SOLID INDUSTRIAL GELATIN WASTE AS A SOURCE OF NITROGEN FERTILIZATION IN MASSAI GRASS

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ABSTRACT

This study aimed to evaluate the effect of gelatin sludge as a source of nitrogen fertilization in Massai grass. The experiment was developed in the greenhouse of the Federal University of Mato Grosso, with three doses of sludge (DGS: 0, 200, 300 m^3 ha^{-1}), an organomineral dose and an of mineral fertilizer and 6 repetitions. Sowing was performed directly in pots. The uniformity cut occurred 30 days after sowing. The variables evaluated were: number of tillers, number of leaves, plant and stem height, chlorophyll index, dry mass (leaves, roots and residue), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), macronutrients and micronutrients contents. Root mass and SPAD value were not influenced by the doses of sludge compared to mineral fertilizer. There was no difference (P>0.05) in the contents of NDF, ADF and iNDF in all treatments. The organomineral fertilizer, recorded the highest number of tillers. The dose of 300 m^3 ha^{-1}, promoted the highest number of green leaves and leaves per tiller. Leaf dry mass and dry mass production were influenced (P<0.05) by the nitrogen fertilization. The average contents of K, B, Cu, Fe, Mn and Zn in the leaf tissue, were not affected (P>0.05). Different doses of gelatin sludge alter the structural characteristics, nutritive value and yield of Massai grass. Besides influencing the stem height, number of leaves and chlorophyll index, being more responsive the dose of 300 m^3 ha^{-1}.

Key words: Forage; *Megathyrsus maximus*; Nitrogen; Sludge.

RESÍDUO INDÚSTRIAL SOLIDO DE GELATINA COMO FONTE DE ADUBAÇÃO NITROGENADA EM CAPIM MASSAI

RESUMO

Objetivou- se avaliar o efeito do lodo de gelatina como fonte de adubação nitrogenada em capim Massai. O experimento foi desenvolvido em casa de vegetação da Universidade Federal de Mato Grosso, com três doses de lodo (DLG: 0, 200, 300 m^3 ha^{-1}), uma dose organomineral e uma de adubo mineral e 6 repetições. A semeadura foi realizada diretamente em vasos. O corte de uniformização ocorreu 30 dias após a semeadura. As variáveis avaliadas foram: número de perfilhos, de folhas, altura de planta, de colmo, índice de clorofila, massa seca (folhas, raízes e resíduo), teores de proteína bruta (PB), fibra em detergente neutro (FDN) e ácido (FDA), macronutrientes e micronutrientes. A massa de raiz e o valor SPAD não foram influenciados pelas doses de lodo em relação ao adubo mineral. Não houve diferença (P>0,05) nos teores de FDN, FDA e FDNi em todos os tratamentos. A adubação organomineral, registrou o maior número de perfilhos. A dose de 300 m^3 ha^{-1}, promoveu maior número de folhas verdes e folhas por perfilho. A massa seca da folha e produção de massa seca foram influenciadas (P<0,05) pela adubação nitrogenada. Os teores médios de K, B, Cu, Fe, Mn e Zn no tecido foliar, não foram afetados (P>0,05). Diferentes doses de lodo de gelatina alteram as características estruturais, o valor nutritivo e a produção do capim Massai. Além de influenciar na altura de colmo, número de folhas, e índice de clorofila, sendo mais responsiva a dose de 300 m^3 ha^{-1}.

Palavras chave: Forrageira; Lodo; *Megathyrsus maximus*; Nitrogênio.

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INTRODUCTION

The sludge, from the gelatin industry, is obtained from the leather and bones of cattle, considered an organic waste, with a high content of nitrogen (N) that could be applied in pasture areas, as an alternative fertilization, in order to increase soil fertility, which translates into increased forage productivity.

Brazil is considered the second country with the largest cattle herd in the world. Currently, it has 224,602,112 head, with livestock being one of the most important pillars in the country's economy (IBGE, 2021). However, there is also a growing increase in activities, especially those related to industry, generating large amounts of solid and liquid waste that could be pollutants of the environment and wastewater.

Until 2005, Brazil produced about 33 thousand tons of gelatin per year, generating 198 thousand tons of sludge (REIMANN, 2005). Currently, in Acorizal - MT, PB LEINER Brazil (Gelatin Industry and Trade) produces 5,520 tons of gelatin per year, being the leading company in the gelatin and hydrolysates market (EMPAER, 2019). Thus, a large amount of waste is available for use as fertilizer in agricultural soils, considering that globally, the application of nitrogen fertilizers has increased rapidly and is expected to increase four to five times by 2050, with two-thirds of this application in developing countries (NADARAJAN; SUKUMARAN, 2021).

According to Wang et al. (2016) and Guimarães et al. (2012), gelatin industry sludge (GIS) contains nutrients for plants, which can improve soil fertility and crop productivity, presented from the physicochemical analyses made, high levels of organic matter, pH, buffering capacity, N and P, while low concentrations of heavy metals and other organic pollutants, which becomes a possibility of use as fertilizer in agricultural areas.

