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Eduardo André Ferreira [1], Joadil Gonçalves de Abreu [2], Wininton Mendes da Silva [1,*], Danielle Helena Müller [1], Dalilhia Nazaré dos Santos [1], Cassiano Cremon [3], Oscarlina Lúcia dos Santos Weber [2], Aaron Kinyu Hoshide [4,5], Daniel Carneiro de Abreu [5,6], Maybe Lopes Gonçalves [7] and José Advan Pereira Pedrosa Júnior [8]

[1] Empresa Mato-Grossense de Pesquisa, Assistência e Extensão Rural (EMPAER-MT), Centro Político Administrativo, Cuiabá 78049-903, MT, Brazil; eduardoagritrop@gmail.com (E.A.F.); winintonsilva@empaer.mt.gov.br (W.M.S.); daniellemuller@empaer.mt.gov.br (D.H.M.); dalilhiasantos@empaer.mt.gov.br (D.N.S.)
[2] Programa de Pós-Graduação em Agricultura Tropical, Universidade Federal de Mato Grosso, Cuiabá 78060-900, MT, Brazil; joadil.abreu@ufmt.br; oscarlinaweber@gmail.com (O.L.S.W.)
[3] Universidade do Estado de Mato Grosso, Rua São João, s/n., Caceres 78216-596, MT, Brazil; cassiano.cremon@unemat.br
[4] The University of Maine, Orono, ME 04469, USA; aaron.hoshide@ maine.edu
[5] AgriSciences, Universidade Federal de Mato Grosso, Sinop, MT 78555-267, Brazil
[6] Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso, Campus Universitário de Sinop, Avenida Alexandre Ferronato, 1200, Sinop 78555-267, MT, Brazil; abreu@ufmt.br
[7] PB Leiner Brazil, Estrada Vicinal Acorizal Aldeia, KM03, Zona Urbana Acorizal 78480-000, MT, Brazil; maybelopesgoncalves@gmail.com
[8] Departamento de Agronomia, Universidade do Estado de Mato Grosso, Av. Tancredo Neves 1095, Caceres, Cavalhada II, Mato Grosso, 78200 000, Brazil; junior.jose@unemat.br
* Correspondence: winintonsilva@empaer.mt.gov.br; Tel.: +55-(65)-999241470

Abstract: Gelatin industry residues are increasingly used as fertilizer and soil conditioner. However, correct residue dosage is critical for grass development and minimizing environmental impacts. This randomized block design study determined adequate dosage of Mixed Gelatin Sludge (MGS) for Marandu grass production in wet/dry seasons in Brazil. Five MGS levels (0%–200% of required nitrogen) were compared to mineral fertilizer. Agronomic/productivity characteristics, bromatological composition, macro/micro-nutrient composition of leaves, and soil chemical attributes were evaluated. Agronomic/productivity characteristics were influenced by MGS dose in both dry/rainy seasons, except for leaf blade pseudostem ratio and percentage of leaves/pseudostem. Bromatological composition was influenced by MGS doses in dry/rainy seasons except for dry/mineral material quantities. Marandu leaf tissue chemical composition was significantly influenced by MGS dose, except for potassium, boron, and iron. Chemical composition of four soil layers between 0–50 cm influenced GMS dose, except for pH, organic matter, magnesium, copper, manganese, and zinc. GMS dose for Marandu production should be 200% of nitrogen (N) requirement. GMS application increased productivity/quality of Marandu grass. Macronutrients (N, phosphorus) and micronutrients (Ca, Mg, S, Cu, and Zn) increased in Marandu grass and in the soil (Ca, S, and Na). Increased sodium (Na) level was not limiting.

Keywords: *Brachiaria brizantha*; pasture management; nitrogen; residue management; sustainability; wastewater

1. Introduction

Increasing urbanization and industrialization result in an increasing volume of residuals from anthropic activities and industrial processes, making sustainable development more challenging. The

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large volume of wastewater produced by industries requires more than adequate policies, treatment technologies, management and safe disposal [1]. The treatment of industrial wastewater produces solid residuals (or sludge) as a by-product [2]. In 2017, the estimated production of industrial sludge was 45 million tons of dry mass [3]. Inadequate use of industrial sludge has been considered wasteful [4]. This waste can be used as a source of energy and can also be used as a substrate for fertilization and soil correction, since it is rich in organic materials and nutrients [5,6].

Gelatin is an important industrial product, used in food items, cosmetics, pharmaceuticals and photography and is produced via controlled hydrolysis of collagen from animal skin and bones. During this process, large quantities of organic waste are produced. The liquid residues from this process are sent to a sewage treatment station from which solid residuals (sludge) result, rich in organic material. The solid residuals are classified based on their treatment: the first is referred to as primary gelatin sludge (PGS). The second, after processing through anaerobic digestion and aeration pools, is called biological gelatin sludge (BGS); the mixture of these two materials is called mixed gelatin sludge (MGS).

Both PGS and BGS are composed of chemical elements that may or may not be nutrients for plants, which vary in their composition depending on the industrial process applied. Their use in agricultural soils is a promising alternative to the continued treatment and disposal of these residuals, for both environmental and economic reasons. This process promotes recycling, recuperating elements that are plant nutrients and necessary correctives for agricultural soils, benefitting rural producers with decreased costs for fertilizers and lime, and also decreasing pressure on landfills [7].

Ribeiro 2007 highlights that sludge has a composition that is well suited to correct soil acidity, as it has a high pH, or to be used as a fertilizer, due to high levels of nitrogen and calcium [8]. Guimarães et al. 2012 evaluated the use of six doses of biological sludge from the gelatin industry in varying soil types, showed increases in pH, effective cation exchange capacity (CEC), phosphorus (P), calcium (Ca^{2+}), sodium (Na^{+}), and inorganic nitrogen (N) and reduction in levels of aluminum (Al^{3+}) and hydrogen ions (H^{+}) without altering levels of organic material in the soil [9]. A study evaluating the use of gelatin sludge in the production of Piatã grass, showed increases in Ca^{2+}, P, and base saturation in the 0 centimeter (cm) to 5 cm soil layer, but no change in pH or base saturation at depths past 5 cm [10].

Despite the positive results found by these studies, the elevated levels of sodium present in MGS suggest practical limits on its application, as this may cause serious risks to the development of plants or to the health of humans and animals [7]. Therefore, sludge must be applied within previously established limits, as the application without technical knowledge or in sensitive areas may cause contamination of soil or subsurface water, as well as public nuisance by way of strong odors and the amplification of disease vectors [11–13]. Therefore it is necessary to determine appropriate limits for agricultural doses of solid residuals [14,15]. Currently, there is no conclusive published research on the use of mixed gelatin sludge (MGS) and its impacts on soil and forage plants. Therefore, the objective of this study is to determine the appropriate dose of MGS for use in the production of Marandu grass during the dry and rainy seasons in Brazil.

2. Materials and Methods

The experiment was installed at the Research Station of the Empresa Mato-Grossense de Pesquisa, Assistência e Extensão Rural (Mato Grosso Business for Research, Assistance and Rural Extension - EMPAER-MT). This research station is located in the municipality of Acorizal, Mato Grosso (15° 11.136″ S 56º 23.059″W) at 257 meters altitude (Figure 1A and 1B). Field experiments were conducted between September 2019 and February 2021, with cutting cycles grouped by rainy season (October through May) and dry season (June through September) over both production years.

