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Survival of *Haemonchus contortus* larvae in forage species in the Eastern Amazon

Abstract – The objective of this work was to evaluate the survival of third-stage larvae (L3) of *Haemonchus contortus* in forage species in the Eastern Amazon. Four paddocks composed of *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu', and *Urochloa humidicola* were used and divided into 13 plots each. Sheep feces containing about 10,000 eggs of *H. contortus* were deposited in each plot. Grass, feces, and soil samples were collected on the seventh, fifteenth, and thirtieth day post-contamination (DPC), and, then, they were sequentially collected every 30 days until the three-hundredth and thirtieth DPC. The following data were determined for the grass species: microclimatic, such as temperature, relative humidity, soil moisture, and grass luminosity, as well as macroclimatic data for rainfall, temperature, relative humidity, and solar radiation. L3 were recovered on all grasses and soil samples, in all plots, from the seventh to the three-hundredth and thirtieth DPC. The microclimatic parameters show correlations between the L3 recovery on grass and in the soil, and the macroclimatic parameters, between the L3 recovery in feces and on grass. *Urochloa humidicola* and *M. maximus* 'Massai' favor the development and survival of L3 of *H. contortus*, while *U. brizantha* 'Marandu' and *M. maximus* 'Mombaça' show a lower bioavailability of these larvae.

Index terms: *Brachiaria*, *Megathyrsus maximus*, *Panicum*, *Urochloa brizantha*, *Urochloa humidicola*, haemonchosis.

Sobrevivência de larvas de *Haemonchus contortus* em espécies de forrageiras na Amazônia Oriental

Resumo – O objetivo deste trabalho foi avaliar o desenvolvimento e a sobrevivência de larvas de terceiro estágio (L3) de *Haemonchus contortus* em forrageiras na Amazônia Oriental. Foram utilizados quatro canteiros compostos por *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu' e *Urochloa humidicola*, os quais foram divididos em 13 parcelas cada um. Fezes de ovinos com cerca de 10.000 ovos de *H. contortus* foram depositadas em cada parcela. Amostras de capim, fezes e solo foram coletadas no no sétimo, no décimo quinto e no trigésimo dia pós-contaminação (DPC) e, sequencialmente, a cada 30 dias até o trecentésimo trigésimo DPC. Foram determinados os seguintes dados das gramíneas: microclimáticos, como temperatura, umidade relativa do ar, umidade do solo e luminosidade do capim, e macroclimáticos, como pluviosidade, temperatura, umidade relativa do ar e radiação solar. As larvas do terceiro estágio foram recuperadas em todas as amostras de gramíneas e solo, em todas as parcelas, do sétimo ao trecentésimo trigésimo DPC. Os parâmetros microclimáticos mostram correlações entre a recuperação de L3 na grama e no solo, e os parâmetros macroclimáticos, entre a recuperação de L3 nas fezes e na grama. *Urochloa humidicola* e *M. maximus* 'Massai' favorecem o desenvolvimento e a sobrevivência de L3 de *H. contortus*, enquanto *U. brizantha* 'Marandu' e *M. maximus* 'Mombaça' apresentam menor biodisponibilidade dessas larvas.

Termos para indexação: *Brachiaria*, *Megathyrsus maximus*, *Urochloa brizantha*, *Panicum*, *Urochloa humidicola*, hemoncose.

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Introduction

Parasitic diseases are one of the main health problems affecting sheep (*Ovis aries*) all over the world, especially the haemonchosis, that is caused by the nematode *Haemonchus contortus*. This abomasal parasite is highly pathogenic and infects mainly small ruminants, more frequently in tropical and subtropical regions (Almeida et al., 2020). In the Eastern Amazon, haemonchosis is one of the main limiting factors in the husbandry of small ruminants, as the lack of knowledge of efficient control alternatives, associated with the indiscriminate use of anthelmintics, makes it difficult to control the disease in the region. In addition, the humid equatorial climate, associated with high rainfall and high temperatures throughout the year, are optimal environmental conditions for the free-living stage of *H. contortus* (Helmer et al., 2020).

