Australian Journal of Crop Science

AJCS

AJCS 13(02):294-299 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.02.p1459

Application of slaughterhouse residues as nitrogen source replacing commercial fertilizers on mombasa grass (*Megathyrsus maximus*)

Rubens Ribeiro da Silva[1], Rubson da Costa Leite[2*], Jefferson Santana da Silva Carneiro[3], Gilson Araújo de Freitas[1], Antônio Carlos Martins dos Santos[1], Antonio Clementino dos Santos[2], Luiz Alberto Kuyumjian[1]

[1]Federal University of Tocantins, Department of Plant Production, Gurupi-TO, Brasil
[2]Federal University of Tocantins, Department of Tropical Animal Science, Araguaína-TO, Brasil
[3]Federal University of Lavras, Department of Soil and Plant Nutrition, Lavras-MG, Brasil

***Corresponding author: rubsonif@gmail.com**

Abstract

We hypothesized that the use of the residues of hull and horn flour and blood flour from the cattle slaughtering industry could provide a similar effect of nitrogen fertilizers when applied in pastures. For this reason, the objective was to evaluate the agronomic performance of mombasa grass fertilized with alternative nitrogen sources. A randomized block design in a 3 x 4 + 1 factorial scheme was used with four replications. Three sources of nitrogen were studied (hoof and horn flour, blood flour and urea) and four nitrogen doses (100, 200, 300 and 400 kg ha^{-1} N), plus a control with no nitrogen application. The experimental units consisted of plastic pots with a capacity of 5.0 dm^3, and 4.0 dm^3 of soil was used. The application of the alternative sources - hull and horn flour and blood flour - promoted an increase in attributes such as leaf area, number of tillers, crude protein and forage mass. The organic sources have the potential to be applied as nitrogen sources in soils under pasture, which would promote an eco-friendly destination for these residues.

Keywords: Alternative sources, blood flour from cattle, hoof and horn flour, meatpackers, tropical forage.
Abbreviations: AEN_agronomic efficiency of nitrogen, BF_blood flour, CC_chlorophyll content, CP_crude protein, FM_forage mass, HHF_hoof and horn flour, LA leaf area and NT number of tillers.

Introduction

The world cattle herd in 2016 was 1.4 billion head, and, only in Brazil, nearly 27 million animals were slaughtered. The estimates show that by 2026 this number could reach 48 million animals (ABIEC, 2017). Thus, the high rate of cattle slaughter in the country results in great amounts of generated residues (Pereira et al., 2016), which can cause environmental impacts if not properly destined.

Meatpackers and slaughterhouses are responsible for the processing and treatment of residues caused by animal slaughter, with the responsibility of avoiding incorrect disposal in the environment (Demattê et al., 2016). Transforming these residues into value-added materials such as fertilizers, specifically for pastures, allows the return of part of the nutrients extracted by cattle slaughter process to the soil.

Several studies have reported the application of alternative sources of commercial fertilizers (Demattê et al., 2016; Zwetsloot et al., 2014; Carneiro et al., 2017), and as soil corrective agents (Freitas et al., 2015). Studies showed that the use of by-products from the refrigeration industry reduces environmental impacts, promotes economic gains, maintains fertility levels, increases productivity and improves soil chemical and physical properties (Araújo et al., 2011; Carneiro et al., 2017).

Residues from slaughterhouses have large amounts of nitrogen (Bonchenari et al., 2017) and high concentration of biodegradable organic matter (Amanatidou et al., 2015). Therefore, it was hypothesized that the use of hoof and horn flour (HHF) and blood flour (BF) residues from the cattle slaughtering industry could provide a similar effect of a nitrogen fertilizer when applied to pasture.