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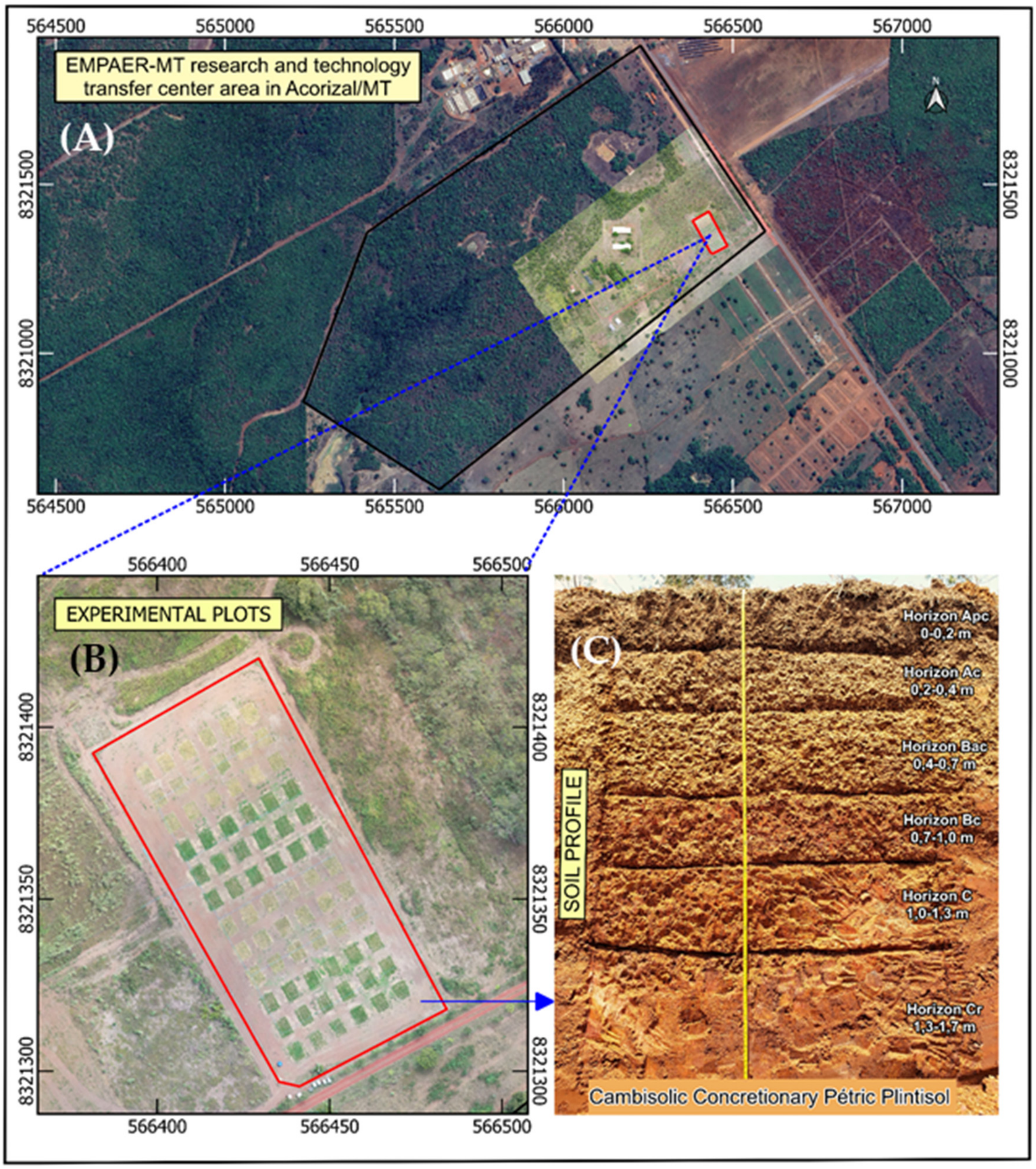

Figure 1. Research Station of the Empresa Mato-Grossense de Pesquisa, Assistência e Extensão Rural experimental. **(A)** area, **(B)** plots, and **(C)** soil profile.

The climate of the region is tropical wet-dry or Köppen classification type Aw. This type of climate is characterized by two well-defined seasons: dry (June through September) and rainy (October through May). Climate data for the experimental period was obtained from Brazil's National Institute of Meteorology or Instituto Nacional de Meteorologia (INMET) [16], where mean temperature varied between 24.35°C and 30.67°C with 1673.80 millimeters of rainfall (Figure 2). The soil of the experimental area (Figure 1C) was classified as a Cambisolic Concretionary Pétric Plintisol [17,18].

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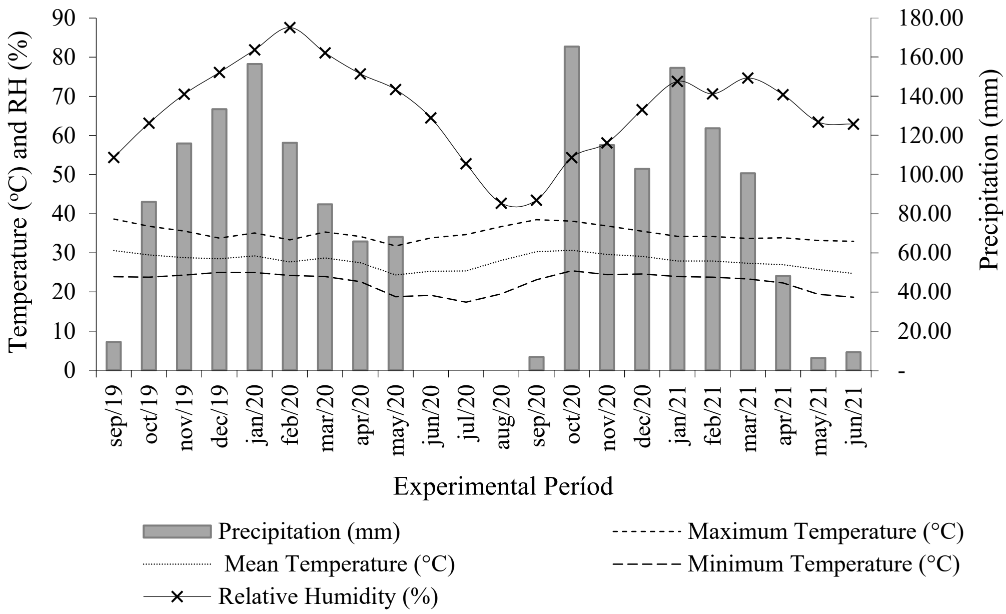

Source: Inmet, 2023 [16]

Figure 2. Monthly means of minimum, mean, and maximum temperature (°C), relative humidity (%), and precipitation (mm), during the experimental period of September 2019 through June 2021 in Acorizal, Mato Grosso state, Brazil.

Before installing the experiment, plant primary nutrient and chemical and granulometric characterization of the soil (Table 1) as well as plant secondary and micro-nutrient analyses (Table 2) were completed for all soil profile layers. The soil was corrected with lime (power of reactivity and total neutralization = 5%) in an effort to increase base saturation to 60%. After application of lime, corrections for phosphorus and potassium were completed, following the recommendations of Sousa and Lobato [19]. Specifically, 90 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} K_2O were used as sources of single super phosphate and potassium chlorate. Seeding of *Urochloa brizantha* Marandu was completed in January of 2020. In March 2020 at 70 days after seeding, a leveling cut was made to experimental plots (Figure 1B) before evaluation. This was followed by fertilizing with mixed gelatin sludge (MGS) and mineral fertilizer following the establishment of experimental treatments.

The Mixed Gelatin Sludge (MGS) is a mixture of primary sludge and biological sludge from the gelatin industry. Both primary and biological sludge types result from the treatment of wastewater from the process of converting collagen in animal bones and skins into gelatin [20]. The MGS used in this study came from a gelatin industry treatment plant in Acorizal, Mato Grosso state, Brazil. Before application in soils, one sample of sludge was collected for chemical analysis in February 2020 and the quantities of each element applied in each treatment of the experiment are summarized in Table 3.

Table 1. Primary nutrient, chemical, and granulometric analyses of soil layers prior to installing experimental design.

Sample	**Profile**	**pH**		**P**	**K**	**Ca+Mg**	**Ca**	**Mg**	**Al**	**H**	**M.O.**	**Silt**	**Sand**	**Clay**	**SB**	**CEC**	**V**	**Na**	**Sat Al**
	cm	H_2O	$CaCl_2$	mg dm^{-3}		cmol$_c$ dm^{-3}					g dm^{-3}	g kg^{-1}	kg^{-1}		cmol$_c$ dm^{-3}		%	mg	
A1	0–20	6.4	5.7	6.3	71.7	3.31	2.40	0.91	0	1.88	21.3	723	56	221	3.50	5.38	65.1	0	21.0

A2	20–40	5.2	4.5	1.9	37.4	0.94	0.65	0.29	0.45	2.77	10.2	690	66	244	1.04	4.26	24.4	30.2
B1	40–70	5.0	4.2	1.2	23.2	0.85	0.60	0.25	0.68	2.57	9.7	523	117	360	0.91	4.16	21.9	42.8	22.0
B2	70–100	4.8	4.0	0.9	22.2	0.66	0.45	0.21	0.75	2.75	10.7	456	134	410	0.72	4.22	17.1	51.0
C1	100–130	4.7	4.0	0.6	26.3	0.57	0.40	0.17	1.32	2.56	11.8	290	156	554	0.64	4.51	14.2	67.4	18.0
C2	130–170	4.7	4.1	1.2	24.2	0.47	0.30	0.17	1.05	2.98	11.2	456	130	414	0.53	4.55	11.7	66.5

Table 2. Secondary nutrient and micro-nutrient analyses of soil layers for experiment prior to installing experimental design.

Sample	Profile cm	Ca Mg	K	Cu	H	Zn		Fe	Mn	B	S	N	Fe_2O_3	SiO_2	Al_2O_3	P_2O_5	TiO_2
		Saturation (%)							mg dm^{-3}						g kg^{-1}		
A1	0–20	44.61	3.46	16.91	34.85	12.4	2.0	117	162.3	0.48	7.1	0.85	56.7	48.2	39.8	0.09	2.01
A2	20–40	15.26	2.28	6.81	65.14	2.5	1.1	234	34.4	0.35	7.3						
B1	40–70	14.42	1.45	6.01	61.78	2.0	0.6	136	17.0	0.32	7.3	0.33	69.1	59.6	62.5	0.05	3.66
B2	70–100	10.66	1.37	4.98	65.17	1.9	0.4	128	9.8	0.19	7.7						
C1	100–130	8.87	1.51	3.77	56.65	1.3	0.5	105	4.7	0.21	4.9	0.29	132.1	128.6	136.8	0.06	2.49
C2	130–170	6.59	1.38	3.74	65.38	1.7	0.7	156	5.4	0.18	5.2						

Table 3. Results of the chemical analysis of mixed gelatin sludge at the start and end of the experiment and doses applied to each treatment. Values below the lowest detectable quantity are labeled (LQ).

