

Agro-industrial residues in the control of gastrointestinal nematodes of small ruminants and fertilization of forages

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ABSTRACT: Verminosis in small ruminants can render rural activity impractical, which is still controlled through the administration of anthelmintics. The present study evaluated four agro-industrial residues as fertilizer in the control of GIN of small ruminants in the freeliving stage. Crab shell, *manipueira*, biochar, and organomineral residues were used in pots (5.0 kg of soil) and cultivated with Massai grass (*Megathyrsus maximum* cv. Massai). Further, the pots were contaminated with feces from sheep carrying a natural multispecific infection by GIN. Next, the residues were applied individually to the soil, with 50 mL/pot for liquid and 50 g/pot for solid residues. Treatment with *manipueira* showed the lowest number of L3.kg MS⁻¹ recovered from the grass (202.44), when compared with treatments using organomineral (823.89) and biochar (689.34). However, there was no statistically significant difference (P < 0.05) between the four treatments as compared to the control group. These agro-industrial residues can be used as organic fertilizers; however, these will not help in the control of GIN in sheep. **Key words**: worm control, infective larvae, sheep nematodiasis, environmental contamination.

Resíduos agroindustriais no controle de nematóides gastrointestinais de pequenos ruminantes e adubação de forrageiras

RESUMO: A verminose em pequenos ruminantes é uma doença que pode inviabilizar a atividade rural. Embora o sucesso no controle dos nematóides gastrointestinais de pequenos ruminantes dependa da adoção associada de diferentes estratégias e tecnologias, ainda é possível notar a escolha, por parte de alguns ovinocaprinocultores, pelo tratamento exclusivamente baseado na administração de anti-helmínticos aos animais. Contudo, é no ambiente que encontramos um elevado número de formas imaturas de nematóides gastrointestinais. Objetivou-se com o presente trabalho avaliar quatro resíduos agroindustriais como adubo no controle dos nematóides gastrointestinais. Objetivou-se com o presente trabalho avaliar quatro resíduos agroindustriais como adubo no controle dos nematóides gastrointestinais de pequenos ruminantes, em estádio de vida livre. Casca de caranguejo, manipueira, biochar e organomineral foram os resíduos empregados nos vasos com capacidade média de 5,0 kg de solo, onde foi cultivado o capim Massai (*Megathyrsus maximus* cv. Massai). Após corte de uniformização do capim, os vasos foram contaminados utilizando-se fezes de ovinos portadores de infecção natural multiespecífica por nematóides gastrointestinais. E, na sequência, os resíduos foram aplicados individualmente no solo, na quantidade de 50 mL/vaso para o resíduo líquido e 50 g/vaso para os sólidos. O tratamento com a manipueira foi o que apresentou o menor número de larvas infectantes de terceiro estádio (L3) recuperado do capim (202,44 L3.kg MS⁻¹) quando comparado com os tratamentos utilizando organomineral (823,89 L3.kg MS⁻¹) e biochar (689,34 L3.kg MS⁻¹). Contudo, não houve diferença estatística significativa (P < 0,05) entre os quatro tratamentos testados quando comparado ao grupo controle. Os resíduos agroindustriais podem ser utilizados como adubos orgânicos, mas não vão auxiliar no controle dos nematóides gastrintestinais dos ovinos.

Palavras-chave: controle parasitário, larva infectante de terceiro estádio, nematodiose ovina, contaminação ambiental.

INTRODUCTION

Goat and sheep production is an extremely important economic activity in Brazil, which has a herd of 29 million animals, with the Northeast region accounting for the largest share, 67% for sheep and 93% for goats (HOLANDA FILHO et al., 2019).

Producing small ruminants in a pasture system is a challenge for tropical regions because

nematode diseases are a major cause of mortality in goats and sheep, especially in young animals, and are a barrier to this economic activity (ALMEIDA et al., 2005). Among the main gastrointestinal nematodes (GIN) parasitizing goats and sheep in Brazil is *Haemonchus contortus*, which is the most prevalent in countries and regions with tropical and subtropical climates. It is also the most pathogenic and can cause acute infection resulting in death.