In Brazilian soils, nitrogen fertilization is necessary, which increases production costs (Canto et al., 2016), has a short-term effect (Hungary et al., 2016) and its majority comes from fossil fuels (Morais et al., 2012). The use of the HHF and BF residues generated by the cattle production industry could reduce the use of commercial fertilizer and provide an appropriate destination for these wastes. Thus, the objective of this study was to evaluate the agronomic performance of Mombasa grass fertilized with alternative nitrogen sources.

Results

Response of variables to interaction between factors

The results of the analysis of variance showed an effect of the nitrogen sources for the following variables: leaf area (LA), number of tillers (NT), crude protein (CP), agronomic efficiency of nitrogen (ANE) and forage mass (FM) (Fig. 1). Regarding the interaction between sources and nitrogen doses, only the chlorophyll content (CC) did not show any kind of interaction (Fig. 1C).

Leaf area (LA) did not present difference between the sources applied at doses of 100 and 400 kg ha^{-1} of N. However, in the intermediate doses, there was an increase as a function of the supplied source (Fig. 1A). At the dose of 200 kg ha^{-1} of N, urea presented a 28% reduction in LA when compared to and horn flour (HHF) and 25% in relation to blood flour (BF). At this dose, the HHF and BF sources presented similar behavior.

At the dose 300 kg ha-^{1} of N, BF presented a similar result of HHF, which was higher than urea. When compared to urea, BF promoted a 19% increase in plant LA. By comparing BF with the control treatment (without nitrogen), the increase in LA was 53%.

For numbers of tillers (NT) at the dose of 100 kg ha^{-1} of N, urea was higher than BF and similar HHF (Fig. 1B). The urea source promoted 36% of NT increments when compared to plants fertilized with BF. The BF source promoted NT similar to plants that did not receive nitrogen (19 tillers).

At the dose 200 kg ha^{-1} of N, urea was higher than the HHF and BF sources, promoting increases of 45 and 52%, respectively. This behavior was observed for the doses 300 and 400 kg ha^{-1}, reaching an increase of up to 58% in the dose of 400 kg ha^{-1} of N with urea compared to HHF.

For the chlorophyll content (CC), there was no difference between the evaluated nitrogen sources (Fig. 1C). Regardless of the source evaluated, the plants presented a CC of 26 ICF, which was 13% higher than the control plants.

The crude protein (CP) at the dose of 100 kg ha^{-1} of N was higher for HHF than for the BF and urea sources, which promoted increases of 13 and 7%, respectively (Fig. 1D). When compared to the control treatment, the HHF increment in CP was 24%.

At the dose 200 kg ha^{-1} of N, there was no difference between the evaluated sources, producing an average CP of 8.85%. In the dose 300 kg ha^{-1} of N, the BF source was higher than HHF (increase of 16% in CP) and was similar the urea (mean CP of 10%).

At the dose 400 kg ha^{-1} of N, the plants that received cover urea application presented higher CP value than the plants that received the HHF and BF sources (increase in CP of 34 and 15%, respectively). The BF source presented the second highest evaluated CP value, with a value 16% higher than the HHF source.

For the agronomic efficiency of nitrogen (AEN) at the dose 100 kg ha^{-1} of N, urea was higher than HHF (up to 71%) and like BF (Fig. 1E). The HHF source, although inferior to urea, was similar to BF.

At the dose 200 kg ha^{-1} of N, urea remained higher than HHF and similar to BF; and the BF source differed from HHF, presenting an increase of 152%. With the reduction in AEN values (Fig. 1E) as a function of the increase in N doses (300 and 400 kg ha^{-1}), the sources presented similar AEN values, varying from 1.49 to 2.75. Regarding the forage mass (FM) of the mombasa grass plants at the dose 100 kg ha^{-1} of N, the HHF source was higher than BF and similar to urea (Fig. 1F). The HHF source presented FM 70% higher than BF, while urea produced FM 44% higher than BF.