Quantities of Elements in mixed gelatin sludge (MGS) at the start and end of the experiment						Doses of MGS by treatment (kg ha^{-1})			
Element	**Unit**	**Feb/20**	**Feb/21**	**Mean**	**CV (%)**	**50%**	**100%**	**150%**	**200%**
pH $CaCl_2$		7.1	7.1	7.1	0.1	-	-	-	-
Nitrogen (N)	%	4.0	4.2	4.1	3.1	59.1	118.1	177.2	236.2
Phosphorus (P)	%	1.1	1.2	1.2	2.4	16.7	33.3	50.0	66.7
Potassium (K)	%	0.7	0.1	0.4	97.6	6.0	12.1	18.1	24.1
Calcium (Ca)	%	4.7	6.7	5.7	24.2	82.0	163.9	245.9	327.9
Magnesium (Mg)	%	0.1	0.1	0.1	32.6	0.9	1.9	2.8	3.7

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Sulfur (S)	%	0.7	0.5	0.6	26.8	8.3	16.7	25.0	33.3
Sodium (Na)	%	0.1	1.1	0.6	122.4	8.5	17.1	25.6	34.2
Boron (B)	mg kg^{-1}	43.1	30.2	36.7	25.0	0.1	0.1	0.2	0.2
Iron (Fe)	mg kg^{-1}	1283.6	1646.7	1465.1	17.5	2.1	4.2	6.3	8.4
Manganese (Mn)	mg kg^{-1}	20.7	33.7	27.2	33.6	< LQ	0.1	0.1	0.2
Copper (Cu)	mg kg^{-1}	15.6	20.0	17.8	17.6	< LQ	0.1	0.1	0.1
Zinc (Zn)	mg kg^{-1}	104.7	75.7	90.2	22.7	0.1	0.3	0.4	0.5
Nickel (Ni)	mg kg^{-1}	0.1	3.2	1.6	137.1	< LQ	< LQ	< LQ	< LQ
Cadmium (Cd)	mg kg^{-1}	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
Mercury (Hg)	mg kg^{-1}	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
Chromium (Cr)	mg kg^{-1}	12.6	8.7	10.6	26.0	< LQ	< LQ	< LQ	0.1
Organic Carbon	g kg^{-1}	14.8	16.8	15.8	9.0	< LQ	< LQ	< LQ	< LQ
Total Carbon	g kg^{-1}	515.1	473.9	494.5	5.9	0.1	0.1	0.2	0.3
Cation Exchange Capacity	cmolc kg^{-1}	26.5	20.0	23.3	19.8	0.7	1.4	2.2	2.9
Total Organic Material	g kg^{-1}	888.0	817.0	852.5	5.9	33.4	66.8	100.2	133.6
Compostable Organic Material	g kg^{-1}	25.6	28.9	27.3	8.6	1224.9	2449.7	3674.6	4899.4
Compost Resistant Organic Material	g kg^{-1}	862.5	788.2	825.4	6.4	39.2	78.3	117.5	156.6
Soluble Mineral Residue	g kg^{-1}	107.9	173.4	140.7	32.9	1185.8	2371.7	3557.5	4743.4
Insoluble Mineral Residue	g kg^{-1}	4.0	9.6	6.8	58.2	202.1	404.2	606.3	808.3
Total Mineral Residue	g kg^{-1}	112.0	183.0	147.5	34.0	9.8	19.5	29.3	39.1
Total Humidity	g kg^{-1}	807.1	809.8	808.5	0.2	211.9	423.9	635.8	847.7
Grease Oils	g kg^{-1}	89.1	78.0	83.6	9.4	1161.6	2323.1	3484.7	4646.3
Carbon/Nitrogen Ratio		44,939.0	44,937.0	44,938.0	11.8	-	-	-	-

Based on the nitrogen content of the mixed gelatin sludge (MGS) and considering the nitrogen (N) requirements of the specific culture proposed by Souza and Lobato [19], the dose of sludge to be applied was calculated following equation 1:

$$Application\ Rate = \frac{\text{N recommended (kg ha}-1)}{\text{N available in MGS (kg t}-1)} \qquad (1)$$

where the N recommended is specific to Marandu grass. Six treatments included five doses of MGS (Table 4) based on percentages (0%, 50%, 100%, 150%, and 200%) of the recommended total yearly dose of nitrogen for irrigated Marandu grass production (300 kg ha^{-1} per year). A sixth treatment that received mineral fertilization exclusively was added that met all fertilization requirements compared with other treatments (50 kg of N ha^{-1} per cutting cycle (total of six cuts per year) and 300 kg N ha^{-1} per year). The experiment was conducted using a randomized complete block design with three repetitions. The dimensions of each experimental field plot was 4 × 5 meters = 20 square meters.

Table 4. Mixed gelatin sludge applied (kg ha^{-1}) per cycle and annually by treatment.

Treatment*	**Dose per application cycle (kg ha^{-1} per cycle)**	**Annual Dose (kg ha^{-1} per year)**
0%	0	0
50%	2.601	15.606
100%	5.202	31.212
150%	7.803	46.818
200%	10.404	62.424

*Considering a requirement of 50 kg N ha^{-1} per cycle for Marandu grass.

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After a leveling cut, MGS was applied with doses manually weighed. Doses were diluted in water using a proportion of 1 liter of water for each kilogram of residue in order to facilitate spraying. The diluted sludge was evenly mixed with a mechanical rotating mixer. After dilution and homogenization, the residue was placed in irrigators and manually sprayed on research plots.

Height, tillering, morphological composition, leaf blade: pseudostem ratio and dry mass were measured when the 100% treatment reached the recommended pasture height of 35 centimeters (cm). These measurements were made within a 1 × 1 meter metal frame, thrown randomly three times within each plot. Grass was cut to 20 cm from the soil surface upon collection to reflect the post-grazing height recommended for Marandu grass.

Samples collected were stored in paper bags, weighed, identified, and transported to the Forage Lab of the Federal University of Mato Grosso (Laboratório Forragicultura). In the laboratory, the morphological separation of the plants involved three parts: leaf, stem and sheath, and senescent material. The samples were then dried in a forced air oven at 55°C until achieving constant mass and were weighed to determine the estimated aboveground dry mass, consistent with grazing.

To determine the bromatological composition of the grass, dried samples were ground in a Willey grinder, conditioned, and analyzed. Ground material was scanned to determine near infrared reflectance using a EspectraAnalyzer manufactured by Zeutec Opto Elektronic Gmbh with 19 filters. Reflectance from 1100 and 2500 nanometers was reported as log1/R (reflectance) per wavelength. The variables estimated from the near infrared (NIR) reflectance are dry matter (DM), mineral material (MM), crude protein (CP), neutral detergent fiber (NDF), and indigestible neutral detergent fiber (iNDF).

To determine the absorption of macronutrients and micronutrients by the grasses in each treatment, the mineral composition of the leaves was measured next, following the methodology of developed by researchers at Embrapa [21]. Based on this analysis, the recovery rate for each nutrient was determined. The recovery rate is calculated as the amount of a given nutrient extracted per unit applied, independent of whether the source is inorganic or mineralized from crop residue. This represents the fraction of the nutrient that is made available to the plants. The recovery rate was calculated for nitrogen (N), phosphorus (P), and potassium (K) following Equation 2:

$$\text{Rec } (\%) = [(\text{Fertilized Treatment - Control}) / \text{Residue}] \qquad (2)$$

in which the "Fertilized Treatment" is the quantity of nutrients available in grass tissues in the treatments fertilized with residue. The "Control" is the quantity of nutrients available in plants in plots without fertilization from crop residues. "Residue" is the quantity of nutrients applied by crop residues.

The extraction of N, P, and K were calculated using Equation 3:

$$\text{Extraction (kg ha-1)} = 0.001 \text{ DM x TN} \qquad (3)$$

where dry matter (DM) produced is measured in kg ha^{-1} and the total nutrient (TN) level in the plant is measured in g kg^{-1}. The quantity of nutrients in grass within the unfertilized plots was used to estimate the supply of nutrients from the soil and atmosphere for nitrogen. The exchangeable sodium percentage was calculated based on Equation 4:

$$\text{ESP } (\%) = (\text{Na}/\text{CEC}_{\text{total}})*100 \qquad (4)$$

where the exchangeable sodium percentage (ESP, %) is a function of the sodium (Na) is concentration in the soil (cmolc dm^{-3}) and the total cation exchange capacity (CEC_{total}) measured in cmolc dm^{-3}.

Soil samples were collected in four soil layers: 0–10 centimeters (cm), 10–20 cm, 20–40 cm, and 40–60 cm. Each soil sample was tested to determine pH and the contents of primary and secondary macronutrients, micronutrients, and sodium. The soil analyses were carried out at the Solo Certo commercial laboratory in the city of Nova Mutum, Mato Grosso state, Brazil.

