Original Article

Infective larvae of *Haemonchus contortus* found from the base to the top of the grass sward

Larvas infectantes de *Haemonchus contortus* são encontradas da base até o topo da forragem

Jennifer Mayara Gasparina[1]; Renata Grasieli Baby[1]; Lidiane Fonseca[3]; Patrizia Ana Bricarello[4]; Raquel Abdallah da Rocha[1,2]* (iD)

[1] Programa de Pós-graduação em Zootecnia, Universidade Estadual de Ponta Grossa – UEPG, Ponta Grossa, PR, Brasil
[2] Departamento de Zootecnia, Universidade Estadual de Ponta Grossa – UEPG, Ponta Grossa, PR, Brasil
[3] CONSIPA – Consultoria em Sistemas Integrados de Produção Agropecuária, Ponta Grossa, PR, Brasil
[4] Departamento de Zootecnia e Desenvolvimento Rural, Centro de Ciências Agrárias – CCA, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil

Abstract

The resistance of gastrointestinal nematodes (GIN) of sheep to anthelmintic treatment has motivated researchers to seek alternatives to reduce the use of these drugs in sheep farming and decontaminate pastureland based on knowledge about the survival dynamics of larvae. The aim of this work was to evaluate the migration of the infective larvae (L3) of *Haemonchus contortus* at different times of the day, strata, and sward heights, with and without shade after the deposition of contaminated sheep feces. The grass species used here was *Cynodon dactylon* cv. Tifton 85 in four treatments: low sward height shade; low sward height sunshine; high sward height shade; and high sward height sunshine. The number of L3 recovered from the pasture at different times of the day did not differ. The highest number of L3 recovered was in shade. The number of L3 at different times and strata occurred uniformly, confirming that L3 remain in the same place after migrating from dung at the hottest times of the day. Infective larvae of *H. contortus* were able to migrate across all the strata regardless of the time of day in the summer season in humid subtropical climate.

Keywords: Gastrointestinal nematodes, parasite, pasture management, sheep farming.

Resumo

A resistência dos nematódeos gastrintestinais (NGI) de ovinos aos tratamentos com anti-helmíntico tem estimulado os pesquisadores a buscar alternativas para reduzir o uso desses medicamentos na ovinocultura e descontaminar pastagens com base no conhecimento sobre a dinâmica de sobrevivência das larvas. O objetivo do trabalho foi avaliar a migração das larvas infectantes (L3) de *Haemonchus contortus* em diferentes horários do dia, estratos, e alturas do pasto, com e sem sombra, após a deposição de fezes de ovinos contaminadas. A espécie forrageira utilizada foi *Cynodon dactylon* cv. Tifton 85 em quatros tratamentos: pasto baixo sombra; pasto baixo sol; pasto alto sombra; e pasto alto sol. Não houve diferença na recuperação de L3 no pasto nos diferentes horários. As maiores recuperações foram encontradas na sombra. O número de larvas recuperadas nos diferentes horários e estratos ocorreu de forma uniforme, confirmando que as L3 podem permanecer no mesmo local, após migrar do bolo fecal, nos horários mais quentes do dia. Larvas infectantes de *H. contortus* foram capazes de migrar por todos os estratos, independentemente da hora do dia no verão em clima subtropical úmido.

Palavras-chave: Nematódeos gastrintestinais, parasitas, manejo do pasto, criação de ovinos.

Introduction

Gastrointestinal nematodes (GIN) can reduce the profitability of sheep farms (Charlier et al., 2014) by directly impacting the productive performance of flocks, diminishing growth, and affecting the finishing period of animals (Pinheiro et al., 2000). The anthelmintic resistance of nematode parasites in sheep due to intensive drug use (Salgado & Santos, 2016) is an important factor to be considered in their management.

Approximately 95% of the parasite population is present in pasture and only 5% in animals (Bowman et al., 2003). An understanding of the vertical migration of parasite larvae and of the grazing behavior of herbivores could contribute to improve pasture management, aiming to reduce L3 intake and the use of anthelmintics to control parasites (Silva et al., 2008, Tontini et al., 2015). However, the number of *H. contortus* L3 in *Brachiaria decumbens* grass forage strata remained relatively constant throughout the day. This indicates there is no particular period of the day when grazing sheep are at higher risk of infection (Silva et al., 2008).

Several management practices have been tested in the attempt to reduce the damage caused by GIN in animals. However, as yet there is no single established recommendation that can be applied worldwide, because variations in climate, soil, and vegetation can affect the development and migration of larvae (van Dijk & Morgan, 2011). Even so, some practices are widely recommended by technicians, such as animals having access to pasture only after the dew has evaporated (Costa et al., 2011). This recommendation makes sense if L3 die or descend to the base of the plant as the due evaporates.

In fact, the availability of L3 in the pasture depends on the presence of free water, rain, or fog and condensed dew (van Dijk & Morgan, 2011). Free water moistens feces, allowing L3 to migrate from fecal matter to the pasture. Moreover, the formation of a continuous film of water on grass blades favors the migration of larvae to the top of the plant (Amaradasa et al., 2010). Santos et al. (2012) found *H. contortus* L3 at the top of *Brachiaria decumbens* even with low relative humidity (autumn). The authors stated that the moisture film produced by dew sufficed for L3 to reach the highest parts of the plant.