At the doses 200 kg ha^{-1} and 300 kg ha^{-1} of N, the plants presented similar production, independently of the used nitrogen source. For the 200 kg ha^{-1} of N dose, the mean FM of the sources was 3.7 g pot^{-1}. At the dose 300 kg ha^{-1} of N, the mean FM value was 4.6 g pot^{-1}.

For the dose 400 kg ha^{-1} of N, the urea source was higher than the alternative tested sources (Fig. 1F). When compared to HHF, the increment with urea use was 31% in FM; in relation to BF this increase corresponded to 87%. The alternative sources did not show any difference at this dose.

Response to Nitrogen doses

Regarding the nitrogen sources at the applied doses, it was possible to determine the appropriate dose for each evaluated source (Fig. 2). For the LA variable, the HHF source presented an adjustment to the linear positive model as a function of the applied doses (Fig. 2A), with plants producing LA of 0.06 cm^2 to each kilo of applied nitrogen, with a production of 99.3 cm^2 at the dose 400 kg ha^{-1}. The BF source had maximum LA production (103.5 cm^2) at the maximum efficiency dose of 287 kg ha^{-1} of N. The urea source had no adjustment to the regression model, with mean LA of 92.4 cm^2.

Comparing the LA of the applied sources to the plant production with no nitrogen application, there was an increase of 39, 45 and 30% for HHF, BF and urea, respectively.

The number of tillers presented a linear positive fit for all evaluated sources (Fig. 2B). With the use of HHF, the plants presented an increase of 0.05 tiller pot^{-1} to each kilo of applied nitrogen, reaching 35 tillers pot^{-1} at the dose of 400 kg ha^{-1} of N. The BF source promoted an increase of 0.07 tiller pot^{-1} per kilo of N applied, with the production of 46 tillers pot^{-1} in the highest evaluated dose (400 kg ha^{-1}). Urea promoted increased LA of 0.1 tiller pot^{-1} per kg of N applied, with the production of 65 tillers pot^{-1} at the dose of 400 kg ha^{-1}.

The chlorophyll content presented adjustment to the linear positive model for all the evaluated sources (Fig. 2C). The plants presented values of 27, 32 and 35 ICF for HHF, BF and urea, respectively.

When comparing to the absence of nitrogen fertilization (control plants), plants presented an increase of 13, 34 and 47% for HHF, BF and urea, respectively.

For crude protein, only the HHF source presented no adjustment to the regression model (Fig. 2C), with a mean of 8.8%. BF provided an increase of 0.008% in crude protein to each kilo of N applied, yielding plants with 10.8% protein at the maximum evaluated dose (400 kg ha^{-1}). The urea source produced 11.4% protein with the highest applied dose.

The use of nitrogen sources, when compared to the absence of nitrogen fertilization, allowed an increase of 41 and 48% for BF and urea, respectively, at the dose of 400 kg ha^{-1}.

For the agronomic efficiency of nitrogen, the HHF source presented no adjustment to the regression model, with a mean of 2.25 (Fig. 2E). However, the BF and urea sources presented an adjustment to the negative linear model, with a reduction of 0.02 and 0.03 in the AEN value to each kilogram of applied N, respectively.

Regarding forage mass, the HHF and urea sources presented an adjustment to the positive linear model, whereas the BF source did not fit the model, with an average value of 2.4 grams pot^{-1} of FM (Fig. 2F).

Table 1. Chemical and physical characterization of the soil used in the experiment.

pH	OM(1)	P	K	Ca	Mg	Al	H+Al	SB(2)	CEC(3)	V%(4)	m%	Sand	Silt	Clay
$CaCl_2$	g/dm³	Mg/dm³		cmolc/dm³							(%)			
5.9	1.9	4.3	21.5	2.0	0.6	0.0	2.9	2.6	5.5	47.3	0.0	69.5	7.3	23.2

(1) OM: Organic matter; (2) SB: Sum of Bases; (3) CEC: Cation exchange capacity at pH 7.0; (4) V%: Base Saturation.