Despite the climatic characteristics and the importance of ruminant husbandry in the Amazon, epidemiological studies that allow for establishing measures to control gastrointestinal helminths in the region are unusual (Helmer et al., 2020). Several forage species are used in the husbandry of small ruminants in the Amazon region, such as those belonging to the genera *Megathyrsus* and *Urochloa*.

Megathyrsus maximus 'Massai' is a small clump-forming plant with 60 cm average height, brittle leaves, and a high yield potential (Martuscello et al., 2015). Pastures of *M. maximus* 'Massai' remained contaminated with third-stage larvae (L3) trichostrongylids throughout the year in the municipality of Lageado, in the state of Tocantins, Brazil (Benavides et al., 2017). *Megathyrsus maximus* 'Mombaça' is a bunch-growing plant with good mass production and can reach up to 2 m height. It exhibits a high concentration of long, wide (3 cm), and upright leaves (Catanio et al., 2021). In the state of Bahia, Brazil, a higher recovery of infective *Haemonchus* sp. larvae occurred in the basal stratum of *M. maximus* 'Mombaça' pastures, in the rainy season (Quadros et al., 2010).

Urochloa humidicola is classified as a perennial, prostrate plant, with a dense grass field, and excellent ground coverage. *Urochloa brizantha* 'Marandu' features good digestibility, large size, perennial vegetative cycle, and growth in clump form (Crispim & Branco, 2002).

The survival of nematodes in pasture has a direct interference both from the climatic environmental effects and the structure of the different types of grasses, so this binomial has to be studied jointly. Due to the peculiar climatic conditions detected in the Eastern Amazon, the results of epidemiological studies on gastrointestinal helminth infections conducted in other regions in Brazil may not be applicable to this Amazon region (Iliev et al., 2018).

The objective of this work was to evaluate the survival of infective third-stage larvae of *Haemonchus contortus* in the forage species *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu', and *U. humidicola*, in the Eastern Amazon.

Materials and Methods

The experiment was carried out from January to December 2019, at the Instituto de Medicina Veterinária of Universidade Federal do Pará, in the municipality of Castanhal (01°17'38"S, 47°55'35"W), in the state of Pará, Brazil. Castanhal shows Am_i climatic type, according to the Köppen-Geiger's classification, with 2,200 mm average annual rainfall. The minimum annual mean temperature ranges from 19.2 to 24.2ºC, and the maximum temperature ranges from 30.1 to 32.7ºC. The mean annual relative humidity ranges between 85 and 90% (INMET, 2020). According to the Brazilian soil classification system, the soil at the experimental site was classified as Latossolo Amarelo distrófico (Santos et al., 2018), i.e., Oxisol. The experimental area was 88 m^2 and has never been used as pasture.

The area was prepared conventionally with plowing and harrowing. Grass sowings were performed in October/2018 in plastic bags (35 x 45 cm) for seedlings containing black earth. The grass species were transplanted to the experimental beds at the beginning of the rainy season, and the experiment started on January 26, 2019.

The experimental area was established with four paddocks of 6.75 m^2 each, composed of *Megathyrsus maximus* 'Massai' (Syn. *Panicum maximum* 'Massai'), *Megathyrsus maximus* 'Mombaça' (Syn. *Panicum maximum* 'Mombaça'), *Urochloa brizantha* 'Marandu' (Syn. *Brachiaria brizantha* 'Marandu'), and *Urochloa humidicola* (Syn. *Brachiaria humidicola*), and divided into 13 parcels (50 x 50 cm each), using nylon thread

and wooden stakes. Each parcel was used to collect a single sample of soil, feces, and pasture. There was a 50 cm border space between parcels, to avoid contact of one grass species with the others.

The research was developed within the principles of animal ethics, approved by the Ethics Committee on the Use of Animals of the Universidade Federal do Pará (Proc. no. 7765110718).

