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Article

Agronomic performance of palisade grass under different doses of liquid blood waste and chemical composition of soil

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Abstract:

The aim of the present study was to assess the agronomic performance and chemical composition of soil cultivated with palisade grass (*Urochloa brizantha* cv. Marandu) subjected to growing doses of liquid blood waste. The experiment followed the completely randomized blocks design with six treatments and four repetitions. The following doses of processed liquid blood waste were applied to test palisade grass' yield: 0, 150, 300, 450 and 600 m^3 ha^{-1}. In addition, it was used in conjunction with chemical fertilization at a rate of 50 kg ha^{-1} of P_2O_5 and 100 kg ha^{-1} of N (this treatment was not managed with liquid blood residue). Palisade grass forage yield was influenced by the fertilization strategy ($P<0.001$) – the highest values observed for this variable were recorded under blood waste doses of 450 m^3 ha^{-1} and 600 m^3 ha^{-1}. The 0.0 – 0.20 m soil layer affect the organic matter fraction. On the other hand, phosphorus (P) content presented differences between fertilization strategies; thus, it was possible observing that the waste dose of 450 m^3 ha^{-1} accounted for the highest availability of nutrients. The application of blood liquid waste as alternative source of organic fertilizers can be feasible, because it promotes significant increase in forage mass.

Keywords: Cerrado, Organic fertilization, Forage Mass, Sustainability, *Urochloa brizantha.*

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Introduction

Urochloa brizantha cv. Marandu (Syn. *Brachiaria brizantha* cv. Marandu), commonly known as palisade grass, is a forage species broadly used by the Brazilian livestock sector, because it shows excellent foraging potential for beef and milk production(1,2,3). However, forage yield in the Brazilian savanna region, also known as Goiás State's Cerrado, suffers with challenges related to abiotic factors, mainly with soil issues, since these soils are featured by low natural fertility, low nutrient contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), as well as by low ability to retain water due to their low organic matter contents(4,5).

Therefore, it is essential to replenish nutrients through chemical fertilization to alleviate this situation. However, this nutrient replenishment strategy goes against the sustainability of an agricultural production system. For this reason, currently, this type of fertilization should be considered as minimally as possible. However, inorganic sources are quite expensive; moreover, the global crisis caused by the pandemic scenario, in association with the on-going wars, can impair food security and the economic feasibility of the production system(6).

The use of organic sources can be an alternative to the aforementioned issues, because it can provide essential nutrients for plants' good development, Oliveira *et al*(7) observing that blood liquid waste from slaughterhouses present the essential nutrients for plants in its chemical composition (*e.g.*, P, K, Ca, Mg and S). Besides, these authors also observed that using this waste type as P source in sunflower culture (*Helianthus annuus* L.) led to good plant morphological development.

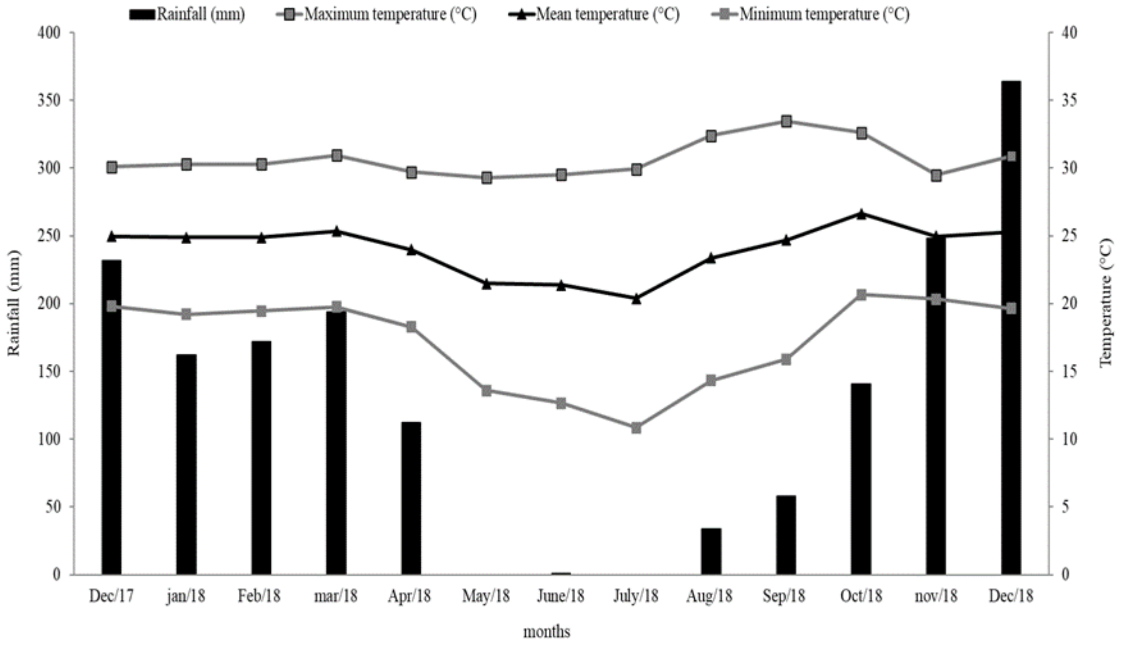
In addition, it is possible to suggest the following hypothesis: by using liquid blood waste as an alternative source of N and P in pastures of palisade grass grown in the Brazilian Cerrado, it is possible to increase the availability of forage mass and improve the chemical composition of the soil. Therefore, the aim of the present study was to assess the agronomic performance of palisade grass and chemical properties of soil cultivated with palisade grass, subjected to growing doses of liquid blood waste.

