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Growth bioestimulant in marandu grass cultivated in the amazon biome

Bioestimulante em pasto de marandu cultivado no bioma amazônico

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Highlights:
1 and 2 L ha^{-1} of biostimulant with 50 kg N ha^{-1} increased forage production.
Biostimulant did not increase forage production with 150 kg N ha^{-1}.
Biostimulant did not change the chemical-bromatological characteristics of Marandu grass.

Abstract

This study aimed to evaluate the effects of a biostimulant on the morphogenesis, structure, productivity, and chemical composition of *Urochloa brizantha* cv. Marandu, managed under two nitrogen fertilization levels. Collections were carried out during the dry (June to September) and the rainy season (September to April). The experimental area was divided into 48 plots of 8 m^2 each, using a randomized block design in a 4 × 2 factorial arrangement (0, 1, 2, and 3 L ha^{-1} of biostimulant × 50 and 150 kg N ha^{-1} $year^{-1}$), and subdivided over time into dry and rainy seasons. Treatments were applied in a single dose. An interaction was observed between biostimulant and nitrogen fertilization for the total forage dry matter production and daily forage dry matter production, in which an increase of 30.1 and 25.3% was observed in the total dry matter production and 33.7 and 27.6% in the daily dry matter production when using 1 and 2 L ha^{-1} of biostimulant, respectively, compared to the non-application of biostimulant and with fertilization of 50 kg N ha^{-1} $year^{-1}$. The leaf life-span showed a triple interaction (biostimulant × nitrogen fertilization × season), and a decomposition of the interaction showed that the fertilization of 150 kg N ha^{-1} $year^{-1}$ provided a shorter leaf life-span during the dry season, with no difference for the other combinations of factors. No interactions were found for the chemical-bromatological composition, with no differences for the biostimulant application. Biostimulant doses of 1 and 2 L ha^{-1} increase the

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dry matter production per hectare in the nitrogen fertilization of 50 kg N ha^{-1} $year^{-1}$, but its action is not effective with the highest nitrogen fertilization (150 kg N ha^{-1} $year^{-1}$).
Key words: Auxin. Bioregulators. Foliar fertilization. Forage production. Urochloa brizantha.

Resumo

Objetivou-se avaliar os efeitos de um bioestimulante sobre a morfogênese, estrutura, produtividade e composição química do capim de *Urochloa brizantha* cv. Marandu, manejado sob dois níveis de adubação nitrogenada. As coletas foram realizadas no período de seca (junho até setembro) e período chuvoso (setembro a abril). A área experimental foi dividida em 48 parcelas de 8 m² cada, sendo utilizado o delineamento em blocos ao acaso em arranjo fatorial 4×2 (0, 1, 2 e 3 L ha^{-1} de Bioestimulante x 50 e 150 kg de N ha^{-1} ano^{-1}) e subdividida ao longo do tempo, em período de seca e período chuvoso. As aplicações dos tratamentos foram feitas em dose única. Houve interação entre bioestimulante e adubação nitrogenada para a Produção de massa seca de forragem total e produção de massa seca de forragem diária, em que observou-se aumento de 30,1% e 25,3% na produção de matéria seca total e de 33,7% e 27,6% na produção de matéria seca por dia, ao utilizar 1 e 2 L ha^{-1} de bioestimulante, respectivamente, quando comparado a não aplicação de bioestimulante e com adubação de 50 kg de N ha^{-1} ano^{-1}. A duração de vida das folhas (DVF) apresentou interação tripla (Bioestimulante x Adubação nitrogenada x Período) sendo que o desdobramento da interação demonstrou que a adubação de 150 kg de N ha^{-1} ano^{-1} proporcionou menor duração de vida das folhas, no período de seca sem apresentar diferença para as demais combinações de fatores. Não foi observado interações para composição químico-bromatológica e não houve diferenças com a aplicação do bioestimulante. As dosagens de 1 e 2 L ha $^{-1}$ do bioestimulante, aumenta a produção de matéria seca por hectare na adubação nitrogenada de 50 kg de N ha -1 ano $^{-1}$, porém com a adubação nitrogenada mais alta (150 kg ha ano $^{-1}$ de N) a sua ação não é efetiva.
Palavras-chave: Adubação foliar. Auxina. Biorreguladores. Produção de forragem. *Urochloa brizantha*.

Introduction

The maximum productive potential of a forage plant is genetically determined. However, adequate environmental (e.g., temperature, humidity, luminosity, and availability of nutrients) and management conditions must be met for this to be achieved. Among these conditions, the low availability of nutrients in tropical regions is certainly one of the main factors that interfere with forage productivity and quality (Duarte, Paiva, Fernandes, Biserra, & Fleitas, 2019). Thus, nutrient application in adequate quantities and proportions, through the use of fertilizers, is an essential practice to increase pasture production, and many alternatives have been studied, mainly to increase the efficiency of growth and production of plants.

In this sense, biostimulants have been used mainly in systems that aim for precision agriculture (Oliveira et al., 2019; Lima et al., 2019). Biostimulants are complexes that contain substances such as auxins, gibberellins, cytokinins, and their analogs, which act mainly in the regulation of growth, elongation, and cell division (Santos et al., 2017), favoring the expression of the genetic potential of the plant and, consequently, stimulating the shoot and root system growth (Magalhães et al., 2016).

These substances have been used in several crops, especially annual crops (Castro, Lobo, Rodrigues, Backes, & Santos, 2016). For instance, they can provide an increase of 12.38% in the productivity of sugarcane stalks (Silva, Cato, & Costa, 2010) and a daily increase of 24.66% in the initial growth rate of the root dry matter of corn plants (V. M. dos Santos et al., 2013).