Tontini et al. (2015), who evaluated the distribution of L3 during summer in the various strata of the tropical grass *Panicum maximum* cv. Aruana, found larvae distributed evenly across the strata. On the other hand, some studies have shown that L3 concentrate in the lower strata in some seasons (Vlassoff, 1982). Rocha et al. (2007) found a higher concentration of *Trichostrongylus colubriformis* in the upper strata of *Panicum maximum* cv. Aruana and in the lower strata of *Brachiaria decumbens* in autumn. However, in spring, the same authors found larvae in the upper portions of both forage species. These findings demonstrate that the location of L3 cannot be pinpointed, given that their migration can be facilitated or hindered by a number of factors.

Allowing animals access to well-managed pastures so they can graze the upper parts of plants of higher nutritional quality (Marchesan et al., 2013), combined with adequate nutrition planning, can reduce parasitism (Almeida et al., 2020). Nutrition plays an important role in the immune response to GIN (Coop & Kyriazakis, 2001). Animals that received high protein diets were able to better withstand parasitism (Bricarello et al., 2005; Louvandini et al., 2006). This fact, allied to knowledge about the grazing behavior of herbivores (which graze approximately 50% of the top of plants in the first mouthful) has been used to reduce L3 intake (Cangiano et al., 2002).

Based on the above findings, the purpose of this work was to evaluate the migration of L3 of *Haemonchus contortus* in Tifton 85 Bermuda grass (*Cynodon* spp.), in shade and sunshine, to different pasture heights at different times of the day, strata, and days after deposition of contaminated feces.

Material and methods

Study site and climate data

The experiment took place during February 2017 (28 days) in Ponta Grossa, Paraná, Brazil (25° 05′ 49″ S, 50° 03′ 11″ W; altitude 990 m). The region's climate is humid subtropical (Cfb), according to the Köppen classification, with moderately hot summers and relatively cold winters. Meteorological data (temperature, humidity, solar radiation, rainfall and dew) were provided by the meteorological station located one kilometer from the experimental site. The presence of dew was observed through a variable obtained from the weather station called leaf wetness duration (LWD), which corresponds to the time during which condensation water droplets remain on the leaf surface (Sentelhas et al., 2008). Figure 1 illustrates the climatic variations that occurred during the experimental period.
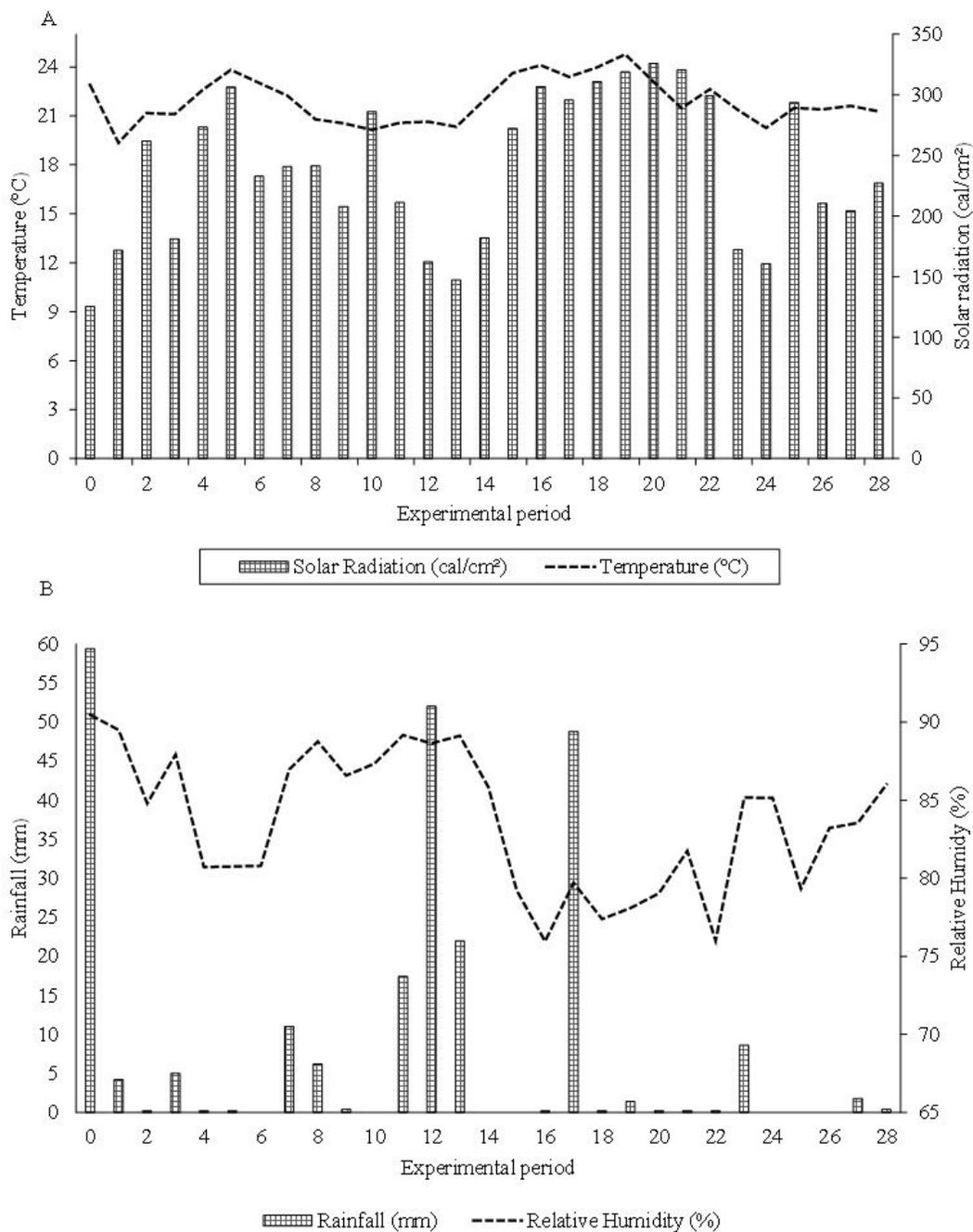
Figure 1. Meteorological data: Mean daily temperature (°C), Solar radiation (cal/cm²) (A), Rainfall (mm) and Relative Humidity (%) (B) during the experimental period in February 2017. The climate of Ponta Grossa, PR, Brazil, is humid subtropical (Cfb), according to the Köppen classification, with moderately hot summers.