Material and methods

Study site

The experiment was installed in a site by SANEAGO's (*Saneamento de Goiás*) sewage treatment station (ETE), in São Luís dos Montes Belos County, Goiás State (coordinates 16º 32' 30" S, 50º 25' 21" W; and altitude: 535 m). The experiment began in December 2017 and concluded in December 2018. This region is featured by Aw climate, according to Köppen's classification, with mean temperature of 23.5 ºC, ranging from 20.7 ºC (June) to 25.0 ºC (December), and mean annual rainfall of 1,785 mm – 87% of it is concentrated between October and March, but there are 4 mo of water shortage every year, on average(8). Temperature and rainfall data recorded during the experiment are shown in Figure 1.

Figure 1: Maximum, medium and minimum temperature, and monthly rainfall rates from December 2017 to December 2018, in the study site - São Luís de Montes Belos County



Experimental design

The study site was properly fenced and the grass was cut to the height of 25cm for treatment application purposes. After it was cut, 16m² (4 x 4 m) plots were set with 1m hallways between them.

The experiment followed the completely randomized blocks design, with six treatments and four repetitions. The treatments consisted of doses of 0 m^3 ha^{-1} (control treatment, without the use of any P and N source), 150 m^3 ha^{-1} (equivalent to 39.60 kg ha^{-1} of N and 27.10 kg ha^{-1} of P_2O_5), 300 m^3 ha^{-1} (equivalent to 79.30 kg ha^{-1} of N and 54.10 kg ha^{-1} of P_2O_5), 450 m^3 ha^{-1} (equivalent to 118.90 kg $ha^{-1}$of N and 81.20 kg ha^{-1} of P_2O_5), and 600 m^3 ha^{-1} (equivalent to 158.60 kg ha^{-1} of N and 108.20 kg ha^{-1} of P_2O_5) of liquid blood processing residue obtained from cattle slaughterhouses, as a source of N and P. Additionally, it was used in conjunction with chemical fertilization (CF) at a rate of 50 kg ha^{-1} of P_2O_5 and 100 kg ha^{-1} of N, according to the crop's needs and soil analysis(9). The CF treatment did not receive any dose of liquid residue.

Soil physical composition and fertilization

Palisade grass (*Urochloa brizantha* cv. Marandu) pasture was set approximately 15 years ago, and it was not subjected to fertilization management. Before implementing the experiment, soil chemical and physical properties were assessed based on samples collected from soil layer 0.0 to 0.20 m. Subsequently, a compound sample was collected and sent to the laboratory for analysis, based on the method described by Raij *et al*(10). Soil was classified as Eutrophic Red Latosol(11); its texture was clayey with 360, 250 and 390 g kg^{-1} sand, silt and clay, respectively; chemical composition was 5.1 active acidity (pH in $CaCl_2$); 23.00 g kg^{-1} organic matter (OM); 100 mg dm^{-3} phosphorus (P in Mehlich I); 2.80 cmol$_c$ dm^{-3} potential acidity (H+Al); 0.400 cmol$_c$ dm^{-3} of K; 2.50 cmol$_c$ dm^{-3} of Ca; 0.700 cmol$_c$ dm^{-3} of Mg; 56 % base saturation (V%).

The CF treatment comprised 100 kg ha^{-1} of N and 50 kg ha^{-1} of P_2O_5 deriving from urea and triple superphosphate, respectively. K_2O was not applied because it was not necessary, according to the soil featuring analysis. P was applied after the plots were set and N fertilization was split in two applications: the first application was carried out in December 2017 along with P and the second one was conducted in January 2018.

Organic fertilizer: blood waste

The herein used waste type came from bovine-blood processing carried out by a company located in São Luís de Montes Belos County, Goiás State. The blood is sent to this company in tank trucks from several slaughterhouses in the region. After it is received, plasma and red cells' physical separation is carried out in high-rotation centrifuge. Then, both the red cells and plasma are subjected to drying process to be used in feed fractions for small animals or in products for the pharmaceutical industries. The liquid waste resulting from this process is treated for its proper disposal. The herein used waste presented the following composition: Acidity (pH) of 7.41; ammoniacal nitrogen (NH_4^+) of 264.30 mg L^{-1}; P_2O_5 of 180.40 mg L^{-1}. The waste was manually applied, at once, with the aid of buckets, according to each treatment, on December 15th, 2017.

Forage canopy assessment

The 40-d time base was applied; it means five days more than the time base suggested by Costa and Queiroz(12) – it was done because defoliation was mechanical, rather than being done through conventional grazing. Every time plants subjected to this treatment did not reach entrance height within 40 d, plant height was used as basis (30 cm). Assessments were carried out on January 25th, 2018; March 07th, 2018; July 05th, 2018 and on November 25th, 2018 (40 d after the beginning of the rainy season).