However, despite the lack of research results, it is hoped that biological sludge can also be used for agriculture. Although, the viability of this sludge needs to be evaluated to define appropriate agronomic and environmental criteria for its application (GUIMARÃES et al., 2012), considering that the effects of alternative practices can be long-term and that depend on the management of pastures and the doses used so that they can reflect on forage productivity.

However, the fertilization of pastures represents a great investment for the cattle rancher and, therefore, understanding the dynamics of pasture growth in response to fertilization for the rational use of fertilizers becomes important to obtain high productivity. Therefore, it requires paying attention to determine the influence exerted by nitrogen fertilization or fertilization with N, P and K on forage productivity (RIBELATTO et al., 2019). Thus, the aimed of this study was to evaluate the effect of gelatin sludge as a source of nitrogen fertilization in Massai grass.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse of the Federal University of Mato Grosso (UFMT). The study consisted of the establishment of the forage specie, *Megathyrsus maximus* (Syn. *Panicum* sp. cv. Massai), with three doses of sludge from the gelatin industry (DGS: 0; 200 and 300 m^3 ha^{-1}), a dose of organomineral fertilizer (FOM) and a dose of mineral fertilizer (MiF) with 6 replications. Pots with a capacity of 6 dm^{-3} filled with Red-Dystrophic Latosol, clayey texture, were used.

Soil collection

In the field, an area with no history of use for agricultural crops was defined for sampling in the layer from 0 to 20 cm deep, in an area belonging to the Federal Institute of Mato Grosso (IFMT), in the municipality of São Vicente – MT.

Soon after, the analysis of the chemical and granulometric composition of the soil was made (TEIXEIRA et al., 2017), having the following characteristics: pH (CaCl2) = 4.2; O.M (g kg^{-1}) = 22.7; P (mg dm^{-3}) = 1; K (mg dm^{-3}) = 14.1; Ca (cml_c dm^{-3}) = 0.65; Mg (cml_c dm^{-3}) = 0.33; H + Al (cml_c dm^{-3}) = 5.3; Al (cml_c dm^{-3}) = 0.6; SB (cml_c dm^{-3}) = 1.02; V (%) = 16.14; S (mg dm^{-3}) = 6.1; B (mg dm^{-3}) = 0.18; Cu (mg dm^{-3}) = 0.8; Fe (mg dm^{-3}) = 42; Mn (mg dm^{-3}) = 4.5; Zn (mg dm^{-3}) = 0.5; Clay (g kg^{-1}) = 534; Silt (g kg^{-1}) = 143; Sand (g kg^{-1}) =323.

Soil incubation with sludge

The gelatin sludge (GS) used came from the PB LEINER industry, located in the municipality of Acorizal (MT), being used as a source of nitrogen fertilization, in addition to other minerals (Table 1).

Table 1. Chemical characterization of gelatin sludge

pH $CaCl_2$	**N-total**	**Ca**	**Mg**	**COM$^{(1)}$**	**HUM$^{(2)}$**	**OC$^{(3)}$**	**P- Total**	**K**	**S**
					%				
8.10	1.94	4.55	0.09	4.97	73.76	2.88	1.35	0.20	0.62
Fe	**Mn**	**Cu**	**Zn**	**B**	**Na**	**Ni**	**Cr**	**Cd**	**CTC**
		(mg kg^{-1})			%		(mg kg^{-1})		($mmol_c$ kg^{-1})
4775.86	84.97	47.61	104.01	154.15	0.09	0.01	9.98	<LQ	250

$^{(1)}$ Compostable organic matter; $^{(2)}$ Humidity; $^{(3)}$ Organic carbon.

Sludge rates were calculated based on vessel area (A= π x r^2) and sludge density (1.00 g cm^3). The soil was incubated in the 5 cm layer with the doses of gelatin sludge (DSGI), respective to each treatment, then they were placed in plastic canvas and mixed, reacting for a period of 20 days, keeping the humidity at 60% of its field capacity.

After the incubation period, a sample of 300g of each dose was collected for soil analysis to later determine the need for limestone (Table 2 and 3). Then the 5 cm of soil were placed, previously incubated to complete with the rest of the soil in the pots.

The liming was determined by the base saturation method (V) to reach the value of 50% with the incorporation of dolomitic limestone (PRNT= 90%; CaO: 28% and MgO: 16%), with the exception of treatments with sludge doses of 300 m^3 ha^{-1} that reached the BS value after the incubation period (soil and sludge) when compared to the grass requirement (SOUZA; LOBATO, 2004). The incubation of the soil with the limestone reacted for 15 days and kept the moisture at the maximum capacity of water retention in the soil.

Table 2**.** Chemical and granulometric characterization of the soil after incubation of gelatin sludge (DGS).

Attributes	**Levels DGS (m^3 ha^{-1})**			*** Classification**
	100	**200**	**300**	
pH ($CaCl_2$)	4.8	5.00	5.40	High acidity (100, 200), Medium (300)
OM (g kg^{-1})	12.90	13.50	13.40	Very Good
SB (cml_c dm^{-3})	1.88	2.17	2.77	Medium
T (cml_c dm^{-3})	4.73	4.84	4.79	Good
V (%)	39.75	44.83	57.83	Low (100), Medium (200), Good (300)
Clay (g kg^{-1})	554	578	584	-
Silt (g kg^{-1})	156	149	160	-
Sand (g kg^{-1})	290	273	256	-

pH: Hydrogenionic potential; SB: sum of bases; T: cation exchange capacity; V: base saturation; OM: organic matter. *(RIBEIRO et al., 1999).