Experimental design

The experimental design involved *Cynodon dactylon* var. dactylon cv. Tifton 85 Bermuda grass (summer pasture). The area used for the experiment was not intended for grazing. The design consisted of sixteen 16.2 m² plots, each of which was subdivided into 18 subplots of 30 x 30 cm. The subplots were delimited with wooden stakes and string. The plots were separated by 0.5 m gaps to allow for circulation of researchers on the site.

The area of the experiment was divided into two parts: with and without the presence of trees (shade and sunshine). The forage heights were defined as high (20 cm) and low (10 cm). Thus, the following treatments were applied: low sward height – shade (LSH – shade), LSH – sunshine, high sward height – shade (HSH – shade) and HSH – sunshine.

Deposition of feces

The experimental plot was contaminated using feces obtained from two male sheep naturally infected with GIN eggs. The use of rams (male sheep) was approved by the Ethics Committee on Animal Use at the State University of Ponta Grossa (Protocol 006/2016). The collection of fecal matter started on 24 Jan 2017 and the feces were stored in a refrigerator at 10°C. On the day of deposition, all the collected feces were weighed, homogenized, and six counts of eggs per gram of feces (EPG) were randomly conducted on the total volume of feces, involving a total of 288 samples, at 2.5 grams of feces, each containing an average of 60,000 eggs, following the procedure described by Silva et al. (2008). All the plots were contaminated (in the center of the plots) on 31 Jan 2017, using one fecal sample (2.5 grams) on each plot.

Recovery of infective larvae from forage and feces

Before collecting fecal matter and grass to evaluate larval migration, the height of the grass was determined based on the average height measured at five random points in each subplot, using a sward stick (Barthram, 1985).

To evaluate the vertical migration of L3 on the sward, the grass was cut manually and divided into three strata: upper stratum (A), consisting of 50% of the initial grass height, followed by an intermediate stratum (B) 25% below stratum A, and a final cut close to the ground to collect the lower grass (C). The samples of pasture were cut to simulate the grazing horizons of the animals. Ruminants use the strategy of preferentially grazing the first layers of forage, in order to maximize the quality of the diet and intake rate, since the upper layers user have more and better quality grass blades (Baumont et al., 2004).

L3 were recovered from both feces and pasture on days 7 (D7), 14 (D14), 21 (D21), and 28 (D28) after the deposition of feces and at different times of day (6 a.m., 12 p.m., and 6 p.m.).

Samples of fecal matter and grass were collected manually from the subplots and stored in labelled plastic bags until they were processed in the laboratory. The L3 were extracted using the technique described by Baerman and modified by Ueno & Gonçalves (1998). After 24 h in a Baermann apparatus, the supernatant was removed and the sediment transferred to graduated tubes. Subsequently, the L3 recovered from the samples were identified and quantified (Keith, 1953). The results are expressed as L3 per kilogram of dry matter (L3/kg DM).

After the L3 extraction procedure, the grass and feces samples were dried at 60°C for 72 h to determine the dry matter (DM) content.

Statistical analysis

The study used block randomization with a 6 x 2 x 2 factorial design of treatments with six replicates at each time point (06:00 a.m., 12:00 p.m., and 06:00 p.m.), two treatments of luminance levels (shade vs. sunshine), and two treatments of grass height (high vs. low).

Larvae recovery data were log transformed (Log (x+1)) to account for non-homogeneous variance and were analyzed using the generalized linear model (GLM) of Minitab® version 18.1 software (Minitab Inc., 2017). The data were analyzed per day of collection. Only the recovery of L3 in fecal matter was compared between the collection days, in order to determine how long the feces remained a viable reservoir for larvae. Collection time, stratum, and sward height were included in the model. The interaction between time and stratum was analyzed. However, to make them easier to understand, they are expressed as arithmetic means ± standard deviation. The means were compared by the Tukey test with a 5% level of significance.

Results

Only *Haemonchus contortus* counts were used, since this was the most abundant species of nematode. In this study, larvae of the genera *Haemonchus* (93.1%), *Trichostrongylus* (4.6%), and *Oesophagostomum* (2.3%) were recovered from Tifton grass.