An adult Brazilian Santa Inês sheep showing clinical signs of haemonchosis was donated to the university, and *H. contortus* was collected from the abomasum. The animal was euthanized and, during necropsy, the contents of the abomasum were dispensed into a plastic tray for research and collection of *H. contortus*. Some collected specimens were fixed in 5% formaldehyde for identification according to Sambodo et al. (2018). The other specimens were placed in test tubes containing 4 mL of phosphate buffer saline solution and kept for 10 hours at 40ºC in a water bath, after which they were dissected with hypodermic needles for egg extraction. The extracted eggs were incubated with autoclaved equine feces, adding 10 mL of brain heart infusion medium with *Escherichia coli*, and kept in stool culture flasks for about 10 days, until they reached the third-stage larvae (L3). The larvae were kept in distilled water at 4°C, until the time of infection of the donor sheep.

A 10-month-old female sheep was used as donor, which was dewormed with 2.5 mg kg^{-1} of monepantel orally, in a single dose. The animal was kept in a suspended pen with a slatted floor which was cleaned daily, receiving cut grass and water ad libitum. The grass offered was collected from an area where there was no access by other animals.

After the deworming, feces were collected every two days for four weeks, to confirm that the donor animal was completely free of gastrointestinal helminths, and to determine the concentration of fecal eggs count (FEC), according to the method by Gordon & Whitlock (1939) modified. Two days after the last FEC, the sheep was infected with 10,000 infective *H. contortus* third-stage larvae (Hupp et al., 2018).

Forty days after infection, fecal samples were collected for three consecutive days using geriatric diapers that were changed three times a day. Feces were stored at 4°C, until they were deposited on the experimental plots, that is, three days after the last collection. All fecal samples were homogenized, and five aliquots were removed to be processed using the modified Gordon & Whitlock (1939) technique. The mean FEC obtained from these samples was used to estimate the amount of feces needed to obtain ten thousand eggs of *H. contortus*.

Thirty-seven grams of feces were deposited in the center of each plot, containing approximately 10,000 eggs of *H. contortus*. Before deposition, forage was lowered with pruning shears to 25 cm height for *U. humidicola,* 30 cm for *U. brizantha* 'Marandu', 45 cm for *M. maximus* 'Massai', and 90 cm for *M. maximus* 'Mombaça'. These forage species are used for the entry of animals in the pasture for a grazing system under rotational stocking (Dias-Filho, 2012).

Thirteen samples were collected from each grass, from the 7th to the 330th day post-contamination of feces (DPC), amounting to 52 samples for the four grass species. Grass, feces, and soil samples were collected at the 7th (01/26/2019), 15th (02/02/2019), 30th (02/18/2019), 60th (03/20/2019), 90th (04/19/2019), 120th (05/19/2019), 150th (06/18/2019), 180th (07/18/2019), 210th (08/17/2019), 240th (09/16/2019), 270th (10/16/2019), 300th (11/15/19), and 330th (12/15/2019) days post-contamination of feces (DPC).

For the L3 recovery on the grass species, the aerial parts of the plants were cut with pruning shears at 5 cm the heights for *U. humidicola*, 15 cm for *U. brizantha* 'Marandu', 20 cm for *M. maximus* 'Massai', and 30 cm for *M. maximus* 'Mombaça', thus simulating the consumption of pasture by the animals. The samples were processed according to Demeler et al. (2012), with modifications, for the identification and counting of L3.

Each grass sample was transferred to a properly identified PVC bucket containing 4 L of water and 0.5 mL of neutral detergent (Extran MA 02 Neutro, Merck S.A., Rio de Janeiro, RJ, Brazil) and kept immersed for 6 hours. From the120th DPC on, 1 L of water was added to the sample processing, for each collection, to ensure the total immersion of grass because of the higher rate of grass growth.

Then the samples were washed with 1 L of water on a fine mesh, with an opening of approximately 0.5 mm, in a tray, and left for 1 hour in a glass beaker to remove 100 mL of the sediment, which was transferred to a glass funnel with a stem and sealed with rubber band.

