

Humic and fulvic acid derived from slaughterhouse waste compost as biostimulants in Marandu grass¹

Ácidos húmicos e fúlvicos derivados de composto de resíduo de frigorífico como bioestimulantes para o capim Marandu

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HIGHLIGHTS:

Humic substances derived from slaughterhouse waste compost have biostimulant effects on Marandu grass.

Humic acids from slaughterhouse waste compost exhibit greater biostimulation than their fulvic counterparts.

Both sources modified the physiology of Marandu grass, increasing forage yield, particularly at 1 L ha⁻¹.

ABSTRACT: Humic and fulvic acids promote the growth and development of different cash crops through biostimulation. However, when extracted from alternative sources, their effects on forage crops are unknown. Thus, the present study aimed to assess the effect of foliar spraying humic and fulvic acids derived from composted slaughterhouse waste compost on the morphophysiology and forage yield of *Urochloa brizantha* cv Marandu grass. The experiment used a randomized block design in a (2 × 2) + 1 factorial scheme, with five replicates. The first factor was humic and fulvic acids, and the second two doses (1 and 2 L ha⁻¹), in addition to a control treatment. Humic acids increased shoot fresh mass and forage yield by 10 and 20%, respectively, in relation to the control. Fulvic acids obtained lower results for these variables than their humic counterparts in these variables, differing from the control only for forage yield. Similar responses were observed for the CO₂ assimilation rate, transpiration, chlorophyll index, and photosynthetic forage surface. Both the univariate and multivariate statistical approaches confirmed that foliar spraying at 1 L ha⁻¹ produced superior results in Marandu grass than those obtained at 2 L ha⁻¹, regardless of the source. Humic and fulvic acids derived from slaughterhouse waste compost have biostimulant effects on Marandu grass. Humic acids exhibit greater biostimulation than fulvic acids in morphophysiological and forage yield, except for leaf area and specific leaf area.

Key words: *Urochloa brizantha*, humic substance, intensification pastures, bioinput

RESUMO: Ácidos húmicos e fúlvicos bioestimulam o crescimento e desenvolvimento de diversas culturas comerciais. No entanto, não se sabe os seus efeitos quando extraídos de fontes alternativas para culturas forrageiras. Desta forma, o objetivo foi avaliar os efeitos da aplicação foliar dos ácidos húmicos e fúlvicos derivados de resíduo de frigorífico compostados na morfofisiologia e rendimento de forragem do capim *Urochloa brizantha* cv. Marandu. O experimento foi conduzido em esquema fatorial (2 × 2) + 1, dispostos em blocos casualizados, com cinco repetições. O primeiro fator foram os ácidos húmicos e fúlvicos, e o segundo composto por duas doses (1 e 2 L ha⁻¹), além de um tratamento controle (com apenas aplicação foliar de água destilada). Os ácidos húmicos aumentaram a matéria fresca em 10 e 20% no rendimento de forragem comparado ao controle. Os ácidos fúlvicos foram inferiores aos húmicos, nestas variáveis, com diferença com o controle apenas no rendimento de forragem. Respostas semelhantes ocorreram na taxa de assimilação de CO₂, transpiração, índice de clorofila e na superfície fotossintética da forrageira. Tanto a abordagem estatística uni e multivariada, confirmaram que a aplicação foliar de 1 L ha⁻¹ foi superior a 2 L ha⁻¹, independente das fontes no capim Marandu. Os ácidos húmicos e fúlvicos derivados de composto de resíduo de frigorífico possuem efeitos bioestimulantes para o capim Marandu. Ácidos húmicos apresentaram maior bioestimulação comparado aos ácidos fúlvicos na morfofisiologia e rendimento de forragem, exceto para a área foliar e área foliar específica.

Palavras-chave: *Urochloa brizantha*, substâncias húmicas, intensificação de pastagens, bioinsumos

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INTRODUCTION

Livestock farming is important to the Brazilian economy, with 51 million cattle slaughtered in 2023, generating over US\$ 179.2 billion per year (8.2% of gross domestic product) (USDA, 2024; ABIEC, 2024). This results in approximately 1.1 billion metric tons of ruminal waste in slaughterhouses in the country. When poorly managed, waste becomes a potential source of environmental liabilities (Tullo et al., 2019), making it impossible to sustain the production chain (Phillips, 2010). The bioconversion of these residues is a recycling alternative, since the composting process decomposes, converts, and stabilizes the organic matter in waste (Ceci et al., 2019; Freitas et al., 2020).

After composting, humic substances (HS), which form part of humified organic matter, can be extracted and divided into three organic fractions: humin, and humic (HA) and fulvic acids (FA) (Mendonça & Matos, 2005). In plants, several studies have reported that HA and FA exhibit bioactivity and affect structural characteristics (García et al., 2019; Savarese et al., 2022) according to the source material and processing time (Jindo et al., 2020; Baltazar et al., 2021; Nardi et al., 2021).

Studies show that these substances affect crop metabolism (Du Jardin, 2015) and physiology (García et al., 2019) and cause molecular-level changes (Shah et al., 2018; Nunes et al., 2019) that influence vegetative growth (Amorim et al., 2015) by inducing resistance to or recovery from abiotic and/or biotic stresses and facilitating nutrient assimilation, translocation and use (Rouphael & Colla, 2018). This bioactivity, induced by plant biostimulants (PBs), can improve forage yield (Pinheiro et al., 2018; Neves et al., 2019; Capstaff et al., 2020).