L3 recovery and weight of fecal matter

The recovery of L3 from feces at the evaluated times did not differ ($p>0.05$). In general, the feces recovered in different times presented 7,217±24,644 L3/kg DM; 6,251±28,345 L3/kg DM, 8,927±30,897 L3/kg DM at 6:00

am, 12:00 noon and 6:00 pm, respectively. The same applies to the treatments, i.e., with no differences in the number of larvae recovered from feces between treatments (*p*>0.05) on the different days of collection (Table 1).

On D7, more L3 were recovered from fecal matter (29,960±50,427 L3/kg DM) than on any other day (*p*<0.05; Table 1). With regard to the weight of feces, on D7 the weight of LSH - shade was lower than that of the other treatments (*p*<0.05). In this treatment, fecal matter underwent 90.6% degradation. The maximum and minimum temperatures on the day of collection were 28.1ºC and 19.9ºC, respectively. The climatic conditions on D7 were 11 mm of rain, 87% RH, and 240.8 cal/m^2 of solar radiation.

On D14, both LSH and HSH - shade treatments had lower fecal weights than treatments exposed to sunshine (*p*<0.05). As expected, the treatments with the lowest fecal weights were also those with the highest percentage degradation (91.12% and 89.36%, LSH - shade and HSH - shade, respectively). On this day, the number of L3 in fecal matter were already quite low (28.80±215.5 L3/kg DM – Table 1).

On D21, the LSH - shade presented lower fecal weight (*p*<0.05) and 92.24% degradation compared to the high treatments (sunshine and shade), but did not differ from the LSH - sunshine treatment. The number of larvae recovered on D21 was 82.60±612.9 L3/kg DM. Lastly, on D28, the high sunshine treatment resulted in higher fecal weight (*p*<0.05) than the other treatments, and the lowest degradation rate, i.e., 83.2%. On D28, larvae were no longer recovered from feces.

Table 1. Mean fecal weight ± standard deviation, percent (%) degradation of feces, and number of *Haemonchus contortus* third stage larvae per kilogram of dry matter (arithmetic means ± standard error) in feces recovered on different days.

Days after fecal deposition*	Treatments**	Feces (g)	% degradation	L3/kg DM feces (1)	L3/kg DM feces (2)
7	LSH - shade	0.235±0.14[b]	90.60	45,615±81,405	29,960±50,427[A]
	LSH - sunshine	0.508±0.18[a]	79.68	11,679±23,278	
	HSH - shade	0.420±0.17[a]	83.20	56,621±50,928	
	HSH - sunshine	0.526±0.12[a]	78.96	17,137±31,757	
14	LSH - shade	0.222±0.12[c]	91.12	0.00±0.00	28.80±215.50[B]
	LSH - sunshine	0.416±0.15[ab]	83.52	0.00±0.00	
	HSH - shade	0.266±0.15[bc]	89.36	0.00±0.00	
	HSH - sunshine	0.447±0.20[a]	82.12	115±431	
21	LSH - shade	0.194±0.14[b]	92.24	0.00±0.00	82.60±612.90[B]
	LSH - sunshine	0.318±0.18[a]	87.28	284.00±1,136	
	HSH - shade	0.406±0.16[a]	83.76	0.00±0.00	
	HSH - sunshine	0.424±0.13[a]	83.04	0.00±0.00	
28	LSH - shade	0.271±0.11[b]	89.16	0.00±0.00	0.00±0.00[B]
	LSH - sunshine	0.305±0.12[ab]	87.80	0.00±0.00	
	HSH - shade	0.224±0.08[b]	91.04	0.00±0.00	
	HSH - sunshine	0.420±0.15[a]	83.20	0.00±0.00	

*Days after deposition of sheep feces contaminated with *Haemonchus contortus*; **Treatments: LSH – shade = low sward height shade; LSH – sunshine = low sward height sunshine; HSH – shade = high sward height shade; HSH – sunshine = high sward height sunshine; Different lowercase letters in the feces column indicate significant statistical differences as a function of day per treatment, and different uppercase letters in the L3/kg DM column indicate significant statistical differences as a function of day (Tukey test; *p*<0.05); (1) mean L3/kg DM recovered from feces per day of treatment; (2) mean L3/kg DM recovered from feces per day.

Pasture

Recovery of L3 from pasture

There was no difference in the number of larvae recovered from the pasture at the different times of day ($p>0.05$). In general, 46±278 L3/kg DM, 105±762 L3/kg DM and 82±371 L3/kg DM were recovered at 6:00 am, 12:00 noon and 6:00 pm, respectively. No significant interaction was found between time and stratum ($p>0.05$).

Evaluating the days separately, there was a significant difference in the recovery rate of L3/kg DM between treatments and strata on D7 ($p<0.05$). No L3 were recovered in LSH - sunshine on D7 (Table 2). On that day, the mean number of L3 recovered (180 L3/kg DM) from stratum C was higher than the mean number recovered from other strata (Table 2). On the other days of collection, the recovery of L3/kg DM did not differ between plant strata ($p>0.05$).

The recovery of L3/kg DM from the pasture also differed significantly between treatments ($p<0.05$) on D14. On that day, treatments LSH - shade (324±1037 L3/kg DM) and HSH - shade (153±352 L3/kg DM) showed higher larvae recovery rates than treatments in sunshine (Table 2). On D21 and D28, the number of L3 recovered from pasture, in both periods, was minimal ($p>0.05$).