The use of biostimulants in forage grasses seems to be promising, as there are elongation and cell division in the growth of forage grasses (Costa et al., 2016). Thus, the stimulation of these factors can provide higher productivity to forage plants. Currently, few studies have used biostimulants in forage grasses, being required more studies evaluating applications and doses. An improvement in the Marandu production has already been observed during its establishment using auxin-based biostimulants, reaching values of 5.75% of accumulated DM in the pasture (Oliveira et al., 2019). Another experiment, also with auxin-based biostimulant, presented an increase of 36.34% of accumulated DM in the pasture when applying 1.25 L ha^{-1} on a hybrid of the genus *Urochloa* (Lima et al., 2019).

Thus, this study is based on the hypothesis that the use of biostimulant improves the productive characteristics of *Urochloa brizantha* cv. Marandu regardless of the used nitrogen fertilization level. Consequently, the objective was to evaluate the effects of a biostimulant on the productivity, nutritional value, and morphogenesis of Marandu grass, evaluated under two nitrogen fertilization levels.

Material and Methods

Experiment location

The experiment was carried out at the Federal Rural University of the Amazon, Campus of Parauapebas, Pará, Brazil, under the coordinates 6°04′24.32″ S and 49°49′04.69″ W and 245 m of altitude. The soil is classified as a sandy clay loam-textured Ultisol (C. M. Santos et al., 2018). The climate of the region is classified as Awi according to the Köppen classification (Peel, Finlayson ,& McMahon, 2007), with the dry season from May to October and a very accentuated wet season, with torrential rains from November to April. Precipitation and air temperature data during the experimental period are shown in Figure 1.

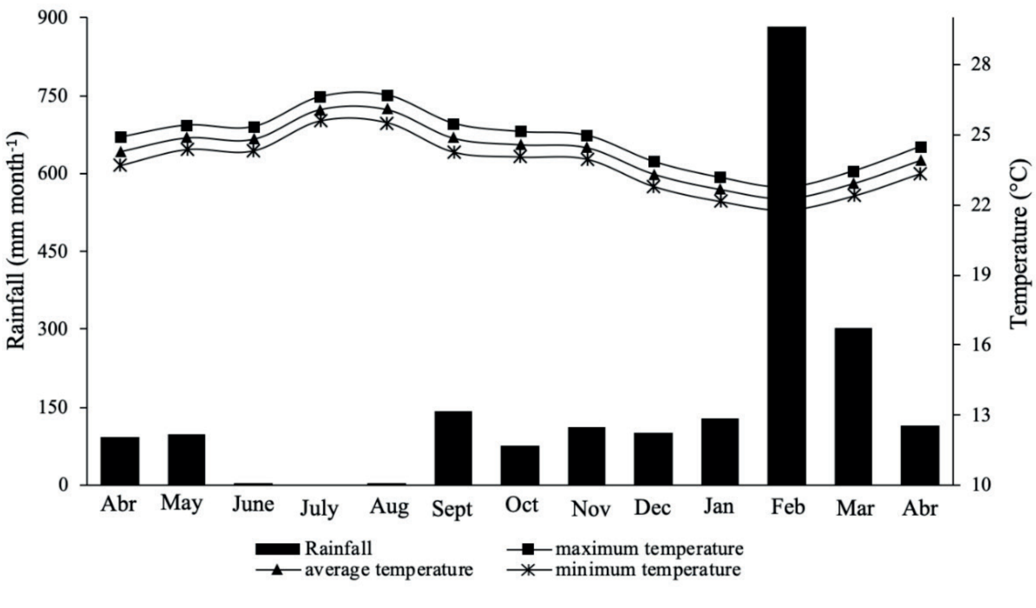

Figure 1. Accumulated monthly precipitation and average, maximum and minimum temperatures from April 2016 to April 2017.

Soil samples were collected at a depth of 0-20 cm before the beginning of the experiment from eight points to form a composite sample for chemical and organic matter (OM) characterization of the experimental area, which had the following results : pH (H_2O) = 5.8, sodium (Na) = 1.5 mg dm^{-3}, potential acidity (H+Al) = 1.9 cmol$_c$ dm^{-3}, boron (B) = 0.2 mg dm^{-3}, aluminum (Al) = 0 cmol$_c$ dm^{-3}, iron (Fe) = 200 mg dm^{-3}, calcium (Ca) = 1.8 cmol$_c$ dm^{-3}, manganese (Mn) = 8 mg dm^{-3}, magnesium (Mg) = 0.7 cmol$_c$ dm^{-3}, copper (Cu) = 0.5 mg dm^{-3}, potassium (K) = 0.21 cmol$_c$ dm^{-3}, zinc (Zn) = 1 mg dm^{-3}, P in Mehlich (P Meh) = 2 mg dm^{-3}, OM = 1.5%, sum of exchangeable bases (SB) = 2.71%, carbon (C) = 9 mg dm^{-3}, cation exchange capacity (CEC) = 4.61 mg dm^{-3}, sulfur (S) = 9.8 mg dm^{-3}, and base saturation (V%) = 58.69%.

The experiment was set up in a pasture module that had been cultivated with *U. brizantha* cv. Marandu for approximately five years. This area was used in a cattle production system under continuous grazing before the treatment application, with a low stocking rate (lower than 0.8 AU ha^{-1}).

Implementation, experimental design and treatments

The pasture module was divided into 48 plots of 8 m^2 (4.0 × 2.0 m), separated by 0.5 m tracks on all sides of each plot. The experimental design used was randomized blocks (divided into four blocks according to the slope) composed of eight treatments, with six replicates per treatment, totaling 48 experimental plots.

Treatments consisted of a 4 × 2 factorial arrangement, with four biostimulant doses (0, 1, 2, and 3 L ha^{-1} of biostimulant) and two conventional fertilization levels (50 and 150 kg N ha^{-1} year^{-1}). Evaluations were conducted throughout the experimental time, being subdivided into two seasons (repeated measures), i.e., dry and rainy seasons. Collections carried out until the beginning of rain intensifications, which occurred in September 2016, characterized the dry season (Figure 1). From then on, collections were characterized as being from the rainy season.