Table 3. Soil macro and micronutrient contents after gelatin sludge incubation.

Attributes	Levels DGS (m^3 ha^{-1})			* Classification
	100	200	300	
Macronutrients				
P (mg dm^{-3})	2.70	3.40	2.30	Very Low
K (mg dm^{-3})	29.30	34.30	28.30	Low
Ca (cml_c dm^{-3})	1.30	1.50	1.95	Medium
Mg (cml_c dm^{-3})	0.50	0.58	0.75	Medium
S (mg dm^{-3})	14.40	15.20	16.50	Medium
Micronutrients (mg dm^{-3})				
Fe	102.00	61.00	64.00	High
Mn	3.70	2.90	4.1	Low
Zn	0.20	0.30	0.7	Very Low (100,200) Low (300)
Cu	1.30	1.20	1.00	Good (100), Medium (200, 300)
B	0.36	0.39	0.44	Medium

*(RIBEIRO et al., 1999).

Analysis of soil chemical attributes after soil and sludge incubation

The routine chemical analysis followed the procedure of Teixeira et al. (2017), performing a soil sampling at the beginning (before sowing, Table 3 and 4) and at the end of the experiment for determination of pH, OM, sum of bases (SB), cation exchange capacity (CEC), saturation per base and the contents of P, K, Ca, Mg, S, Fe, Mn, Zn, B and Cu.

Sowing of Massai grass

After the soil incubation period, sowing was carried out directly in the pots. Then, the implantation fertilization was made; applying 300 mg dm^{-3} of phosphorus (simple superphosphate: 18% P_2O_5) (CABRAL et al., 2018; BONFIM-SILVA; MONTEIRO, 2010). After eight days of emergence, the pre-thinning was carried out (LOPES et al., 2014), remaining ten plants per pot, which were reduced to five after the final thinning, carried out fifteen days after emergence, the criterion being the uniformity and vigor of the plants.

Next, nitrogen and potassium fertilization were performed for the treatments with mineral fertilizer at doses of 50 and 20 mg dm^{-3}, respectively, in the form of ammonium sulfate (20% N) and potassium chloride (58% K_2O). For the dose of 0 m^3 ha^{-1} (without GIS application) only potassium fertilization was performed. For the doses of 100, 200 and 300 m^3 ha^{-1} it was not necessary to apply K_2O because the gelatin sludge met the need for K.

Uniform cutting

Twenty days after final thinning, the uniform cut was made at 15 cm from the residue height and the complementary nitrogen and potassium fertilization was performed (using the same sources) for the treatments with MiF with doses of 100 and 100 mg dm^{-3} of N and K respectively (TEIXEIRA et al., 2018; SILVA et al., 2016; CABRAL et al., 2018), and potassium (100 mg dm^{-3}) for doses of 0; 100; 200 and 300 m^3 ha^{-1}, applied in dilution with water.

Organomineral fertilization (FOM)

About 15 days after the cutting of uniformization, the gelatin sludge was reapplied in the respective doses for each treatment, uniformizing the plants again, initiating in this period the experimental phase of the cuts of use of the grass. After 15 days of sludge reapplication, complementary chemical fertilization was performed (Urea: 100 mg dm^{-3} and K_2O: 100 mg dm^{-3}), being necessary only for the dose of 100 300 m^3 ha^{-1} since it did not supply the amount of N that the plant needs for its development.

Gelatin sludge and mineral fertilization were reapplied after each cut (GALINDO et al., 2018; CABRAL et al., 2018) in the same doses. Irrigations were performed every two days, performing water control by individual weighing of the pots, keeping the soil at its field capacity.

Growing periods and grass cutting intervals

The grass was cultivated in three growing periods at intervals of 30 days. The first occurred 30 days after cutting the uniform and the next, 30 days after each cut (25 cm from the height of the residue).

In each pot, three tillers were randomly identified and marked with colored ribbons. On the day before each cut, the SPAD value (chlorophyll index) was determined in the leaves of the marked tillers, performing three measurements per pot on the newly expanded leaf blades (BONFIM-SILVA; MONTEIRO, 2010; JÚNIOR et al., 2008), using the portable chlorophyllometer SPAD-502 (Soil-Plant Analysis Development) to define the chlorophyll contents. The readings were taken in the central region of the leaf limb.

At the beginning of each period and at the end of the experiment, the tiller number (TIN) was counted and the plant height (PH) was measured per pot with the aid of a graduated ruler, considering the distance from the ground to the average height of the canopy from the base to the apex. Stem height (SH) was also measured. The number of green leaves (NGL) were quantified as the number of expanded and expanding leaves.

The number of leaves/tillers (NLTI) were estimated by the ratio of the number of leaves (NLE) by the TIN.

Production of dry mass of forage

The mass of the shoot was placed in paper bags, identified for weighing and later taken to the laboratory for separation of the fractions in leaf and stem + sheath, and quantified the fresh mass (FM), in addition to counting the number of leaves (NLE). These fractions were dried separately in a forced ventilation oven (55ºC for 72 h).