Table 2. Number of *Haemonchus contortus* third stage larvae per kilogram of dry matter ± standard deviation (minimum and maximum in parenthesis) recovered from different herbage strata in different treatments on different days.

Days after fecal deposition*	Treatments**	Herbage strata			Overall Mean
		A	B	C	
7	LSH - shade	117±496	573±2430	180±587	290±1458 (0-10309)
	LSH - sunshine	0±0	0±0	0±0	0 (0-0)
	HSH - shade	0±0	18±75	269±144	96±370 (0-2343)
	HSH - sunshine	13±53	0±0	270±647	94±388 (0-2454)
Overall Mean		32±249[b] (0-2105)	148±1215[b] (0-10309)	180±533[a] (0-2454)	120±778# (0-10309)
14	LSH - shade	576±1631	114±292.8	282±702	324±1037[A] (0-6548)
	LSH - sunshine	0±0	122±518	228±969	117±629[B] (0-4109)
	HSH - shade	223±309	136±445	99±289	153±352[A] (0-1818)
	HSH - sunshine	19±83	56±166	0±0	25±107[B] (0-602)
Overall Mean		205±846 (0-6547)	107±373 (0-2198)	152±612 (0-4110)	155±639# (0-6547)
21	LSH - shade	0±0	47±198	0±0	15±114 (0-840)
	LSH - sunshine	0±0	0±0	0±0	0 (0-0)
	HSH - shade	0±0	0±0	0±0	0 (0-0)
	HSH - sunshine	15±63	101±429	19±79	45±253 (0-1818)
Overall Mean		4±32 (0-269)	37±235 (0-1818)	5±40 (0-338)	15±139 (0-1818)
28	LSH - shade	0±0	45±190	22±94	22±121 (0-806)
	LSH - sunshine	0±0	0±0	0±0	0 (0-0)
	HSH - shade	0±0	68±220	46±196	38±170 (0-893)
	HSH - sunshine	14±61	58±247	0±0	24±146 (0-1049)
Overall Mean		4±30 (0-258)	43±189 (0-1049)	17±108 (0-833)	21±127 (0-1049)

*Days after deposition of sheep feces contaminated with *Haemonchus contortus*; **Treatments: LSH – shade = low sward height shade; LSH – sunshine = low sward height sunshine; HSH – shade = high sward height shade; HSH – sunshine = high sward height sunshine; Different lowercase letters in the same line indicate significant statistical differences as a function of strata/day and different uppercase letters in the same column indicate significant statistical differences as a function of treatment/day (Tukey test; $p<0.05$); Means followed by # indicate a statistical difference between the collection days.

Sward heights

The sward structure was evaluated within each day and differed over the experimental period. On D7, all the treatments differed (*p*<0.05 – Table 3). On this day, the LSH - sunshine (15.50±3.3 cm) had the lowest height and the HSH - sunshine (25.94±2.5 cm) the highest height. On D14, HSH - sunshine (21.02±2.3 cm) differed from the other treatments (*p*<0.05). HSH - shade differed from LSH - sunshine, but not from LSH - shade. This, in turn, did not differ from LSH - sunshine. D21 followed the same trend as D7, with differences between all heights, LSH - sunshine (14.05±2.7 cm) had the lowest and HSH - sunshine the highest height (30.75±3.1 cm). Lastly, on D28, the HSH - sunshine (28.52±2.9 cm) was higher than all the other treatments, and the treatments in shade (high and low) did not differ from each other (*p*>0.05), but differed from the LSH - sunshine, which presented the lowest height (15.46±2.4 cm).

Table 3. Tifton 85 grass sward height in different treatments on different collection days.

Days after fecal deposition*	Treatments**			
	LSH - shade	LSH - sunshine	HSH - shade	HSH - sunshine
7	18.24±3.7[c]	15.50±3.3[c]	22.82±2.7[b]	25.94±2.5[a]
14	16.53±2.4[bc]	15.55±2.8[c]	17.98±2.5[b]	21.02±2.3[a]
21	23.27±1.8[c]	14.05±2.7[d]	27.94±3.8[b]	30.75±3.1[a]
28	25.56±2.1[b]	15.46±2.4[c]	26.40±3.1[b]	28.52±2.9[a]

*Days after deposition of sheep feces contaminated with *Haemonchus contortus*; **Treatments: LSH – shade = low sward height shade; LSH – sunshine = low sward height sunshine; HSH – shade = high sward height shade; HSH – sunshine = high sward height sunshine; Means followed by different letters on lines differ by Tukey test (*p*<0.05).

Discussion

GIN larvae successfully migrated through all the plant strata, regardless of the time of day. Seven days after the fecal deposition in the environment, a fraction of the L3 were recovered in fecal pellets. The wet weather that prevailed from the day of contamination until the first collection (D7) favored the development of eggs to larvae due to frequent rainfall, moderate temperatures and high humidity (Figure 1). *H. contortus* eggs developed rapidly to L3 (three to four days) within a temperature range of 20°C to 25.8°C, like the conditions reported by Hsu & Levine (1977). Temperature, humidity, and rainfall in the first seven days after the deposition of feces were important factors for the development of the free-living stages of *H. contortus* (O'Connor et al., 2007; Reynecke et al., 2011).