The biostimulant was composed of 22% nitrogen, 8% sulfur, 0.1% molybdenum, 400 ppm auxin complex, and 210 ppm cytokinins. It was applied using a CO_2-pressurized knapsack sprayer with constant pressure, a 2.0-meter boom, and XR 110.02 spray tips. The equipment was set to distribute 200 L ha^{-1} of spray solution. An association of selective herbicides picloram (76 g L^{-1}) and 2,4-D (289 g L^{-1}) was used in the same spray solution to control dicots.

The grass was cut (17 cm high) on March 30, 2016, to standardize the area, with subsequent division of the experimental plots. A new grass cut also at 17 cm high was performed on April 27, 2016, characterizing the beginning of the experiment. Doses of biostimulant and nitrogen fertilization with urea (50 and 150 kg N ha^{-1} year^{-1}) were applied at 20 days after the grass cut (May 17, 2016) to obtain a higher leaf contact surface.

Morphogenic evaluations

The morphogenic evaluations consisted of identifying three tillers in each experimental unit (plot) in each cycle after the cutting process and evaluating them throughout the growth cycle. Each tiller was identified with a different colored ring for later monitoring. The evaluations were carried out by measuring the total length of mature (visible ligule) and expanding leaf blades (non-visible ligule) and pseudostem (stem + sheath) length.

The leaf blade length was measured according to the leaf development stage. Mature leaves were measured from the leaf tip to the ligule, while expanding leaves were measured from the leaf tip to the ligule of the youngest, fully expanded leaf (Duru & Ducrocq, 2000). The pseudostem length was determined from the last exposed ligula to the tiller base.

The following morphogenic variables were calculated from this information: leaf appearance rate (LAR, leaves tiller^{-1} day^{-1}), i.e., the average number of appearing leaves per tiller divided by the number of days in the evaluation interval; phyllochron (PHY, days leaf^{-1} tiller^{1}), i.e., the inverse of the leaf appearance rate; leaf elongation rate (LER, cm tiller^{-1} day^{-1}), i.e., the sum of all leaf blade elongation per tiller divided by the number of days in the evaluation period; stem increment (SI, cm); stem elongation rate (SER, cm tillers^{-1} day^{-1}), i.e., the sum of the stem elongation of each tiller divided by the number of days in the evaluation period; number of live leaves per tiller (NLL, leaves tiller^{-1}), i.e., the average number of expanding, expanded, and senescent leaves per tiller, excluding those with more than 50% of the leaf blade in the senescence process; number of leaves in expansion (NLE, leaves tiller^{-1}), i.e., the average number of leaves without visible ligule; number of mature leaves (NML, leaves tiller^{-1}), i.e., the average number of leaves with visible ligule; and leaf life-span (LLS, days), i.e., the interval between the leaf appearance until its total senescence, estimated by equation LLS = NLL × Phyl (Lemaire & Chapman, 1996).

Productivity and structural characteristics evaluation

Forage height measurements were performed every 5 days and collections were carried out when the experimental plots reached an average height of 40 cm. Therefore, the number of collections was dependent on the number of cutting cycles (simulating grazing). The field data collection lasted a total of 1 year.

The pasture dry matter was measured by cutting and weighing the experimental plot, but excluding the 0.50-m borders on each side. The total collected area was 3 m^2 at an average height of 17 cm for each experimental unit. The collections were performed using a rectangular metal frame with the same dimensions as the total collected. The samples were homogenized in the laboratory and an aliquot was taken to estimate the dry matter (DM) content (Detmann et al., 2012).

Aliquots were taken from the properly homogenized samples of each plot and subjected to the morphological separation of the leaf blade, stem (stem + leaf sheath), and dead material fractions. Subsequently, these samples were placed in an air-circulation oven at 55 °C for 72 hours.

Productivity and structural characteristics of the pasture were measured in the plots as follows: total forage dry matter production (TFDMP, kg ha^{-1} season^{-1}), based on the total produced per season in each experimental plot; percentage produced in the season relative to the total production (% produced); daily forage dry matter production (DFDMP, kg ha^{-1} day^{-1}), based on the total produced per day in each experimental plot; interval between cuts (IBC, days), i.e., the sum of days between two cuts; tiller population density (Tillers), obtained by the total sum of tillers per square meter of each plot; percentage of leaves (% DM) percentage of stems (% DM), and percentage of dead material (% DM), obtained by separating the total amount of the constituent concerning the forage canopy; and leaf to stem ratio (Leaf:Stem), obtained by the product of the proportion of leaves divided by the proportion of stems of the forage canopy in each experimental plot.

Chemical analysis

Samples from each cycle were taken at each collection, taken to an air-circulation oven at 55 °C for 72 hours, and then homogenized. Two composite samples per experimental plot, one corresponding to the dry season and the other to the rainy season, were prepared at the end of the experiment. Subsequently, the samples were analyzed for dry matter (DM), ash (AS), crude protein (CP), neutral detergent insoluble fiber (NDF), and ethereal extract (EE) following the methods recognized by INCT-

CA and described by Detmann et al. (2012). The content of non-fibrous carbohydrates (NFC) was calculated using the formula described by Detmann and Valadares (2010): NFC = OM − (CP + EE + NDF).

Statistical analysis

The parameters were grouped and divided into dry (data collected from June 2016 to September 2016) and rainy seasons (September 2016 to April 2017).