Then, the plant material was weighed on a precision scale and the dry mass (LDM) was quantified, considered the mass of the leaves (g pot^{-1}). The dry mass production (DMP) was estimated, considered as the accumulation of dry mass of the three cuts (BARNABÉ et al., 2007).

Leaf analysis, residue mass and roots

After drying and weighing, the samples of the plant material were crushed in a Willey mill with a 1.0 mm sieve and packed in plastic bags and stored in hermetic plastic pots, duly identified by each cut to later determine the contents of crude protein (CP), neutral detergent fiber (NDF) and acid (ADF) and indigestible (iNDF) (SILVA and QUEIROZ, 2004). While the nutrient contents (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn) in the leaf tissues were analyzed according to the method of Malavolta et al. (1997).

The last cut was made close to the soil to quantify the dry mass of residue (DMRes) and roots (DMRo). The roots were washed and separated by sieving into two 4 mm meshes to remove all the soil. Then the residue and the roots were separated, then placed in an oven for drying (same procedure for the leaves).

Experimental design and data analysis

The experimental design was completely randomized with five treatments, consisting of doses of organic fertilizer (three DGS: 0, 200 and 300 m^3 ha-[1]), a FOM and a MiF with 6 replications. The data obtained were submitted to analysis of variance and test of means (Tukey test at 5% probability).

RESULTS AND DISCUSSION

Productive characteristics and chlorophyll index

LDM and DMP were influenced ($P<0.05$) by nitrogen fertilization with gelatin sludge and other fertilization sources, with higher values in these variables in MiF and FOM treatments (Table 4). This is probably related to the higher NLE and NGL, promoted by N, increasing mass production in these variables. Martuscello et al. (2015) verified this same fact, as nitrogen fertilization was added to the same Massai grass.

Table 4. Leaf dry mass (LDM), residue (DMRes), root (DMRo) and total dry mass production (DMP) (g pot^{-1}) and chlorophyll index of Massai grass as a function of DGS, FOM and MiF.

	DGS (m^3 ha-[1])					
Variables	0	200	300	FOM	MiF	CV(%)
LDM	0.91 e	3.61 d	5.91 c	7.85 b	11.41 a	11.98
DMP	2.74 e	10.83 d	17.75 c	23.57 b	40.55 a	11.38
DMRes	13.57 c	42.62 b	64.22 b	96.66 a	97.82 a	25.11
DMRo	26.16 b	161.16 a	181.16 a	126.67 a	213.66 a	40.09
Chlorophyll index	17.17 c	27.52 ab	34.30 a	28.94 ab	26.66 b	16.17

Means followed by the same lowercase letter on the line do not differ from each other by Tukey's test at 5% probability

Other researchers, evaluating different nitrogen sources under the bromatological composition of *Brachiaria decumbens*, with swine wastewater and mineral fertilizer (using urea, simple superphosphate, and potassium chloride as a source), observed higher productivity with the mineral fertilizer, when compared with the doses of swine wastewater (SILVA et al., 2015).

However, Monteiro et al. (2014) studied doses of organic wastes (poultry litter: 4 g dm^{-3} and balloon powder: 10 g dm^{-3}) with levels of N (0, 50, 100, 200 mg dm^{-3} of urea, and of P_2O_5 (0, 100, 200, 300 mg dm^{-3}) in *P. maximum* cv. Mombasa, verified that doses of organic wastes with N levels significantly influenced ($p<0.05$) the forage mass production of this cultivar, providing higher production (22.81 g pot^{-1}) with the use of poultry litter.

In research of evaluation of the effects of nitrogen fertilization on forage production in Massai grass in a greenhouse, Costa et al. (2016) observed increases in dry matter production, which was increased by nitrogen fertilization whit doses of up to 160 mg N per kg of soil. Marques et al. (2016) also verified an increase in forage production, with the increasing application of N in the first and third cut with four levels of N application (0, 40, 80 and 120 mg dm^{-3}), during the evaluation of structural characteristics and bromatological composition in Massai grass.

The lack of N (DGS: 0 m^3 ha^{-1}) had effects on the different variables studied, providing the lowest values in the productive characteristics, directly influencing in the LDM and DMP, when compared to other treatments. However, the MiF presented the highest values in the same variables (Table 4). It is noteworthy that the stem mass was not determined, because the aerial part of the plant was constituted in greater proportion by the leaves.

In the residue mass (DMRes), there was no statistical difference (P>0.05) in the treatments with FOM and MiF, being higher when compared to the other treatments. There was also no difference between the DGS. The increase in DMRes may be associated with the higher TIN promoted by the availability of N and the particular characteristic of this cultivar. The Massai grass is one of the grasses that presents the highest number of tillers in relation to other cultivars of the same genus (COSTA et al., 2020).

The root mass (DMRo) and the SPAD value (chlorophyll index) were not influenced (P>0.05) by the fertilization sources (Table 4). However, MiF provided higher DMRo. Martuscello et al. (2019) also reported that root dry mass was not influenced by nitrogen fertilization when they evaluated forage production and morphogenic and structural characteristics of BRS Tamani grass.