Kolmogorov-Smirnov and Levene tests were applied to the data to determine the homogeneity of variance and normality of errors, followed by regression. Regression models were tested for the significance of equation parameters. To test the variation of each element, ANOVA followed by a Tukey-test at 5% was applied to each dose for each grass cycle. Statistical calculations were conducted using the SPSS 20.0 - Statistical Package for Social Sciences from IBM Corp (2011) [22].

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3. Results

Morphological characteristics of Marandu grass in this experiment are summarized in Table 5. These morphological characteristics were influenced by the dose of mixed gelatin sludge (MGS) during the dry and rainy seasons. The leaf blade to pseudostem ratio and the percentage of leaves and stems did not show significant effects during the periods evaluated (Table 5). The variables tillering and height show linear relationships with MGS dose during the dry season, with increments of 232 tillers per m² and 11 cm, equivalent to increases of 32.91% and 35.19% for tillers and height of Marandu grass, respectively (Table 5). The greatest number of tillers were observed with the highest dose of MGS (Table 5). Tiller number positively impacts the composition of the grass canopy with an increase in apical buds.

Similar results compared to our study were where MGS was applied to Piatã grass with increases of 12.88% and 11.43% in tillers in the 3rd and 4th evaluation cycles of treatments with the largest quantity of MGS applied compared to treatments without fertilization [23]. Santos et al. 2013 [24] also observed increases in tillering with increased doses of dairy residue to Mombaça grass. The increase in tillering with increased dose of MGS is likely related to the improved nutrition of the plant, taking into account the increase in organic matter in the soil resulting from the MGS applied.

Table 5. Morphological characteristics and Marandu grass productivity under the effect of varying doses of GMS during dry and rainy seasons.

Marandu Grass Morphological Characteristics	Dose								
	Season	0%	50%	100%	150%	200%	NPK	Regression Equation[Rf]	R^2
Tillering (tillers m²)	Rainy	637.86	546.91	619.34	701.23	640.74	562.14	ŷ=629.22	
	Dry	672.84	683.95	761.11	791.36	809.88	708.02	ŷ=704.815+1.16x**	0.49
Height (cm)	Rainy	37.03	38.57	43.15	44.91	44.05	37.75	ŷ=37.468+0.041x**	0.46
	Dry	28.71	32.57	35.09	35.57	37.25	27.65	ŷ=31.258+0.055x**	0.49
Leaves (%)	Rainy	73.60	73.03	72.04	74.57	75.05	74.73	ŷ=73.66	
	Dry	76.96	75.19	73.40	74.70	76.43	77.70	ŷ=79.23	
Pseudostem (%)	Rainy	26.40	26.97	27.96	25.43	24.95	25.27	ŷ=26.34	
	Dry	23.04	24.81	26.60	25.30	23.57	22.30	ŷ=27.77	
Leaf Blade / Pseudostem Ratio	Rainy	2.82	2.77	2.76	3.08	3.12	3.04	ŷ=2.91	
	Dry	3.37	3.23	2.93	2.98	3.33	3.53	ŷ=3.94	
Dry Matter (t ha⁻¹)	Rainy	1.41	1.90	2.63	3.23	3.52	1.92	ŷ=1.427+0.011x**	0.93
	Dry	1.36	1.79	2.37	2.44	2.51	1.55	ŷ=0.878+0.010x**	0.85
Crude Protein (kg ha⁻¹)	Rainy	161.88	239.82	367.97	440.81	486.92	240.30	ŷ=169.268+1.702x**	0.93
	Dry	151.81	223.95	325.05	382.42	392.15	198.55	ŷ=57.902+1.574x**	0.93

R^2: Coefficient of determination. ** Significance of model constants at 0.01. [Rf] Indicates that nitrogen, phosphorus, potassium (NPK) was not considered in the regression model as it is the standard treatment studied (50 kg ha^{-1} of N).

Plant height also showed a linear correlation with MGS dose during the dry season with a maximum height observed of 42.26 cm for the maximum dose applied (Table 5). During the rainy season no significant response to MGS dose was observed. The height of plants observed in the treatment fertilized with NPK was 12.51% higher during the rainy season, and 21.29% lower during the dry season. The lower values of plant height during the dry season are likely due to the water stress and high temperatures during this period that increase ammonia volatility (NH_3) related to the use of urea [25].

Productivity of dry material also showed a significant linear response with increasing MGS dose for both the rainy season and dry season of 3.63 and 2.88 t ha^{-1}, respectively. During the rainy season, increases of 35.55%, 87.28%, 130.12%, and 150.82% were observed for treatments of 50%, 100%, 150%, and 200% in comparison with the treatment that received no fertilization. During the dry season,

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increases of 31%, 73.47%, 79%, and 84.23% were observed for the same treatment levels when compared with no fertilization. It was expected that treatments with mineral fertilizer would show greater production of dry matter when compared to those fertilized with MGS, given that organic sources require more time for mineralization and availability to plants. However, this tendency was not observed in the present study, which further supports the potential for use of this residue as a pasture fertilizer.

A linear tendency was also observed in levels of crude protein (CP) with the increasing dosage of MGS during both observation periods (Table 5). The highest values observed were 486.92 kg CP ha^{-1} during the rainy season, and of 392.15 km CP ha^{-1} during the dry season. These values are 200.78% and 158.32% higher than those observed in the control treatment during the seasons in question. The 100% dose produced CP 53.13% and 68.72% higher than the mineral fertilizer treatment (NPK) during the rainy season and dry season respectively. These results are similar to those observed in the production of dry matter. The increasing tendency of CP with increasing dose of MGS is promising as it is reflected in animal weight gain and profitability of rural properties.

One of the key elements responsible for the increased productivity of CP with increasing doses of MGS is N, which leads to increased leaf area and also stimulates the production of tillers [26]. Nitrogen is an essential component to biomolecules, such as enzymes, proteins, chlorophyll, nucleic acids, porphyrins, alkaloids, amino acids and various other molecules. It also plays a crucial role in physiological plant processes, and as such is the most necessary to fertilization for pasture management [27].

3.1. Bromatological Composition

Doses of MGS influenced the bromatological composition of Marandu grass, both during the wet and dry seasons, except for total dry material and mineral material (Table 6). The levels of crude protein (CP) show a positive linear response to MGS dose, during both the rainy and dry season, with maximum levels of 14.62% and 13.25% respectively for the highest dose (200%). These levels represent an increase of 40.95 and 99.06% in CP of Marandu grass when compared with the treatment without fertilization. For CP, Marandu grass showed a consistent increase until the maximum dose of MGS tested, demonstrating that higher doses could be applied to verify the maximum obtainable.

Table 6. Bromatological characteristics of Marandu grass under the effect of varying doses of mixed gelatin sludge during dry and rainy seasons.

Marandu Grass Bromatological Characteristics	Season	Dose 0%	50%	100%	150%	200%	NPK	Regression Equation[Rf]	R^2
Dry Matter (%)	Rainy	27.80	28.31	28.86	27.70	28.31	27.54	ŷ=27.7983	
	Dry	28.17	29.06	28.00	28.30	29.97	28.33	ŷ=28.1657	
Mineral Matter (%)	Rainy	8.07	7.95	7.92	7.83	7.83	7.33	ŷ=8.0692	
	Dry	7.99	7.52	7.44	8.24	8.24	6.68	ŷ=7.9944	
Crude Protein (%)	Rainy	10.23	11.90	13.04	13.72	14.42	11.74	ŷ=10.620+0.020x**	0.74
	Dry	6.41	12.48	13.91	12.36	12.76	12.04	ŷ=6.451+0.034x**	0.84
Neutral Detergent Fiber (%)	Rainy	65.00	63.84	63.17	62.72	62.23	65.37	ŷ=64.724-0.013x**	0.57
	Dry	66.87	64.40	63.36	61.78	61.63	65.86	ŷ=66.477-0.027x**	0.61
Acid Detergent Fiber (%)	Rainy	30.10	30.21	30.63	30.52	30.48	30.12	ŷ=30.0996	
	Dry	28.87	30.50	30.59	30.02	30.19	29.88	ŷ=29.210+0.005x**	0.22
iNDF (%)	Rainy	20.65	20.54	21.02	21.18	21.31	19.93	ŷ=20.548+0.004x*	0.12
	Dry	17.43	19.90	20.10	19.42	19.54	19.10	ŷ=17.454+0.011x**	0.58

R^2: Coefficient of determination. * and ** indicate levels of significance of constants within regression models of 5% and 1% respectively; [Rf] indicates that nitrogen, phosphorus, potassium (NPK) was not considered in the

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regression model as it is the standard treatment studied (50 kg ha^{-1} of N) and iNDF: indigestible neutral detergent fiber.

Similar to the present study, past research showed increases up to 150% for CP in *Brachiaria decumbens* for the highest doses of liquid swine waste when compared to the control treatment without fertilization [28]. Santos et al. 2013 [24] tested liquid dairy residue in Mombaça and also observed linear increases in CP with increasing dose of residue. Another study that applied liquid meatpacking waste to Marandu grass saw smaller increases in CP, with levels of CP varying between 9.4% and 11.1% [29].

