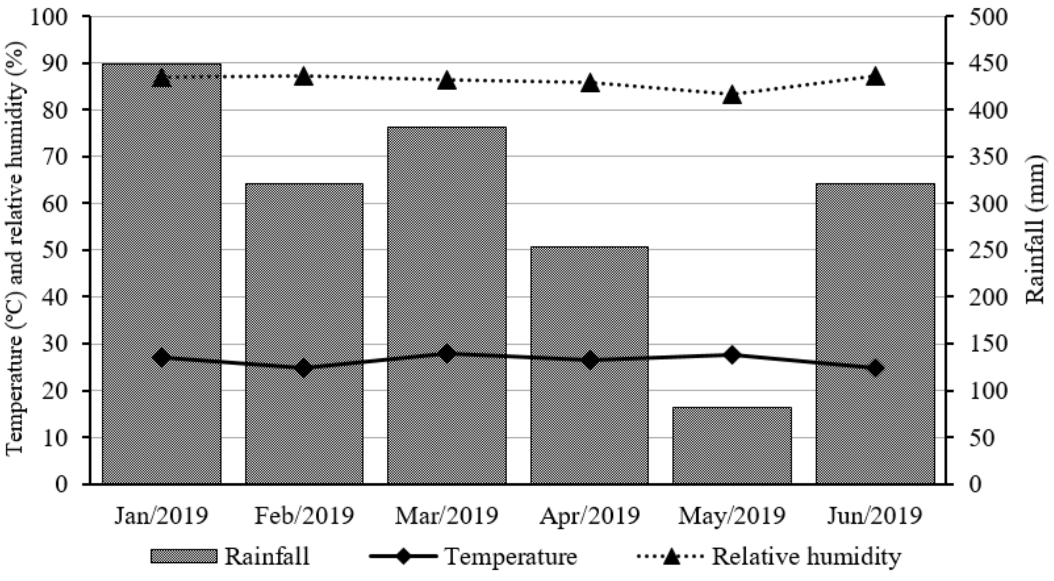

Figure 1. Temperature, relative humidity and rainfall during the experimental period.

Treatments were applied using a back-loaded CO_2 sprayer (pressure, 2 bar) equipped with a DG11002-VS drift guard spray tip (spray volume, 100 L·ha^{-1}). At the time of application, the adjacent plots were protected with plastic sheets to prevent drift.

The phytotoxicity of herbicide against Marandu grass was evaluated visually at 7 and 14 days after application based on a scale from 0 to 100, where 0 corresponds to no injury and 100 corresponds to death (SBCPD, 1995).

Adopting a defoliation frequency of 21 days, assessments were performed on the 15^{th} day after treatment application, totaling four evaluations: first evaluation at 92 DAS, second evaluation at 113 DAS, third evaluation at 134 DAS, and fourth evaluation at 155 DAS. Foliage at 20 cm height from the soil surface was harvested and separated into two subsamples: (1) leaves without stalk + sheath for determining the leaf/stalk ratio (LSR) and forage production (FP) and (2) tiller + leaves for laboratory analysis.

The samples were pre-dried in a forced ventilation oven at 55°C for 72 hour using the INCT-CA G-001/1 method (Detmann et al., 2012). Then, forage subsamples were processed in a Wiley knife mill with a 1 mm mesh to obtain dry mass in an oven at 105°C (INCT-CA G-003/1). Crude protein (CP) content was determined by the Kjeldahl method (INCT-CA N-001/1). Neutral detergent fiber (NDF) and acid detergent fiber (FDA) contents were determined using the Ankom® Filter Bag Technique (INCT-CA F-002/1 and INCT-CA F-004/1, respectively). Lignin (LIG) content was determined based on permanganate oxidation (INCT-CA F-006/1; Detmann et al., 2012).

Data were subjected to analysis of variance using the F test in Sisvar (Ferreira, 2014). When significant, the effects of sublethal glyphosate doses and interactions among factors were evaluated by regression analysis. Results of different evaluations were compared by the Tukey test at a 5% significance level.

Results and discussion

Sublethal glyphosate doses affected FP, LSR, and LIG content at different evaluation time points (Table 1). All interactions were significant, indicating interdependence among factors. NDF and ADF differed across evaluations. CP content was not affected by any treatment.

Sublethal doses of glyphosate did not promote FP (Figure 2A). In the mathematical model, the FP response was inversely proportional to glyphosate dose. A previous study indicated that the presence of manganese in a glyphosate-based spray solution did not alter the effectiveness of the herbicide (Bernards, Thelen, Penner, Muthukumaran, & McCracken, 2005), which is consistent with our results. However, the deleterious effects of glyphosate, such as chlorosis or phytotoxicity, were not observed during the experimental period.