Urochloa is an important genus of forage species, with *U. brizantha* cv. Marandu the most used widely used in the Cerrado biome due to its rusticity, high yield potential, adaptation to soils with low to medium fertility, and responsiveness to fertilization (Gonçalves et al., 2018). Research on biostimulation in tropical forage grasses remains limited (Capstaff et al., 2020), particularly regarding the underlying physiological mechanisms of plant responses. Moreover, studies exploring alternative sources for humic and fulvic acid extraction, such as organic waste-derived composts, are scarce. The aim of this study was to assess the effect of foliar spraying of humic and fulvic acids from composted slaughterhouse waste compost on the morphophysiology and forage yield of *Urochloa brizantha* cv Marandu.

MATERIAL AND METHODS

The experiment was conducted in a plastic-covered greenhouse with side shading and no climate control, between

January and March 2019 at the Gurupi Campus of Universidade Federal do Tocantins (UFT), in Tocantins state, Brazil (11° 44' 44.16" S and 49° 03' 04.17" W, at 280 m.a.s.l.). The regional climate is humid with moderate water deficiency, classified as type B1wA'a' according to Koppen's classification, with average annual rainfall of 1600 mm, concentrated from November to May, and an average annual temperature of 27 °C (Alvares et al., 2013).

The experiment used a randomized block design in a (2 × 2) + 1 factorial scheme, with five replicates. The first factor was humic (HA) and fulvic acids (FA), and the second two doses (1 and 2 L ha⁻¹), in addition to a control treatment (only foliar spraying of distilled water). HA and FA were diluted in distilled water and applied via foliar spraying (spray volume of 100 L ha⁻¹) using handheld sprayers, four days after each cut (grazing simulation). HA and FA were fractionated according to Mendonça & Matos (2005), with KOH (0.1 mol L⁻¹) as the extractor. The HA and FA used contained 16 and 5 g L⁻¹ of organic carbon, respectively. The extraction material was generated after composting (120 days) bovine ruminal residue obtained from the wastewater stream (green line) of the municipal slaughterhouse. Further details are available in Freitas et al. (2020).

Urochloa brizantha cv. Marandu grass was grown in pots containing 13 dm³ of Latossolo Amarelo (Santos et al., 2018) or Oxisol (United States, 2022) (soil class representative of the region), collected (0-20 cm) in a degraded pasture area. Soil chemical and physical attributes were determined according to Teixeira et al. (2017) (Table 1). Acidity was corrected using 1.5 t ha⁻¹ of limestone filler (PRNT = 97%) and 0.5 t ha⁻¹ of gypsum, 30 days before experiment onset. In line with Ribeiro et al. (1999), mineral fertilization was performed at sowing for a moderate pasture management level, with 50 kg ha⁻¹ of FTE BR12 and 22 kg ha⁻¹ of P via MAP (20% P and 9% N).

The seeds were sown at a depth of 4 cm, and the soil was maintained at 80% field capacity. Thinning was performed 30 days after emergence (DAE), leaving only 4 plants pot⁻¹, which were cut at a height of 20 cm using pruning shears. The treatments were always applied after four days of growth in developing leaves. The shoots were cut three times in 25-day cycles to simulate grazing, with topdressing performed three days after each cut, using 50 and 18 kg ha⁻¹ of N (Urea) and K (KCl), respectively.

The following traits were determined after each cut: plant height (PH), measured with a ruler (cm) from the ground to the tip of the last leaf; and number of tillers (n) by direct counting. Shoot fresh mass (SFM, g) was measured before the leaves lost turgidity, and the leaves and stems were then separated. Next, the samples were dried in a forced-air oven at 55 °C for 72 hours or until constant weight and then weighed on a scale to

Table 1. Chemical and textural attributes of the soil used in the experiment

Ca ²⁺	Mg ²⁺	Al ³⁺	H + Al	CEC	ECEC	SB	
(cmol _c dm ⁻³)						(%)	
0.6	0.4	0.0	2.5	3.55	1.05	30	
P	K	S	OM	pH	Texture (g kg ⁻¹)		
(mg dm ⁻³)				CaCl ₂	Sand	Silt	Clay
0.7	18	5.0	1.3	4.9	475	50	475

CEC - Cation exchange capability; ECEC - Effective cation exchange capability; SB - Base saturation; OM - Organic matter

determine leaf (LDM, g) and tiller dry mass (TDM, g) (Morais et al., 2018, Pinheiro et al., 2018).

Forage yield consisted of the total LDM and TDM for all three 25-day cycles, and the leaf-to-tiller ratio (TLR, g g⁻¹) was calculated based on the mean value of all three cycles. Leaf area (LA, cm²) was determined using the leak disk method, according to Gomes et al. (2011), and specific leaf area (SLA, cm² g⁻¹) based on the ratio between LA and LDM.

Twenty days after biostimulant application, photosynthetic pigments were estimated in fully expanded leaves using a FCI 1030 chlorophyll meter[®], with results expressed as Falker chlorophyll index (FCI) (FALKER, 2022). CO₂ assimilation (A - $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and transpiration rate (E - $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were determined using an open photosynthesis system equipped with a CO₂ analyzer, and water vapor via infrared radiation (LCiSD Infra-Red Gas Analyzer - IRGA, ADC System, UK). These analyses were performed on sunny days between 9 and 11 a.m., with irradiance $\sim 1100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and external CO₂ concentration of $\sim 400 \mu\text{mol mol}^{-1}$.