The treatments fertilized with MGS showed higher levels of CP than those observed in the treatment fertilized with mineral fertilizer (urea). Values 1.41%, 11.11%, 16.93%, and 22.90% higher during the rainy season and 3.65%, 15.49%, 2.69%, and 5.95% higher during the dry season for treatments 50%, 100%, 150%, and 200% respectively. This result was unexpected as mineral sources of fertilizer have greater solubility of nutrients, with higher levels of immediate availability to plants while organic sources require more time for mineralization and availability of nutrients [28].

All treatments present in this study show levels of CP above limiting levels for pasture animals, which are between 6.0% and 8.5% to allow for better fermentation in the rumen [30]. For animals in the fattening phase, the value of CP should approach 10% and for dairy cows with 15% or more CP is suggested. Lower levels of CP reduce digestibility of grass and lower voluntary consumption due to the inadequate quantity of nitrogen for rumen microorganisms [31].

Levels of neutral detergent fiber (NDF) showed a negative linear relationship with increasing dose of SMG for both the dry and rainy seasons. The lowest values of NDF were 62.23% and 61.63% for the highest doses applied during the rainy and dry seasons, respectively (Table 6). These results corroborate with a prior study that showed NDF between 64.2% and 69.7% for *Brachiaria decumbens* cut at 35 days and fertilized with varying doses of swine waste [28].

Acid detergent fiber (ADF) was positively correlated with MGS dose during the dry season (Table 6). However, values shown in this study are lower than those presented by the same past study that showed levels of ADF in *Brachiaria decumbens* between 37.5% and 41.7%, though differences may be related to species-specific characteristics of the grasses studied [28]. These results have implications for pasture management since lower levels of fiber in grasses result in greater digestibility. The lower the level of ADF, the lower the lignin, and consequently, the better the digestibility of the feed [32].

Levels of insoluble neutral detergent fiber (iNDF) showed a positive linear relationship with MGS dose, with highest values at 21.35% and 19.65% for the rainy season and dry season, respectively (Table 6). Despite the linear effect observed, the maximum doses of MGS do not cause modifications to the fibrous tissue of the forage. High rates of fiber in pasture grasses may alter the voluntary consumption by ruminant livestock, with lower rates of NDF related to higher grass consumption [33].

3.2. Plant Chemical Composition

The chemical composition of the plant tissue of Marandu grass was influenced by the dose of MGS, with the exception of potassium, boron, and iron which did not show significant change. Significant response was shown to dose for nitrogen (N), phosphorus, calcium, magnesium, sulfur, copper, manganese, and zinc in the leaf tissue of Marandu grass (Table 7). Nitrogen showed a positive linear response to increasing MGS dose during the rainy season. The highest value of N (15.89 g kg^{-1}) in leaf tissue corresponded to the highest dose of MGS, and was 27.83% higher than the unfertilized treatment. Previous research evaluated the effect of household waste water on forage and also found a linear increase in N capture with increasing dosage for all forage cuts [34]. These researchers observed that grasses Tifton 85 and Pojuca showed the greatest accumulation of N in their structure, whereas Marandu grass did not show significant change in N accumulation as household waste water applications increased. The largest increase was for Tifton 85 and is attributed to the higher

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nutritional needs of this species, which has higher nutritional value and a greater capacity to absorb nutrients [34].

Table 7. Chemical characteristics of leaf tissue of Marandu grass under varying dosage of GMS and mineral fertilization during the rainy and dry seasons.

Marandu Grass Chemical Characteristics	Season	Doses 0%	50%	100%	150%	200%	NPK	Regression Equation^{Rf}	R^2
Nitrogen (N in g kg^{-1})	Rainy	12.43	13.39	14.44	15.42	15.89	13.11	ŷ=12.527+0.018x**	0.56
	Dry	8.55	11.80	11.83	11.27	11.22	11.53	ŷ=10.93	
Phosphorus (P in g kg^{-1})	Rainy	1.42	1.44	1.62	1.70	1.82	1.28	ŷ=1.391+0.002**	0.56
	Dry	1.13	1.22	1.58	1.48	1.80	1.32	ŷ=1.123+0.003x**	0.62
Potassium (K in g kg^{-1})	Rainy	23.57	25.98	24.48	24.04	26.51	22.23	ŷ=24.92	
	Dry	17.82	22.13	21.30	20.22	23.25	16.78	ŷ=20.94	
Calcium (Ca in g kg^{-1})	Rainy	2.51	2.60	2.84	3.20	3.11	2.84	ŷ=2.493+0.004**	0.59
	Dry	2.60	2.50	3.00	3.30	3.27	2.53	ŷ=2.507+0.004*	0.50
Magnesium (Mg in g kg^{-1})	Rainy	4.78	4.76	4.91	5.51	5.27	4.82	ŷ=4.698+0.003x*	0.41
	Dry	4.17	4.12	4.50	4.80	4.92	4.03	ŷ=4.063+0.004x*	0.38
Sulfur (S in mg kg^{-1})	Rainy	1.09	1.27	1.36	1.57	1.48	1.11	ŷ=1.136+0.002**	0.65
	Dry	1.23	1.35	1.50	1.60	1.73	1.25	ŷ=1.233+0.002**	0.89
Boron (B in mg kg^{-1})	Rainy	9.95	8.17	8.12	8.19	8.27	8.30	ŷ=8.54	
	Dry	7.35	6.67	6.06	5.58	10.43	6.47	ŷ=7.22	
Copper (Cu in mg kg^{-1})	Rainy	8.12	6.80	7.37	8.03	7.74	7.27	ŷ=7.61	
	Dry	3.54	3.82	3.97	4.96	5.10	3.54	ŷ=3.428+0.009**	0.81
Iron (Fe in mg kg^{-1})	Rainy	971.00	320.00	398.00	309.00	305.00	492.00	ŷ=460.60	
	Dry	453.00	412.50	381.00	600.00	361.50	396.00	ŷ=441.60	
Manganese (Mn in mg kg^{-1})	Rainy	126.31	127.40	132.84	137.20	111.07	111.07	ŷ=129.96	
	Dry	125.77	125.77	122.50	124.13	93.10	101.27	ŷ=131.647-0.134x*	0.30
Zinc (Z in mg kg^{-1})	Rainy	15.74	14.36	16.22	17.03	18.01	15.09	ŷ=16.27	
	Dry	11.56	14.48	16.18	15.21	18.13	12.29	ŷ=12.337+0.028**	0.56

R^2: Coefficient of determination. * and ** indicate levels of significance of constants within regression equation of 5% and 1% respectively; ^{Rf} indicates that nitrogen, phosphorus, potassium (NPK) was not considered in the regression model as it is the standard treatment studied (50 kg ha^{-1} of N).

Only doses of 150% and 200% during the rainy season showed mean values of N above adequate levels at 15.42 and 15.89 g kg^{-1}, respectively. Other study treatments did not reach the minimum suggested level of 15 to 25 g kg^{-1} for both dry and wet seasons [35]. The lowest levels of N were observed during the dry season and are likely related to the lower production of dry matter (DM), which varied from 2.42 to 2.00 t ha^{-1} during this period. This decreased production is related to edaphoclimatic factors, principally related to variation in temperature, light levels, and radiation.

The doses tested showed a positive linear effect on levels of phosphorus (P), with maximum values of 26.51 and 23.25 g kg^{-1} observed for the highest dose of MGS during the rainy season and dry season, respectively (Table 7). Other studies tested nitrogen fertilization with urea and ammonium sulfate found varying effects on P. For example, Costa et al. 2009 [36] varied the nitrogen dose from 0 to 300 kg ha^{-1} yr^{-1} and found positive linear effects on nitrogen (N), potassium (K), and magnesium (Mg) in the leaf tissue of Marandu grass, but a negative linear effect on P. Another past study varied the nitrogen dose from 0 to 800 kg ha^{-1} yr^{-1} and found positive linear effects on N and K, while for P and Mg, there was a positive linear effect until 400 kg ha^{-1} yr^{-1} [37].

The Ca levels also showed a positive linear relationship with increasing MGS dose. Maximum levels of 3.29 and 3.31 g kg^{-1} were found for rainy and dry seasons, respectively. The Mg levels within

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leaf tissue showed a positive linear relationship with increasing MGS dose with all values observed above recommended amounts of 1.5 to 4.0 g kg^{-1} [38].

The levels of sulfur (S) in leaf tissue showed a positive linear relationship with increasing dose of MGS for both seasons evaluated. Maximum values of 1.54 g kg^{-1} in the rainy season and 1.63 g kg^{-1} in the dry season were found for maximum doses of MGS (Table 7) which are within adequate levels (0.8 to 2.5 g kg^{-1}) established by Werner et al. [38]. For S, it is also necessary to consider the ratio of nitrogen to sulfur (N:S) in plant tissue [39]. In the current study, the N:S ratio in Marandu grass tissue varied between 6.6 and 13.9. Scott et al. 1983 [40] assert that ratios of N:S above 14:1 induce S deficiency in plants and Batista 2002 [41] further affirms that Marandu grass dry matter productivity is sensitive to the balance between these nutrients.