The data were analyzed using the procedure PROC MIXED of SAS (9.4), according to a randomized block design in a split-plot scheme with repeated measures, and following the model:

where is the overall constant, is the random block effect, is the fixed effect of the nitrogen level, is the fixed effect of biostimulant levels, is the fixed effect of taken repeated measure, is the fixed effect of interaction between the nitrogen and biostimulant levels, is the fixed effect of interaction between repeated measure and nitrogen level, is the fixed effect of interaction between biostimulant levels and repeated measure, is the effect of interaction between nitrogen and biostimulant levels and repeated measure, is the random error between blocks, is the random error between repeated measures within each experimental unit, and is the overall random error. The Kenward-Roger method was used to calculate the degrees of freedom of the residue. The structures of compound symmetry covariance, heterogeneous compound symmetry, and variance components were tested for repeated measures, with the best fit being selected according to the corrected Akaike criterion. The effects of significant interaction ($P<0.05$) were decomposed through the CONTRAST statement, using polynomial contrast of linear and quadratic effects for BC levels. The critical significance level adopted in all comparisons was 0.05.

Results and Discussion

The double interactions ($p>0.05$; biostimulant × nitrogen fertilization, biostimulant × season, and fertilization × season) presented no effect for the evaluated morphogenic variables (Table 1). A triple interaction (biostimulant × nitrogen fertilization × period; $p>0.05$) was observed for leaf appearance rate (LAR) and leaf life-span (LLS; $p<0.05$; Table 2). LAR showed a decreasing linear effect with an increase in the biostimulant dose only with the fertilization of 50 kg N ha^{-1} $year^{-1}$ in the dry season (Figure 2a). LLS showed an increasing linear effect ($p<0.05$) when increasing the biostimulant doses with fertilization of 50 kg N ha^{-1} $year^{-1}$ and a decreasing linear effect ($p>0.05$) with an increase of biostimulant doses with fertilization of 150 kg N ha^{-1} $year^{-1}$ only in the dry season (Figure 2b).

The increased leaf appearance rate (LAR) is positively associated with an increase in the number of tillers in the forage canopy, as a new bud with potential for development and formation of a new tiller is formed for each new leaf (Martuscello et al., 2019). The biostimulant provided higher total forage dry mass production (TFDMP) and daily forage dry mass production (DFDMP) even with a reduction in LAR in the dry period when applying the biostimulant at the dose of 50 kg N ha^{-1} $year^{-1}$. A decrease in LLS is associated with high growth rates, provided by higher tissue renewal (Luna et al., 2014), which may have occurred in response to the fertilization of 150 kg N ha^{-1} $year^{-1}$. However, the biostimulant application at the dose of 50 kg N ha^{-1} $year^{-1}$ possibly provided higher conservation of the nutrients already assimilated, causing them to have a longer leaf life-span.

Table 1
Morphogenic characteristics of Marandu grass submitted to biostimulant and different levels of nitrogen fertilization in the dry and rainy season

Item[(1)]	Nitrogen fertilization (kg N ha^{-1} year^{-1})		Biostimulant (L ha^{-1})				SEM[(2)]
	50	150	0	1	2	3	
Dry season							
LAR	0.039	0.041	0.044	0.039	0.039	0.039	0.006
PHY	29.14	27.54	28.15	28.47	27.05	29.68	3.68
LER	1.779	1.930	1.988	1.764	1.825	1.840	0.310
SI	7.481	7.272	7.114	6.297	8.468	7.626	1.858
SER	0.093	0.109	0.095	0.091	0.122	0.095	0.034
NLL	3.097	2.839	2.945	2.858	3.066	3.004	0.197
NLE	2.295	2.265	2.278	2.289	2.274	2.279	0.152
NML	1.862	1.929	1.882	1.733	2.127	1.840	0.094
LLS	87.753	88.551	85.518	90.917	88.138	88.034	17.56
Rainy season							
LAR	0.060	0.063	0.064	0.061	0.059	0.062	0.006
PHY	19.80	19.26	17.56	20.58	20.09	19.88	3.68
LER	3.347	3.599	3.619	3.329	3.603	3.341	0.310
SI	16.85	14.96	15.14	18.19	14.89	15.4	1.858
SER	0.488	0.503	0.471	0.541	0.506	0.464	0.034
NLL	1.469	1.419	1.483	1.402	1.474	1.418	0.197
NLE	2.139	2.187	2.09	2.144	2.242	2.177	0.152
NML	1.944	1.883	1.801	1.863	2.036	1.955	0.094
LLS	22.57	19.96	18.22	23.6	24.07	19.18	17.56
P - value[(3)]	BIO	Nitrogen.	BIO x Nitrogen	Period	BIO x Period	Nitrogen x Period	BIO x Nitro-gen x Period
LAR	0.36	0.36	0.88	<0.01	0.96	0.69	<0.01
PHY	0.80	0.52	0.85	<0.01	0.83	0.75	0.06
LER	0.51	0.15	0.85	<0.01	0.92	0.72	0.19
SI	0.95	0.41	0.41	<0.01	0.55	0.52	0.28
SER	0.78	0.64	0.61	<0.01	0.82	0.99	0.28
NLL	0.74	0.11	0.38	<0.01	0.92	0.28	0.85
NLE	0.86	0.89	0.54	0.07	0.85	0.54	0.36
NML	0.04	0.96	0.40	0.77	0.34	0.30	0.91
LLS	0.83	0.84	0.06	<0.01	0.97	0.68	0.03

(1) LAR: Leaf appearance rate, leaves tiller^{-1} day^{-1}; PHY: Phyllochron, days leaf^{-1} tiller^{-1}; LER: Leaf elongation rate, cm tiller^{-1} day^{-1}; SI: Stem increment, cm; SER: Stem elongation rate, cm tiller^{-1} day^{-1}; NLL: Number of live leaves per tiller, leaves tiller^{-1}; NLE: Number of leaves in expansion, leaves tiller^{-1}; NML: Number of mature leaves, leaves tiller^{-1}; LLS: leaf life-span, Number of live leaves tiller^{-1} × Phyllochron.
(2) SEM: Standard error mean.
(3) BIO: Bioestimulant effect; Nitrogen: nitrogen fertilization effect; Period: experimental period effect and the interactions.