The mesh used to wash the grass samples was rinsed with 200 mL of water. Water used in the

washing remained for one hour in sedimentation cups. The supernatant was discarded to remove 10 mL of the sample, which was added to the 100 mL that was stored in the funnel.

Water in which the grass sample was immersed remained at rest for 2 hours, and the supernatant was removed through a siphon, leaving only 300 mL.

After 24 hours of sedimentation, the supernatant was removed. The remaining 45 mL were transferred to graduated conical tubes with caps and kept for 20 min. The supernatant was discarded again and, from the remaining 5 mL, 1 mL of larval solution was removed for larvae counting. This volume was divided into 10 parts of 100 µL each that were placed on glass slides for reading under an optical microscope in a 40X objective. To perform the reading, the slides were divided into three quadrants, and 10 µL of Lugol Forte-Parasito (Laborclin, Pinhais, PR, Brazil) was added to facilitate the larvae counting. After the washing process, the grass samples were packed in paper bags and oven-dried at 60 ºC for 72 hours, to determine the mass of dry matter (DM). As from the 120th DPC on, for each collection, an extra hour was added for the drying of the samples, to secure the total drying of the grass.

On the day of sampling, feces were spread in the center of each grass and were manually collected from each grass parcel and placed in a plastic container. To recover L3 from feces, the entire fecal volume collected in each plot was analyzed according to the Baermann technique (Ueno & Gonçalves, 1998).

Soil samples (50 g each) were collected at 2−10 cm away from the center of the plant, around each grass. To recover L3 from the soil, samples were collected and processed according to the technique recommended by Roberto et al. (2020).

Grass microclimate was based on a combination of aboveground interactions and the aerial part of the grass species, including changes in temperature, relative humidity, and shading. In the sampling days, the microclimatic data for grass species were measured by placing the devices in the basal stratum of each grass. A hygrometer (7663, Incoterm, Porto Alegre, RS, Brazil) was used to determine the average temperature (ºC) and relative humidity (%); an HH2 meter (HH2, Delta T Devices, Cambridge, UK) was used to determine the soil moisture; and a digital luxmeter (LD 540, Icel, Manaus, AM, Brazil) was used to measure the intensity of light in the environment.

Climatic data such as rainfall (mm), temperature (ºC), relative humidity (%), and solar radiation (KJ m^{-2}) were provided by the Instituto National de Meteorologia (INMET) in Castanhal. Climatic data for each day of collection were obtained through the average of the period between collections.

The χ-square test was used to verify the recovery percentage of L3 in each forage in different days of collection. To evaluate the macroclimatic and microclimatic variables and the number of L3 present on the grass species, feces and soil samples, the Pearson's correlation was used. All analyses were performed using the BIOESTAT 5.0 software with 1% and 5% level of significance (Ayres et al., 2007).

Results and Discussion

There was a difference in the recovery frequency of L3 in pastures, feces, and soil according to the collection periods. The recovery of L3 occurred in pasture and soil samples in all plots, from the 7th to the 330th DPC (Table 1). Total recovered larvae was significantly higher on the following grass pastures: *U. humidicola* at the 7th DPC, 15th DPC, and 30th DPC; *M. maximus* 'Massai', on which the larvae increase was verified in the15th DPC and 30th DPC; *U. brizantha* 'Marandu', in the 7th DPC and the 180th DPC; and *M. maximus* 'Mombaça', in the 180th DPC and the 240th DPC. The first decline occurred in the 60th DPC. In the samples collected from the 210th to the 330th DPC, there was a new reduction of L3 in pasture, except for the sample of *M. maximus* 'Mombaça' collected at the 240th DPC.