For micronutrients in all treatments, iron (Fe) stands out as it has levels above what is considered adequate at 50 to 250 mg kg^{-1} [38]. In grasses, the absorption of Fe by roots is dependent on phytosiderophores, which are compounds with high affinity for iron that are secreted within the rhizosphere where they join with Fe^{3+} to form a chelate compound. This compound is carried within the cell by the specialized Yellow Stripe transporter [42,43]. The high availability of this element in the soil assists in absorption by the plant.

The micronutrients copper (Cu) and zinc (Zn) show significant linear relationships with increasing levels of MGS during the dry season, with maximum values of 5.10 and 18.13 mg kg^{-1}, respectively, for the highest dose tested (Table 7). Primavesi et al. [37] also observed a linear effect on the absorption of Cu and Zn with an increase in nitrogen availability from urea and ammonium nitrate. In out experiment, the comparative increases in Cu and Zn between the highest dose of MGS and the treatment without fertilization are 44.07% and 56.83%, respectively, for the dry season. Compared to the mineral fertilization treatment, the increases were 44.07% and 47.51%, respectively, also for the dry season. The micronutrients Cu and Zn are critical to plant functions, as they are necessary in the structure of proteins and take part in the processes of photosynthesis, respiration, hormonal regulation, nitrogen fixation (indirectly), and metabolism of secondary compounds. For some plants, Cu also takes part in chlorophyll synthesis [44].

3.3. Soil Chemical Attributes

Soil chemistry was analyzed in four layers: 0–10 centimeters (cm), 10–20 cm, 20–40 cm, and 40–60 cm to determine the effect of MGS dose. Significant effects were found for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), sodium (Na), total cation exchange capacity (CEC), boron (B), and iron (Fe) as summarized in Tables 7 and 8. The level of N in the soil layer from 10–20 cm shows a negative linear relationship with increasing mixed gelatin sludge (MGS) dose, with values decreasing from 0.65 to 0.45 g kg^{-1} (Table 8). This may be explained by the linear increase in nitrogen absorption by the grass with increasing MGS dose, as this soil layer is where the highest root volume of forage grasses is concentrated. This indicates the use of fertilizers increases output of N from the 10–20 cm soil layer beyond the replenishment capacity of this layer.

Table 8. Chemical characteristics with soil depth by mixed gelatin sludge dose at the conclusion of the experiment for macronutrients and secondary nutrients for plants.

Chemical Characteristics	**Doses**							
Soil Depth (cm)	**0%**	**50%**	**100%**	**150%**	**200%**	**NPK**	**Regression Equation[Rf]**	**R^2**
Nitrogen (N in g kg^{-1})								
0–10	0.6 a	0.7 a	0.6 a	0.8 a	0.7 a	0.4 a	ŷ=0.68	
10–20	0.7 a	0.6 a	0.5 a	0.5 b	0.5 a	0.6 a	ŷ=0.6467-0.001x*	0.3
20–40	0.3 b	0.4 a	0.3 a	0.4 b	0.4 a	0.4 a	ŷ=0.36	
40–60	0.6 a	0.4 a	0.4 a	0.4 b	0.5 a	0.4 a	ŷ=0.46	
Phosphorus (P in mg dm^{-3})								

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0–10	14.6 a	24.7 a	20.0 a	26.0 a	16.6 a	9.6 a	ŷ=20.38	
10–20	9.7 a	3.8 b	9.1 a	4.9 b	4.6 b	5.0 b	ŷ=6.42	
20–40	3.3 b	3.4 b	2.9 b	2.2 b	2.8 b	3.3 bc	ŷ=3.3633-0.0045x*	0.3
40–60	2.7 b	2.7 b	2.2 b	1.7 b	2.2 b	2.4 c	ŷ=2.68-0.004x*	0.3
Potassium (K in g kg^{-1})								
0–10	66.7 a	54.0 a	55.3 a	39.3 a	43.7 a	43.0 a	ŷ=63.933-0.1213x**	0.4
10–20	44.7 b	42.0 ab	39.0 b	26.0 a	31.3 b	26.0 b	ŷ=45.133-0.0853x*	0.3
20–40	35.7 b	32.3 ab	32.0 b	22.7 a	27.0 b	26.0 b	ŷ=35.333-0.054x*	0.3
40–60	28.7 b	22.0 b	29.0 b	27.3 a	27.3 b	23.3 b	ŷ=26.86	
Calcium (Ca in cmolc dm^{-3})								
0–10	2.6 a	2.1 a	3.0 a	3.4 a	2.9 a	3.0 a	ŷ=2.4133+0.004x*	0,3
10–20	2.2 ab	1.2 b	2.3 b	2.1 ab	1.9 b	1.8 b	ŷ=1.94	
20–40	1.6 bc	1.3 b	1.6 c	1.6 b	1.2 b	1.7 b	ŷ=1.46	
40–60	1.4 c	1.2 b	1.1 d	1.4 b	1.0 b	1.5 b	ŷ=1.22	
Magnesium (Mg in cmolc dm^{-3})								
0–10	1.0 a	0.9 a	0.9 a	0.8 a	0.8 a	1.1 a	ŷ=0.88	
10–20	0.7 b	0.6 ab	0.6 b	0.5 ab	0.6 b	0.5 b	ŷ=0.60	
20–40	0.5 b	0.4 b	0.5 b	0.4 b	0.4 bc	0.4 b	ŷ=0.44	
40–60	0.6 b	0.4 b	0.5 b	0.5 b	0.5 c	0.4 b	ŷ=0.50	
Sulfur (S in mg dm^{-3})								
0–10	5.7 a	5.8 a	6.4 a	8.1 a	7.3 a	5.1 a	ŷ=5.596+0.0106x*	0.2
10–20	5.3 ab	5.8 a	6.2 a	5.7 a	6.4 ab	5.7 a	ŷ=5.88	
20–40	5.2 ab	6.0 a	5.2 ab	5.5 a	5.3 b	5.0 a	ŷ=5.44	
40–60	5.0 b	5.6 a	4.5 b	5.3 a	5.1 b	4.9 a	ŷ=5.10	

Lower case letters in a single column indicate significant differences between soil depths for each dose of waste evaluated according to a Tukey test with 5% probability. R^2: Coefficient of determination; * and ** indicate the level of significance of constants within regression models of 5 and 1%, respectively. ^{Rf} indicates that nitrogen, phosphorus, potassium (NPK) was not considered in the regression model as it is the standard treatment studied (50 kg ha^{-1} of N.

This is based on the fact that N is an important component of proteins, maximizes production of dry matter in forage grasses, and is the primary nutrient for maintaining productivity [45]. When added to soil it is assimilated by plants. Therefore, N is associated with carbon chains resulting in an increase in cell components. This leads to increases in the vigor of re-sprouting plants, the total production of dry mass given favorable climatic conditions [46], and greater nutrient absorption, as shown in the current study.

Due to the increased output of N to plants, there was a significant reduction in P and K in the 20–40 cm and 40–60 cm soil layers as the dose of MGS increased. In the soil layer from 20-40 cm, the levels of P increased from 3.3 to 2.8 mg dm^{-3}, and in the soil layer from 40–60 cm, P increased from 2.2 to 2.7 mg dm^{-3}. In the area treated with NPK mineral fertilizer, the lowest amount of P was found at the greatest depth. The level of K showed a negative linear effect with increasing dose of MGS for soil layers 0–10 cm, 10–20 cm, and 20–40 cm. In the 0–5 cm soil layer, the amount of K decreased from 66.7 to 43.7 mg dm^{-3} and in the 15–30 cm layer, the decrease was from 35.7 to 27.0 mg dm^{-3} (Table 8).

The decrease in K levels in the soil with increasing doses of MGS may occur due to the increase in N availability driving greater activity within the root systems resulting in greater K absorption. A well-developed root system makes use of greater soil volume and tends to absorb more nutrients, which in turn benefits plant growth. The increase in dry matter production deriving from increased nutrient availability from MGS and absorption of N and K, may have also stimulated absorption of P from the soil to plants.

Previous studies on the effects of MGS and dairy waste showed positive linear relationships between dosage and soil P and K. For example, Araujo et al. [10] showed increasing levels of P and K with increasing dose of MGS, with maximum levels of 2.38 and 2.07 mg dm^{-3} for the maximum

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dose of MGS within the 0–10 cm and 10–20 cm soil layers, respectively. Santos et al. [24], also observed an increase in the level of P in the 0–20 cm soil layer with increasing dairy waste. This was attributed to the limited mobility of P that stays fixed in the surface layer of the soil.

Both Ca and S showed a positive linear relationship with increasing dose of MGS for the 0–10 cm soil layer. The greatest quantity of each was found for the highest dose of MGS at 3.2 and 7.7 cmolc dm^{-3}, respectively (Table 8). There was a relative increase in Ca and S of 33.14% and 37.88% when compared to the treatment without fertilization, suggesting that these nutrients are introduced to the soil by MGS. The NPK treatment of mineral fertilizer, showed levels of Ca equal to the 100% treatment, whereas S was 7.73% lower. Araújo et al. [10] and Santos et al. [24] also found positive linear effects on Ca with increasing MGS dose and increasing dairy waste dose, respectively. However, Araújo et al. [10] observed a significantly higher maximum concentration of 4.41 cmolc dm^{-3}.