FP obtained in the first evaluation differed significantly from that obtained in subsequent evaluations, particularly the fourth one (Table 2). Sublethal glyphosate and control doses reduced FP by 58.71% in the second, third, and fourth evaluations. In a previous study evaluating the effects of different glyphosate doses (90, 180, 360, 720, and 1440 g·a.e.·ha^{-1}) on the forage dry weight of *B. ruziziensis*, a linear decrease in forage dry weight with increase in herbicide dose was observed, without any increase in yield (Costa, Peres, Ritter, Silva, & Fey, 2013), which is consistent with our findings.

Sublethal doses of glyphosate reduced LSR in a concentration-dependent manner (Figure 2B). In the first and second evaluations, LSR was inversely proportional to glyphosate dose. In the third and fourth evaluations, the relationship between LSR and glyphosate dose followed a second-order polynomial. In the third evaluation, FP was the highest at the glyphosate dose of 42.25 g·e.a.·ha^{-1}. In the fourth evaluation, FP was the lowest at the glyphosate dose of 70.00 g·e.a.·ha^{-1}, with an LSR of 1.54.

Table 1. Analysis of variance for forage production (FP); leaf/stem ratio (LSR); and contents of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG) in Marandu grass as a function of sublethal glyphosate and control doses (D) and evaluation time points (E).

Variable	Sublethal dose (D)	Evaluation (E)	D × E	$CV^{(1)}$%	Mean
	F value				
FP (kg·ha^{-1} DM)	10.470^{**}	29.806^{**}	4.411^{**}	15.20	7,261.02
LSR	10.902^{**}	60.160^{**}	3.541^{**}	13.76	2.61
LIG (% DM)	9.371^{**}	131.160^{**}	6.896^{**}	11.44	1.99
NFD (% DM)	0.096^{ns}	6.339^{*}	1.359^{ns}	4.10	62.95
AFD (% DM)	0.255^{ns}	2.103^{*}	0.9436^{ns}	6.74	32.40
CP (% DM)	2.11^{ns}	0.643^{ns}	1.282^{ns}	8.04	16.79

$^{(1)}$Coefficient of variation. **significant at 1%, *significant at 5%, ^{ns}non-significant (F test).