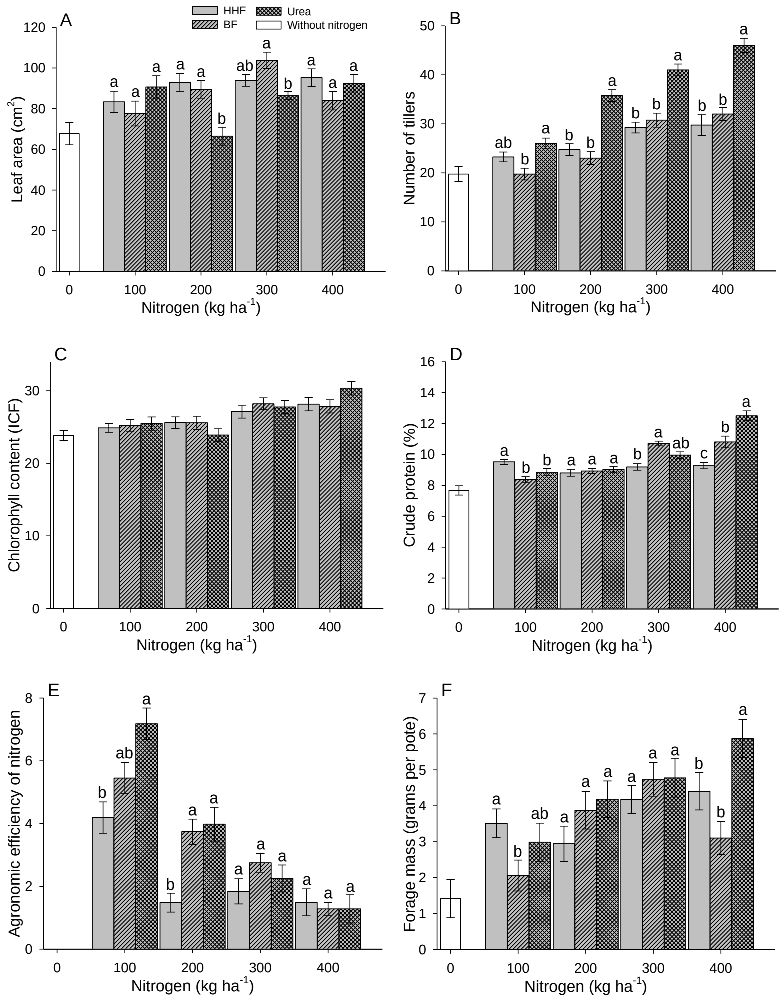

Fig 1. Leaf area (A), number of tillers (B), chlorophyll content (C), crude protein (D), agronomic efficiency of nitrogen (E) and forage mass (F) of mombasa grass plants in relation to nitrogen doses with the following sources: hoof and horn flour (HHF), blood flour (BF) and urea; Averages followed by the same letter at the doses did not significantly differ by the Tukey test (p≤0.05).

Table 2. Chemical and physical characterization of the studied nitrogen sources.

Nutrients		HHF	BF	Urea
N		124.5	139.0	450.0
P	$g\ kg^{-1}$	2.6	2.5	_
K		35.0	6.6	_
Ca		2.6	8.7	_
Mg		0.6	0.2	_
S		2.4	_	_
Zn		115.0	20.0	_
Mn	$mg\ kg^{-1}$	23.0	_	_
Fe		731.0	930.0	_
Cu		12.0	20.0	_

HHF: hoof and horn flour; BF: blood flour.