Table 2
Productivity and structural characteristics of Marandu grass submitted to biostimulant and different levels of nitrogen fertilization in the dry and rainy season

Item[(1)]	Nitrogen fertilization (kg N ha^{-1} year^{-1})		Biostimulant (L ha^{-1})				SEM[(2)]
	50	150	0	1	2	3	
Dry season							
TFDMP	1638	1965	1522	1906	2011	1768	1200
%Produced	11.38	12.45	10.03	12.10	13.60	11.93	3.77
CI	104.07	107.86	98.87	105.9	119.6	99.5	9.40
DFDMP	16.12	18.3	15.45	18.67	16.73	17.98	5.48
Tillers	168.5	164.33	164.38	186.57	165.5	176.83	28.78
%leaf	82.96	76.98	85.91	83.71	73.2	77.07	6.62
%stem	17.37	21.59	14.12	16.46	25.20	22.13	6.51
%Senescence	0.69	0.86	1.13	0.64	0.56	0.76	0.90
Leaf:Stem	5.38	4.11	6.02	5.86	2.99	4.12	1.23
Rainy season							
TFDMP	13058	13729	12873	14578	12947	13177	1200
%Produced	88.62	87.55	89.97	87.90	86.40	88.07	3.77
CI	35.66	40.08	35.75	36.01	41.63	38.10	9.40
DFDMP	51.89	51.12	50.10	57.47	47.85	50.61	5.48
Tillers	230.71	267.92	247.33	231.17	261.83	256.92	28.78
%leaf	70.04	74.44	76.23	67.38	71.48	73.87	6.62
%stem	22.44	23.94	23.13	19.06	26.19	24.39	6.51
%Senescence	3.58	1.04	1.81	4.43	1.30	1.69	0.90
Leaf:Stem	4.15	3.76	5.28	4.04	2.94	3.57	1.23
P - value[(3)]	BIO	Nitrogen	BIO x Nitrogen	Period	BIO x Period	Nitrogen x Period	BIO x Nitrogen x Period
TFDMP	0.45	0.27	0.03	<0.01	0.51	0.70	0.16
%Produced	0.28	0.52	0.70	<0.01	0.21	0.39	0.48
CI	0.07	0.28	0.14	<0.01	0.33	0.93	0.87
DFDMP	0.25	0.77	0.02	<0.01	0.56	0.50	0.60
Tillers	0.82	0.25	0.79	<0.01	0.42	0.01	0.17
%leaf	0.12	0.77	0.42	0.01	0.27	0.05	0.74
%stem	0.07	0.24	0.57	0.02	0.15	0.36	0.16
%Senescence	0.63	0.12	0.31	0.04	0.68	0.07	0.35
Leaf:Stem	0.03	0.13	0.28	0.15	0.56	0.41	0.90

[1)] TFDMP: total forage dry mass production (kg ha^{-1} period^{-1}); %Produced: Percentage produced in the dry or rainy season; CI: cutting interval, days; DFDMP: Daily forage dry mass production, kg ha^{-1} day^{-1}; Tillers: tiller density, tillers by m²; %leaf: percentage of leaves, %Stem: percentage of stems, % Senescence: percentage of senescence; Leaf:Stem: Leaf: stem ratio.
[(2)] SEM: Standard error mean.
[(3)] BIO: Bioestimulant effect; Nitrogen: nitrogen fertilization effect; Period: experimental period effect and the interactions.

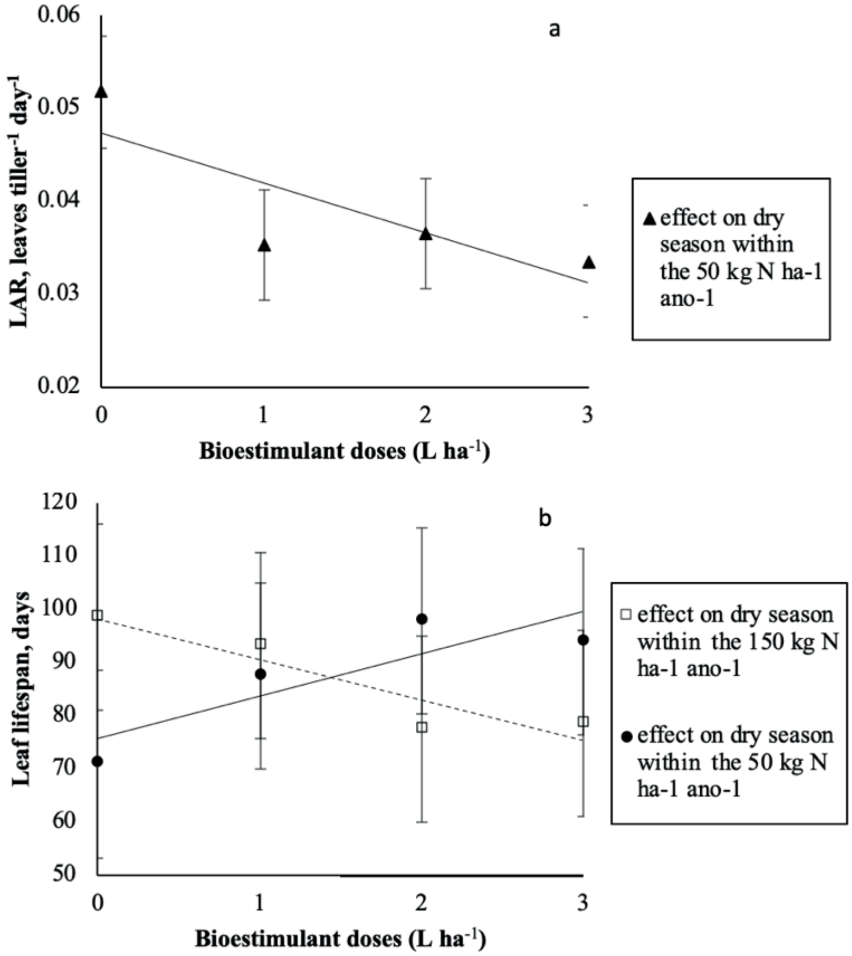

Figure 2. Effect of the split of the interaction between bioestimulante, nitrogen fertilization and period on the Leaf appearance rate (LAR) and leaf lifespan of Marandu grass.