The macroclimatic parameter solar radiation showed a positive correlation with the recovery of L3 in feces on pasture of *U. brizantha* 'Marandu' (r = +0.903) (Table 2). The action of solar radiation increased the recovery of L3 in feces. There was also a positive correlation with maximum (r = +0.890) and minimum (r = +0.941) temperatures on the recovery of L3 in feces on 'Mombaça' grass. Wang et al. (2018) reported the impact of temperature on the horizontal migration of L3 of gastrointestinal nematodes out of feces, thus achieving a lower migration rate at temperatures from 25 to 30ºC to avoid exposure to solar radiation.

The macroclimatic parameter rainfall showed a negative correlation with the L3 recovery from feces on *M. maximus* 'Massai' grass (r = -0.979), as the

more it rains, the lower is the recovery of L3 in feces (Table 2). Moisture is needed for the development and migration of larvae from feces to pasture, thus, rainfall is a limiting factor (Morgan & van Dijk, 2012).

The accumulated L3 population on forages was above 50% at the 30th DPC on pastures of *U. humidicola* and *M. maximus* 'Massai'. From the 180th DPC until the end of sampling in all pastures, for all grasses, the accumulated L3 population was above 50%. As to the L3 population recovered in the last two collections, the accumulated percentage was observed to be higher than 95%, except for those that had already been

Table 1. Percentage of third-stage larvae (L3) of *Haemonchus contortus* recovered from *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu', and *U. humidicola*, on crescent days post contamination (DPC) in 2019, in the city of Castanhal, in the state of Pará, Brazil.

DPC	L3 kg^{-1} DM							
	M. maximus Massai		*M. maximus* Mombaça		*U. brizantha* Marandu		*U. humidicola*	
	L3	%	L3	%	L3	%	L3	%
7th	515	8.7	473	11.8	600	13.5**	1410	27.3**
15th	1,485	25.0**	117	2.9	572	12.8	929	17.9**
30th	1,886	31.8**	197	4.9	310	6.9	625	12.0**
60th	158	2.6	355	8.9	44	0.9	119	2.3
90th	145	2.4	207	5.1	368	8.2	343	6.6
120th	256	4.3	91	2.2	124	2.7	306	5.9
150th	158	2.6	77	1.9	61	1.3	118	2.2
180th	676	11.4	708	17.7**	1,471	33.1**	583	11.2
210th	65	1.1	29	0.7	315	7.1	94	1.8
240th	112	1.8	1,063	26.6**	71	1.6	95	1.8
270th	103	1.7	256	6.4	148	3.3	88	1.7
300th	209	3.5	189	4.7	160	3.6	244	4.7
330th	151	2.5	225	5.6	195	4.3	227	4.3
Total	5,919	100	3,987	100	4,439	100	5,181	100

DM, dry matter. **Significant at 1% probability by the χ-square test.

Table 2. Pearson's correlation (r) between microclimatic and macroclimatic variables, and the amount of third-stage larvae (L3) recovered on grass, sheep feces, and soil from pastures of *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu', and *U. humidicola* in 2019, in the city of Castanhal, in the state of Pará, Brazil.

Variable	Sample	Pasture	r
Microclimatic			
Minimum temperature (°C)	Soil	*M. maximus* 'Massai'	+0.554*
		U. brizantha 'Marandu'	+0.623*
		M. maximus 'Mombaça'	+0.602*
Minimum relative humidity (%)	Grass	*U. humidicola*	+0.583*
Maximum relative humidity (%)	Grass	*U. humidicola*	+0.712**
Macroclimatic			
Minimum temperature (°C)	Feces	*M. maximus* 'Mombaça'	+0.941*
Maximum temperature (°C)	Feces	*M. maximus* 'Mombaça'	+0.890*
Solar radiation (kJ m^{-2})	Feces	*U. brizantha* 'Marandu'	+0.903**
	Grass	*U. humidicola*	- 0.629*
Rainfall (mm)	Feces	*M. maximus* 'Massai'	- 0.979**

The positive sign indicates that the variables are directly proportional. The negative sign indicates that the relationship between the variables is inversely proportional. **, *Significant at 1% and 5% by the Pearson's test, respectively.

collected in the pasture of *M. maximus* 'Mombaça' which was 94.3% (Table 3).