Normality (Shapiro-Wilks) and homoscedasticity of variance (Bartlett) were assessed and variables failing to meet these assumptions were subsequently transformed. The results were submitted to analysis of variance ($p \leq 0.05$), with post-hoc

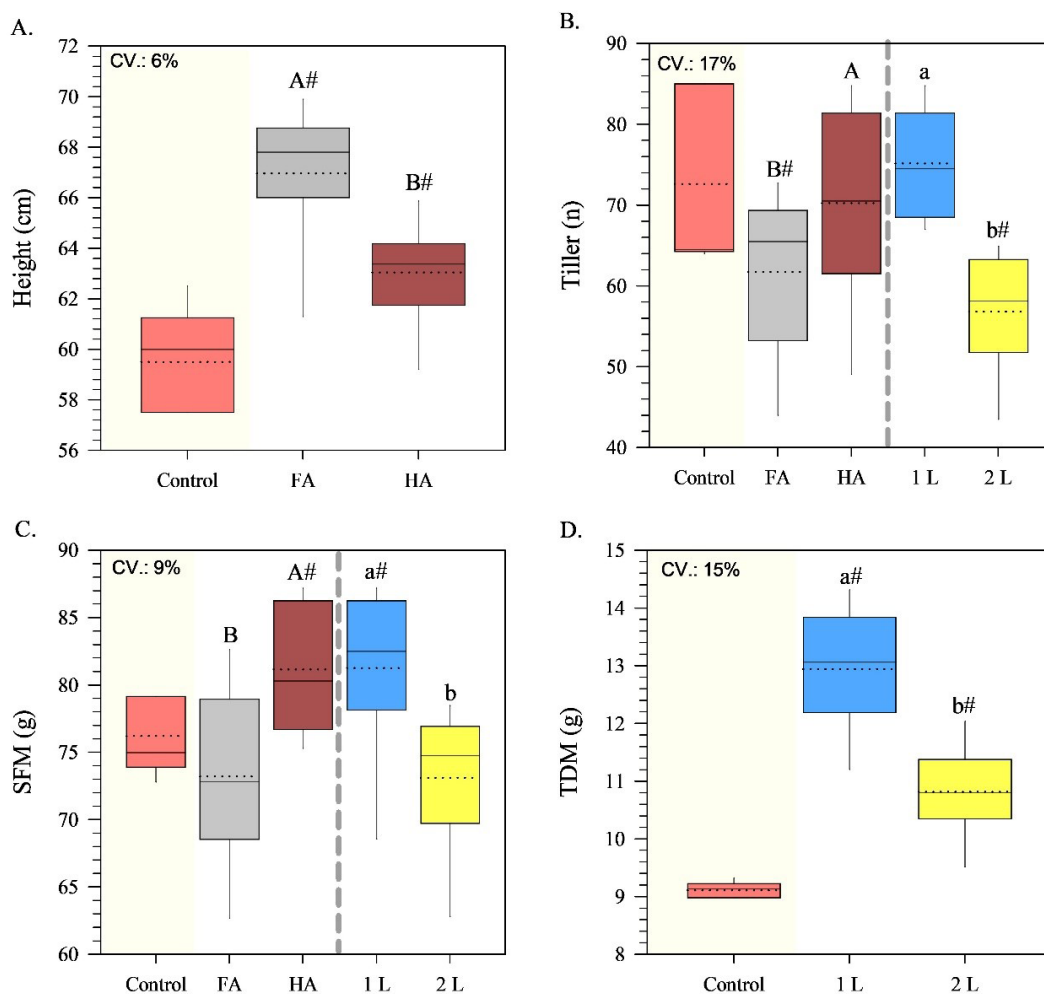
comparisons by the LSD test ($p \leq 0.05$). The treatments and control were compared by Dunnett's test ($p \leq 0.05$).

Principal component analysis (PCA) was used to reduce the dimensionality of the agronomic and morphophysiological traits of Marandu grass. Hierarchical cluster analysis was performed, using a heatmap to compare treatment similarity according to the expression of the variables. Statistical analyses and graphs were produced using R[®] software (CRAN, 2024) and the Tratamentos.ad, FactoMineR, and pheatmap packages.

RESULTS AND DISCUSSION

Humic and fulvic acids derived from the decomposition of ruminal residue promoted significant changes ($p \leq 0.05$) in the morphophysiology and yield of *Urochloa brizantha* cv. Marandu, demonstrating the effects of biostimulation on forage grass grown under the soil and climate conditions of the Brazilian Cerrado. Interaction was observed between LA, SLA, and CO₂ assimilation rate, while the other traits studied showed only simple effects.

Plant height exhibited only a source effect ($p = 0.00054$) (Figure 1A), with FA resulting in plants 6% taller than those



Different letters, uppercase for source effect and lowercase for doses, differ according to the LSD test ($p \leq 0.05$). Means with # indicate differences from the control treatment according to Dunnett's test ($p \leq 0.05$). For clarity and esthetic purposes, letters are only displayed where significant differences were observed. FA - Fulvic acids; HA - Humic acids. 1 L and 2 L: 1 and 2 L ha⁻¹ application; SFM - Shoot fresh mass; TDM - Tiller dry mass. The vertical dashed bar indicates the results of the simple effects of the factors.