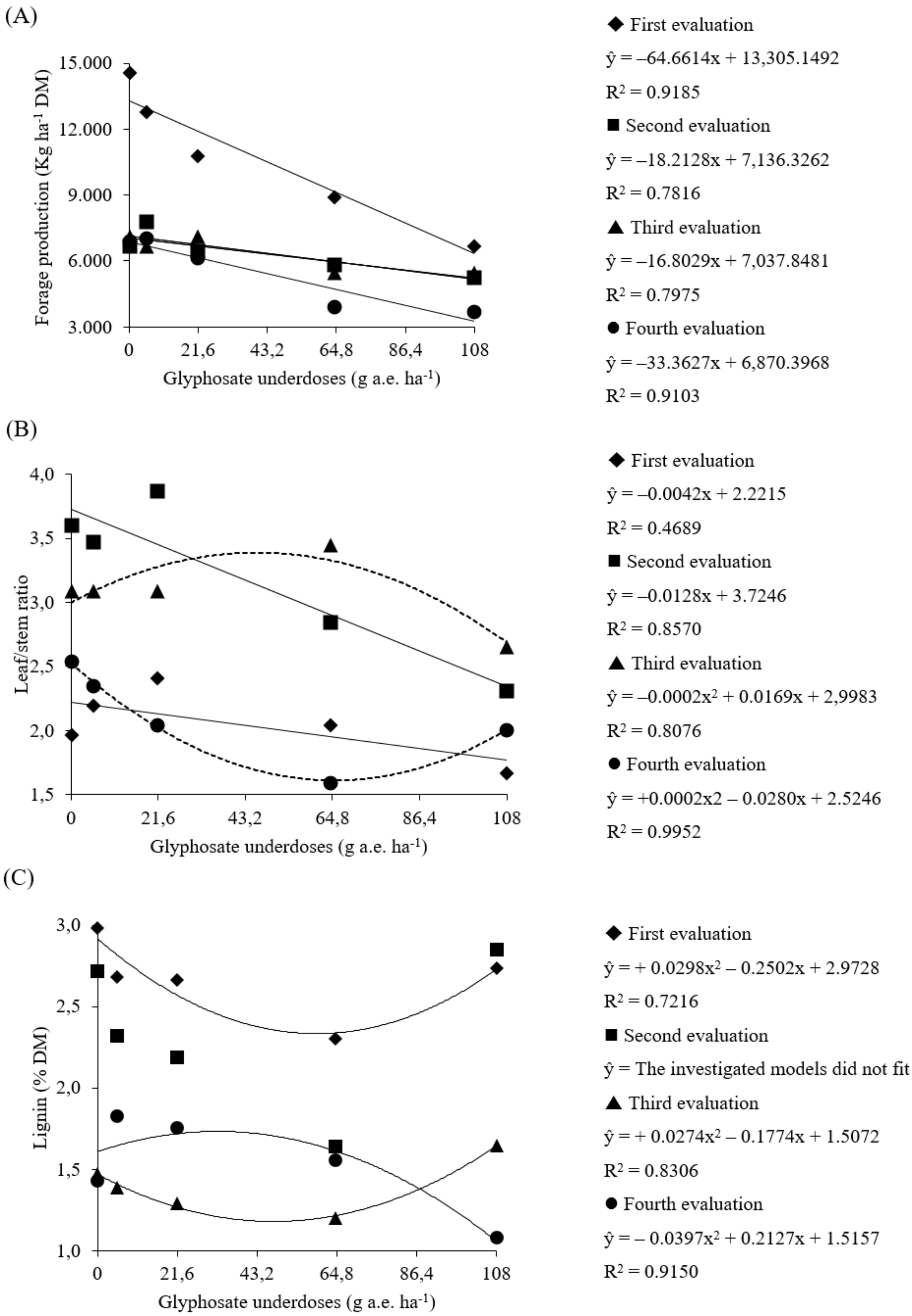

Figure 2. Effects of glyphosate and control treatments on the forage production (A), leaf/stem ratio (B), and lignin (C) content of Marandu grass in four evaluations.

The effects of sublethal doses of glyphosate on LSR were more evident in intermediate (second and third) evaluations than in others. However, there was no significant relationship between LSR and evaluation (1.0). The F values recorded in this study were higher than those reported in a previous study in which the mathematical model indicated an F value of 1.11 for glyphosate doses of 54 and 108 g·a.e. ha^{-1} and control

(Lima, Pereira, Sousa, Oliveira, & Jakelaitis, 2019). LSR is an important parameter for animal weight gain (Euclides et al., 2009), as a greater proportion of leaf blades is consumed during grazing (Bauer, Pacheco, Chichorro, Vasconcelos, & Pereira 2011). Leaves constitute over 80% of bovine diet, particularly in pastures (Forbes & Hodgson, 1985).

In sequential evaluations, the response of LIG content to glyphosate dose followed a second-order polynomial (Figure 2C). Under very low glyphosate doses (4.20 and 3.24 g·a.e. h^{-1}), the maximum LIG contents (2.45 and 1.22% DM, respectively) were obtained in the first and second evaluations. In the fourth evaluation, the glyphosate dose of 2.68 g·a.e. h^{-1} produced 1.80% DM LIG content. In the second evaluation, analysis of variance of regression coefficients indicated significant deviation, although the models studied were not accurate.

In general, the maximum recorded LIG content did not exceed 3.00% DM level. Meschede et al. (2011) analyzed LIG content of *B. decumbens* in pastures treated with sublethal doses of glyphosate and observed 55.83% decrease in LIG content following treatment with 36 g·a.e.·ha^{-1} glyphosate compared with that following control treatment at 30 days after application. They also reported overall high LIG contents and found that glyphosate significantly changed yield at 30 and 60 days after application (13.61 and 14.67% DM, respectively) of glyphosate at doses of 36, 72, and 180 g a.e. ha^{-1}.

LIG contents following the application of glyphosate differed across evaluations and doses (Table 2). In the first evaluation, LIG contents were significantly different between intermediate glyphosate doses of 21.60 and 64.40 g·a.e.·ha^{-1}. Meanwhile, application of glyphosate at doses of 5.40 and 108.00 g·a.e.·ha^{-1} significantly reduced LIG content in the third and fourth evaluations, respectively. Thus, contrary to the data obtained by Meschede et al. (2011), LIG content decreased in sequential evaluations in our study. Therefore, changes in LSR (Table 2) with sequential evaluations contributed to reduced LIG contents.