The chlorophyll index was higher with DGS of 300 m^3 ha^{-1}. This higher value in the chlorophyll index promoted an increase in the intensity of the green color of the leaves. Similar results were recorded by Gonçalves et al. (2020) when evaluating the development and production of *P. maximum* cv Mombasa, as a function of N doses and inoculation with diazotrophic bacteria, in which the chlorophyll index had a rise with increasing doses, intensifying the color in the leaves.

However, Emereciano Neto et al. (2016) studying the use of organic fertilizers (poultry, sheep and pigs) and mineral (urea) in the structure and forage production of Massai grass, did not find significant differences between the nitrogen sources under the chlorophyll contents in the leaf blades. It is important to note that still, being the Massai grass of the present study, the chlorophyll contents were lower (19.43; 16.63; 17.28 and 22.99) with organic sources (poultry, sheep and pigs) and inorganic (urea) respectively, in comparison with this work. The chlorophyll index was higher with DGS, FOM and MiF, registering higher values with the dose of 300 m^3 ha^{-1} (Table 4).

According to Santos et al. (2022) plants with higher chlorophyll index have higher photosynthetic efficiency, providing higher production. This is due to the fact that the N, participates in the synthesis and structure of chlorophyll molecules, so that with the supply of this nutrient, plants can increase the content of chlorophyll, therefore, the green color in the leaves (GONÇALVES et al., 2020)) and also, the content of CP, there is a correlation between this content and the total digestible nutrients (TDN) (PARIZ et al., 2011).

Some other authors have stated that the application of nitrogen fertilizers is the most significant in terms of higher forage yields (COBLENTZ et al., 2017), being essential for the maintenance of productivity and sustainability of pastures, contrary to its deficiency that could be the cause to trigger degradation processes (HAJIGHASEMI et al., 2016).

Structural characteristics

The absence of nitrogen fertilization caused lower PLH, NTI, NLE and NLTI in Massai grass. The PLH was influenced (P<0.05) by the treatments and the highest height was obtained with MiF. The gelatin sludge (DGS) doses of 200 and 300 m^3 ha^{-1} showed no statistical difference in PLH (P>0.05) with FOM. A similar situation was observed for stem height (SH) with significant effect, only for MiF (Table 5). Nitrogen sources and defoliation intensity are considered the factors that can influence the development of forage plants (MARTUSCELLO et al., 2019; CASTRO et al., 2016) , such as growth in height and dry biomass production (BITTAR; SOUZA, 2021).

Table 5. Structural characteristics of Massai grass as a function of gelatin sludge (DGS), organomineral (FOM) and mineral fertilizer (MiF) doses.

	DGS (m^3 ha^{-1})					
Variables	0	200	300	FOM	MiF	CV(%)
PLH (cm)	31.19 c	45.16 b	45.5 b	48.44 b	57.92 a	6,29
SH (cm)	9.23 b	10.69 b	11.18 ab	10.54 b	14.68 a	19,94
NTI (tillers pot^{-1})	47.33 c	84.66 b	102.11 b	128.94 a	127.44 a	10,98
NLE (leaves pot^{-1})	32.11 e	108.33 d	137.44 c	191.11 b	231.44 a	11,05
NGL (leaves pot^{-1})	3.50 b	7.30 a	9.77 a	9.71 a	9.60 a	25.78
NLTI (leaves/tillers)	1.16 b	2.43 a	3.26 a	3.23 a	3.20 a	25.77

PLH: plant height; SH: stem height; NTI: number of tillers; NLE: number of leaves; NGL: number of green leaves, NLTI: number of leaves per tiller.
Means followed by the same lowercase letter on the line do not differ from each other by Tukey's test at 5% probability.

In the study by Carvalho et al. (2021) under evaluation in the development and production of three cultivars of *P. maximum* in response to the application of manipueira, in a protected environment, they observed the effect of the doses of manipueira (0; 22; 44; 88 and 176 m^3 ha^{-1}), for the height of the plant, providing the highest averages for the grasses Massai (43.13 cm) and Mombasa (23.87 cm) with the dose of 44 m^3 ha^{-1}. Taking into account that it is not the same nitrogen source used in Massai grass, however, the averages of plant height were similar to the present study, in relation to the DGS applied (Table 5). Other researchers, always using organic fertilizers, making applications of cattle manure in *U. brizantha* cv. Marandu and *P. maximum* cv. Mombasa, recorded maximum heights between 46 and 78 cm, respectively, at doses of 26.5 and 30.7 Mg ha^{-1} (CASTRO et al., 2016).

Regarding the number of tillers (NTI), there was a statistical difference in the DGS in relation to the treatments with FOM and MiF. However, within the same treatments (DGS with each other and comparison of FOM and MiF) were not influenced ($P>0.05$) by the sources of nitrogen fertilization. The highest NTI was observed in plants fertilized with FOM. This fact may be related to the complement of gelatin sludge with mineral fertilizer (FOM), increasing the concentrations of N and the supply of this nutrient, favors the development of the plant. The N, acts on the formation of axillary buds, generating a greater number of buds and new tillers. These factors cause forage grasses to increase their production, reaching the moment of defoliation more quickly (MARTUSCELLO et al., 2015; MEDICA et al., 2107) .

