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Infection with gastrointestinal nematodes in lambs in different integrated crop-livestock systems (ICL)

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ABSTRACT

Lamb performance was evaluated in four integrated crop-livestock (ICL) systems. The ICL areas were previously planted with maize and marandu palisade grass in December 2013. ICL systems 1 and 2 were also seeded with pigeon pea. After harvesting in April 2014, black oats were sown in rows (ICL 1 and 3) or by broadcast seeding (ICL 2 and 4). Each ICL area was divided into 12 paddocks of 225 m^2 to be grazed by young sheep from July 23 to September 30, 2014. To determine if the pasture remained contaminated by free-living stages of sheep gastrointestinal nematodes (GIN) after approximately ten months without animals in the area, 12 worm-free "tracer" lambs (n = 3/ ICL system) grazed each ICL pasture for 14 consecutive days in July 2015 and were later housed in pens, where they remained for another 14 days. The tracer lambs acquired no worm infections, demonstrating that the area was cleared after 300 days without animals. To evaluate GIN infection and uncastrated male lamb performance, we used 60 (n = 15/ system) Poll Dorset x Corriedale (crossbred) sheep with mean body weights of 24.4 ± 3.4 kg from July to September 2015 and 48 (n = 12/ system) Texel x Corriedale sheep with body weights of 26.4 ± 3.5 kg from June to September 2016. Lambs were allocated to the following groups: Group 1 rotated on the 12 ICL1 paddocks; Group 2 rotated on the 12 ICL2 paddocks; Group 3 rotated on the 12 ICL3 paddocks; and Group 4 rotated on the 12 ICL4 paddocks. Each paddock was grazed by sheep twice for three days, with a 33-day interval between grazing. At the end of the day the lambs were supplemented with concentrate plus silage. Groups 1 and 2 received mixed silage made of maize, marandu palisade grass and pigeon pea and groups 3 and 4 received mixed silage made of maize and marandu palisade grass. Faecal and blood samples were taken from all the animals every two weeks, and body weight was recorded on the same occasion. Nematode faecal egg counts (FEC), packed cell volume and total plasma protein means did not differ ($P > 0.05$) between the four lamb groups. In 2015, throughout the experimental period, the FEC decreased in all animals, with 6733 and 1407 eggs per gram (EPG) on average at the beginning and end of the trial, respectively. At the beginning of the trial in 2016, the animals had mild GIN infections (1077 EPG on average), and the individual faecal egg counts did not exceed 10,000 EPG during the trial. In decreasing order, *Haemonchus* spp., *Trichostrongylus* spp. and *Cooperia* spp. third stage larvae were found in the faecal cultures in both years. The performance of lambs were similar in the four ICL systems ($P > 0.05$), in the first and second years, the daily weight gains were 0.192 ± 0.05 kg and 0.221 ± 0.06 kg, respectively. In conclusion, a period of withdrawal of contaminated sheep results in plots free of infective larvae. The use of clean pastures during the dry season, in the different ICL systems, associated with a good nutrition plan, resulted in progressively declining degrees of GIN infections and satisfactory daily weight gain of young sheep.

1. Introduction

Traditionally, sheep have been reared in extensive pasture systems at low stocking rates, often in areas unsuitable for crop production. However, with the developing sheep industry, production has intensified with higher animal stocking rates employed in areas of high forage production. An adverse consequence is the increased risk of parasitic gastroenteritis due to increased pasture contamination by the

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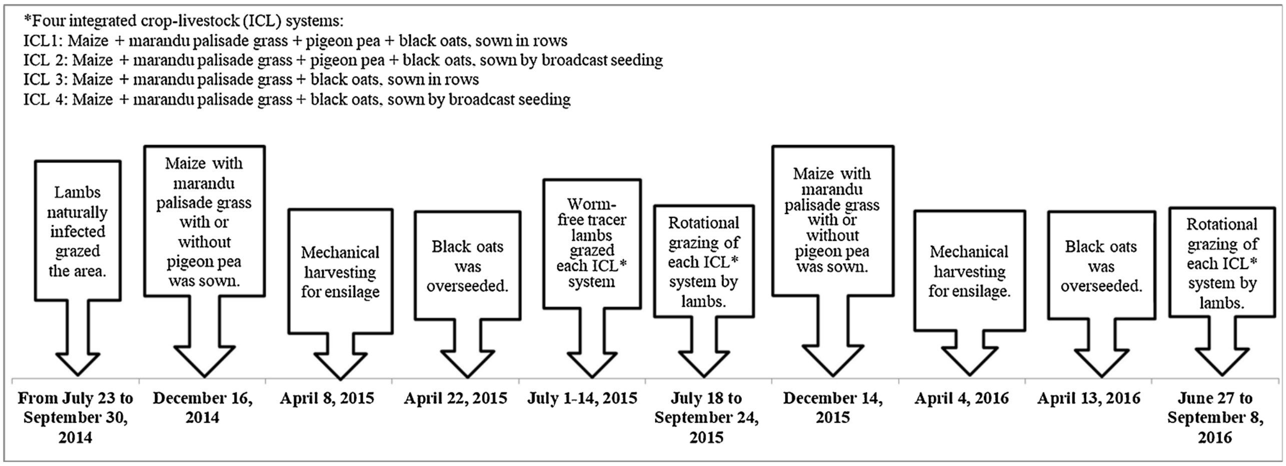