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Another important GIN genus associated with infections in small ruminants is Trichostrongylus (GYELTSHEN et al., 2022). The presence of the genus Teladorsagia is observed in Brazilian states, especially in the south, where winter is characterized by low temperatures (ECHEVARRIA et al., 1996). In addition to the previously mentioned GIN, MACIEL et al. (2014) reported a high prevalence of Cooperia sp. and Oesophagostomum columbianum in sheep raised in southeastern Brazil. In northeastern Brazil, H. contortus, Trichostrongylus axei (AROSEMENA et al., 1999), and Oesophagostomum sp. (CHARLES, 1995) were the most common GIN parasitizing sheep. It is estimated that only 5% of GIN are reported in the digestive tract of animals, while 95% contaminate the environment (BENAVIDES & SOUZA, 2020). Therefore, pastures represent an important source of GIN infection for all animals in the herd, which ingest the infective third stage larvae (L3) along with the pasture during feeding. Nematodes in different free-living stages can remain in the pasture in the fecal bulk for many months, especially under conditions of adequate moisture, temperature, and shading (microclimate). According to ROCHA et al. (2014), the fecal bulk acts as a reservoir for GIN L3 in pasture, where larvae can remain viable for up to 16 weeks, depending on the local environment and climatic conditions.

The use of agro-industrial residues for GIN control can provide environmental benefits (reducing environmental liability-residues and reducing anthelmintic use), economic benefits (reducing mineral fertilizer use-soluble in pastures and, again, anthelmintic use), and productive benefits (increasing productive efficiency of pastures by improving soil fertility). In this context, some studies using agroindustrial residues as fertilizers in an area with Megathyrsus maximus cv BRS Tamani report a decrease in GIN and satisfactory forage production when replacing urea to castor bean cake, which is a residue of the biodiesel industry with an efficiency of 63.41% to control worms (MARANGUAPE et al., 2020).

Soil control of L3 is a strategy that can be used in regions where agro-industrial residues are present, which also have the potential to improve the forage. However, it is important to consider that the availability of residues is limited by the amount of source material, which justifies the search for regionalized by-products (SALES et al., 2019).

This study evaluated agro-industrial residues that have the potential to control the development of GIN in small ruminants in the free-living stage and to serve as fertilizers when applied to the soil.

MATERIALS AND METHODS

The experiment was conducted in a 50% shaded greenhouse in Teresina, PI, located at geographic coordinates 5° 02' 21.36" S and 42° 47' 22.44" W, from November 2020 to March 2021. According to the Koppen–Geiger classification, the climate of the region is type Aw, tropical climate, with an average annual temperature and rainfall of 27.9 °C and 1,451 mm, respectively. Table 1 shows the chemical properties and texture of the soil used for the experiment after autoclaving.

Experimental design and treatments

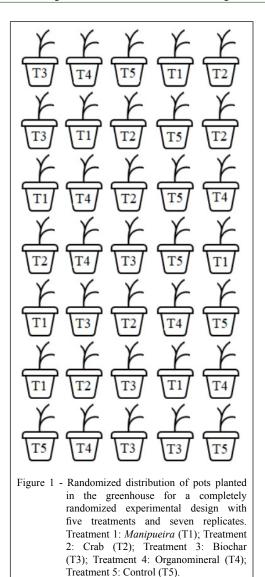
The experimental design chosen was completely randomized with five treatments and seven replicates (Figure 1). The treatments correspond to the application of four by-products: cassava production residue—*manipueira* (fluid from the production of cassava starch) (T1), crab waste (shells from the consumption of crabs) (T2), biochar (material from the pyrolysis of eucalyptus) (T3), organomineral whose organic matrix consists of residues from the production and slaughter of small ruminants and the mineral matrix by monoammonium phosphate

Table 1 - Chemical properties and texture of the soil used in the test.