Within micronutrients, Fe level stands out, as it shows a negative linear relationship in the 0–5 cm layer, indicating a reduction in Fe with increasing dose of MGS (Table 8). The Fe in plants is related to metabolic activities, particularly enzyme production (catalase, peroxidase, cytochrome oxidase and xanthine oxidase). It is also critical to the processes of respiration, photosynthesis, N_2 fixation, and electron transfer between Fe^{2+} and Fe^{3+} [43,47]. Our present study shows the increase in nutrients by MGS, stimulating production of DM and increasing the demand for Fe from the soil, which also occurred in the NPK treatment. MGS has a considerable amount of available Fe, and the soil is also rich in Fe. We observe that the addition of fertilizers and the increase of photosynthetic plant material also causes the stimulation of absorption of Fe by forage grasses.

The total cation exchange capacity (CEC) showed a negative linear effect with increasing MGS dose in the 40–60 cm soil layer, decreasing from 4.2 to 3.7 cmolc dm^{-3} (Table 9). In the 0–40 cm soil layer, total CEC did not vary with MGS dose. This is likely related to balancing the base supply from MGS application and extraction with H^+. Similarly, Santos et al. [24], also did not see alterations in soil CEC when using more dairy waste.

Table 9. Chemical characteristics with soil depth by mixed gelatin sludge dose at the conclusion of the experiment for soil quality measures.

Chemical Characteristics	**Doses**							
Soil Depth (cm)	**0%**	**50%**	**100%**	**150%**	**200%**	**NPK**	**Regression Equation**^{Rf}	R^2
				pH				
0–10	6.3 a	6.2 a	6.2 a	6.2 a	6.2 a	6.2 a	ŷ=6.22	
10–20	5.9 b	5.6 b	6.0 a	6.0 ab	5.8 ab	5.8 a	ŷ=5.86	
20–40	5.8 b	5.5 b	5.6 b	5.8 ab	5.7 b	5.8 a	ŷ=5.68	
40–60	5.8 b	5.6 b	5.6 b	5.9 b	5.8 ab	5.8 a	ŷ=5.74	
				Organic matter (OM in dag kg^{-1})				
0–10	1.7 a	1.9 a	1.8 a	1.8 a	1.8 a	2.0 a	ŷ=1.80	
10–20	1.5 b	1.6 a	1.5 b	1.3 ab	1.6 a	1.3 b	ŷ=1.50	
20–40	1.1 c	0.9 b	1.1 c	1.0 bc	0.8 b	1.1 b	ŷ=0.98	
40–60	0.9 c	0.7 b	0.8 d	0.7 c	0.7 b	0.8 b	ŷ=0.76	
				Hydrogen+Aluminum (H^+Al cmolc dm^{-3})				
0–10	1.9 a	1.9 b	1.9 b	1.9 a	1.9 b	2.1 a	ŷ=1.9	
10–20	2.4 a	2.5 a	2.6 a	2.4 a	2.7 a	2.4 a	ŷ=2.52	
20–40	2.3 a	2.4 ab	2.6 a	2.2 a	2.6 ab	2.1 a	ŷ=2.42	
40–60	2.2 a	2.2 ab	2.3 ab	1.9 a	2.1 ab	1.9 a	ŷ=2.14	
				Sum of Bases (SB in cmolc dm^{-3})				
0–10	3.7 a	3.2 a	4.1 a	4.4 a	3.9 a	4.2 a	ŷ=3.86	
10–20	3.0 ab	1.9 b	3.1 b	2.7 b	2.6 b	2.4 b	ŷ=2.66	
20–40	2.2 bc	1.7 b	2.1 b	2.1 b	1.7 bc	2.2 b	ŷ=1.96	

40–60	2.0 c	1.6 b	1.6 b	2.0 b	1.5 c	2.3 b	ŷ=1.74	
Total Cation Exchange Capacity (CEC in cmolc dm^{-3})								
0–10	5.6 a	5.1 a	6.0 a	6.2 a	5.7 a	6.2 a	ŷ=5.72	
10–20	5.4 ab	4.4 b	5.7 b	5.1 b	5.2 b	4.8 b	ŷ=5.16	
20–40	4.5 bc	4.1 b	4.7 c	4.3 b	4.3 b	4.3 b	ŷ=4.38	
40–60	4.2 c	3.8 b	3.9 c	3.9 b	3.7 b	4.0 b	ŷ=4.0707-0.0019x*	0.3
Bases Saturation (V in %)								
0–10	66.9 a	63.8 a	68.6 a	70.2 a	67.5 a	66.7 a	ŷ=67.4	
10–20	54.7 ab	43.7 b	54.9 b	53.2 ab	48.7 b	49.6 b	ŷ=51.04	
20–40	49.9 b	41.5 b	45.1 c	48.4 b	39.5 bc	49.5 b	ŷ=44.88	
40–60	48.3 b	42.8 b	41.3 c	50.5 ab	41.6 c	56.9 ab	ŷ=44.90	

Lower case letters in a single column indicate significant differences between soil depths for each dose of waste evaluated according to a Tukey test with 5% probability. R^2: Coefficient of determination; * and ** indicate the level of significance of constants within regression models of 5 and 1%, respectively. ^{Rf} indicates that nitrogen, phosphorus, potassium (NPK) was not considered in the regression model as it is the standard treatment studied (50 kg ha^{-1} of N).

Na^+ showed a positive linear relationship with increasing doses of MGS in the upper three soil layers analyzed (0–40 cm; Table 10). The increase of Na^+ resulting from the highest dose of MGS were 74%, 76% and 52% higher than the non-fertilized treatments for the 0–10 cm, 10–20 cm, and 20–40 cm layers, respectively. The absence of a significant linear effect in the 40–60 cm layer indicates that the Na^+ available in the MGS is concentrated in the upper soil layers. Other results from the literature show that the Na^+ contained in the MGS applied is easily weathered to deeper soil layers due to low affinity for the exchange complex, remaining principally in the soil solution [48].

Table 10. Chemical characteristics with soil depth by mixed gelatin sludge dose at the conclusion of the experiment for micronutrients for plants.

Chemical Characteristics	**Doses**							
Soil Depth (cm)	0%	50%	100%	150%	200%	NPK	Regression Equation^{Rf}	R^2
Sodium (Na in mg dm^{-3})								
0–10	4.7 a	9.0 a	13.7 a	17.7 a	18.0 a	6.3 a	ŷ=5.5333+1.359x**	0.9
10–20	3.0 b	5.0 b	10.0 a	8.0 b	12.3 b	3.7 b	ŷ=3.3333+0.0433x**	0.7
20–40	1.7 c	2.0 c	2.5 b	4.0 bc	3.5 c	1.3 c	ŷ=1.6+0.0113x**	0.6
40–60	1.0 c	1.0 c	1.0 b	1.0 c	1.0 c	1.0 c	ŷ=1.0	
Boron (B in mg dm^{-3})								
0–10	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	ŷ=0.20	
10–20	0.2 a	0.2 a	0.2 ab	0.2 a	0.2 a	0.2 a	ŷ=0.20	
20–40	0.2 a	0.2 a	0.2 b	0.2 a	0.2 a	0.2 a	ŷ=0.194-0.0002x*	0.3
40–60	0.2 a	0.2 a	0.2 b	0.2 a	0.1 a	0.2 a	ŷ=0.18	
Copper (Cu in mg dm^{-3})								
0–10	0.6 ns	0.6 ns	0.7 ns	0.6 ns	0.6 ns	0.6 ns	ŷ=0.62	
10–20	0.6 ns	0.7 ns	0.7 ns	0.6 ns	0.6 ns	0.6 ns	ŷ=0.64	
20–40	0.7 ns	0.7 ns	0.7 ns	0.7 ns	0.7 ns	0.6 ns	ŷ=0.70	
40–60	0.7 ns	0.7 ns	0.6 ns	0.6 ns	0.6 ns	0.6 ns	ŷ=0.64	
Iron (Fe in mg dm^{-3})								
0–10	80.0 a	90.5 a	53.0 b	41.3 a	59.0 b	53.5 a	ŷ=83.00-0.1823x*	0.3
10–20	90.0 a	120.0 a	70.3 ab	63.0 a	73.3 ab	57.5 a	ŷ=83.32	
20–40	77.0 a	78.7 a	83.7 a	66.0 a	71.3 ab	36.5 a	ŷ=75.34	
40–60	67.7 a	75.3 a	70.3 ab	58.7 a	86.7 a	51.3 a	ŷ=71.74	

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	Manganese (Mn in mg dm^{-3})						
0–10	61.9 a	61.1 a	67.5 a	65.3 a	63.7 a	62.9 a	ŷ=63.90
10–20	48.8 b	38.0 b	54.9 a	48.5 b	48.8 ab	37.2 b	ŷ=47.8
20–40	34.7 c	26.4 bc	39.5 b	32.3 c	23.2 b	26.8 bc	ŷ=31.22
40–60	24.8 c	19.2 c	22.1 c	24.0 c	25.9 b	15.6 c	ŷ=23.2
	Zinc (Zn in mg dm^{-3})						
0–10	1.0 a	1.3 a	1.1 a	1.1 a	1.0 a	0.9 a	ŷ=1.10
10–20	0.8 a	0.7 b	0.7 ab	0.6 b	0.6 b	0.7 a	ŷ=0.68
20–40	0.4 b	0.3 bc	0.4 bc	0.4 b	0.3 c	0.3 a	ŷ=0.36
40–60	0.3 b	0.2 c	0.3 c	0.3 b	0.3 c	0,5 a	ŷ=0.28

Lower case letters in a single column indicate significant differences between soil depths for each dose of waste evaluated according to a Tukey test with 5% probability. R^2: Coefficient of determination; * and ** indicate the level of significance of constants within regression models of 5 and 1%, respectively. [Rf] indicates that nitrogen, phosphorus, potassium (NPK) was not considered in the regression model as it is the standard treatment studied (50 kg ha^{-1} of N).