Fig. 1. Experimental design.

free-living stages of gastrointestinal nematode (GIN) parasites (Amarante, 2001). To reduce economic losses caused by GIN infections, anthelmintics have been intensively used, resulting in the selection of parasite populations with multiple anthelmintic resistance, a widespread problem in Brazil (Salgado and Santos, 2016).

In some tropical areas, sheep may acquire massive GIN infections year-round (Wilmsen et al., 2014). In such conditions, grazing management to decontaminate the pasture might be a feasible option for controlling GIN parasitism. Observations in sub-tropical conditions of Santa Catarina state, Brazil, indicate that spelling a grazing area for 42–126 days may decontaminate a pasture (Souza et al., 2000). This long time for spelling is unfeasible, since it is incompatible with properly managing the forages, which aim to qualitatively and quantitatively increase production. However, in areas managed by integrating agricultural crops and livestock, known as integrated crop-livestock (ICL) systems, a long time without animals during crop production can eliminate the free-living GIN stages. This occurred in a trial in Rio Grande do Sul state in southern Brazil, where an area that had been grazed by beef cows and their calves during the winter and spring was ploughed and seeded with a soybean [*Glycine max* (L.) Merr.] crop in mid-November. The crop was harvested at the end of May, and the soil was then lightly ploughed and seeded with black oats. This winter/spring grass was "clean" and ready for grazing by late August (Echevarria et al., 1993).

In addition, ICL systems afford ecological interactions among the different land-use systems to make agricultural ecosystems more efficient at cycling nutrients, preserving natural resources and the environment, improving soil quality and enhancing biodiversity (Macedo, 2009; Lemaire et al., 2014).

Producing maize intercropped with tropical perennial grass and legumes (for example, pigeon pea) is an interesting alternative for improving and diversifying agricultural activities and represents an alternative for the producer to implement nitrogen biological fixation in the soil (Oliveira et al., 2011; Pariz et al., 2017). Maize silage production intercropped with palisade grass and pigeon pea evaluated in the present study, can promote areas with different soil covers, which generates different microclimates, which may influence the population of free-living GIN stages (Besier et al., 2016). Thus, it is important to evaluate systems using different combinations of plants and seeding techniques.

Diverse field studies are needed with ruminant livestock, including sheep, to adequately characterize the impacts of ICL systems on animal performance, because stocking rates and management approaches can alter crop yield and forage quantity and quality. In most areas of Brazil, such as São Paulo state, no detailed research has been conduct to verify whether the ICL system favours preventing GIN infections in grazing lambs. There are only some studies evaluating bovine infection and pasture contamination by GIN in the silvopasture system that combines forestry and livestock production (Faria et al., 2016; Mendonça et al., 2014; Oliveira et al., 2017a).

The purpose of the trial was to evaluate the performance and GIN infection of the lambs finished on four integrated crop-livestock systems formed by maize, marandu palisade grass, pigeon pea and black oats. In addition, the objective of the trial was to evaluate whether the ICL system after ten months without sheep, which is the time required for crop production and subsequent pasture seed and growth, leads to eliminating the free-living GIN stages from the pasture.

2. Materials and methods

This study was conducted in accordance with the local Ethics Committee on Animal Use (protocol number 716-CEUA, IBB-UNESP).