Forage canopy height (CH, cm), tiller population density (TPD, m^2), forage dry matter (DM, Mg ha^{-1}) and forage dry matter yield (FDM, Mg ha^{-1}) (sum of all cuts) were quantified for forage canopy featuring.

Canopy height was measured in each plot with the aid of a ruler, in five different points; soil level was measured up to the mean level of the curve of fully expanded superior leaf blades. TPD was determined by counting the three points in the experimental unit with the aid of an iron frame (0.25 x 0.25 cm in dimension).

Yield was measured through DM by using a metal frame (1x1 m in dimension), supported 0.25 m from soil surface. This equipment was randomly placed in the plot and all forage inside it, at height $\geq$ 0.25 m, was collected and quantified, a 300 g fresh sample was separated for dry matter determination in forced air circulation oven at 65 °C until it reached constant weight. Grass leaves (the two ones recently expanded from the tiller) were collected to find leaf contents of N (g kg^{-1}), P (g kg^{-1}), K (g kg^{-1}), Ca (g kg^{-1}), Mg (g kg^{-1}), S (g kg^{-1}), Cu (mg kg^{-1}), Fe (mg kg^{-1}), Mn (mg kg^{-1}) and Zn (mg kg^{-1}). In order to do so, 10 representative plants were randomly collected in the plot and selected; it totaled 20 leaves. Subsequently, they were washed in running water, followed by washing in deionized water, dried in forced air circulation oven at 65 °C for 72 h and ground in Willey type mill(13).

Soil chemical composition

At the end of the assessment cycle, in September 2018, compound samples formed by five simple samples resulting from random points in each plot were prepared with the aid of metallic type probe from layers 0.00-0.20 and 0.20-0.40 m to observe likely soil chemical changes caused by the waste application.

Soil was sieved after its collection and identification, and the following features were analyzed: OM (g kg^{-1}), pH ($CaCl_2$), H+Al ($cmol_c$ dm^{-3}), CEC ($cmol_c$ dm^{-3}), P (mg dm^{-3}), K (mg dm^{-3}), Ca ($cmol_c$ dm^{-3}), Mg ($cmol_c$ dm^{-3}), S (mg dm^{-3}), Na (mg dm^{-3}), B (mg dm^{-3}), Cu (mg dm^{-3}), Fe (mg dm^{-3}), Mn (mg dm^{-3}) and Zn (mg dm^{-3}), according to the methodology described by Teixeira *et al*(14).

Statistical analysis

Information related to pasture was subjected to split-plot model in time:
$y_{ijk} = \mu + E_i + Bj + \varepsilon_{ij} + C_k + T_i*C_k + \varepsilon_{ijk}$
wherein,

y_{ijk}: observed value;
μ= general constant;
Ti: treatments' effect (i = 0, 150, 300, 450, 600 m^3 ha^{-1}, and CF);
B_j= Block's effect (j = I, II, II and IV);
C_k: cuts' effect (k = 1st, 2nd, 3rd and 4th);
ε_{ij}: waste at plot level;
T_i*C_k: interaction effect;
ε_{ijk}: experimental waste.

After this procedure was over, Tukey's average test was applied at 5 % probability level.

Data related to blood waste doses and chemical fertilization were analyzed through the randomized block design model:

Y_{ijk} $= \mu + T_i + B_j + \varepsilon_{ijk}$; wherein,

Y_{ijk}: observed value;
μ= general constant;
T_i: treatments' effect (i= 0, 150, 300, 450, 600 m^3 ha^{-1}, and CF);
B_j: Block's effect (j= I, II, II and IV);
ε_{ijk}: random error associated with each observed value.

After the completion of the aforementioned procedure, Tukey's average test was applied at 5% probability level, whenever applicable, at 5% significance level.

Waste doses were subjected to first ($Y_{ij} = \beta0 + \beta1*X + \varepsilon_{ij}$) and second degree ($Y_{ij} = \beta0 + \beta1*X + \beta2*X^2 + \varepsilon_{ij}$) regression analysis; the model presenting 5 % significance effect and the highest determination coefficient ($R^2 \geq 70$ %) was the chosen one. Variance and regression analyses were carried out in R software, version 4.2.1.

Results

Forage canopy structure

Palisade grass forage CH presented a significant blood waste and cut interaction ($P<0.001$); thus, in the first and second cuts, the highest CH values were obtained when doses of 150 m^3 ha^{-1} and 600 m^3 ha^{-1} were used. The highest CH values under doses 300 m^3 ha^{-1} and 450 m^3 ha^{-1}, and chemical fertilization, were only recorded at the first cut. Then, the first, second and the third cuts after dose 0 m^3 ha^{-1} accounted for the lowest CH values. The fourth cut did not show difference between fertilization strategies. Mean height of 21.55 cm was recorded for this fourth cut (Table 1).

If one only takes into account the blood waste doses, the first cut generated a second degree equation; thus, the use of 144 m^3 ha^{-1} liquid waste led to height of 47.81cm. Doses were adjusted to the first degree equation at the second and third cuts; thus, based on the inclination parameters, it was possible inferring that increased offer of liquid waste increases forage canopy height (Table 1).