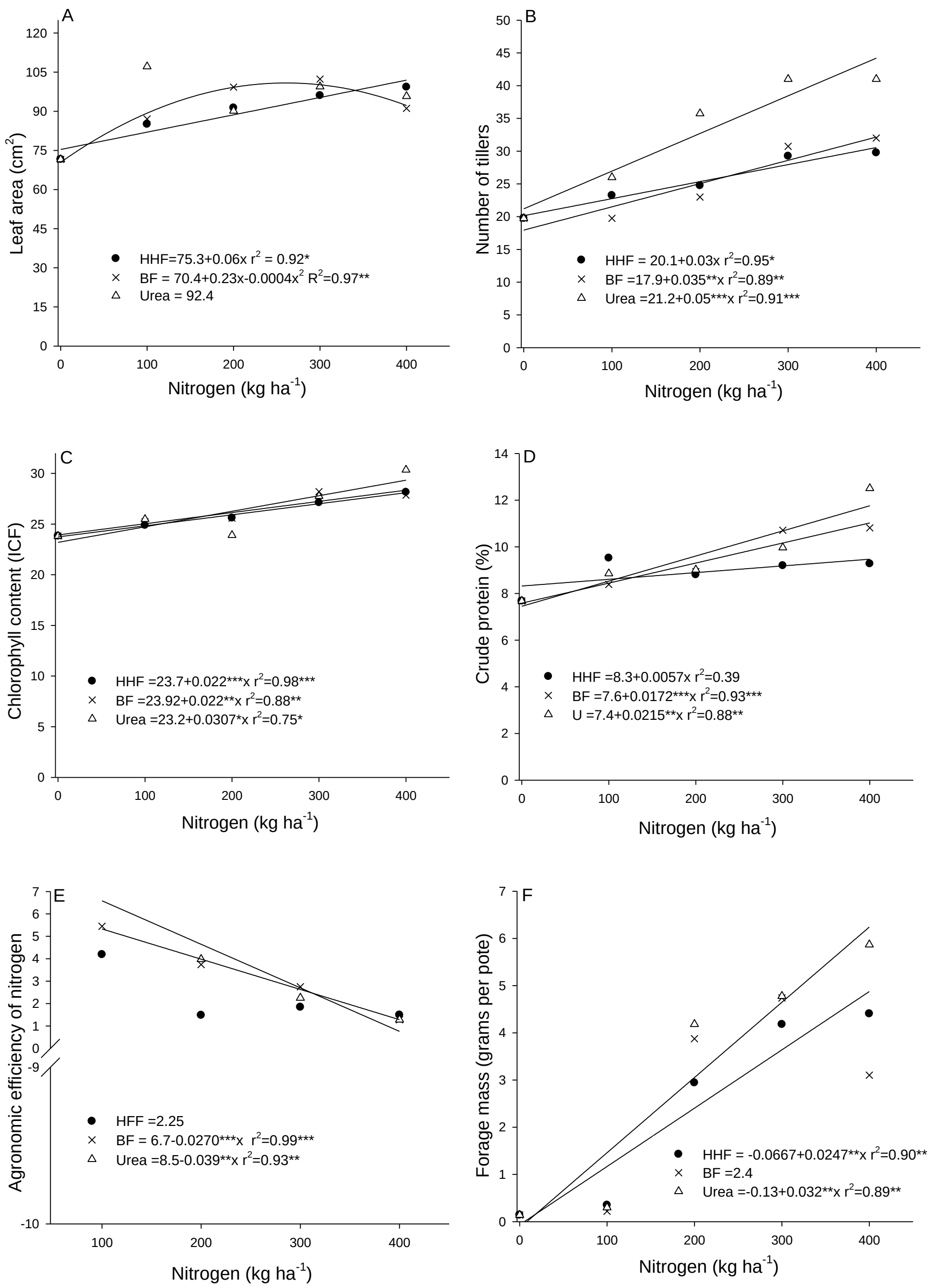

Fig 2. Leaf area (A), number of tillers (B), chlorophyll content (C), crude protein (D), agronomic efficiency of nitrogen (E) and forage mass (F) of mombasa grass plants in relation to nitrogen fertilization with the following sources: hoof and horn flour (HHF), blood flour (BF) and urea.