For the number of leaves (NLE) there was a significant effect ($P<0.05$) for all treatments. The highest NLE was obtained with MiF, followed by FOM. Probably, the increase was due to the increase in the concentration of N and the functions of this nutrient, as a controlling agent of the growth and development processes in plants, allowing a greater production of biomass through carbon fixation (VASCONCELOS et al., 2020).

Martuscello et al. (2019) also showed the effect of nitrogen fertilization on this variable in *Panicum maximus* cv. BRS Tamani. The appearance of leaves is related to high elongation rates, providing a greater number of leaves and a greater amount of tillers, however, they will be dependent on fertilization (MARTUSCELLO et al., 2019; MARTUSCELLO et al., 2015).

NGL and NLTI were not influenced ($P>0.05$) by nitrogen fertilization (Table 5). Abreu et al. (2020) found that fertilization with N promoted the growth of *M. maximus* cv. BRS Zuri, with an increase in the number of live and total leaves per tiller, with doses close to 500 kg ha^{-1} of N, and higher leaf size per tiller, population density of tillers and dry matter production, with variations of doses from 600 to 647 kg ha^{-1} of N.

However, in Tamani grass, submitted to increasing doses of fertilization, using urea as a nitrogen source (0, 300, 600 and 1,200 kg ha^{-1} of N per year), increased the rates of leaf and stem elongation and increments of 704% in the biomass of green leaves with applications of up to 1,200 kg ha^{-1} of N per year, in addition to having significant effects on plant height and population density of tillers (VASCONCELOS et al., 2020).

Even though in most of the variables evaluated, the greatest effect was observed with MiF and FOM, in relation to the application of DGS (Table 5), these results can be attributed to the immediate availability of N to the plant, with the application of inorganic fertilizers in relation to organic ones, in which the organic matter must be decomposed for the use of nutrients by the plant. According to Gutser et al. (2005), organic fertilizers present slow release of organically bound N, promoting little effect on the growth and development of the crop in the same year of application.

This fact was also observed in the study by Emereciano Neto et al. (2016) evaluating the effect of the use of organic and mineral fertilizers on the structure and forage production of Massai grass, verifying that the mass of leaf blade and stem were higher in treatments with urea applications. A similar result was presented for height, being higher in plants fertilized with urea (50.97 cm) and lower with sheep, pig and poultry manure (25.11; 29.92; 34.37 cm, respectively). These authors stated that the release of N in mineral fertilizer is always higher than in organic compounds.

The effect of nitrogen fertilization on plant growth and development is evidenced, especially when the sources used are mineral fertilizers, because N is immediately available. Martuscello et al. (2015); Abreu et al. (2020) and Vasconcelos et al. (2020) evaluating the same genus of *P. maximus* observed similar results with positive effects on morphogenic, structural and productive characteristics.

Although studies with Massai grass under nitrogenous doses with gelatin sludge are still incipient, in the present study it was observed that the structural and productive characteristics were influenced by nitrogen fertilization (Table 4 and 5), and it is important to consider it in pasture management by the contribution of nutrients from the sludge, especially N. This nutrient provides a greater renewal of tissues and the structure of the forage canopy (VALOTE et al., 2021) .

Similar results were observed by Bittar and Souza (2021) and Valote et al. (2021) in the study of Zuri grass with N, where there was an increase in tillering and biomass production. Silva et al. (2015) also verified, increase in the productivity of dry mass with organic wastes, with the application of swine wastewater after 60 days in the grass *B. decumbens*. This result was probably associated with greater mineralization of organic matter present in swine wastewater.

Bromatological composition

For crude protein (CP) and mineral matter (MM), plants fertilized with DGS (200 and 300 m^3 ha^{-1}) showed no statistical difference compared to FOM and MiF. The levels of NDF and ADF increased and CP decreased with increasing doses of 200 and 300 m^3 ha^{-1} and in the absence of DGS, compared to FOM and MiF. (Table 6).

Table 6. Percentages of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), mineral matter (MM), indigestible neutral detergent fiber (iNDF) of Massai grass, as a function of DGS, FOM and MiF doses.

	DGS (m^3 ha^1)					
Variables	0	200	300	FOM	MiF	CV (%)
CP	7.31 b	8.46 b	8.50 b	10.34 a	10.33 a	6.85
NDF	67.48 a	64.77 ab	64.25 ab	60.94 b	61.26 b	3.24
ADF	33.53 a	31.58 ab	32.16 ab	30.09 b	30.77 ab	4.71
MM	7.07 c	7.50 b	7.78 b	8.18 a	8.31 a	2.27
iNDF	24.02 a	24.13 a	24.95 a	22.86 a	23.07 a	4.41

Means followed by the same letter on the lines do not differ by Tukey's test at 5% probability.

Contrary results were verified by Oliveira et al. (2020), when evaluating the effects of increasing doses of N, applying as a source ammonium sulfate (150, 300 and 450 kg ha^{-1} of N $year^{-1}$) in Mombasa grass, where the CP content increased at the highest level of N ($P <0.05$) and the levels of NDF and ADF, decreased with the increase of N doses.