It was possible observing the effect of interaction between fertilization strategy and cuts ($P=0.002$) in TPD; therefore, CF after the dose of 0 m^3 ha^{-1}, at the first cut, led to the lowest values. The 300, 450 and 600 m^3 ha^{-1} doses had an impact on the increase in TPD in the second cut, respectively. Dose 600 m^3 ha^{-1} accounted for the highest TPD at the third cut. The fourth cut did not show difference between fertilization strategies; mean value of 437 tillers m^{-2} was recorded for palisade grass canopy on this fourth cut (Table 1).

Blood waste doses at the first cut have led to a quadratic equation; thus, 759 tillers m^{-2} were measured when 590 m^3 ha^{-1} organic fertilizer was applied. The second cut reached a first-degree equation with positive inclination; therefore, increase in organic fertilization doses had impact on palisade grass TPD increase. Doses did not have any effect at the third and fourth cuts (Table 1).

DM was affected by the interaction between fertilization strategy and cuts ($P<0.001$); thus, the dose of 450 m^3 ha^{-1} blood waste generated the highest forage mass values at the first cut. Dose 600 m^3 ha^{-1} led to the highest DM values at the fourth cut. Blood waste doses at the first and fourth cuts were the ones presenting adjustment to the quadratic equation; therefore, doses 417 m^3 ha^{-1} and 500 m^3 ha^{-1} organic fertilizer led to DM yield of 5.42 Mg ha^{-1} and 6.22 Mg ha^{-1}, respectively (Table 1).

Palisade grass forage yield was influenced by fertilization strategies ($P<0.001$), the highest forage-yield values were recorded at blood waste doses of 450 m^3 ha^{-1} and 600

m^3 ha^{-1}. If one only takes into account waste doses; it is possible observing the best adjustment to the quadratic equation under the dose 583 m^3 ha^{-1} blood waste to obtain 14.77 Mg ha^{-1} (Table 1).

Nutrients’ content in palisade grass leaf blades

There was blood waste effect on leaf N ($P<0.001$) and P ($P= 0.013$) content at dose 600 m^3 ha^{-1}, which led to the highest N values. Fertilization also affected K ($P= 0.015$), S ($P<0.001$), Fe ($P= 0.001$) and Mn ($P<0.001$) concentration; the highest values recorded for these elements were recorded at doses 450 m^3 ha^{-1} and 600 m^3 ha^{-1} (Table 2).

The Ca content in the leaf was affected by the fertilization strategies ($P=0.002$), where the highest concentrations were observed in the 300 m^3 ha^{-1} and 600 m^3 ha^{-1} doses. Mg was also influenced by the treatments tested ($P=0.019$), with the highest concentrations recorded at the 450 m3 ha^{-1} dose. Copper (Cu) was not influenced by fertilization strategies ($P=0.05$); mean copper value of 9.25 g kg^{-1} was recorded (Table 2).

N, P, Ca, Mg, S, Fe Mn and Zn contents in leaf blades were affected by blood waste doses; it was possible observing their best adjustment to first degree equations. So, the higher the waste dose the higher the leaf concentration of these elements (Table 2).

Soil chemical composition at layer 0.00 -0.20 m

Layer 0.00 -0.20 m did not show any effect of fertilization strategies ($P>0.05$) on OM, pH, K, Mg, S, Na, B, Cu and Mn. Thus, the following mean values were recorded: 32.17 g kg^{-1}, 5.05 $CaCl_2$, 127.17 mg dm^{-3}, 0.713 cmolc dm^{-3}, 3.63 mg dm^{-3}, 2.04 mg dm^{-3}, 0.204 mg dm^{-3}, 1.25 mg dm^{-3} and 54.58 mg dm^{-3}, respectively (Table 3).

Fertilization strategies influenced CEC ($P=0.013$), Ca ($P<0.001$) and Fe ($P<0.001$); dose 600 m^3 ha^{-1} led to the highest CEC means. Fertilization also affected H+Al ($P= 0.039$); dose 0 m^3 ha^{-1} accounted for the lowest mean H+Al value. Phosphorus (P) contents present difference between fertilization strategies ($P=0.001$); thus, dose 450 m^3 ha^{-1} recorded the highest availability of this nutrient. Chemical fertilization led to the lowest Zn values ($P=0.006$) (Table 3).

Blood waste doses accounted for the best adjustment to second degree equations when it comes to P, Ca and Fe; therefore, doses 400 m^3 ha^{-1}, 500 m^3 ha^{-1} and 575 m^3 ha^{-1} generated contents of 2.32 mg dm^{-3}, 2.37 $cmol_c$ dm^{-3} and 33.46 mg dm^{-3} of these elements, respectively (Table 3).

Soil chemical composition at layer 0.20 -0.40 m

Soil layer 0.20 - 0.40 m did not show any effect of fertilization strategy (*P*>0.05) on OM, pH, P, K, Ca , Mg, S, Na, B, Cu, Mn and Zn. Thus, it was possible reaching mean values of 22.69 g kg^{-1}, 5.19 in $CaCl_2$, 1.18 mg dm^{-3}, 85.39 mg dm^{-3}, 2.00 cmolc dm^{-3}, 0.708 cmolc dm^{-3}, 3.67 mg dm^{-3}, 2.04 mg dm^{-3}, 0.200 mg dm^{-3}, 1.25 mg dm^{-3}, 36.13 mg dm^{-3} and 0.492 mg dm^{-3} for these elements, respectively (Table 3).