Attribute	Unit	Value	
pН	CaCl ₂	4.10	
OM	dagkg ⁻¹	1.8	
Р	mg dm ⁻³	6.2	
Κ	cmol _c dm ⁻³	0.1	
Ca	cmol _c dm ⁻³	1.9	
Mg	cmol _c dm ⁻³	0.5	
H+Al	cmol _c dm ⁻³	3.7	
SB	cmol _c dm ⁻³	2.5	
CEC	cmol _c dm ⁻³	6.2	
BS	%	41	
Coarse Sand	%	23	
Fine sand	%	37	
Total Sand	%	60	
Clay	%	15	
Silt	%	25	

pH, potential of hydrogen; OM, organic matter; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; H+Al, potential acidity; SB, sum of bases; CEC, cation exchange capacity; BS, base saturation.

Source: Soil and Plant Laboratory, Embrapa Mid-North.



(MAP) (T4), and the control without the application of residues (T5).

The agro-industrial residues used in the study were chosen because they are abundant and important in the Northeast Region and in the State of Piauí, such as *manipueira* from mills and agro-industries in many municipalities of the State of Piauí. Crab shell residues come from the consumption of the meat of this crustacean, which is abundant on the coast of Piauí. Biochar is a product obtained from the biomass of eucalyptus, which is widely grown in the Cerrado of Piauí. The organomineral was produced by combining two matrices, the mineral (MAP) and the organic (compound from residues from the production and slaughter of small ruminants) and was selected because the mineral source served as a positive control. The analyzed parameters of the residues used in the study are shown in table 2. Samples were subjected to nitric acid perchloric digestion, with phosphorus (P) determined colorimetrically; potassium (K) and sodium using flame photometry; S turbidimetrically; and Ca and Mg using atomic absorption spectrophotometer (ABREU ET AL., 2006); nitrogen (N) was determined using the Kjeldahl method and carbon by wet (Walkley–Black method).

Massai grass (*Megathyrsus maximus* cv. Massai) was grown in pots with an average capacity of 5.0 kg of soil. Seeds were sown at a depth of 2.0 cm. Thinning was done 14 days after planting (DAP) using pruning shears, leaving four plants per pot. After planting, two fertilizations were applied, the first at 17 DAP with N, P, and K at respective doses of 20, 20, and 24 kg ha⁻¹ and the second at 38 DAP with N and K at doses of 60 and 24 kg ha⁻¹, diluting the fertilizers in 50 mL of water. The humidity in the pots was kept constant (at least 70% of the total pore volume) throughout the experimental period by manual watering.

Fifty-six DAP the Massai grass in the pot, the standardization cut was performed at a 15cm height above the ground. Sixty-five DAP, 50 g of sheep feces from animals with natural multispecific infection by GIN (presence of Haemonchus sp., Trichostrongylus sp., and Oesophagostomum sp. in previous stool cultures in the herd) was introduced. Only feces from animals that had an egg count per gram of feces (EPG) of at least 1500, as determined by the McMaster method (GORDON & WHITLOCK, 1939), were homogenized in a container from which aliquots were taken to be later added to the pots, but without counting the number of eggs they contained. Immediately thereafter, the agro-industrial residues were applied individually to each of the pots according to the experimental design (Figure 1). The amount of solid waste applied to each of the five pots of each treatment was 50 g, while the amount of manipueira was 50 mL. A total of 50 mL of water was used for the control group.

Parasitological analysis and forage yield

After 25 days of contamination of the pots with feces, the grass was cut 5 cm from the ground, packed in buckets, individually identified and immediately sent to the laboratory for parasitological analyses. The grass cutting process took place between 7:00 and 8:00 am, a less hot and humid period of the day, to enable the recovery of infective larvae.

Residue	Ν	Р	K	Na	Ca	Mg	S	С	C/N
g kg ⁻¹									
Biochar	5.6	1.3	6.5	1.5	18.6	3.2	0.86	347	62.4
Crab	13.0	10.5	0.7	5.7	281.2	18.2	0.36	50	3.8
Organomineral	44.5	103.5	8.4	7.0	13.34	5.6	3.45	81	1.8
g L ⁻¹									
Manipueira	4.88	0.29	2.29	0.42	0.45	0.32	0.06	22	4.5

Table 2 - Chemical properties of the residues used in the test.