This experiment showed that on D7, larvae were still present in feces and in the lower strata, since seven days did not suffice for the larvae to leave the feces and migrate long distances from the ground.

The presence of dew in the pasture was observed only in the early hours of the morning. This indicates that even after evaporation/reduction of dew, the larvae remain in the same place after migrating from the feces. Larvae need free water only to abandon the dung, but not to climb up grass blades (van Dijk & Morgan, 2011). For larvae to migrate through the canopy of the plant, the moisture of dew is sufficient (Santos et al., 2012). In addition, larvae that reach the highest parts of the grass are able to remain there due to anhydrobiosis, a mechanism used by GIN larvae to enable them to survive the absence of water (Lettini & Sukhdeo, 2006).

Larvae exposed directly to solar radiation are subject to high mortality rates (van Dijk et al., 2009; Silva et al., 2008). This fact was observed in our study on D7 in LSH - sunshine and D14 in LSH and HSH - sunshine, when low numbers of L3 were recovered. The sward height in full sunshine, especially HSSH - sunshine, was found to provide a more favorable microclimate than LSSH - sunshine, as it probably reduced the intensity of solar radiation reaching the base of the grass sward, enabling some larvae to survive for longer periods (Moss & Bray, 2006).

Significant numbers of L3 were recovered on D14, possibly due to high RH (88.3%) and frequent rainfalls recorded from the day of deposition up to D14. The rainfall and RH that prevailed in the period caused an increase in the humidity of the feces, leading to higher degradation and favoring the migration of larvae to the grass sward (Almeida et al., 2020). Although we did not measure the climatic variables in each treatment (with and without the presence of trees – shade), the shade certainly contributed to retain more moisture in the environment than the

treatments in sunshine (Almeida et al., 2018). Faria et al. (2016), who evaluated integrated crop-livestock-forestry systems, also reported higher recovery rates of GIN larvae than in systems without the presence of trees (sunshine).

Fourteen days after deposition of the feces in the environment, L3 were already present in the upper stratum, it indicates that the larvae contained in feces on D7 successfully migrated to the top of the plant after leaving the dung. The presence of plant cell extensions called trichomes in different forage species can influence larval migration (Niezen et al., 1998). Trichomes can hinder the movement of L3, or facilitate their migration by accumulating dew moisture (Niezen et al., 1998; Rocha et al., 2007). In our experiment, we did not measure the number of trichomes, but Tifton grass contains trichomes, albeit in very small quantities (Ahmad et al., 2016). The paucity of trichomes, allied to the shade produced in treatments with trees, facilitated the migration of larvae to the top of the plant, providing a microclimate with ideal conditions for larval development. Marley et al. (2006) reported that red clover negatively affected the migration of *H. contortus* larvae due to the presence of trichomes on its leaves and stems.

There is a positive correlation between rainfall and the emergence of larvae in pastures, since rain probably favors the development and survival of larvae in the field (Amaradasa et al., 2010). However, in this study, some L3 may have been removed from the pasture by a heavy rain that dumped 48.8 mm of water over a one-hour period, three days prior to collection on day 21. This intense rainfall may have carried some L3 away from the pasture (Grønvold & Høgh-Schmidt, 1989), which could explain the low L3 recovery on days 21 and 28 after fecal deposition. In addition, the moderate temperatures and high relative humidity recorded throughout the experimental period enabled larvae to develop and migrate to the sward in the first 14 days. However, this rapid development of larvae also resulted in their lower survival time in the environment (Rocha et al., 2014; Carneiro & Amarante, 2008).

Parasitological studies that evaluate the survival and migration dynamics of gastrointestinal nematodes in their developmental stages are important but increasingly meager. Such studies involve time-consuming but low-cost methods that allow for the choice of forages with strategic potential in the overall control of gastrointestinal nematodes reduced use of anthelmintics, thanks to the lower occurrence of L3, and the high nutritional quality and availability of forage during the months of greatest nutritional demand of animals susceptible to nematode infections.

In our experiment, the presence of shade and pastures with greater sward heights in full sunshine provided better climatic conditions for larval development and migration. In addition, *H. contortus* L3 were able to migrate across all the strata regardless of the time of day in the summer season. Therefore, there is no evidence that could help establish grazing schedules when animals are at lower risk of becoming infected by reducing larvae-host contact in regions of humid subtropical climate.

References

Ahmad KS, Hameed M, Deng J, Ashraf M, Hamid A, Ahmad F, et al. Ecotypic adaptations in Bermuda grass (*Cynodon dactylon*) for altitudinal stress tolerance. *Biologia (Bratisl)* 2016; 71(8): 885-895. http://dx.doi.org/10.1515/biolog-2016-0113.

Almeida FA, Albuquerque ACA, Bassetto CC, Starling RZC, Lins JGG, Amarante AFT. Long spelling periods are required for pasture to become free of contamination by infective larvae of *Haemonchus contortus* in a humid subtropical climate of São Paulo state, Brazil. *Vet Parasitol* 2020; 279: 109060. http://dx.doi.org/10.1016/j.vetpar.2020.109060. PMid:32143013.