With the use of the HHF source, an increase of 0.012 g pot^{-1} was added to each kilo of N applied, producing plants with FM of 4.74 grams of pot^{-1} at the dose of 400 kg ha^{-1}. Urea presented a behavior similar to HHF, yielding 0.015 g pot^{-1} to each kilo of applied N, generating plants with 5.9 g pot^{-1} at the highest evaluated dose (400 kg ha^{-1}).

Discussion

Studies have proven the influence of the nutritional status of the forage plant on tillering and other morphological attributes, such as leaf area (Lavres Jr and Monteiro, 2003).

The results showed that the alternative sources HHF and BF positively influenced the evaluated attributes in mombasa grass plants. Additionally, Demattê et al. (2016), show that there is a significant nutrient (C, N, P, Ca, Mg, K, Na, and S) increase.

The increase of mass in the cultivated area depends on the development of leaf area (Pedreira and Pedreira, 2007), by applying urea and the alternative sources (HHF and BF), it was possible to promote higher LA for the mombasa grass plants when compared to the control plants that did not receive any nitrogen.

According to the regression graph for the number of tillers (Fig. 2B), regardless of the nitrogen source, there was an increase in the number of tillers as the nitrogen dose increased. These results demonstrate the efficiency of the alternative sources for this variable and corroborate with the results found by Benett et al. (2008), who mentioned that nitrogen is one of the main nutrients to provide better tillering and plant production.

Organic fertilizers, such as the studied sources, when compared to mineral fertilizers, such as urea, have a slower mineralization rate. However, they imply a longer effect of constant availability of the nutrients to the plant, favoring its development over several cycles (Silva et al*.,* 2013).

Practical solutions for soil conservation and maintenance or improved crop productivity are needed to ensure sustainable food production (Ranaivoson et al., 2017). It is estimated that the Brazilian cattle production is mostly derived from pasture production (Carneiro et al., 2017). With these results, it was possible to increase the production of mombasa grass plants (Fig. 1), which was similar to the use of nitrogen fertilizer, promoting a suitable and sustainable destination for wastes with low or no economic value (eg.: HHF and BF). For Demattê et al. (2016), if properly used, slaughterhouse residues have potential to be used as fertilizers due to their considerable amounts of nutrients.

Considering that the standard fertilization in mombasa grass is equivalent to 50 kg ha^{-1} of nitrogen (Sousa and Lobato, 2004), with the use of 79 kg ha^{-1} of N, through the HHF source, it was possible to obtain similar production when compared to standard fertilization using urea (Fig. 2F). Among the main problems associated with the use of organic sources, such as slaughterhouse residues, as substitutes of commercial fertilizers applied to crops is the need for high dosages of residues (Nunes et al., 2015), usually occurring in tons. Due to the high N content of the alternative HHF source (12.4%), high dosage was not required to obtain a productivity result similar to urea, making it feasible for soil application.

Considering conservative estimates that 1.0 kg of N equals to 4.5 kg of CO_2_equivalents (Hungria et al. 2013), the substitution of standard fertilizers by one of the tested sources tested (Fig. 2F) could avoid the emission of 225 kg ha^{-1} CO_2_equivalents.

According to Dias_Filho (2014), degraded pasture is an area that drastically reduced its productive capacity, with the accumulation of biomass with ideal indicator for forage. Moreira et al. (2009) adds that the low availability of nitrogen to the forage entails a reduction in the production of forage, initiating the process of degradation. With the use of alternative sources (HHF and BF) in comparison to the non_use of nitrogen fertilization, there was an increase in the production of the forage grass (Fig. 2F), which could mitigate the degradation of pastures and promote the conservation of the environment.

Materials and methods

Location, Climate and characterization of soil

The study was carried out in an experimental area of the Federal University of Tocantins (UFT), at the coordinates 11º44'44'' S and 49º 03'04'' W, in Gurupi. The region is classified as Brazilian Cerrado, and presents a Aw climate (hot and humid), according to the Köppen International Classification (Alvarez et al., 2013).