N, nitrogen; P, phosphorus; K, potassium; Na, sodium; Ca, calcium; Mg, magnesium; S, sulfur; C, carbon; C/N, carbon-nitrogen ratio. Source: Soil and Plant Laboratory, Embrapa Mid-North.

The freshly harvested grass samples were individually weighed and, subsequently, the forage larvae recovery technique was applied as described in the Manual of Veterinary Parasitological Laboratory Techniques (1977). After L3 recovery, the grass was packed in brown paper bags, individually identified and taken to the air re-circulation oven at 65 °C for three days (constant weight), for new weighing and determination of dry matter (DM). The estimated amount of L3 in the grass grown in the pots was expressed by L3.kg MS⁻¹.

The L3 were observed under an optical microscope using a 4x and 10x objective for simultaneous counting and sex identification, according to KEITH (1953). Specimens of L3 (non-rhabditiform esophagus) with impaired morphology or covered by dirt, making it impossible to identify the genus, were classified as "L3 not identified" during counting.

Soil fertility analysis

After recovering the forage larvae, a soil sample was collected with a Dutch auger and the following chemical analyses were performed: hydrogen ion potential (pH_{CaCl2}), organic matter (OM, Walkley–Black method), phosphorus (P, Melichl extractor), K (Melichl extractor), sodium (Na, Melichl extractor), calcium (Ca, KCl extractor 1M), magnesium (Mg, KCl extractor 1M), potential acidity (H+Al, calcium acetate extractor), sum of bases (SB = K+Ca+Mg+Na), cation exchange capacity (CEC = SB+H+Al), and base saturation (BS = SB /CEC*100), following TEIXEIRA et al. (2017).

Statistical analysis

Statistical analysis was performed using the BioEstat program (version 5.3, Instituto de

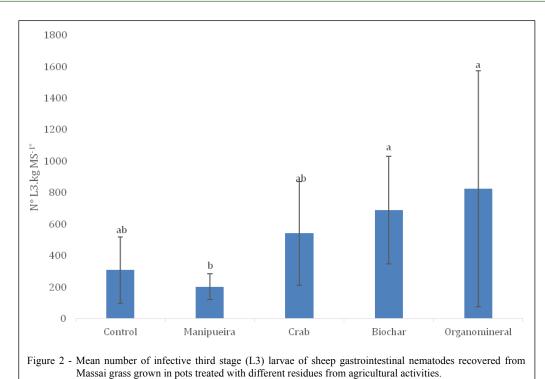
Desenvolvimento Sustentável Mamirauá, Belém, Pará, Brazil). Analysis of variance of larval count data (L3. kg MS^{-1}) and Tukey's test for comparison of means were performed with significance levels of 5% and 7%, respectively. Data were transformed into log (x) to correct for heterogeneity of variances, but these were expressed as arithmetic mean \pm standard deviation.

RESULTS

Parasitological analysis

None of the treatments tested showed a statistically significant difference compared to the control group (Figure 2). Treatment with *manipueira* has the lowest number of L3.kg MS⁻¹recovered from the grass compared to the organomineral and biochar treatments. However, the difference between the means of the treatments observed for the *H. contortus* species (P < 0.05) (Table 3) and for the total number of L3 (P < 0.07) was not sufficient to exclude random variation.

Table 3 shows the percentages of occurrence and absolute frequency of each genus of GIN recovered from grass grown in pots treated with various agricultural residues. Haemonchus contortus was the most abundant species in all treatments, followed by Trichostrongylus sp. In pots not treated with residues (control), only the presence of these two species was detected. In the manipueira treatment, the amount of L3 of H. contortus and Trichostrongvlus sp. was equivalent, with relative frequencies of 43% and 41%, respectively, and the presence of the genus Bunostomum (5%) was recorded, which was not present in any other treatment. Infectious larvae of the genus Oesophagostomum were detected in the pots treated with crabs (2%), biochar (3%), and organomineral (1%), but in low percentages.