Figure 1. Plant height (A), tiller (B), shoot fresh mass - SFM (C), and tiller dry mass - TDM (D) of Marandu grass with foliar humic and fulvic acid application

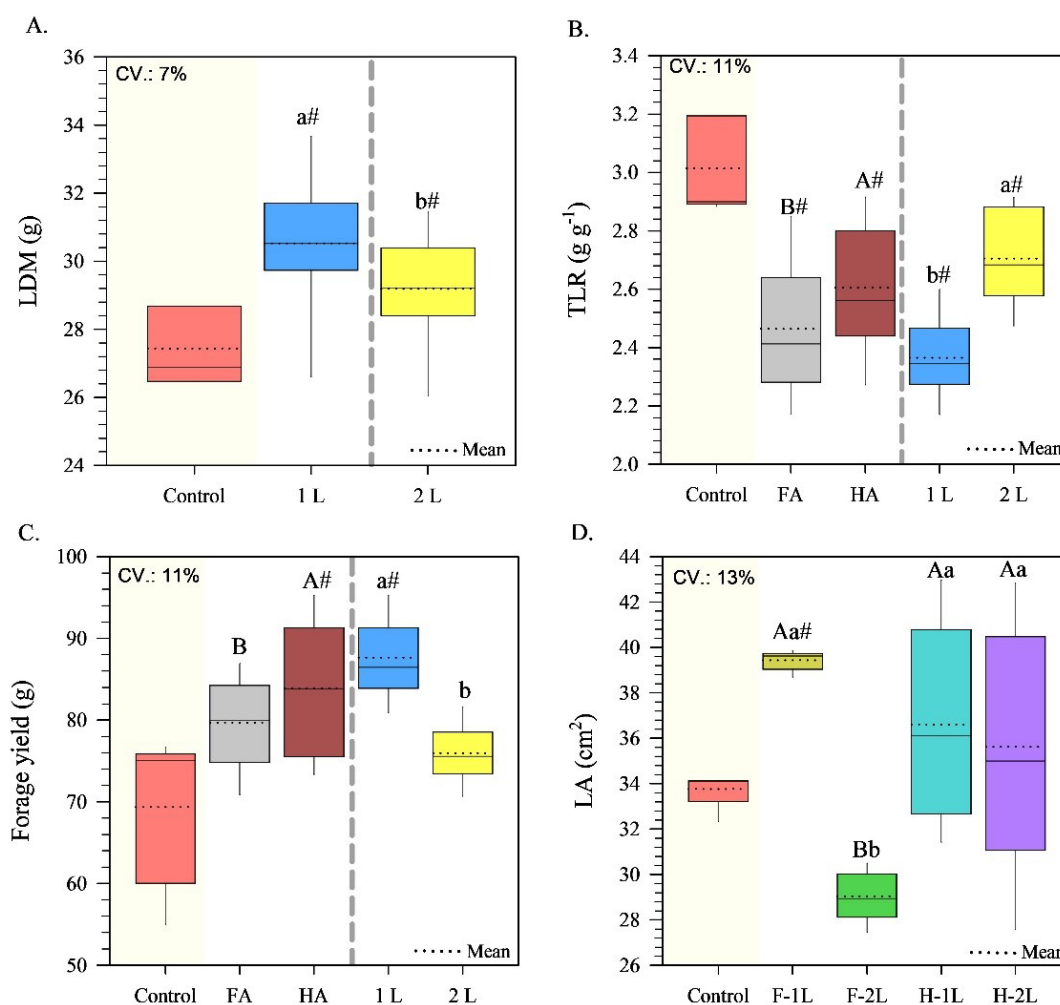
treated with HA (66.9 vs. 63.4 cm), and both differing from the control (59.6 cm, $p = 0.00006$). However, differences between the sources and control are small, with 7.5 and 3.5 cm for FA and HA, respectively. In intensive pasture management systems based on plant height (95% light interception), higher FA and HA doses may represent forage yield gains. In these systems with Marandu grass, animals enter the pasture at a canopy height of 35 cm and are removed at 15 cm, enabling optimal forage quality and grazing cycle gains (Morais et al., 2018).

Tillering exhibited only a simple effect for the factors (Figure 1B), with HA (70.2 un) 14% higher ($p = 0.018$) than FA (61.7 un), and 1 L ha⁻¹ (75.2 un) 24% superior ($p = 0.0005$) to 2 L ha⁻¹ (56.8 un). These increases were significant for simple effects but did not differ from the control. Compared to the control treatment, FA and a dose of 2 L ha⁻¹ (regardless of source) resulted in reductions of 10.8 and 15.8 un plant⁻¹, respectively. Similar results were obtained for SFM, except for control performance (Figure 1C). HA and 1 L ha⁻¹ exhibited similar mean values (~81.2 g), the highest SFM ($p = 0.000006$), and a significant increase ($p = 0.001$) of 5 g compared to the control. FA (73.2 ± 6.3 g) and 2 L ha⁻¹ (73.1 ± 5.1 g) did not

differ from the control, with an average reduction of ~11% compared to HA and 1 L ha⁻¹.