The higher the CP content, and the lower NDF and ADF, the nutritional value of the forage will be higher (PACIULLO et al., 2017). However, in this study, the levels of CP were higher than the minimum recommendation of 7% by Van Soest (1994) for the functioning of ruminal metabolism. A study by Pedreira et al. (2014) on the productivity and ruminal degradability of *P. maximum* grass forage found similar levels to the present study, with Massai grass for ADF (32.2%), CP (12%) and NDF (75.5%).

Regarding NDF, ADF, and iNDF, there were no statistical differences ($P>0.05$) between treatments. However, with the application of gelatin sludge there was a reduction in the levels for NDF and ADF, in relation to the treatments without nitrogen fertilization, in which these values increased (Table 6). A similar situation was observed by Marques et al. (2016) in the evaluation of structural and bromatological characteristics in Massai grass, in which ADF levels decrease with the addition of N doses, presenting mean values of 36.5%.

This fact improves the nutritional value of forages (PACIULLO et al., 2017), contrary to the absence of N, which impairs the synthesis of proteins, pigments of plant tissues, related to photosynthesis, causing a reduction in production (GOMES et al., 2020), being verified at the dose without fertilization.

The moment of application of N and some structural variables and bromatological composition of Massai grass indicated that the NDF and ADF content decreased and the MM content increased with the increase in N doses. The fibrous constituents are related to the plant cell structure, increasing the levels of NDF and ADF. In this sense, the higher the contents of these fractions, there will be a reduction in the amount of nutrients because they are related to ruminal microbial metabolism and the consumption of DM by animals, which are indicators in forage quality (MARQUES et al., 2016; GARCEZ et al., 2020) .

NDF levels above 60% are negatively correlated with forage consumption (VAN SOEST, 1994). However, in this study, these fractions are within the means of research conducted on Massai and Mombasa grass (HARE et al., 2015; MARQUES et al., 2016.; SIMONETTI et al., 2016; CARVALHO et al., 2017; BRAGA et al., 2018)

However, for MM there was an effect ($P<0.05$) only for treatments in absence of fertilization (lower content). Nevertheless, MiF and FOM presented higher levels because N was more readily available to the plant. The higher the availability of N, the higher the values obtained.

Mineral composition

The P and Mg content varied ($P<0.05$) in the DGS of 0, 200 and 300 m^3 ha^{-1} when compared to FOM and MiF and that between them, the P content did not present statistical differences ($P>0.05$) (Table 7). The DGS promoted the increase of the P content, becoming available to the plants, this due to the mineralization process originated by the microorganisms after decomposition of the organic matter. Thus, organic P can be a potential source of P for plants, since microorganisms and plant roots transform organic P into inorganic P by the synthesis and exudation of enzymes known as phosphate (ROSOLEM et al., 2014).

Table 7. Average leaf contents of macro (g kg^{-1}) and micronutrients (mg $kg^{-1)}$ of Massai grass fertilized with DGS, FOM and MiF.

Variables	**DGS (m^3 ha^1)**			FOM	MiF	CV (%)
	0	200	300			
P	1.71 bc	2.74 a	2.34 ab	1.40 c	1.21 c	13.72
K	19.03 ab	19.09 ab	19.62 ab	20.90 a	18.07 b	4.09
Ca	3.80 b	3.60 b	4.08 b	4.06 b	4.80 a	6.54
Mg	1.93 c	2.21 c	2.11 c	3.02 b	4.50 a	5.74
S	1.0 b	0.95 b	0.99 b	1.13 ab	1.28 a	7.38
B	7.65 a	10.14 a	6.06 a	6.76 a	10.43 a	34.75
Cu	6.51 a	3.82 a	5.24 a	4.53 a	4.39 a	28.08
Fe	253.50 a	166.50 b	164.25 b	226.50 ab	189.75 ab	16.99
Mn	42.46 a	22.86 c	24.50 bc	31.03 b	29.40 bc	10.94
Zn	8.03 a	6.26 ab	6.63 a	6.20 ab	3.83 b	18.25

Means followed by the same letter on the lines do not differ by Tukey's test at 5% probability.

On the other hand, the recalcitrant P that can be linked to Fe and Al, is returned to the soil surface, through the mineralization of its wastes (in this particular case of DGS), increasing the concentration of P-labile forms in the most superficial layers of the soil (ALMEIDA; ROSOLEM, 2016).

In the present study, it is possible to prove what was stated by these researchers, verifying that as the Fe contents increased, the P contents decreased, resulting contrary to the DGS applications, that after the mineralization process, the Fe contents decreased, but the P contents increased when compared to the FOM, MiF and in the absence of nitrogen fertilization (0 m^3 ha^{-1} DGS). However, the P contents are in the recommended ranges (0.8 to 1.2 g kg^{-1}) for grasses of the genus *Panicum* (MALAVOLTA et al., 1997).