Almeida FA, Piza MLST, Bassetto CC, Starling RZC, Albuquerque ACA, Protes VM, et al. Infection with gastrointestinal nematodes in lambs in different integrated crop-livestock systems (ICL). *Small Rumin Res* 2018; 166: 66-72. http://dx.doi.org/10.1016/j.smallrumres.2018.07.009.

Amaradasa BS, Lane RA, Manage A. Vertical migration of *Haemonchus contortus* infective larvae on *Cynodon dactylon* and *Paspalum notatum* pastures in response to climatic conditions. *Vet Parasitol* 2010; 170(1-2): 78-87. http://dx.doi.org/10.1016/j.vetpar.2010.01.026. PMid:20149541.

Barthram GT. Experimental techniques: the HFRO sward stick. In: Hill Farming Research Organization. *Biennial Report of the The Hill Farming Research Organization, 1984/1985*. Penicuik: HFRO; 1985. p. 29-30

Baumont R, Cohen-Salmon D, Prache S, Sauvant D. A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. *Anim Feed Sci Technol* 2004; 112(1-4): 5-28. http://dx.doi.org/10.1016/j.anifeedsci.2003.10.005.

Bowman DD, Lynn RC, Eberhard ML, Alcaraz A. *Georgi's parasitology for veterinarians*. 8th ed. Philadelphia: Saunders; 2003.

Bricarello PA, Amarante AFT, Rocha RA, Cabral SL Fo, Huntley JF, Houdijk JGM, et al. Influence of dietary protein supply on resistance to experimental infections with *Haemonchus contortus* in Ile de France and Santa Ines lambs. *Vet Parasitol* 2005; 134(1-2): 99-109. http://dx.doi.org/10.1016/j.vetpar.2005.05.068. PMid:16098676.

Cangiano CA, Galli JR, Pece MA, Dichio L, Rozsypalek SH. Effect of liveweight and pasture height on cattle bite dimensions during progressive defoliation. *Aust J Agric Res* 2002; 53(5): 541-549. http://dx.doi.org/10.1071/AR99105.

Carneiro RD, Amarante AFT. Seasonal effect of three pasture plant species on the free-living stages of *Haemonchus contortus*. *Arq Bras Med Vet Zootec* 2008; 60(4): 864-872. http://dx.doi.org/10.1590/S0102-09352008000400014.

Charlier J, Morgan ER, Rinaldi L, van Dijk J, Demeler J, Höglund J, et al. Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. *Vet Rec* 2014; 175(10): 250-255. http://dx.doi.org/10.1136/vr.102512. PMid:25217603.

Coop RL, Kyriazakis I. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends Parasitol* 2001; 17(7): 325-330. http://dx.doi.org/10.1016/S1471-4922(01)01900-6. PMid:11423375.

Costa VMM, Simões SVD, Riet-Correa F. Controle das parasitoses gastrintestinais em ovinos e caprinos na região semiárida do Nordeste do Brasil. *Pesq Vet Bras* 2011; 31(1): 65-71. http://dx.doi.org/10.1590/S0100-736X2011000100010.

Faria EF, Lopes LB, dos Reis Krambeck D, dos Santos Pina D, Campos AK. Effect of the integrated livestock–forest system on recovery of trichostrongylid nematode infective larvae from sheep. *Agrofor Syst* 2016; 90(2): 305-311. http://dx.doi.org/10.1007/s10457-015-9855-1.

Grønvold J, Høgh-Schmidt K. Factors influencing rain splash dispersal of infective larvae of *Ostertagia ostertagi* (Trichostrongylidae) from cow pats to the surroundings. *Vet Parasitol* 1989; 31(1): 57-70. http://dx.doi.org/10.1016/0304-4017(89)90008-3. PMid:2728328.

Hsu CK, Levine ND. Degree-day concept in development of infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* under constant and cyclic conditions. *Am J Vet Res* 1977; 38(8): 1115-1119. PMid:911077.

Keith RK. The differentiation of the infective larvae of some common nematode parasites of cattle. *Aust J Zool* 1953; 1(2): 223-235. http://dx.doi.org/10.1071/ZO9530223.

Lettini SE, Sukhdeo MVK. Anhydrobiosis increases survival of trichostrongyle nematodes. *J Parasitol* 2006; 92(5): 1002-1009. http://dx.doi.org/10.1645/GE-784R.1. PMid:17152941.

Louvandini H, Veloso CFM, Paludo GR, Dell'Porto A, Gennari SM, McManus CM. Influence of protein supplementation on the resistance and resilience on young hair sheep naturally infected with gastrointestinal nematodes during rainy and dry seasons. *Vet Parasitol* 2006; 137(1–2): 103-111. http://dx.doi.org/10.1016/j.vetpar.2006.01.004. PMid:16495016.

Marchesan R, Paris W, Ziech MF, Prohmann PEF, Zanotti J, Hartmann DV. Produção e composição química-bromatológica de Tifton 85 (*Cynodon dactylon L. Pers*) sob pastejo contínuo no período hibernal. *Semina: Ciênc Agrár* 2013; 34(4): 1935-1942. http://dx.doi.org/10.5433/1679-0359.2013v34n4p1935.