No triple interaction (biostimulant × nitrogen fertilization × period; $p>0.05$) was observed for the productivity and structural characteristics of Marandu grass (Table 2). An interaction was observed between nitrogen fertilization and season ($p<0.05$) only for the variable tiller population density (Figure 3). The number of tillers differed only in the rainy season ($p<0.05$), in which the treatment that received the highest nitrogen fertilization showed the highest population density of tillers. The double interactions between biostimulant × period showed no effect ($p>0.05$) for the studied variables.

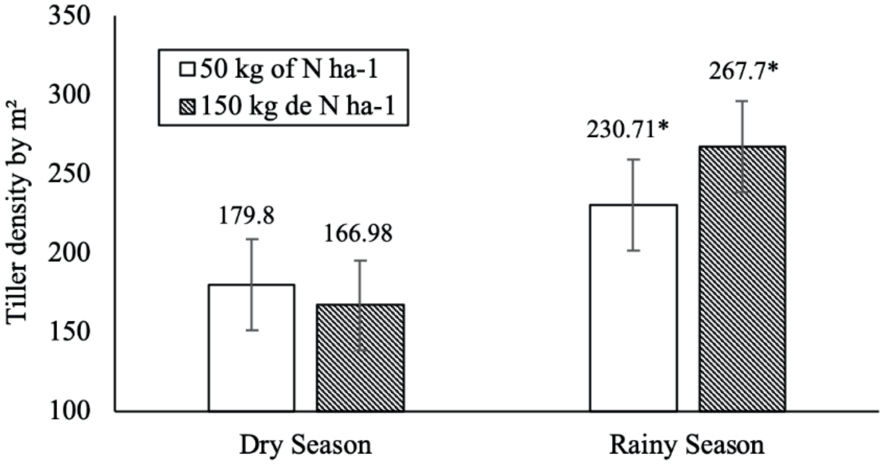

Figure 3. Effect of the split of the interaction between period and nitrogen fertilization on the tiller density of Marandu grass, under different dosages of nitrogen fertilization, evaluated in the dry and rainy season (averages followed by an asterisk (*) are different from each other).

The increased population density of tillers when receiving nitrogen fertilization of 150 kg N ha^{-1} $year^{-1}$ may be associated with the N stimulus in the growth and multiplication of plant cells, as this nutrient is required in large quantities by C4 forages, which express their maximum tillering under favorable growth conditions (water, light, temperature, and nutrients) (Martuscello et al., 2019), as already demonstrated in other studies with the species *U. brizantha* (Paiva et al., 2011; Carvalho et al., 2019).

The interaction between biostimulant and nitrogen fertilization (Table 2) showed an effect ($p<0.05$) for TFDMP and DFDMP (Figures 4 and 5). TFDMP showed a quadratic effect ($p>0.05$) for biostimulant doses only when fertilizing with 50 kg N ha^{-1} $year^{-1}$, with a higher TFDMP when using the biostimulant at doses of 1 and 2 L ha^{-1} (Figure 4). The fertilization with 150 kg N ha^{-1} $year^{-1}$ showed no effect of the biostimulant ($p<0.05$).

The variable DFDMP showed a quadratic effect ($p>0.05$) for biostimulant doses in the fertilizations with 50 and 150 kg N ha^{-1} $year^{-1}$, but with different trends (Figure 5). The fertilization with 50 kg N ha^{-1} $year^{-1}$ led to an increase in DFDMP with biostimulant doses between 1 and 2 L ha^{-1}. On the other hand, the fertilization with 150 kg N ha^{-1} $year^{-1}$ showed a reduction in DFDMP at the same doses.

The variable TFDMP increased by 30.1 and 25.3% and DFDMP increased by 33.7 and 27.6% when using biostimulant doses of 1 and 2 L ha^{-1}, respectively, at lower nitrogen fertilization levels (50 kg N ha^{-1} $year^{-1}$), but DFDMP did not increase with the fertilization of 150 kg N ha^{-1} $year^{-1}$. Higher auxin to cytokinin ratios induces root differentiation (Mercier, 2013). As observed in this study, the biostimulant composition contained 400 ppm of auxin and 210 ppm of cytokinin. In this condition, a probable stimulation of the biostimulants may have provided an increase in root development with the nitrogen fertilization of 50 kg N ha^{-1} $year^{-1}$, mainly at biostimulant doses of 1 to 2 L ha^{-1}, allowing the forage grass to have higher contact area with the soil to capture nutrients and develop.

However, when the fertilization with 150 kg N ha^{-1} $year^{-1}$ is carried out, the biostimulant application provided no positive effects on the plant growth rate possibly related to an N saturation, with no response to the biostimulant. Considering the significant influence on the biomass flow (Nave, Pedreira, & Pedreira, 2010) provided by the use of higher doses of nitrogen fertilizers (Carvalho et al., 2019), the highest dose of nitrogen fertilization possibly provided higher pasture growth to the point of not allowing the biostimulant doses used to be sufficient to express higher productivity, as the demand for nitrogen has already been met by the nitrogen fertilization.