There was no difference between sources for TDM and LDM ($p = 0.17$ and 0.42), with effects only observed for doses, whereby 1 L ha⁻¹ increased ($p = 0.00001$) TDM by 20% compared to 2 L ha⁻¹ (13.1 vs. 10.8 g) and by 29% in relation to the control ($p = 0.00001$) (Figure 1D). The same response was observed for LDM, albeit at a lower magnitude (Figure 2A), with 5% higher values at 1 L ha⁻¹ ($p = 0.044$) when compared to 2 L ha⁻¹, and 10% greater ($p = 0.002$) than the control.

For the tiller-to-leaf ratio (indirect indication of the quality of the forage yield), the control exhibited the best result, with 3.01 g of leaves for every 1 g of tiller (Figure 2B). However, the fixed number of days between forage cuts may have compromised the cumulative increase in leaf production in biostimulant-treated plants, whereas Marandu grass may have quickly reached 95% of light interception before completing the 25-day cycle (Pinheiro et al., 2018, Neves et al., 2019). This is corroborated by the significant differences in plant height (Figure 1A) and SFM (Figure 1C). As such, cumulative forage yield for all three cycles was influenced only by the simple effects of sources ($p = 0.043$) and doses ($p = 0.0001$) (Figure



Different letters, uppercase for source effect and lowercase for doses, differ according to the LSD test ($p \leq 0.05$). Means with # indicate differences from the control treatment according to Dunnett's test ($p \leq 0.05$). For clarity and esthetic purposes, letters are only displayed where significant differences were observed. FA - Fulvic acid; HA - Humic acid. 1 L and 2 L: application of 1 and 2 L ha⁻¹; F-1L, F-2L, H-1L, H-2L: interaction between sources and doses, TDM - Tiller dry mass; LDM - Leaf dry mass; TLR - Tiller-to-leaf ratio. The vertical dashed bar indicates the results of the simple effects of the factors.

Figure 2. Leaf dry mass - LDM (A), tiller-to-leaf ratio - TLR (B), forage yield (C), and leaf area - LA (D) of Marandu grass with foliar humic and fulvic acid application

2C). The most significant difference was 18 g (26%) between 1 L ha⁻¹ and the control, followed by 12 g (13%) between 1 L ha⁻¹ and 2 L ha⁻¹. The magnitude of the difference between sources was small (5%) due to result variability, particularly for HA (± 8.4 g), despite differing only from the control ($p = 0.0001$). However, the higher dose significantly reduced forage yield, regardless of the source.

Similar results were reported by Pinheiro et al. (2018) with *U. decumbens* and HA. This reduction may be due to phytotoxic effects at higher concentrations, which may have compromised plant growth or nutrient uptake, thereby decreasing yield. It is also possible that nutrient imbalances or osmotic stress contributed to this effect, since higher doses of HSs can disrupt plant physiology if not carefully optimized (Luz et al., 2021). These effects are similar to those of synthetic hormones in plants (Capstaff et al., 2020; Jindo et al., 2020). These findings underscore the importance of dosage optimization in biostimulant application for tropical forage grasses.

The increase in the fresh biomass, number of tillers (Figure 1C and D), TLR, and forage yield (Figures 2A and C) is associated with the primary and underlying effects of using HS as plant biostimulants. Jindo et al. (2020) argue that direct effects are often recognized as the “auxin effect”, resulting from PM H⁺ATPase activity. This effect promotes morphoanatomical and biochemical changes that increase the formation of lateral roots (Amorim et al., 2015) and root hairs (García et al., 2019), maximizing the root contact surface and favoring ion and water transport and nutrient uptake (Taiz et al., 2021).

Pinheiro et al. (2018) evaluated foliar spraying of lyophilized humic acids in *U. decumbens* under low soil fertility in a greenhouse and reported that 30 mg L⁻¹ applied 45 days after emergence increased shoot and root dry mass by 47.7 and 376.7%, respectively. Similar findings were reported by Amorim et al. (2015) in germination tests with *U. brizantha* cv. MG5 using humic acids derived from vermicomposts. The authors found that applying 0.24 g C of HA pot⁻¹ 14 days after sowing increased SFM and root biomass by 19.5 and 25.6%, respectively, declining after reapplication. In the present study, this magnitude of response was observed with 16 g ha⁻¹ of C from HA (1 L ha⁻¹) or 5 g ha⁻¹ of C from FA (1 L ha⁻¹) over three cycles.

Interaction between the factors ($p = 0.002$) was observed for surface photosynthetic (leaf area - LA), with FA at 1 L ha⁻¹ producing the highest LA (39.4 cm²) and lowest standard deviation (± 0.4 cm), being the only treatment that differed from the control ($p = 0.0004$) (Figure 2D). Increasing the dose from 1 to 2 L ha⁻¹ caused grass toxicity due to the 10.4 cm² (26%) decline in LA. No difference in doses was observed for HA. This can be attributed to the high variability of the results (± 4.4 and ± 5.6 cm² for HA at 1 and 2 L ha⁻¹, respectively), differing significantly from FA at 2 L ha⁻¹, with a ~ 24% increase in LA.

Interaction between factors ($p = 0.007$) was observed for specific leaf area, which demonstrates the efficiency of plant biomass accumulation per cm². Responses for this variable were similar to those of LA, but with more significant data variability (CV: 11%) (Figure 3A). HA was not influenced by the different doses, with an overall average (124 cm² g⁻¹) similar to that of FA at 1 L ha⁻¹. FA at 2 L ha⁻¹ was the most efficient

treatment in terms of biomass accumulation unit⁻¹ of LA and the only treatment that differed from the control ($p = 0.005$), with a reduction of 21.7 cm² g⁻¹. However, 2 L ha⁻¹ produced the worst performance for tiller and leaf biomass (Figures 1D and 2A), and forage yield (Figure 2C), that is, low biomass accumulation and therefore a smaller SLA.