The experiment was conducted at the Lageado Experimental Farm (22°51′01″S, 48°25′28″W; altitude, 777 m), located in the city of Botucatu, State of São Paulo, Brazil. The soil was a clay, kaolinitic, thermic Typic Haplorthox (FAO, 2006) with 630, 90 and 280 g kg^{-1} of clay, silt and sand, respectively. According to the Köppen classification system, the region's predominant climate is the Cwa type (humid subtropical), which is characterized by warm, rainy summers and dry winters (Alvares et al., 2014). The average accumulated monthly precipitation is highest (261 mm) in January and lowest (38 mm) in August. The average monthly temperature ranges from 23.2 °C in February to 17.1 °C in July (Escobedo et al., 2011).

2.1. Experimental area and management of the animals

The study was developed in an experimental area of the integrated crop-livestock (ICL) system established in 2013 for producing mixed silage of maize (*Zea mays*), marandu palisade grass (*Urochloa brizantha* cv. Marandu), and pigeon pea (*Cajanus cajan*) with an overseeding of black oats (*Avena strigosa*), followed by lamb grazing. Before the present study, this area with mixed pasture of palisade grass and black oats was grazed by male lambs from July to September 2014. The lambs were naturally infected with GIN, with an average of 330 (0–3,500) eggs per gram of faeces (EPG) at the end of the fattening period (September 30, 2014). Details of the area's use are presented in Fig. 1.

Maize (with marandu palisade grass or with marandu palisade grass + pigeon pea) was sown in December 2014 and 2015, and mechanical harvesting for ensilage was conducted in March 2015 and 2016. After harvesting in April 2015 and 2016, black oats were overseeded, either in rows (with a seeder-fertilizer) or by broadcast seeding (manually) (Fig. 1). Therefore, the ICL systems evaluated were: ICL 1 -

maize + marandu palisade grass + pigeon pea + black oats, sown in rows; ICL 2 - maize + marandu palisade grass + pigeon pea + black oats, sown by broadcast seeding; ICL 3 - maize + marandu palisade grass + black oats, sown in rows; and ICL 4 - maize + marandu palisade grass + black oats, sown by broadcast seeding. Each ICL system area was divided by an electric fence into 12 paddocks of 225 m^2. Each paddock was grazed twice for three days, with a 33-day interval between grazing (totalling 72 days of grazing).

In the first year (from July 18 to September 24, 2015), we used 60 (n = 15/ ICL system) approximately 8-month-old Poll Dorset × Corriedale (crossbred) uncastrated male sheep with mean body weights of 24.4 ± 3.4 kg. In the second year (from June 27 to September 8, 2016), we used 48 (n = 12/ ICL system) Texel x Corriedale (crossbred) young male sheep of the same age as the animals from the previous year, with body weights of 26.4 ± 3.5 kg. Lambs were allocated to four groups balanced as closely as possible by body weight: Group 1 rotated on the 12 ICL1 paddocks; Group 2 rotated on the 12 ICL2 paddocks; Group 3 rotated on the 12 ICL3 paddocks; and Group 4 rotated on the 12 ICL1 paddocks.

Before starting the experiment, faecal samples were collected to determine EPG, and lambs were tagged, vaccinated against *Clostridium* spp. (Sintoxan® Polivalente T; Merial Saúde Animal Ltda., Paulínia, Brazil). The animals were treated with closantel (10 mg/kg BW, Zuletel®, Microsules Laboratories, Uruguay) on July 18 in 2015 and June 27 in 2016.

Daily at 6:00 a.m., all groups of lambs were allocated to their respective ICL paddocks, and at 4:00 p.m., they were returned to a covered shed where the lambs of each group were housed in 5 m × 5 m (25 m^2) pens and supplemented with concentrate and silage. Groups 1 and 2 received mixed silage made of maize, marandu palisade grass and pigeon pea and groups 3 and 4 received mixed silage made of maize and marandu palisade grass. Details about the ingredients and the chemical composition of experimental diets are presented in the Table of the Supplementary File. The estimated dry matter intake was 4.35% of the body weight for an average daily weight gain of 200 g (NRC, 2007). In all systems, the pasture accounted for approximately 30% of the dry matter intake. The remainder was provided as maize silage (produced previously in the same area) and concentrate per the lambs' daily needs. The lamb diet was computer-formulated using the Small Ruminant Nutrition System (SRNS) program based on the Cornell Net Carbohydrate and Protein System (2000) for sheep. The animals always had free access to water.

Faecal and blood samples were taken from all the animals every two weeks, and body weight was recorded on the same occasion.