*Number of infective larvae recovered from Massai grass (kg DM⁻¹). Means followed by the same letter do not differ

Analysis of forage yield and soil fertility

Productivity of Massai grass was altered by residue application, and the use of organomineral resulted in a higher DM yield to the detriment of other residues and the control (Figure 3). All soil chemical properties were altered by residue application (Table 4). The highest pH values were associated with the application of crab waste and biochar compared to *manipueira*, organomineral, and the control. Even the highest concentrations of organic matter were

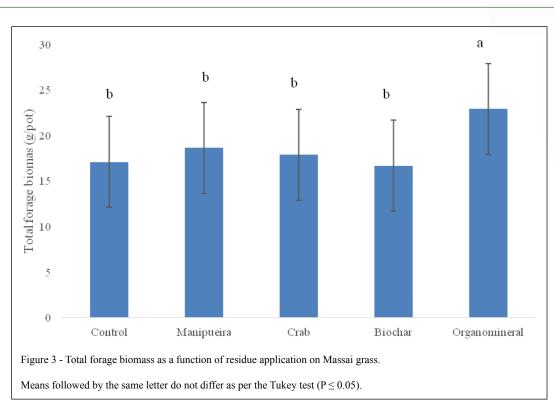
Table 3 - Mean, relative, and absolute frequencies of sheep gastrointestinal nematodes recovered from grass grown in pots treated with different residues from agricultural activities.

	Genus						
	Haemonchus	Trichostrongylus	Oesophagostomum	Bunostomum	Total L3		
Control	81% (250.2 ^{ab})	19% (57.5 ^a)	(0^{a})	(0^{a})	(307.7)		
Manipueira	43% (87.5 ^b)	41% (82.0 ^a)	(0^{a})	5% (9.8 ^a)	(202.4)		
Crab	55% (298.3 ^{ab})	16% (88.4 ^a)	2% (9.5 ^a)	(0^{a})	(540.9)		
Biochar	52% (359.1ª)	20% (138.0 ^a)	3% (22.4 ^a)	(0 ^a)	(689.3)		
Organomineral	67% (549.3 ^a)	19% (156.4 ^a)	1% (9.7 ^a)	(0 ^a)	(823.9)		

L3: third-stage infective larvae. The sum of the relative frequencies (%) of each treatment is 100% if the percentage of unidentified L3 is added to the value. Absolute mean frequencies (L3.kg MS⁻¹) in parentheses, followed by the same letter (columns) do not differ as per the Tukey's test ($P \le 0.05$).

as per the Tukey test ($P \le 0.07$).

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obtained with the application of biochar compared to the control.

The highest concentrations of P and K were obtained with the application of organomineral; however, for K, there was no difference between the organomineral and the application of crab waste and biochar (Table 4). The lowest sodium levels were obtained with organomineral and crab waste which did not differ from biochar (Table 4). For Ca, the use of crab waste provided the highest concentrations and increased SB and BS (%). The combined use with biochar, in turn, resulted in lower H+Al. The organomineral and crab waste increased the Mg concentration in the soil, and the use of the organomineral provided the highest CEC value (Table 4).

DISCUSSION

In a herd, there are animals carrying various parasitic loads of GIN. Few of which, considered susceptible, are often diagnosed with high EPG levels, contributing to environmental contamination and thus transmission to other animals in the herd (CHARLIER et al., 2020). In multispecific infections, which occur naturally in herds, the frequency of occurrence of each species varies. According to BRICARELLO et al. (2021), CHARLIER et al. (2020), and FOX et al. (2013), this distribution is related to differences in host exposure to the parasite, establishment of infection, or survival of the nematode in the host and has important implications for transmission, diagnosis, and control. This fact may explain the large standard deviation observed in the L3 samples obtained from the pots of each treatment, since there was no standardization of the number of GIN eggs, but only the amount of fecal pellet (50 g of feces from animals with an EPG greater than 1500) used to contaminate the pots.