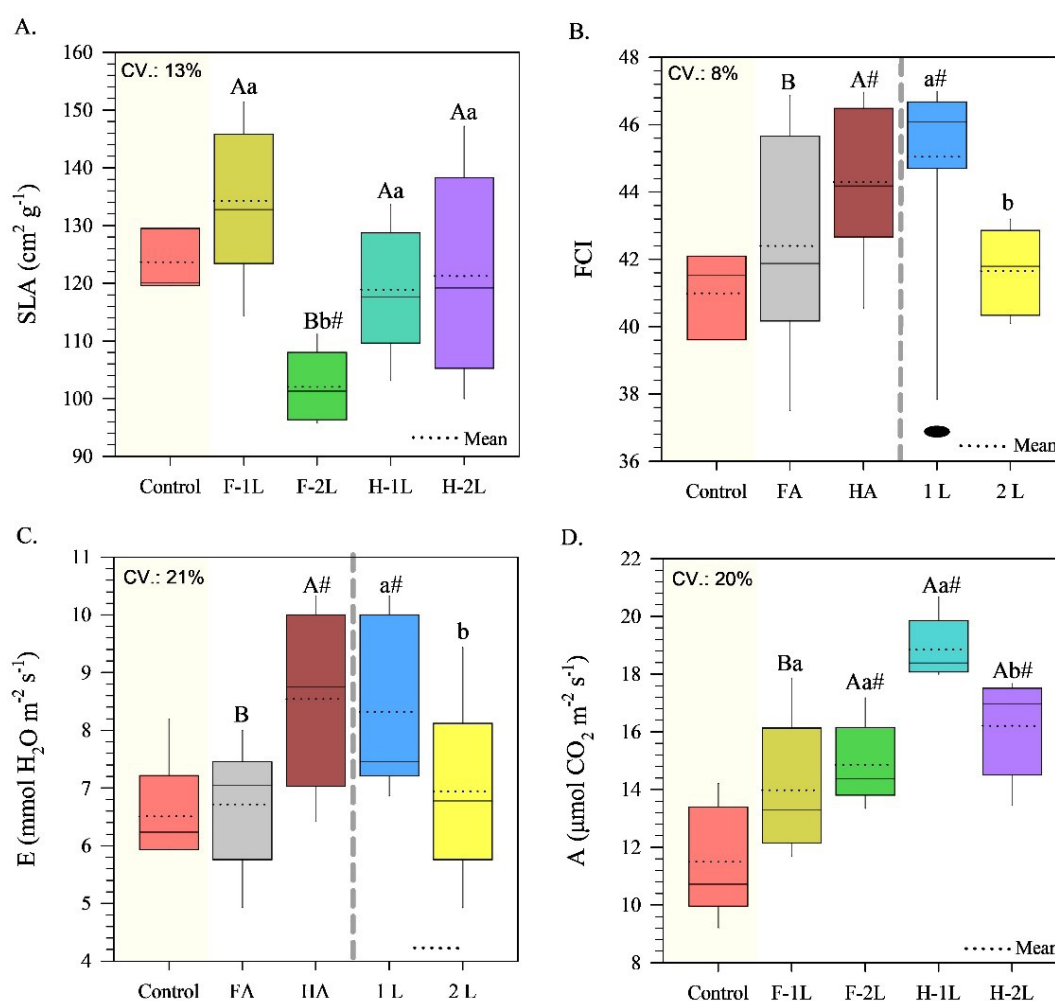
HA provided a higher FCI than that obtained for FA ($p = 0.041$), despite the high source variability observed (Figure 3B). With respect to doses, 1 L ha⁻¹ produced an 8% higher FCI than 2 L ha⁻¹ ($p = 0.001$) and the control ($p = 0.01$). Although measurements were not taken, these results suggest similar fertilization between treatments (50 kg ha⁻¹ of N (urea)) after each cut. Plants with HA or a dose of 1 L ha⁻¹ were more nitrogen-use efficient due to the increase in FCI.

Transpiration rate (E) responses were similar to those observed for FCI (Figure 3C), with HA resulting in a significant increase in E of 2.1 and 1.8 mmol H₂O m⁻² s⁻¹ compared to the control ($p = 0.04$) and FA ($p = 0.001$), respectively. In regard to doses, E was 20% higher at 1 L ha⁻¹ ($p = 0.01$) when compared to 2 L ha⁻¹ and 27% higher than the control.

Interaction between factors was observed for CO₂ assimilation in Marandu grass, demonstrating the effect of the biostimulants on forage physiology ($p = 0.001$) (Figure 3D). HA at 1 L ha⁻¹ showed a higher assimilation rate (18.8 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), which was higher than 2 L ha⁻¹ (both doses differed from the control) and FA (regardless of dose). The lack of a significant response to a higher FA dose suggests that FA may have reached maximum efficacy under the conditions tested. Furthermore, factors such as low soil fertility and plant nutrition could have constrained CO₂ assimilation, since biostimulants do not supply nutrients directly but rather improve the plants' natural processes (Du Jardin, 2015; Shah et al., 2018; Luz et al., 2021). However, the sources differed from the control ($p = 0.0001$) only at 2 L ha⁻¹ (11.5 vs. 14.9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) due to its low variability (± 1.2 standard deviation).