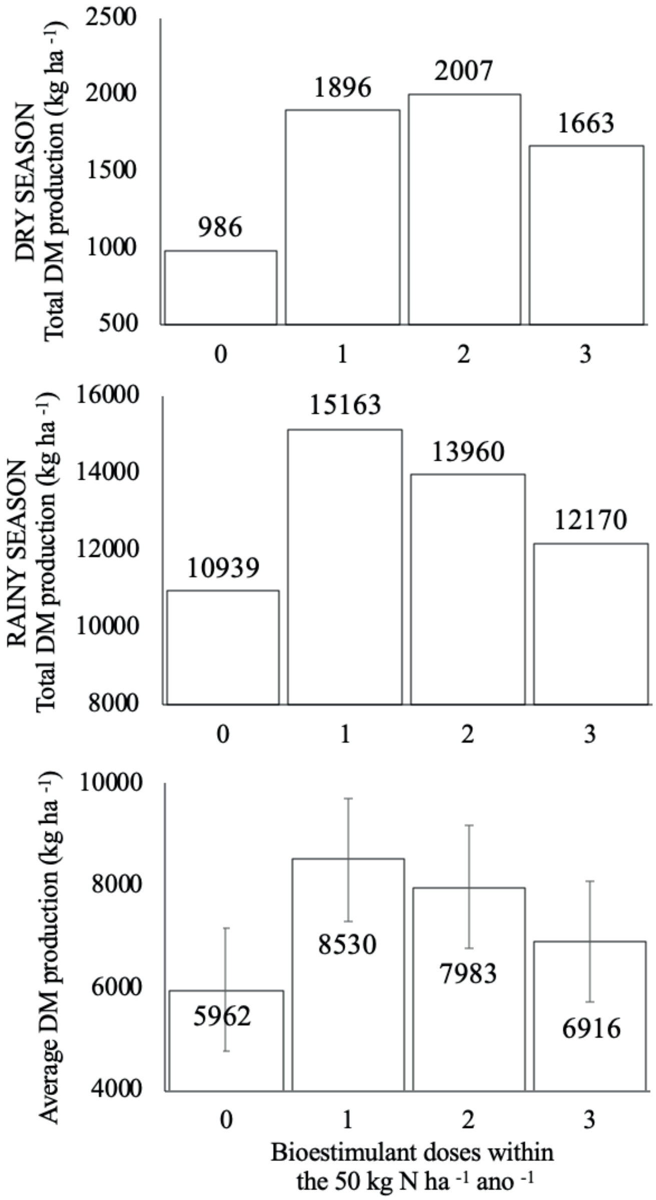

Figure 4. Effect of the split of the interaction between Biostimulant and Nitrogen fertilization on the total forage dry mass production (TFDMP, kg ha^{-1} $period^{-}$) of Marandu grass, under different dosages of nitrogen fertilization and biostimulant, evaluated in the dry and rainy season (quadratic effect in the levels of Biostimulant within the fertilization of 50 kg of N ha^{-1} $year^{-1}$).

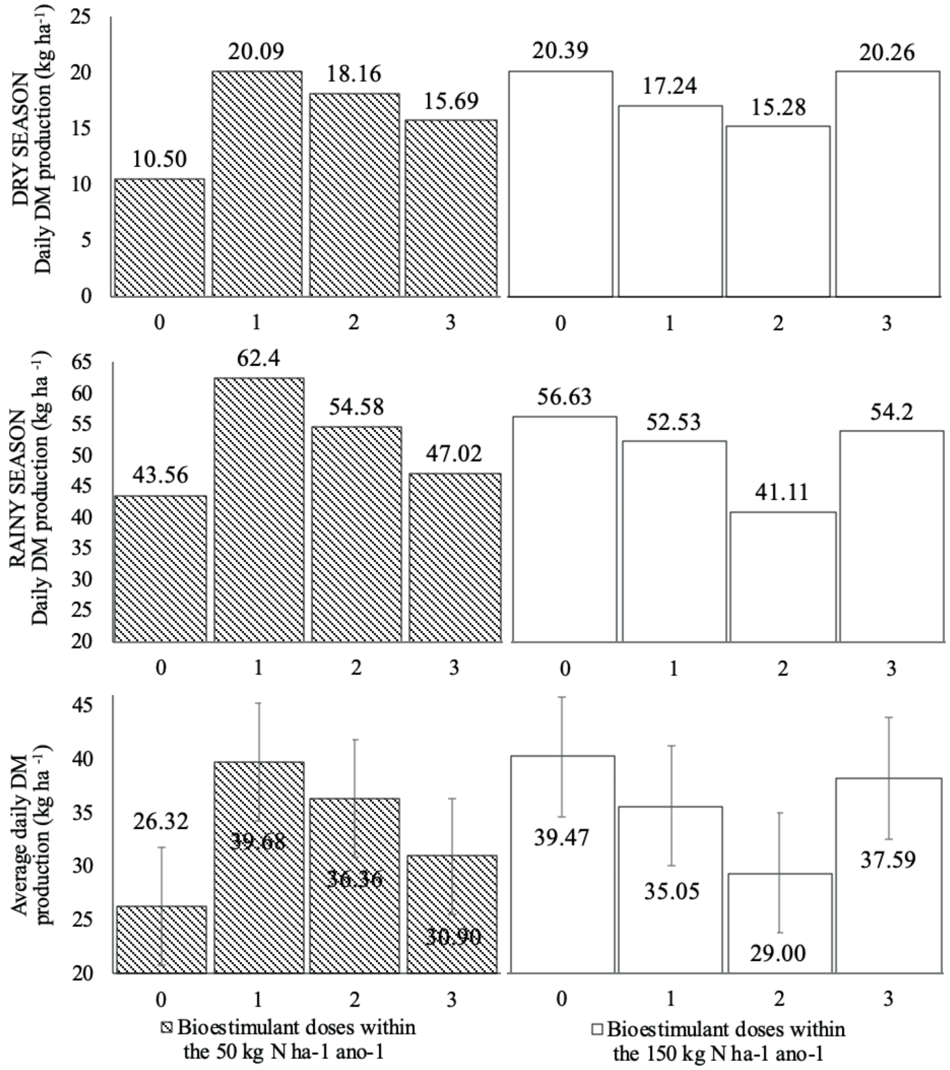

Figure 5. Effect of the split of the interaction between Biostimulant and Nitrogen fertilization on daily forage dry mass production (DFDMP, kg ha^{-1} day^{-1}) of Marandu grass, under different dosages of nitrogen fertilization and biostimulant, evaluated in the dry and rainy season (quadratic effect in the levels of Biostimulant within the 50 and 150 kg of N ha^{-1} $year^{-1}$).