The research was carried out under the greenhouse (4 m wide x 20 m long) covered with transparent plastic of 150 microns and with shadow sides (50% retention capacity of the solar radiation). The experimental units consisted of plastic vessels with a capacity of 5.0 dm^3 and 4.0 dm^3 of soil was used. The soil used to fill the pots was an Oxisol (Soil Taxonomy) with Sandy Clay Loam texture (Table 1).

After soil characterization, soil acidity and fertility were corrected according to the crop demand (Sousa e Lobato 2004). The soil was incubated for 30 days at 70% of the field capacity. A randomized block design in a 4x3 + 1 factorial scheme with four replications was used. Four doses of nitrogen fertilization were studied (100, 200, 300 e 400 kg ha^{-1} de N) and three sources of nitrogen (Hull and horn flour, blood meal and Urea), plus a control without nitrogen fertilization. The HHF and BF residues were obtained from a slaughterhouse (COOPERFRIGU) of Gurupi _ TO, resulting from the slaughter of 600 animals. The chemical and physical characterization of all used nitrogen sources was performed (Table 2).

The studied culture was the forage *Megathyrsus maximus* cv. mombasa. The seeds presented a purity of 62% and germination of 82%, with a cultural value of 50%. Sowing was performed at the 1.0_cm depth in the proportion of 2.0 g pot^{-1} of seeds. After ten days of emergency (DAE), plants were thinned to leave seven vigorous and well distributed plants in the surface area of the pot. The application of the nitrogen sources was divided in three times during the four harvests.

Analyzed variables

The following variables were evaluated: leaf area, number of tillers, chlorophyll content, crude protein, agronomic efficiency of nitrogen and forage mass.

The leaf area (LA) was calculated from the measurement of the height x width of the leaf in cm, considering six leaves per pot. The average data was based on the formula proposed by Benincasa (2003):

$$\mathrm{LA} = \mathrm{H} \times \mathrm{W} \times 0.905$$

Where: H = mean height; W = mean width.

The number of tillers (NT) was obtained by simple counting. The forage was harvested at a height of 30 cm from the soil level in the pot. After the cut, the material was packed in paper bags and oven dried at 60 ºC for 72 hours to obtain the forage mass (FM). The forage samples, after pre_dried and ground in a 1 mm sieve, were subjected to sulfur digestion, distillation (Kjeldahl) and titration for the determination of the crude protein (CP). The chlorophyll content (CC) was determined using a portable digital chlorophyll meter (model CFL_1030). The agronomic efficiency of nitrogen (AEN) was calculated by formula:

$$\mathrm{AEN} = (\mathrm{PD} - \mathrm{PA} \div \mathrm{ND} - 0\mathrm{D}) \times 100$$

Where: PD: production in doses; PA: production in the absence of nitrogen; ND: N applied dose; 0D: Dose zero.

Statistical analysis

Initially, the data were tested for normality (Shapiro_Wilk) and homoscedasticity. The F test was applied for the qualitative data (nitrogen sources), and when significant, the Tukey test was applied ($p \leq 0.05$). The quantitative data (nitrogen doses) were submitted to regression analysis, evaluating the significance of betas and the determination coefficients to obtain the appropriate regression model ($p \leq 0.05$).

Conclusion

The application of the alternative sources _ hull and horn flour and blood flour _ promoted an increase in the following attributes: leaf area, number of tillers, crude protein and forage mass.

The organic sources have potential to be applied as nitrogen fertilizers in soil under pasture, promoting an environmentally adequate destination for the wastes.

The use of slaughterhouse residues promoted mitigation in the use of nitrogen fertilizer.

The use of alternative sources reduced the CO_2_eq emissions by not using urea, without productivity loss.

Acknowledgements

The authors gratefully thank the Federal University of Tocantins (UFT) and the research group on organic waste (NERO) of the UFT.

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