Considering all the treatments and variables, the first two PCA components (PC1 and PC2) explained 64% of total data variance (Figure 4A). PCA revealed that 1 L ha⁻¹ of HA and FA produced the best results due to the more significant dissimilarity of Mahalanobis distance (7.0 and 4.5, respectively) from the control. However, for both sources, the higher dose reduced the distance to 3.2, suggesting possible toxicity of excess foliar HA and FA in Marandu grass across three grazing cycles.

Hierarchical clustering identified two large groups of treatments with considerable similarity (X-axis of the heatmap, Figure 4B). All the 1 L ha⁻¹ repetitions of FA or HA were grouped as similar, suggesting highly consistent results at this dose. In general, this cluster was associated with the greatest expressions of biomass accumulation traits (SFM, forage yield, TDM, and LDM), tillering, and physiological characteristics (LA, SLA, FCI, CO₂ assimilation, and transpiration rate). The second cluster of treatments contained the control and 2 L ha⁻¹ (for both sources), exhibiting low expression of the abovementioned traits, except for plant height and TLR. It is noteworthy that in the grouping of variables (Y axis of the heatmap), the plant height and TLR were identified as outliers, i.e., not good indicators of forage crop responses to biostimulants.



Different letters, uppercase for source effect and lowercase for doses, do not differ according to the LSD test ($p \leq 0.05$). Means with # indicate differences from the control treatment according to Dunnett's test ($p \leq 0.05$). For clarity and esthetic purposes, letters are only displayed where significant differences were observed. SLA - Specific leaf area; FCI - Falker chlorophyll index; E - Transpiration rate; A - CO_2 assimilation rate; FA - Fulvic acids; HA - Humic acids; 1 L or 2 L: Application of 1 or 2 L ha^{-1} ; F-1L and F-2L - Fulvic acid at 1 and 2 L ha^{-1} ; H-1L and H-2L - Humic acid at 1 and 2 L ha^{-1} ; F-1L, F-2L, H-1L, H-2L: Interaction between sources and doses. The vertical dashed bar indicates the results of the simple effects of the factors.

Figure 3. Specific leaf area - SLA (A), Falker total chlorophyll index - FCI (B), transpiration - E (C) and CO_2 assimilation - A (D) of Marandu grass with foliar humic and fulvic acids application

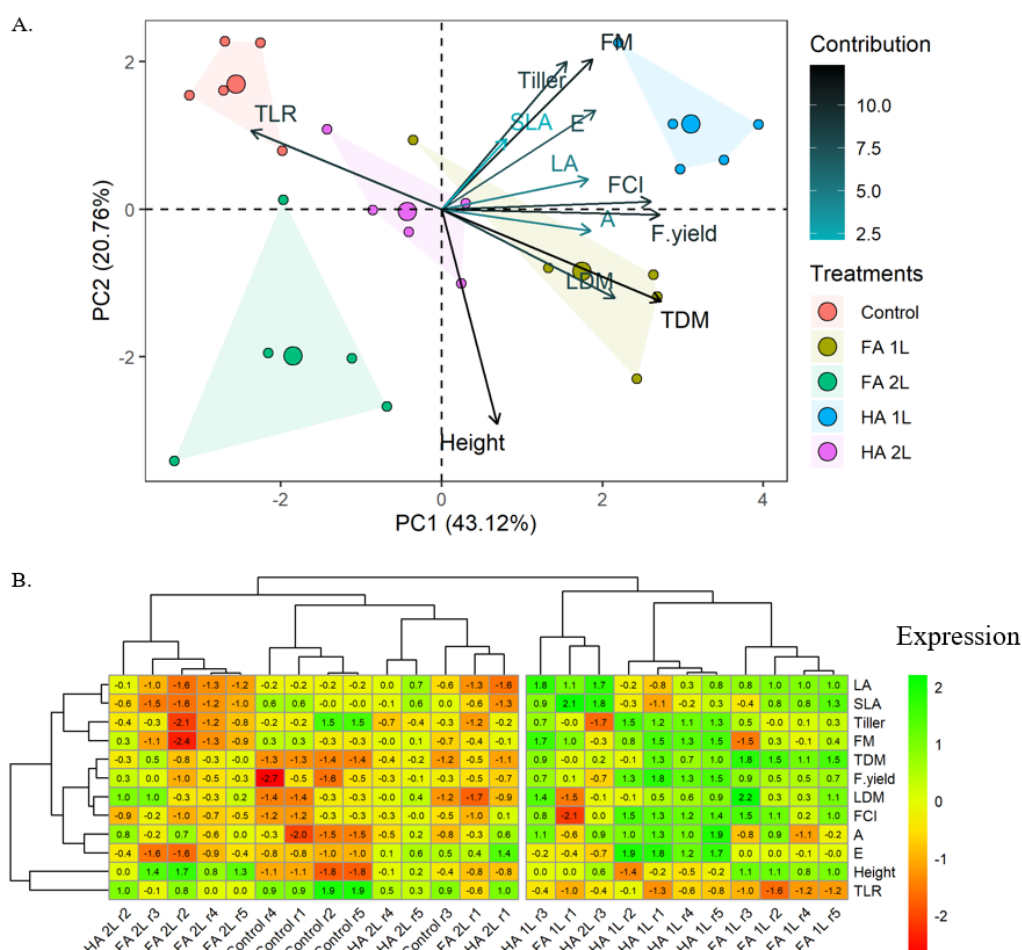
The present study demonstrates the potential of HS as biostimulants in Marandu grass yield, one of the main forage grasses grown in tropical regions. In addition to highlighting a technology that favors pasture crop intensification, this study supports a circular economy by using waste from the slaughterhouse industry as a source for HS's extraction. This mitigates the environmental impact and improves the sustainability of livestock farming in tropical regions (Phillips, 2010; Tullo et al., 2019).