Although there were no changes in morphogenic characteristics (Table 1) that indicated an increase in productivity, the increased TFDMP and DFDMP with a low nitrogen fertilization dose seems to have been caused by an increase in cell size, as the presence of auxin in the biostimulant leads to an increase in the efflux of H^+ ions, with a consequent decrease in the apoplast pH (Mercier, 2013). It initially activates the expansins, which work by breaking the hydrogen bonds of the cross-links between the cellulose microfibrils and hemicelluloses, triggering the activation of other enzymes (hydrolases, pectinases, cellulases, and hemicellulases). These enzymes act on the cell wall components, causing it to loosen and increasing its extensibility (Mercier, 2013), thus allowing an increase in cell size. This mechanism possibly increased the deposition of leaf and stem dry matter, providing an increase in

TFDMP and DFDMP, even without changing the morphogenic characteristics of the forage canopy, except for those already mentioned.

Similar results were observed with the application of a biostimulant that contained an auxinic complex (400 ppm), with an increase of 5.75% in the accumulated DM production and 6.71% in the production of M ha^{-1} day^{-1} in Marandu grass with the application of 2 kg ha^{-1} (Oliveira et al., 2019).

No interaction effect (p>0.05) was observed on the chemical-bromatological characteristics of Marandu grass (Table 3). The independent evaluation of factors showed a difference only for crude protein (CP) content between nitrogen fertilization levels (p>0.05), with a higher CP content for the fertilization level of 150 kg N ha^{-1} $year^{-1}$. It demonstrates how nitrogen fertilization favors an increase in N concentration in the plant and, consequently, CP content, leading to a better forage quality (M. E. R. Santos, Fonseca, Balbino, Silva, & Monnerat, 2010).

Table 3
Chemical characteristics of Marandu grass submitted to biostimulant and different levels of nitrogen fertilization in the dry and rainy season

Item[(1)]	Nitrogen fertilization (kg N ha^{-1} $year^{-1}$)		Biostimulant (L ha^{-1})				SEM[(2)]
	50	150	0	1	2	3	
Dry season							
DM	23.15	24.17	23.91	23.91	22.58	25.52	4.88
CP	8.84	10.49	8.93	8.93	9.75	9.27	0.78
NDF	62.01	61.25	63.03	61.04	60.82	61.63	1.99
EE	2.09	2.07	2.03	2.03	2.15	2.08	0.27
Ash	6.37	6.51	6.58	6.58	6.24	6.52	0.41
NFC	20.78	19.77	19.66	19.89	20.76	20.70	1.90
Rainy season							
DM	21.84	23.52	24.00	24.01	20.62	23.33	4.88
CP	9.77	11.19	9.38	10.36	10.2	11.08	0.78
NDF	62.91	61.09	62.17	62.9	61.36	61.36	1.99
EE	1.91	2.07	2.25	2.25	1.96	1.93	0.27
Ash	8.88	8.69	8.74	8.75	8.49	9.06	0.41
NFC	17.84	17.94	17.91	16.29	18.54	18.88	1.90
P - value [(3)]	BIO	Nitrogen.	BIO x Nitrogen.	Period	BIO x Period	Nitrogen x Period	BIO x Nitro-gen x Period
DM	0.39	0.33	0.12	0.13	0.39	0.60	0.08
CP	0.85	0.01	0.64	<0.01	0.14	0.30	0.42
NDF	0.42	0.08	0.26	0.62	0.52	0.46	0.33
EE	0.61	0.50	0.62	0.35	0.50	0.40	0.63
Ash	0.31	0.99	0.05	<0.01	0.65	0.42	0.61
NFC	0.30	0.53	0.90	<0.01	0.76	0.47	0.60

(1) DM: Dry matter, % of natural matter; CP: crude protein, % of DM; NDF: Neutral detergent fiber, % of DM; EE: Ether extract, % of DM; Ash, % of DM; NFC: Non-fibrous carbohydrate, % of DM.
(2) SEM: Standard error mean.
(3) BIO: Bioestimulant effect; Nitrogen: nitrogen fertilization effect; Period: experimental period effect and the interactions.

A reduction ($p<0.05$) was observed in the leaf to stem ratio as a function of the different biostimulant doses (Table 2), regardless of the nitrogen fertilization level. The decrease in the L:S ratio may be related to the increasing biostimulant doses, which provided a higher dry matter accumulation in the stems compared to the leaves and, consequently, a decrease was found in this relationship. Lima et al. (2019) reported a 5.91% reduction in the L:S ratio when applying 0.75 L ha^{-1} of biostimulant on forage of the genus *Urochloa*.

However, the L:S ratio has great importance for animal nutrition and management of forage grasses, as the higher proportion of leaves or stems in the canopy composition alters the nutritional value of the consumed forage (Januskiewicz, Raposo, Morgado, Reis, & Ruggieri, 2016). Although the biostimulant application caused a decrease in the L:S ratio, the PB and NDF contents showed no difference with the different biostimulant doses (Table 3).

A difference ($p<0.05$) was observed between the dry and rainy seasons for most of the evaluated parameters (Tables 1, 2, and 3). Therefore, it demonstrates how the formation, development, growth, and senescence processes of tillers are influenced by climate conditions, such as temperature and water availability (Carvalho et al., 2018; Rocha et al., 2019).

Conclusion

Biostimulant doses of 1 and 2 L ha^{-1} increase the dry matter production per hectare in the nitrogen fertilization of 50 kg N ha^{-1} $year^{-1}$, but its action is not effective with the highest nitrogen fertilization (150 kg N ha^{-1} $year^{-1}$). The use of biostimulant does not change the nutritional value and morphogenesis of the evaluated nitrogen fertilization levels.

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