Fertilization strategies influenced CEC (*P*=0.049) and H+Al (*P*<0.001); their values have increased at dose 600 m^3 ha^{-1}. The highest Fe contents (*P*=0.003) were observed at doses 450 m^3 ha^{-1} and 600 m^3 ha^{-1}, respectively (Table 3).

Blood waste doses have influenced H+Al and Fe, since they showed the best adjustment to first degree equations; therefore, the rate of potential acidity and minerals that can be toxic in plants at soil layer 0.20 – 0.40m increased, as the organic source also increased (Table 3).

Discussion

Forage canopy structure

The recommended CH for palisade grass pastures is 30-45 cm, as it is the best height to maximize the availability of forage mass; higher CH values indicate an undesirable accumulation of morphological components that can compromise the chemical composition of the forage canopy, such as pseudostem (stem + sheath) and dead material(15,16). The dose of 150 m^3 ha^{-1} of organic fertilizer induces the palisade grass canopy to reach heights that comply with the management recommendation. However, the use of this dose does not promote the maximum potential availability of forage mass.

On the other hand, TPD showed the highest values at the highest blood waste doses; consequently, the highest DM and FDM values were measured under these nutritional management conditions. Véras *et al*(17) assessed five *Urochloa* spp. cultivars (Basilisk, Marandu, BRS Paiaguás, Piatã, Xaraés), was found moderate correlation between CH and DM; however, the correlation between DM and TPD was closer because it ranged from moderate to high. Thus, it is necessary to pay close attention to the pasture’s tiller dynamics at organic fertilization application, since this feature is determining to forage mass yield.

In the fourth cut, it was observed that regardless of the fertilization strategy used, there was proportionality in TPD. This occurred because there were no differences in the management criteria (defoliation frequency and cutting height), which did not alter the tillering dynamics. However, adopting different management strategies can lead to fluctuations in the phenotypic plasticity of the forage canopy(18).

Orrico *et al*(19) grew tufted grass subjected to growing poultry slaughterhouse waste doses and found the highest tiller and forage mass values at higher organic fertilizer doses. According to the findings, the high N content in the organic fertilizer boosts tissue flow in the tillers, and it allows forage canopy to reach the maximum yield potential. Costa *et al*(20) assessed *Megathyrsus maximus* cv. Massai (Syn. *Panicum maximum* cv. Massai) pastures and observed that fertilization management based on using other bio-fertilizer source (deriving from swine farming) increased leaf forage mass in comparison to mineral fertilization.

In this context, the use of organic fertilizers derived from slaughterhouses is highly recommended as a primary fertilization strategy, as these fertilizers enhance the morphological performance of tillers and significantly increase forage production. However, to achieve these results, it is essential that the fertilizer supplied to the soil contains the necessary nutrients to optimize plant production(21).

Nutrient contents in palisade grass leaf blades

The growing doses of liquid waste (0 m^3 ha^{-1}, 150 m^3 ha^{-1}, 300 m^3 ha^{-1}, 450 m^3 ha^{-1}, 600 m^3 ha^{-1}) led to significant increase in N, P, Ca, Mg, S, and Fe fractions in palisade grass leaf blades. According to Tomazello *et al*(22) and Rezende *et al*(23), the adequate supply of nutrients enhances the accumulation of N, P, Ca, S, and Mg in the aboveground part of tropical grasses managed in savanna regions. Furthermore, it enhances the nutritional value of the produced forage. Nitrogen sources (organic or mineral) supply to palisade grass favors its use efficiency and P, K, Ca and S accumulation, respectively.

There is a specific factor about the micro-nutrients (B, Cu, Fe, Mn, Zn) accumulation, namely: soils presenting pH value lower than 6.0 show increased availability of micro-nutrients for plants; on the other hand, if soil acidity increases, one observes undesired Fe increase in it, and this process can be toxic in plants. Yet, Brazilian *Cerrado* soils often present high Fe contents(24,25,26); therefore, it is necessary often assessing soil acidity levels to avoid complications capable of impairing the maximum agronomic performance of the forage canopy.

Soil chemical composition at layers 0.0 – 0.20 m and 0.20 – 0.40 m

Liquid waste doses and mineral fertilization at layer 0-0.20 m did not influence OM, Mg, S, Na, B, Cu, Mn and Zn. On the other hand, the highest liquid waste doses led to increased CEC and Ca contents (Table 3). According to Caovilla *et al*(27), cation content increase forms the sum base, as it happens with Ca; this process increases soil CEC. However, acidic pH soil compromises the availability of other cations. Thus, it is possible suggesting that the continuous use of liquid waste can change the cation fraction in the soil. Nevertheless, it is necessary associating it with liming management to achieve the availability of essential nutrients for plant development.

In order to make changes in soil chemical composition in tropical climate regions, mainly at its deepest layers (0.20 – 0.40 m), it is necessary to continuously apply organic fertilizers, because it is not possible reaching the desired increase in OM and P fractions in the short-term(28). However, cation addition can change the sum of bases in the soil; thus, it is essential carrying out long-term research to analyze the effect of blood waste on pasture yield in tropical regions.