The accumulated percentage of L3 recovered exceeded 50% at the 30th DPC on *M. maximus* 'Massai' and *U. humidicola*, which evidences that these forages showed a higher parasite load earlier than the others (Table 3). This fact is related to the morphological characteristics of each pasture. *U. humidicola* promoted the development of L3 by covering the soil to its greatest extent, thus providing a more humid and shadier microhabitat. The microclimate of grasses, provided by shading and grass density, is important to determine fecal moisture and larval migration (Wang et al., 2018). Stoloniferous grass species feature greater ground cover and closed leaf mass, which improves the microclimatic conditions by promoting a greater interception of solar radiation, favoring the survival of gastrointestinal nematodes in the environment (Santos et al., 2012). In the evaluation of the development and survival of L3 of *H. contortus*, on different pasture species in São Paulo state, Brazil, a greater recovery of L3 was observed on *M. maximus* 'Aruana', since this grass species has a higher forage mass density (Carneiro & Amarante, 2008). Due to its voluminous and clump-shaped leaflet architecture, *M. maximus* 'Massai' also promoted good conditions for protecting larvae from both the action of rain and the direct sunlight.

On the 60th DPC, there was a reduction of L3 recovery on forages, except for larvae on *M. maximus* 'Mombaça'. The increase of rainfall in March/2019, with accumulation of 294 mm and, possibly, the intense attack of an insect called spittlebugs (*Deois flavopicta*), may have influenced the recovery decrease of L3 in this period. The attack of spittlebugs, a very common fact in forages in the Amazon, started between the 60th DPC and the 150th DPC, during the rainy season. The leafhopper released a frothy secretion at the base of the pasture, which may have compromised the larval development, negatively influencing the L3 recovery on forage plants. Constant manual control was carried out in the juvenile stage (nymph) of this pest, without using chemical products to avoid compromising the larvae survival in the environment. The only forage that showed tolerance to spittlebugs was *M. maximus* 'Mombaça', a fact also reported by Valério et al. (2013).

During the occurrence of spittlebugs, the L3 may have migrated to the grass base, below the height defined for cutting. This condition, associated with the period of heavy rainfall (60th to 150th), caused the recovery decrease of L3 on the grasses. The eradicating spittlebugs and the reduction of heavy rains contributed to the increased amount of L3 on forages at the 180th DPC. Although 'Mombaça' grass was not visually attacked by spittlebugs, the intense elongation of the stems and open leaf architecture (Cândido et al., 2005)

Table 3. Accumulated percentage of third-stage larvae (L3) of *Haemonchus contortus* recovered from *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu', and *U. humidicola*, in crescent days post contamination (DPC) in 2019, in the city of Castanhal, in the state of Pará, Brazil.

DPC	*M. maximus* Massai (%)		*M. maximus* Mombaça (%)		*U. brizantha* Marandu (%)		*U. humidicola* (%)	
	Simple	Cumulative	Simple	Cumulative	Simple	Cumulative	Simple	Cumulative
7th	8.7	8.7	11.9	11.9	13.5	13.5	27.2	27.2
15th	25.1	33.8	2.9	14.8	12.9	26.4	17.9	45.1
30th	31.9	65.7	4.9	19.7	7.0	33.4	12.1	57.2
60th	2.7	68.4	8.9	28.6	1.0	34.4	2.3	59.5
90th	2.4	70.8	5.2	33.8	8.3	42.7	6.6	66.1
120th	4.3	75.1	2.3	36.1	2.8	45.5	5.9	72.0
150th	2.7	77.8	1.9	38.0	1.4	46.9	2.3	74.3
180th	11.4	89.2	17.8	55.8	33.1	80.0	11.3	85.6
210th	1.1	90.3	0.7	56.5	7.1	87.1	1.8	87.4
240th	1.9	92.2	26.7	83.2	1.6	88.7	1.8	89.2
270th	1.7	93.9	6.4	89.6	3.3	92.0	1.7	90.9
300th	3.5	97.4	4.7	94.3	3.6	95.6	4.7	95.6
330th	2.6	100	5.6	99.9	4.4	100	4.4	100

facilitated the penetration of the rain into the plant, causing the L3 recovery to be reduced in the period of heavy rains. However, an increase of L3 was observed in the soil in this period, which allows of the inference that the L3 migrated from the soil to the grass. In São Paulo, Brazil, heavy rainfall in a short period of time, in the autumn, resulted in low larval recovery in the pasture (Santos et al., 2012).