Marley CL, Cook R, Barrett J, Keatinge R, Lampkin NH. The effects of birdsfoot trefoil (*Lotus corniculatus*) and chicory (*Cichorium intybus*) when compared with perennial ryegrass (*Lolium perenne*) on ovine gastrointestinal parasite development, survival and migration. *Vet Parasitol* 2006; 138(3–4): 280-290. http://dx.doi.org/10.1016/j.vetpar.2006.01.029. PMid:16495015.

Minitab Inc. *Minitab 18.1 statistical software* [online]. State College, PA: Minitab, Inc.; 2017 [cited 2020 Dec 3]. Available from: www.minitab.com

Moss RA, Bray AR. Effect of sward density and size of faecal deposit on the development and persistence of third-stage Trichostrongylid larvae of sheep. *N Z J Agric Res* 2006; 49(4): 475-481. http://dx.doi.org/10.1080/00288233.2006.9513738.

Niezen JH, Charleston WAG, Hodgson J, Miller CM, Waghorn TS, Robertson HA. Effect of plant species on the larvae of gastrointestinal nematodes which parasitise sheep. *Int J Parasitol* 1998; 28(5): 791-803. http://dx.doi.org/10.1016/S0020-7519(98)00019-8. PMid:9650060.

O'Connor LJ, Kahn LP, Walkden-Brown SW. Moisture requirements for the free-living development of *Haemonchus contortus*: quantitative and temporal effects under conditions of low evaporation. *Vet Parasitol* 2007; 150(1-2): 128-138. http://dx.doi.org/10.1016/j.vetpar.2007.07.021. PMid:17920198.

Pinheiro RR, Gouveia AMG, Alves FSF, Haddad JPA. Aspectos epidemiológicos da caprinocultura cearense. *Arq Bras Med Vet Zootec* 2000; 52(5): 534-543. http://dx.doi.org/10.1590/S0102-09352000000500021.

Reynecke DP, Waghorn TS, Oliver AMB, Miller CM, Vlassoff A, Leathwick DM. Dynamics of the free-living stages of sheep intestinal parasites on pasture in the North Island of New Zealand. 2. Weather variables associated with development. *N Z Vet J* 2011; 59(6): 287-292. http://dx.doi.org/10.1080/00480169.2011.610280. PMid:22040333.

Rocha RA, Bricarello PA, da Rocha GP, Amarante AF. Recuperação de larvas de *Trichostrongylus colubriformis* em diferentes estratos de *Brachiaria decumbens* e *Panicum maximum*. *Rev Bras Parasitol Vet* 2007; 16(2): 77-82. PMid:17706008.

Rocha RA, Bricarello PA, Rocha GP, Amarante AFT. Retrieval of *Trichostrongylus colubriformis* infective larvae from grass contaminated in winter and in spring. *Rev Bras Parasitol Vet* 2014; 23(4): 463-472. http://dx.doi.org/10.1590/s1984-29612014075. PMid:25517524.

Salgado JA, Santos CP. Overview of anthelmintic resistance of gastrointestinal nematodes of small ruminants in Brazil. *Rev Bras Parasitol Vet* 2016; 25(1): 3-17. http://dx.doi.org/10.1590/S1984-29612016008. PMid:26982560.

Santos MC, Silva BF, Amarante AFT. Environmental factors influencing the transmission of *Haemonchus contortus. Vet Parasitol* 2012; 188(3-4): 277-284. http://dx.doi.org/10.1016/j.vetpar.2012.03.056. PMid:22521972.

Sentelhas PC, Dalla Marta A, Orlandini S, Santos EA, Gillespie TJ, Gleason ML. Suitability of relative humidity as an estimator of leaf wetness duration. *Agric Meteorol* 2008; 148(3): 392-400. http://dx.doi.org/10.1016/j.agrformet.2007.09.011.

Silva BF, Amarante MRV, Kadri SM, Carrijo-Mauad JR, Amarante AFT. Vertical migration of *Haemonchus contortus* third stage larvae on *Brachiaria decumbens* grass. *Vet Parasitol* 2008; 158(1-2): 85-92. http://dx.doi.org/10.1016/j.vetpar.2008.08.009. PMid:18824304.

Tontini JF, Poli CHEC, Bremm C, De Castro JM, Fajardo NM, Sarout BNM, et al. Distribution of infective gastrointestinal helminth larvae in tropical erect grass under different feeding systems for lambs. *Trop Anim Health Prod* 2015; 47(6): 1145-1152. http://dx.doi.org/10.1007/s11250-015-0841-4. PMid:26003429.

Ueno H, Gonçalves PC. *Manual para diagnóstico de helmintoses em ruminantes*. Tokyo: International Cooperation Agency; 1998.

van Dijk J, de Louw MD, Kalis LP, Morgan ER. Ultraviolet light increases mortality of nematode larvae and can explain patterns of larval availability at pasture. *Int J Parasitol* 2009; 39(10): 1151-1156. http://dx.doi.org/10.1016/j.ijpara.2009.03.004. PMid:19358849.

van Dijk J, Morgan ER. The influence of water on the migration of infective trichostrongyloid larvae onto grass. *Parasitology* 2011; 138(6): 780-788. http://dx.doi.org/10.1017/S0031182011000308. PMid:24650934.

Vlassoff A. Biology and population dynamics of the free living stages of gastrointestinal nematodes of sheep. In: Ross AD. *Control of internal Parasites of sheep*. Lincoln: Lincoln College; 1982.