Considerations on the use of liquid slaughterhouse waste in primary production

Despite being considered a potentially polluting material, when used judiciously, liquid blood residue proves to be a nutrient-rich source, along with an abundance of beneficial microbial populations for the soil, as observed by Bhunia *et al*(29). In agriculture, this factor has a significant impact on increasing primary production. In the specific case of Marandu palisade grass, the results demonstrated that, in a short period of time, there was a considerable increase in forage availability, indicating that pastures reached their maximum productive potential when liquid residue is used as a source of P and N.

Another relevant point to consider when exploring alternative sources of organic fertilizers is the geopolitical conflicts associated with health crises(6), as these conflicts have led to substantial increases in chemical fertilizer prices, increasing risks to food security. Therefore, the partial or complete substitution of chemical fertilizers with organic alternatives can result in a significant reduction in production costs, making primary production less burdensome(22,30).

Conclusions and implications

To maximize the availability of forage mass from Marandu palisade grass produced in the Brazilian Cerrado, doses ranging from 450 m³ ha^{-1} to 600 m³ ha^{-1} of blood residue can be employed. However, concerning the soil's chemical composition, only the dose of 450 m³ ha^{-1} results in significant increases in the phosphorus content in the 0.00-0.20 m layer.

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Table 1: Palisade grass forage canopy featuring based on different fertilization strategies associated with intervals between cuts

	Fertilization Strategy							
Cut	**0 m³ ha^{-1}**	**150 m³ ha^{-1}**	**300 m³ ha^{-1}**	**450 m³ ha^{-1}**	**600 m³ ha^{-1}**	**CF kg ha^{-1}**	**Equation**	**R²**
CH (cm)								
1st	28.75^{Bd}	57.80^{Ab}	92.45^{Aa}	89.00^{Aa}	87.90^{Aa}	44.85^{Ac}	$y = 27.22 + 0.287x - 0.001x^2$	0.966
2nd	43.75^{Ad}	52.10^{Acd}	68.40^{Bb}	66.50^{Bb}	87.60^{Aa}	55.25^{Bc}	$y = 43.25 + 0.068x$	0.919
3rd	27.75^{Bc}	32.70^{Bc}	36.00^{Cbc}	42.00^{Cb}	50.30^{Ba}	29.95^{Cc}	$y = 26.87 + 0.036x$	0.973
4th	19.20^{Ca}	21.25^{Ca}	22.37^{Da}	22.90^{Da}	23.40^{Ca}	20.15^{Da}	$y = 19.81$	-
SEM	2.46							
TPD (m²)								
1st	625^{ABb}	625^{Aab}	696^{Aa}	754^{Aa}	675^{Aa}	676^{Aa}	$y = 410.52 + 1.18x - 0.002x^2$	0.976
2nd	610^{Ab}	533^{ABb}	794^{Aa}	814^{Aa}	802^{Aa}	785^{Aa}	$y = 577.50 + 0.444x$	0.654
3rd	488^{ABa}	488^{Ba}	526^{Ba}	573^{Ba}	516^{Ba}	489^{Ba}	$y = 490.10$	-
4th	421^{Ba}	427^{Ba}	432^{Ba}	435^{Ba}	441^{Ba}	463^{Ba}	$y = 421.45$	-
SEM	15.13							
DM (Mg ha^{-1})								
1st	0.386^{Ce}	2.99^{Bc}	5.63^{Aab}	5.99^{Aa}	5.16^{Bb}	2.14^{Bd}	$y = 0.2145 + 0.025x - 0.00003x^2$	0.985
2nd	1.55^{Bd}	2.60^{Bc}	2.82^{Cc}	3.69^{Cb}	4.49^{Ca}	3.66^{Ab}	$y = 1.63 + 0.005x$	0.974
3rd	0.00^{Db}	0.185^{Cb}	1.22^{Da}	1.65^{Da}	1.73^{Da}	1.28^{Cb}	$y = -0.028 + 0.003x$	0.911
4th	2.64^{Ad}	3.78^{Ac}	3.85^{Bc}	4.71^{Bb}	6.21^{Aa}	3.28^{Acd}	$y = 4.97 + 0.005x - 0.000005$	0.950
SEM	0.189							
FDM (Mg ha^{-1})								
FDM	4.58^{d}	9.56^{c}	13.52^{b}	16.04^{a}	17.69^{a}	9.21^{c}	$y = 4.56 + 0.035x - 0.00003x^2$	0.999
SEM	0.936							

CH= canopy height; TPD= tiller population density; DM= dry matter; FDM= forage dry matter yield.

CF= chemical fertilization with 80 kg ha^{-1} P_2O_5; y= observed value; x= blood waste doses (0 m³ ha^{-1}, 150 m³ ha^{-1}, 300 m³ ha^{-1}, 450 m³ ha^{-1}, 600 m³ ha^{-1}). R²= determination coefficient. SEM= standard error of the mean.

The means followed by the same lowercase letter (row) and uppercase letter (columns) do not differ from each other at the 5% probability level.