The climate remained favorable to the development of larval stages, with an average temperature above 25°C and humidity above 75%, throughout the year. Temperatures around 24.6 and 25.1ºC and relative air humidity from 74.4 to 79.9% positively influenced the parasitic infection in sheep in the state of Rondônia, Brazil, according to Oliveira et al. (2019). There was little variation of microclimatic factors among the forage species. Ground-level forage temperatures ranged from 26 to 31ºC.

U. humidicola exhibited the best microclimate for L3 recovery on the grass, showing the maximum (r = +0.712) and minimum (r = +0.583) relative humidity as a microclimatic parameter, since it promoted survival of L3 on this grass (Table 2). In temperate climates, humidity is the most important factor for trichostrongylid L3 to move from feces to grass (Wang et al., 2014). The action of the solar radiation (r = -0.629) negatively affected the recovery of L3 in the *U. humidicola* grass. Exposure to adverse survival conditions, such as high lethal ultraviolet radiation on grasslands, causes L3 to remain in protected microenvironments with milder temperatures (Van Dijk et al., 2009). The recovery of L3 of *H. contortus* was higher on *Cynodon dactylon* 'Tifton 85' grass with larger shading area, that showed to provide a more favorable microclimate for larval development, as there was a reduction of the intensity of solar radiation directly on the grass, which allowed of some larvae to survive for longer periods (Gasparina et al., 2021).

Under the conditions of the Eastern Amazon biome, a small percentage of third-stage larvae of *H. contortus* showed a survival of up to 330 days. In humid subtropical climate, characterized by hot and rainy weather, the massive contamination of pasture with L3 of *H. contortus* remains up to one year after contamination in the winter (Almeida et al., 2020).

There was a significant increase of L3 recovery in the soil from the 180th DPC on, for all pastures. During this period, solar radiation increased considerably. Solar radiation reaches the forage directly. However, due to its morphology and denser architecture, the forage acts as a natural barrier to protect the soil. Since there were no more feces, the L3 ended up taking refuge in the soil, which also serves as a reservoir in undesirable weather conditions (Santos et al., 2012).

Minimum temperature, as a microclimatic factor of grasses, showed a positive correlation in L3 recovery from soil on *M. maximus* 'Mombaça' (r = +0.602), *U. brizantha* 'Marandu' (r = +0.623), and *M. maximus* 'Massai' (r = +0.554) grasses (Table 2).

U. brizantha 'Marandu' and *M. maximus* 'Mombaça' were considered the densest forages in the present study, and the effect of this density served to dilute the L3, causing them to have the lowest concentrations of L3 per kilogram of dry matter.

Conclusions

1. Soil and *Megathyrsus maximus* 'Massai', *M. maximus* 'Mombaça', *Urochloa brizantha* 'Marandu', and *U. humidicola* act as a reservoir for third-stage (L3) *Haemonchus contortus* larvae throughout the year, increasing the larval longevity.

2. Microclimatic parameters show correlations between the recovery of L3 of *H. contortus* on grasses and in the soil; macroclimatic parameters show correlation between the recovery of L3 of *H. contortus* in feces and on grasses.

3. *U. humidicola* and *M. maximus* 'Massai' promote the development and survival of third-stage larvae of *H. contortus,* while *Urochloa brizantha* 'Marandu' and *Megathyrsus maximus* 'Mombaça' show lower bioavailability of these larvae under the conditions of the experiment.

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