The average levels of Ca and S were not influenced ($P>0.05$) by DGS and FOM when compared to MiF (Table 7). However, the concentrations of Ca in the treatments of 300 m^3 ha^{-1} DGS, FOM and MiF, were above the critical levels (4.0 g kg^{-1}) recommended by Monteiro (2005). Some authors verified a significant effect ($p<0.05$) on the levels of Ca and Mg (CARDOSO et al,, 2016; MONTEIRO et al., 2014) with applications of organic wastes, combined with N and P in the Mombasa grass (MONTEIRO et al., 2014), and Massai, when the macronutrient contents were evaluated, in leaf blades with nitrogen fertilization (0, 20, 40, 80 e 160 kg ha^{-1}) (CARDOSO et al., 2016).

Other authors, evaluating the aerial part of Tanzanian grass fertilized with N and P, observed that the Ca contents decreased with the increase of fertilization (N and P_2O_5), presenting averages of 4.5, 4.3, 3.9 and 4.0 g kg for the doses of 0, 100, 200 and 300 kg ha^{-1} of N (SOUSA et al., 2010), being generally above critical levels, similar to the present study.

For Mg, the influence (P<0.05) of FOM and MiF was verified. Mg concentrations in leaf tissue were within the critical levels (1.5 to 4.2 g kg^{-1}) recommended by Monteiro (2005) for all treatments. McRoberts et al. (2018) evaluating the effects of urea and bovine manure on the yield and nutritional value of *Brachiaria hybrid* cv. Mulatto II verified different results, in which the manure increased the concentrations of K and Mg in relation to the control, also increasing the concentrations of P.

Andrade et al. (2022) stated that for K, even though it is an element easily extracted and absorbed by plants, however, the concentration in the leaves will depend on the concentrations of N. These authors observed the highest concentration of K (18.20 g kg^{-1}) in the leaf tissue, when they studied the forage production and macronutrient contents in the leaves of Tamani grass, fertilized with natural phosphate and inoculated with *Azospirillum brasilense*, presenting similar results with MiF and higher levels in leaf tissue in this study with applications of DGS.

It was observed that the average contents of K, B, Cu, Fe, Mn and Zn in the leaf tissue were not affected (P>0.05) by nitrogen fertilization in all treatments (Table 7). However, the highest concentrations of these elements in the leaves were present in the treatments with applications of FOM (K), MiF (B) and 0 m^3 ha^{-1} DGS, in decreasing sequence of micronutrients (Fe, Mn, Zn and Cu).

The same decreasing order of these nutrients was verified by Braz et al. (2004), evaluating the accumulation of nutrients N, P, K, Ca, Mg, Zn, Cu, Mn and Fe in the leaves as a function of the days after grass emergence, Brachiaria (*B. brizantha* cv. Marandu) and Mombasa (*P. maximum* cv. Mombasa). According to Oliveira et al. (2004), the levels of Fe recorded in this study are at the recommended limit (50 to 200 mg kg^{-1}), being higher only in treatments with 0 m^3 ha^{-1} DGS and for FOM. However, the Mn and Zn content are not in the appropriate range (40 to 200 and 20 to 50 mg kg^{-1}, respectively) for *P. maximum.* The low levels of Zn were evident in this study, because the plants presented deficiency of this element.

Other studies with organic fertilizers in Mombasa grass, under fertilization sources with waste of the siderurgic industry (pó de balão) combined with N and P, under greenhouse conditions, observed that the combinations of organic wastes with N dose, significantly influenced the contents of macrominerals (N, P and K) and micromineral (Mn) (MONTEIRO et al., 2014).

McRoberts et al. (2018) found that the combination of organic compounds with urea is more effective, reflecting both the greater availability of N, as well as the release of P, K, macro and secondary micronutrients during the mineralization process of organic fertilizers. This fact is confirmed in the present study, even though most of the variables evaluated presented the highest levels with applications of MiF, however the complement of sludge with urea (FOM) improved the structural, productive characteristics and nutritional value in Massai grass, presenting similar results with inorganic fertilizers.

Thus, gelatin sludge is an organic waste that contains macronutrients (N, P, Ca) and micronutrients (Fe, B, Zn, Mn, Cu), considered important for forage development, however, it could well be used as an alternative fertilization for fertilization in pasture areas.

However, according to Gonçalves et al.(2022), the use of these wastes and other alternative nitrogenous sources are dependent on the dose and rate of mineralization In addition to considering other factors, such as the high cost of fertilizers, meeting the nutritional needs of the plant.

In this sense, the application of FOM could be an alternative of low-cost fertilization in pastures. The combination of sludge (52%N) with urea (48%N) increases the availability of N, which could be a partial substitute for chemical fertilizer, considering that the results of the variables analyzed in this study were similar to the mineral. This result is affirmed by Galindo et al. (2018b), when the doses of N increase, the tendency is that the availability of N in the pasture also increases.

CONCLUSION

Different doses of gelatin sludge alter the structural characteristics, nutritional value and production of Massai grass.

The gelatin sludge used as a nitrogen source influences the stem height, number of leaves, green leaves, leaves per tiller and the chlorophyll index, being more responsive to doses of 300 m^3 ha^{-1}.

The N content contained in gelatin sludge is insufficient to meet the nutritional needs for the development of Massai grass. The complement of sludge with urea (organomineral) positively influences the structural, productive and nutritional characteristics of Massai grass and the reduction in the use of chemical fertilizer, up to 52%.

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