Table 2: Nutrients’ content in palisade grass leaf blades under different fertilization strategies

	Fertilization Strategy								
Item	**0 m^3 ha^{-1}**	**150 m^3 ha^{-1}**	**300 m^3 ha^{-1}**	**450 m^3 ha^{-1}**	**600 m^3 ha^{-1}**	**CF kg ha^{-1}**	**SEM**	**Equation**	**R^2**
N, g kg^{-1}	17.50^{c}	18.75^{b}	20.00^{ab}	20.00^{ab}	21.5^{a}	20.25^{ab}	0.310	$y = 17.70 + 0.006x$	0.945
P, g kg^{-1}	1.75^{b}	1.80^{b}	1.90^{ab}	2.10^{ab}	2.35^{a}	1.85^{ab}	0.062	$y = 1.68 + 0.001x$	0.925
K, g kg^{-1}	26.00^{ab}	24.70^{ab}	26.30^{ab}	27.80^{a}	27.55^{a}	23.20^{b}	0.473	$y = 25.23$	-
Ca, g kg^{-1}	1.95^{b}	2.15^{b}	2.30^{a}	2.42^{ab}	2.95^{a}	1.82^{b}	0.095	$y = 1.82 + 0.001x$	0.935
Mg, g kg^{-1}	1.10^{b}	1.17^{ab}	1.50^{ab}	1.60^{a}	1.57^{ab}	1.35^{ab}	0.053	$y = 1.11 + 0.001$	0.856
S, g kg^{-1}	0.750^{c}	1.00^{b}	1.15^{ab}	1.45^{a}	1.42^{a}	0.875^{bc}	0.062	$y = 0.795 + 0.001x$	0.931
Cu, mg kg^{-1}	9.00^{a}	9.25^{a}	10.75^{a}	8.25^{a}	9.00^{a}	9.25^{a}	0.590	$y = 9.45$	-
Fe, mg kg^{-1}	88.75^{b}	91.75^{b}	102.00^{ab}	125.00^{a}	122.75^{a}	85.25^{b}	4.03	$y = 85.80 + 0.067x$	0.885
Mn, mg kg^{-1}	41.25^{c}	58.00^{bc}	72.50^{b}	115.75^{a}	125.50^{a}	43.25^{bc}	7.36	$y = 37.35 + 0.150x$	0.955
Zn, mg kg^{-1}	25.75^{b}	31.50^{ab}	35.75^{a}	34.75^{ab}	33.00^{ab}	26.00^{b}	1.14	$y = 28.60 + 0.011x$	0.509

CF= chemical fertilization with 80 kg ha^{-1}P_2O_5; y= observed value; x= blood waste doses (0 m^3 ha^{-1}, 150 m^3 ha^{-1}, 300 m^3 ha^{-1}, 450 m^3 ha^{-1}, 600 m^3 ha^{-1}). N= nitrogen; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; S= sulfur; Cu= copper; Fe= iron; Mn= manganese; Zn= zinc; R^2: determination coefficient; SEM: standard error of the mean.

Means followed by the same lowercase letters in the rows, did not differ from each other at 5% probability level.

Table 3: Chemical composition of the soil in the 0.0 - 0.20 m and 0.20 - 0.40 m layers of soil cultivated with palisade grass subjected to different fertilization strategies

Item	Fertilization strategy						SEM	Equation	R²
	0 m³ ha^{-1}	150 m³ ha^{-1}	300 m³ ha^{-1}	450 m³ ha^{-1}	600 m³ ha^{-1}	CF kg ha^{-1}			
	Layer 0.0-0.20 m								
OM, g kg^{-1}	36.00^{a}	28.00^{a}	31.00^{a}	30.00^{a}	34.00^{a}	34.00^{a}	1.00	$y = 32.20$	-
pH, $CaCl_2$	5.10^{a}	5.10^{a}	5.07^{a}	5.00^{a}	4.95^{a}	5.05^{a}	0.020	$y = 5.12$	-
CEC, cmol$_c$ dm^{-3}	5.19^{b}	5.46^{b}	6.24^{ab}	6.42^{ab}	7.10^{a}	6.24^{b}	0.191	$y = 5.13 + 0.003x$	0,969
H+Al, cmol$_c$ dm^{-3}	2.22^{b}	2.30^{a}	2.82^{a}	2.92^{a}	3.42^{a}	2.27^{a}	0.129	$y = 2.13 + 0.002x$	0.405
P, mg dm^{-3}	1.00^{b}	1.25^{b}	2.25^{ab}	3.25^{a}	2.00^{ab}	1.50^{b}	0.197	$y = 0.721 + 0.008x – 0.00001x^2$	0.706
K, mg dm^{-3}	153.50^{a}	113.50^{a}	130.00^{a}	124.00^{a}	129.00^{a}	113.00^{a}	5.63	$y = 137.70$	-
Ca, cmol$_c$ dm^{-3}	1.87^{d}	2.17^{c}	2.42^{abc}	2.47^{ab}	2.57^{a}	2.20^{bc}	0.055	$y = 1.87 + 0.002 – 0.000002x^2$	0.989
Mg, cmol$_c$ dm^{-3}	0.700^{a}	0.700^{a}	0.675^{a}	0.725^{a}	0.775^{a}	0.700^{a}	0.036	$y = 0.680$	-
S, mg dm^{-3}	3.75^{a}	3.75^{a}	3.50^{a}	3.75^{a}	3.50^{a}	3.50^{a}	0.157	$y = 3.75$	-
Na, mg dm^{-3}	1.75^{a}	2.50^{a}	2.25^{a}	1.50^{a}	2.75^{a}	1.50^{a}	0.164	$y = 1.95$	-
B, mg dm^{-3}	0.200^{a}	0.150^{a}	0.225^{a}	0.200^{a}	0.175^{a}	0.275^{a}	0.017	$y = 0.176$	-
Cu, mg dm^{-3}	1.10^{a}	1.20^{a}	1.27^{a}	1.47^{a}	1.12	1.35^{a}	0.047	$y = 1.17$	-