According to BENAVIDES & SOUZA (2020), approximately 95% of the parasite population is in the pasture. The environment, which enables the development of the free-living stage of GIN, is of great importance for the implementation of parasite control measures that are not exclusively aimed at the administration of anthelmintics to the animals. Environmental conditions have a direct influence on GIN larval development. Under conditions of high local temperature and humidity in the greenhouse and pots (greater than or equal to 70% of total pore volume), L3 recovery was observed in all treatments tested and in the control, especially in the genera Haemonchus and Trichostrongylus. The great importance of environmental factors such as temperature and humidity is reiterated by GYELTSHEN et al. (2022).

Residue		pH		OM	Р		BS
		CaCl ₂		dagkg ⁻¹	mg dm ⁻³		%
Biochar		4.78a		1.77a	13.4bc		35b
Crab		5.12a		1.50ab	36.8b		48a
Manipueira		4.22b		1.62ab	10.4c		28bc
Organomineral		4.23b		1.62ab	308.6a		29bc
Control		3.95b		1.40b	11.2bc		21c
CV (%)		5.4		12.2	24.8		15.2
Residue	K	Na	Ca	Mg	H+Al	SB	CEC
		c	mol _c dm ⁻³				
Biochar	0.034ab	0.034bc	0.86bc	0.49ab	2.64bc	1.43bc	4.07c
Crab	0.029ab	0.028c	1.52a	0.53a	2.24c	2.11a	4.34bc
Manipueira	0.020b	0.043b	0.76bc	0.39bc	3.07b	1.21bc	4.28c
Organomineral	0.040a	0.013d	0.98b	0.54a	3.86a	1.58b	5.44a
Control	0.020b	0.057a	0.66c	0.29c	3.76a	1.02c	4.78b
CV (%)	34.2	23.2	21.4	18.5	9.0	18.4	6.5

Table 4 - Mean values, F test, and coefficient of variation of soil chemical attributes as a function of residue application in pots grown with Massai grass.

pH, potential of hydrogen; OM, organic matter; P, phosphorus; BS, base saturation; K, potassium; Na, sodium; Ca, calcium; Mg, magnesium; H+Al, potential acidity; SB, sum of bases; CEC, cation exchange capacity. Means followed by the same letter (columns) do not differ as per the Tukey test (P < 0.05).

According to ALMEIDA et al. (2005), specimens of the genera Haemonchus, Trichostrongylus, Cooperia, and Oesophagostomum in the fecal bulk of ruminants could complete the molt of eggs up to L3 and complete the entire nonparasitic biological cycle, including vertical and horizontal migration on pasture, even in the dry season. The authors noted that low precipitation associated with mild temperatures can determine L3 survival for extended periods in the environment. These results corroborated the findings of this study, as the same genera, except for Cooperia sp., were recovered from pots planted with grass after a period of 25 days since contamination with feces. Although, the experiment was conducted in a greenhouse, the temperature and humidity parameters were not controlled. However, the high temperatures and humidity provided by daily manual watering were sufficient for egg development up to L3.

Although, the availability of water is an important factor in the development and maintenance of free-living forms of GIN, excess water can affect the ecology of the parasite since the L3 can be washed away by vegetation (CHAUDARY et al., 2008). When grown in pots, manual watering may contribute

to the variation in the amount of L3 recovered within a few treatments, as a small overflow at the time of deposition on the soil may release the L3 present in the surface or grass outside of the pot.

In a study conducted by GASPARINA et al. (2021), H. contortus was the most abundant species in pasture with a recovery rate of 93.1%, while the frequency of Trichostrongylus sp. and Oesophagostomum sp. was 4.6% and 2.3%, respectively. The results are similar to those of this study, in which a high prevalence was observed for H. contortus (58.4%) and a lower prevalence for Trichostrongylus sp. (37.3%) and Oesophagostomum sp. (2.9%). However, in contrast to GASPARINA et al. (2021), there was a smaller discrepancy between the relative frequencies of H. contortus and Trichostrongylus sp. (93.1:4.6 and 58.4:37.3, respectively). However, the difference in experimental design of the two studies (pot vs. field) must be considered.