According to Capstaff et al. (2020), FA does not act as a biostimulant in *Lolium perene* grass. However, the authors reported a significant positive effect of FA on the growth and nodulation of forage legumes (*Medicago sativa*) under controlled conditions and in the field, with no nutritional effect. In our results, responses to FA were always of a lower magnitude than those observed for HA, except for LA and SLA (Figures 2D and 3A). This response suggests that low-dose FA may be better suited to legumes than forage grasses.

The results obtained for HS's in grasses may also be inconsistent, especially in field conditions. Neves et al. (2019) reported no increase in Marandu grass yield or bromatological

quality over two growing seasons with up to 200 L ha^{-1} of HA extracted from vermicompost. In this study, a negative linear change was only observed for TLR (i.e.; increased leaf differentiation), and the low experimental precision (CV > 24%) explains the lack of response in the forage. Verlinden et al. (2010) studied four sites with three forage grasses over three harvests and concluded that HS's only increased forage yield at the first cut and were associated with increased N absorption. This response confirms our results, since forage yield was strongly associated with FCI variation according to PCA and the clustered heatmap (Figure 4A and B).

Forage yield responses may also be the results of the underlying HS's mechanisms in plant biostimulation (Jindo et al., 2020), which generate a broader electrochemical gradient by inducing ATPase and accelerating nutrient uptake. This was also confirmed by the overexpression of transport genes (Nunes et al., 2019). These changes may interfere with the specialized metabolism by regulating chemical compounds in plant cells, such as those related to the Krebs cycle, nitrate and P metabolism, glycolysis, and photosynthesis (Baltazar et al., 2021; Nardi et al., 2021).



In the heatmap, the color on each line of the matrix represents the normalized Euclidean distance between the Marandu grass traits evaluated in the parametric space. Treatments with the same color for a given variable are close together in the parametric space. PC1 - first principal component; PC2 - second principal component; F-1L and F-2L - Fulvic acid at 1 and 2 L ha⁻¹; H-1L and H-2L - Humic acid at 1 and 2 L ha⁻¹. LA - Leaf area; SLA - Specific leaf area; FM - Shoot freshmass; TDM - Tiller dry mass; LDM - Leaf dry mass; FCI - Falker chlorophyll index; TLR - Tiller-to-leaf ratio; F.yield - Forage yield; A - CO₂ assimilation rate; E - Transpiration rate

Figure 4. Biplot of the main components (A) and heatmap with cluster by Euclidean distance (B) of the effect of humic (HA) and fulvic acid (FA) doses on the agronomic and morphophysiological traits of Marandu grass

The results suggest that HA is more efficient than FA at increasing gas exchange and chlorophyll in Marandu grass (Figure 3), thus explaining the increased forage yield. No studies were found that evaluated the effects of FA or HA on forage gas exchange. In corn, Anjum et al. (2011) observed an increase in CO₂ assimilation, transpiration rate, and chlorophyll content with foliar FA application (1.5 mg L⁻¹) and attributed these responses to greater antioxidant enzyme activity. It has been reported that HA also strongly regulates the content of reactive oxygen species (ROS), which can oxidize organic compounds and are essential in cell signaling (García et al., 2019; Nunes et al., 2019). However, the magnitude of these effects may vary according to the chemical structure of HS, the rate of application, application format, and especially the crop in question (Jindo et al., 2020; Savarese et al., 2022).

A more significant response to FA was expected since its low molecular weight means it can pass through the microspores of biological membrane systems, achieving greater biostimulation than HA. However, due to its high molecular weight, HA has hydrophobic regions that allow conformational changes and form micelle-like structures to penetrate biological membranes (García et al., 2019; Baltazar et al., 2021; Nardi et al., 2021). This means that HA can be

absorbed through the leaf cuticle and FA via leaf stomata (Jindo et al., 2020).

As such, the results indicate that FA and HA obtained from slaughterhouse waste compost have biostimulant effects on Marandu grass grown under moderate pasture management in the Brazilian Cerrado. These effects can potentially intensify Marandu grass pastures due to structural regeneration, and physiological and yield responses in forage. However, further research is needed, particularly under field conditions, in order to adjust doses and assess fertilization levels and the combined use of FA and HA to determine their influence on the bromatological quality of the forage produced.

CONCLUSIONS

1. Humic and fulvic acids derived from slaughterhouse waste compost have biostimulant effects on *Urochloa brizantha* cv. Marandu.
2. Humic acids showed greater biostimulation than their fulvic counterparts for morphophysiology and forage yield, except for leaf area and specific leaf area.
3. Regardless of the source, the dose of 1 L ha⁻¹ increased forage yield; however, significant reduction was observed at 2 L ha⁻¹.

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