Fe, mg dm^{-3}	19.50^{d}	27.75^{bc}	30.5^{abc}	31.32^{ab}	34.00^{a}	24.75^{c}	1.10	$y = 20.23 + 0.046x - 0.00004x^2$	0.956
Mn, mg dm^{-3}	51.00^{a}	50.50^{a}	61.00^{a}	49.25^{a}	60.25^{a}	55.50^{a}	2.24	$y = 50.95$	-
Zn, mg dm^{-3}	0.550^{ab}	0.700^{a}	0.750^{a}	0.675^{a}	0.700^{a}	0.400^{b}	0.032	$y = 0.620$	-
	Layer 0.20-0.40 m								
OM, g kg^{-1}	22.25^{a}	23.32^{a}	24.25^{a}	21.50^{a}	23.32^{a}	21.50^{a}	0.492	$y = 23.33$	-
pH, CaCl$_2$	5.22^{a}	5.22^{a}	5.17^{a}	5.17^{a}	5.15^{a}	5.22^{a}	0.023	$y = 5.23$	-
CEC, cmol$_c$ dm^{-3}	4.82^{ab}	5.06^{ab}	5.28^{ab}	5.28^{ab}	5.73^{a}	4.57^{b}	0.115	$y = 4.83 + 0.001x$	0.233
H+Al, cmol$_c$ dm^{-3}	1.92^{b}	2.07^{b}	2.30^{ab}	2.35^{ab}	2.65^{a}	1.87^{b}	0.067	$y = 1.91 + 0.001x$	0.968
P, mg dm^{-3}	1.10^{a}	1.00^{a}	1.50^{a}	1.25^{a}	1.25^{a}	1.00^{a}	0.077	$y = 1.11$	-
K, mg dm^{-3}	106.00^{a}	94.00^{a}	74.00^{a}	79.00^{a}	81.32^{a}	78.00^{a}	4.99	$y = 99.73$	-
Ca, cmol$_c$ dm^{-3}	1.97^{a}	2.12^{a}	2.10^{a}	1.90^{a}	2.07^{a}	1.85^{a}	0.058	$y = 1.98$	-
Mg, cmol$_c$ dm^{-3}	0.650^{a}	0.625^{a}	0.700^{a}	0.825^{a}	0.800^{a}	0.650^{a}	0.028	$y = 0.620 + 0.001x$	0.202
S, mg dm^{-3}	3.50^{a}	3.75^{a}	3.50^{a}	3.75^{a}	3.50^{a}	4.00^{a}	0.115	$y = 3.60$	-
Na, mg dm^{-3}	2.00^{a}	1.50^{a}	2.25^{a}	2.00^{a}	1.75^{a}	2.75^{a}	0.175	$y = 2.21$	-
B, mg dm^{-3}	0.250^{a}	0.200^{a}	0.200^{a}	0.250^{a}	0.125^{a}	0.175^{a}	0.015	$y = 0.245$	-

Cu, mg dm^{-3}	1.27^a	1.15^a	1.67^a	1.25^a	0.975^a	1.20^a	0.100	$y = 1.36$	-
Fe, mg dm^{-3}	19.57^{bc}	21.5^b	24.5^{ab}	25.32^a	25.75^a	18.75^c	0.721	$y = 20.09 + 0.010x$	0.979
Mn, mg dm^{-3}	35.25^a	35.25^a	38.25^a	33.50^a	39.75^a	34.75^a	0.944	$y = 34.95 + 0.004x$	0.466
Zn, mg dm^{-3}	0.400^a	0.425^a	0.600^a	0.525^a	0.525^a	0.475^a	0.031^a	$y = 0.425$	-

CF= chemical fertilization ;with 80 kg ha^{-1} P_2O_5; y= observed value; x= blood waste dose (0 m^3 ha^{-1}, 150 m^3 ha^{-1}, 300 m^3 ha^{-1}, 450 m^3 ha^{-1}, 600 m^3 ha^{-1}). OM= organic matter; pH in $CaCl_2$= active acidity; CEC= cation exchange capacity; H+Al= potential acidity; P= phosphorus; K= Potassium; Ca= calcium; Mg= magnesium; S= sulfur; B= Boron; Cu= copper; Fe= iron; Mn= manganese; Zn= zinc; R^2= determination coefficient; SEM= standard error of the mean.

Means followed by the same lowercase letters in the rows, did not differ from each other at 5% probability level.