LIG prevents the access of ruminal microorganisms to cellulosic and hemicellulosic fractions as well as other potentially digestible nutrients, such as soluble sugars, proteins, minerals, and vitamins (Velásquez et al., 2010). Young forage plants have a higher rate of leaf elongation, a lower rate of stem elongation, and a greater leaf length, resulting in a more favorable chemical composition, that is, lower proportions of fibrous fractions (Velásquez et al., 2010). The frequency of defoliation used (21 days) in this study was important to control the development of stem and forage canopy structure. Neves Neto, Santos, Alexandrino, and Santos (2015) have recorded average leaf life of 48.61 days in Marandu grass. Therefore, defoliation periods longer than this leaf life span imply senescence losses (Casagrande et al., 2010). During these periods, senescent or dead leaves are immeasurable. In this context, the defoliation interval (21 days) and defoliation intensity (20 cm) used in this study avoided senescence loss.

Sequential evaluations showed reduced NDF and ADF contents (Table 3), corroborating the results observed for LIG (Table 2). However, the obtained NDF content does not limit forage consumption. The limit of 65% DM NDF content in tropical forage plants, at a defoliation frequency of 30 days, indicates good nutritional value (Alencar et al., 2010).

Table 2. Forage production (FP), leaf/stem ratio (LSR), and lignin (LIG) content of Marandu grass treated with sublethal doses of glyphosate and control in four evaluations.

Evaluation	Sublethal glyphosate dose (g a.e. ha^{-1})				
	0.00	5.40	21.60	64.40	108.00
			FP, kg ha^{-1}		
First	14,536.92a	12,736.66a	10,778.19a	8,845.72a	6,734.79a
Second	6,719.29b	7,739.39b	6,506.26b	5,814.89b	5,270.18ab
Third	7,103.03b	6,705.89b	7,140.94b	5,438.42b	5,450.47ab
Fourth	6,947.12b	6,999.17b	6,077.38b	3,948.27b	3,727.53b
			LSR		
First	1.96c	2.20b	2.41bc	2.04b	1.66c
Second	3.59a	3.47a	3.86a	2.84a	2.30bc
Third	3.09ab	3.09a	3.09b	3.45a	2.65a
Fourth	2.54bc	2.35b	2.05c	1.60b	2.01bc
			LIG, % DM		
First	2.98a	2.68a	2.67a	2.30a	2.74a
Second	2.72a	2.32a	2.19b	1.64b	2.85a
Third	1.47b	1.39b	1.29d	1.20c	1.65b
Fourth	1.43b	1.83b	1.76c	1.55bc	1.08c

Means followed by the same letter in the column do not differ significantly ($p > 0.05$, Tukey test).

Table 3. Neutral detergent fiber (NDF) and acid detergent fiber (FDA) contents of Marandu grass in four evaluations.

Evaluations	NDF	ADF
	% DM	
First	64.54a	33.60a
Second	63.10ab	32.03ab
Third	62.08b	33.84a
Fourth	62.08b	30.14b

Means followed by the same letter in the column do not differ significantly ($p > 0.05$, Tukey test).

The obtained ADF contents (Table 3) indicate low levels of lignified components, favoring forage digestibility and assimilation (Oliveira, Bonfim-Silva, Silveira, & Monteiro, 2010). ADF content of ~30% in forage is considered ideal for animal consumption. Meschede et al. (2011) tested the effects of glyphosate on the chemical and bromatological variables of *B. decumbens*. The initial dose of glyphosate (32 g·a.e. ha^{-1}) and control treatment (without application) obtained ADF contents of 40.50 and 42.50% DM, respectively, indicating no effect of herbicide on this variable.

CP content affects the digestibility and consumption of forage (Benett, Buzetti, Silva, Bergamaschine, & Fabricio, 2008). CP content of Marandu grass was not affected by sublethal doses of glyphosate in the presence of manganese and/or sequential evaluations (Table 1). However, fertilizer application and defoliation frequency of 21 days led to high average CP content (16.79% DM). Nitrogen absorption rate of grasses is faster than their growth rate (Carámbula, 1977). Thus, lower the defoliation frequency for grazing, higher the forage CP content (Costa, Gonçalves, Oliveira, Oliveira, & Magalhães, 2004). Therefore, ideal forage should contain high levels of CP but low levels of NDF, ADF, and LIG. Moreover, high LSR increases forage digestibility and consumption, enhancing assimilation and, ultimately, animal performance.

Conclusion

1. Sublethal doses of glyphosate supplemented with manganese and sequential evaluations did not promote the yield of Marandu grass forage, although they increased LSR.
2. Sequential evaluations improved forage quality and reduced NDF, ADF, and LIG contents.
3. Application of sublethal doses of glyphosate supplemented with manganese did not produce phytotoxic effects or leaf chlorosis in Marandu grass.

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