Increases in the concentration of Na^+ in the soil were also reported by Guimarães et al. [9], working with sludge from gelatin production. According to Leal et al., an excess of salt in soils, principally Na^+, increases both the salinity and sodicity and results in the deterioration of soil physical properties [48]. Together with the toxic and osmotic effects of this ion, high salt levels reduce crop production. While Araújo et al. [10] did not observe Na^+ in soils after fertilization with MGS, Santos et al. [24] observed increases in Na^+ with application of dairy waste with totals of 6.75 and 8.5 mg dm^{-3} in the 0–20 cm and 20–40 cm layers, respectively.

To classify soils that are affected by salts, limiting conditions are based on measurements of soil water at saturation: electrical conductivity (EC), exchangeable sodium percentage (ESP), sodium adsorption ratio (SAR) and pH (Table 11). The results of ESP in soil are presented in Table 12. The highest concentration of Na^+ (18 mg dm^{-3}) and the highest ESP (1.39%) were found for the highest dose of MGS at the soil surface layer (0–10 cm). It is important to note that this ESP is well below the limit of 15% considered to indicate sodic soils [48,49].

Table 11. Criteria and limits for the classification of normal, saline, sodic and saline-sodic soil.

Critérios	**Tipos de solos**			
	Normal	**Saline**	**Sodic**	**Saline-sodic**
EC (dS m^{-1} a 25°C)[1]	< 4	> 4	< 4	> 4
ESP (%)[2]	< 15	< 15	> 15	> 15
SAR[3]	< 13	< 13	> 13	> 13
pH	< 8.5	< 8.5	> 8.5	> 8.5

[1] EC = electrical conductivity of paste solution extracted at saturation. [2] ESP = Exchangeable Sodium Percentage. [3] SAR = Sodium Adsorption Ratio; SAR= Na / (Ca + Mg)$^{1/2}$. Source: Richard 1969 [50].

Table 12. Exchangeable sodium percentage (ESP) in different soil layers varying dosage of mixed gelatin sludge and mineral fertilization during the rainy and dry seasons.

Layer	**Dose**					
	0%	**50%**	**100%**	**150%**	**200%**	**NPK**
(cm)	---------------------------------- % ---------------------------------					
0–10	0.37	0.77	0.99	1.24	1.39	0.45
10–20	0.25	0.49	0.77	0.67	1.08	0.33
20–40	0.16	0./21	0.23	0.40	0.36	0.13
40–60	0.10	0.11	0.11	0.11	0.12	0.11

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4. Discussion

Increased tillering from mixed gelatin sludge (MGS) application in our experiment can allow the grass to re-sprout more quickly, reduces the cycling time of the pasture, and allows the pasture to be grazed more frequently per year. Sbrissia & Silva reinforce that the persistence and perpetuity of forage is based on the number of individual tillers present in its structure [51]. A greater quantity of tillers per area also allows for greater photosynthetic efficiency given increased light interception. This results in higher accumulation of photoassimilates that, in practice, reflect an increase in dry matter production [52].

The results obtained in this study show that increasing the dose of MGS promotes increased growth rates in plants for the same fallow period, potentially improving pasture management. The faster pastures re-sprout, the shorter the rotational grazing period, resulting in a greater frequency of grazing and higher productivity for a given area. This result was expected given the N contained in MGS increases the formation of tiller tissues, increasing the rate of elongation and the number of tillers per plant [26].

However, it is important to note that the increase in tillering and height of plants resulting from the highest doses of MGS may be negated over time depending on management. *Brachiaria* spp. adapts to varying management. When managed to maintain taller grass, their tillers are heavier. However, when managed to maintain lower heights, the grass has an increase in number of tillers, but a decrease in tiller weight [51].

Our results demonstrate that MGS contributes to overall plant nutrition and is particularly promising as it increases productivity of Marandu grass compared to mineral fertilization. Other studies that evaluate the application of residue on pasture grasses have found similar results. Araújo 2016 applied MGS to Piatã grass and found similar tendencies of linear increases in the production of dry matter with increasing doses of MGS [53]. Past research showed increased dry matter production in *Brachiaria decumbens* with organic fertilizer when compared to mineral fertilizers and these researchers believed this result was due to the increased mineralization of organic material present in the swine residue applied compared to mineral fertilizer [28].

Nitrogen deficiency causes limitations in protein synthesis and pigmentation of plant tissue causing significant reductions in photosynthetic activity. This leads to diminished growth and affects the production and quality of biomass [54]. In nitrogen-limited environments, forage growth slows and plants are smaller, with fewer tillers, and insufficient crude protein to meet animal needs, compromising the sustainability of the plant-animal system [55]. In this way, the production of dry matter of tropical grasses is directly related to nitrogen fertilization.

Current results demonstrate the potential of MGS fertilization for Marandu grass during both the dry and rainy seasons, as it led to significant increases in dry matter productivity. It is important to note that no signs of phytotoxicity were observed. However, studies with longer durations are important to monitor the continuity of these observed trends.

Our results suggest that the combination of nutrients provided by MGS improved the bromatological composition of forage without compromising the quality of Marandu grass, instead resulting in a feed with greater nutritive value for animals. According to Develatti et al., higher levels of nitrogen fertilization promote increases in crude protein (CP) and proportional reductions in fiber [56]. Magalhães et al. further suggests that MGS promotes increases in N, Ca, P, K, and Mg in soils, and stimulates new leaves, which are the components with the highest levels of CP in the plant [57].

Our results indicate that the use of MGS leads to increases in crude protein (CP), without impacting levels of fiber in Marandu grass. This is promising given that protein is a high cost item in animal supplements. If protein levels are higher in the available pasture, the amount offered as supplement can be reduced, further reducing production costs. Analyzing morphological characteristics and bromatological composition of Marandu grass by way of CP production, it is estimated that the 200% treatment in our study had the best results, both increasing the productivity of Marandu grass and not compromising the quality of the forage.

Based on our results of chemical composition in leaf tissue, MGS has potential as a source of nutrients for Marandu grass, especially during the dry season, maintaining a nutritional balance

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between macro- and micro-nutrients essential for plant growth. It is important to highlight the effect of MGS in maintaining adequate levels of macro-nutrients in the soil even in the face of an increase in forage productivity of around 83% at a MGS dose of 200% compared to mineral fertilization in the rainy season and with an increase of 62% in the dry season. Our results demonstrate the importance of MGS as a low-cost fertilizer in recovering and maintaining soil fertility and promoting increased productivity in pastures. Although sodium (Na+) levels are insufficient to indicate sodicity, it is necessary to evaluate the experiment over a period longer than two years to understand whether accumulation could become problematic for grass production or cause risks to the local environment under similar climatic conditions.

5. Conclusions

The experimental dose of mixed gelatin sludge (MGS) resulting in better productivity and quality of Marandu grass is 200% of the crop's annual requirement for both the rainy and dry periods. This corresponds to an application of 60.4 metric tons of this sludge per hectare per year. The use of MGS in Marandu grass increased the production of dry matter and crude protein, reduced neutral detergent fiber, and increased the levels of nitrogen, phosphorus, calcium, magnesium, sulfur, copper, and zinc in this grass. The use of MGS increases the levels of calcium and sulfur on the surface, the amount of potassium and sodium in the 0 cm to 40 cm soil layer, and the nitrogen and phosphorus in the subsurface, demonstrating an important effect as a carrier of bases for the sub-surface layers of the soil that will guarantee better root development for plants. Increasing sodium in the soil up to the doses used was not harmful to the soil nor to plant growth. This suggests the need for future studies to evaluate the effect of higher doses of this type of sludge on grass growth and maintenance of adequate levels of soil fertility. Mixed gelatin sludge is a promising organic fertilizer for the recovery of degraded pastures and as a conditioner of the chemical properties of the soil, promoting an increase in the levels of macro-nutrients and micro-nutrients in a balanced way and maintaining their adequate levels in the soil even with increased forage productivity. The predominant soil in the Acorizal region has low natural fertility, which leads to low productivity and quality of pasture. In this sense, the use of MGS as a low-cost organic fertilizer can result in increased pasture productivity and producer profitability, contributing to the circular economy of local family farming.

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