As mentioned earlier, under favorable temperature and humidity conditions, the GIN eggs expelled into the environment with the fecal bulk develop from morula to first-stage larva (L1) within 1 to 2 days, soon passing into the second larval stage

(L2). The pre infective larvae (L1 and L2) feed on the bacteria in the feces and soil; hence, they must acquire an adequate energy reserve until they reach the L3 phase, when they can no longer feed on themselves, as they retain the L2 cuticle (BOWMAN, 2010). The L3, which accumulates more lipid reserves, can survive longer in the environment until they are taken up by the host (SIAMBA et al., 2011). Some organic fertilizers can promote the microbiological enrichment of the soil, the bacteria present act as a food source for the pre infective larvae. This may explain the high number of L3 in the biochar and organomineral treatments, which had higher organic matter concentrations (1.62 dag kg⁻¹ and 1.77 dag kg⁻¹, respectively) and, in the case of organomineral, also the highest forage biomass. The increase in organic matter resulting from the application of some residues can be explained by the increase in the dynamics of the relationship between soil and plant, since the interactions and reactions are limited by the volume of the pot used (FERNANDES et al., 2015); thus, we can expect an increase or change in certain bacterial groups in the soil due to the application of residues (LEITE et al., 2021; MIRANDA et al., 2018), contributing to the feeding of L1 and L2.

There are many options for agricultural residues, such as castor bean cake (RESENDE and COSTA, 2016), the by-product of guava-processing agro-industry (SOUZA et al., 2014), and vinasse (COSTA et al., 2021), which can be used as soil conditioners and nutrient sources but deserve to be studied for their potential nematicidal effects in the free-living phase of GIN in small ruminants. Manipueira, derived from cassava processing, contains a toxic substance (linamarin) to many life forms, including nematodes (NASU et al., 2010). According to the authors, treatments in pots with manipueira at concentrations of 10% and 25% were more effective in controlling the tomato nematode Meloidogyne incognita. Studies such as those of NASU et al. (2010) reinforce the interest in new tests with this residue using other experimental setups.

The organomineral has a higher solubility of nutrients (MAP in its composition) and a low C/N ratio (Table 2), which also leads to an improvement in the concentrations of P, K, Mg and even CEC, which translates into a higher production of total forage biomass. The increase in CEC, $5.44 \text{ cmol}_{e} \text{ dm}^{-3}$ (Table 4), can be justified by the use of the organic fraction (organic compound) of the organomineral.

Regarding other residues, crab waste improved the cationic properties of the soil (Ca, SB,

and BS%) and the pH (Table 4). This improvement is related to its chemical composition, which has a high calcium carbonate content (FERREIRA et al., 2011). Biochar led to an improvement in OM content and an increase in pH (Table 4), but its high C/N ratio (Table 2) may not have contributed to translating the increase in organic matter into an increase in biomass of Massai grass. Because of the high C/N ratio, a longer interaction time between the biochar and the soil is required to evaluate the effects of the residue on grass productivity.

CONCLUSION

The results of the study showed that under the conditions of the experiment, agro-industrial residues can be used as organic fertilizers, but without nematicidal effect on the non-parasitic phase of GIN in sheep; although, the treatment with cassava led to a small recovery of the larvae from the grass.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTION

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

APPROVAL OF THE BIOETHICS AND BIOSAFETY COMMITTEE

The Ethics Committee on the Use of Animals (*Comissão de Ética no Uso de Animais*, CEUA) of the Embrapa Meio-Norte approved the experimental protocol of this study (CEUA CPAMN 005/2021) at a meeting held on 07/06/2021, according to 5529779 SEI/EMBRAPA certificate.

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