2.2. Tracer lambs

To determine whether a ten-month period without any animals was enough to decontaminate the pasture, 12 tracer lambs (n = 3/ ICL system) were used in the first year (2015). The animals used were 3-month-old uncastrated male Dorper x Santa Ines crossbreeds, weighing approximately 25 kg. The tracer lambs received monepantel (2.5 mg/ kg BW, Zolvix®, Novartis Animal Health) to eliminate strongyle infection, and albendazole (15 mg/ kg of BW, Valbazen®, Pfizer) for 10 consecutive days to eliminate *Strongyloides* spp. infection.

The tracer lambs entered the experimental area on July 1st, 2015, before the test animals and grazed the paddocks for 14 consecutive days. They were then housed for another 14 days and fed Tifton 85 hay and concentrate with 16% crude protein. After this period, the lambs were slaughtered to verify worm presence in the gastrointestinal tract.

2.3. Laboratory analyses

Faecal samples were taken directly from the animals' rectums for faecal egg counts (FEC) (Ueno and Gonçalves, 1998). Each counted egg represented 100 eggs/g. Composite faecal cultures were performed separately for each group of sheep for production of third stage larvae, which were identified according to the descriptions of Ueno and Gonçalves (1998).

At necropsy, tracer lambs' abomasum and small and large intestines were removed and opened, and the contents were placed in graduated buckets. A 10% aliquot was preserved in 5% formalin to subsequently identify and quantify the helminths (Ueno and Gonçalves, 1998). The small intestine was digested in saline solution (NaCl 0.85%) for 4 h at 37 °C A sample of 10% of the intestinal digested material was collected and preserved in 5% formalin.

Pasture samples were collected to assess the number of infective GIN larvae per kilogram of dry matter (L3/ kg DM). The sampling was performed in each ICL system at the start (July 18, 2015 and June 27, 2016), middle (August 23, 2015 and August 02, 2016) and end (September 25, 2015 and September 07, 2016) of the grazing cycle. In the first year, sampling was performed in paddocks 1, 6 and 11 of each system. These paddocks were selected by their distribution, with the objective of achieving homogeneity in sampling the area. In the second year, all paddocks were sampled. Sampling was conducted per the modified "W" method described by Taylor (1939). The samples were processed per Niezen et al. (1998), and the larvae were separated from the sediment, recovered and quantified as previously described by Carneiro and Amarante (2008).

Blood samples were collected by jugular venepuncture into vacuum tubes containing EDTA (BD Vacutainer®). The packed-cell volume (PCV) was determined by micro-haematocrit centrifugation and the total plasma protein (TPP) was estimated using a refractometer (Refractometer SPR-N, Atago). In the last sampling, plasma samples were stored at −20 °C to be used in an enzyme-linked immunosorbent assay (ELISA) to access the IgG antibody levels against the total third-stage larvae (L3) antigens of *Haemonchus contortus*. Antigen production was previously described by Amarante et al. (2009), and the protocol used to measure the parasite-specific plasma IgG levels as described by Silva et al. (2012) with the following modifications: the plates were coated with 2 μg of antigen/ml; washed three times, rotated 180° and re-washed three more times, and the negative control (NC) sample was from a worm-free animal, as previously described by Santos et al. (2014). The plasma positive control (PC) sample was from an animal artificially infected with *H. contortus* and *Trichostrongylus colubriformis* every three days for 84 days, and the peroxidase-conjugated rabbit anti-sheep IgG (A130-101 P, Bethyl Laboratories, Inc., USA) was diluted at 1:40,000.

2.4. Statistical analysis

All data were initially tested for normality using the Shapiro-Wilk test from the UNIVARIATE procedure (version 9.3; SAS Inst. Inc., Cary, NC, USA), and the results indicated that PCV, TPP, daily weight gain (DWG) and IgG data were normally distributed (W = 0.90). The EPG data were transformed using log (x + 1) prior to the analysis. Data were analysed using the SAS PROC MIXED procedure with Satterthwaite approximation to determine the denominator degrees of freedom for the fixed effect tests. 'Animal' was considered the experimental unit, and the model statements for EPG, PCV, TPP, DWG and IgG levels analyses contained 'ICL system' and 'collection period' as the fixed effects. A repeated statement was used, with collection period specified as the repeated variable. An autoregressive covariance structure was used in the analyses, which provided the best fit per the Akaike information criterion. The results were reported as least square means and separated by preplanned pairwise comparisons (PDIFF). Means were compared by using Fisher's Least Significant Difference (LSD) test. Effects were considered statistically significant at $P \leq 0.05$. Relationships between variables (EPG × DWG and EPG × IgG anti-L3) were assessed by linear regression and Pearson's correlation using Graph Pad Prism 5. Descriptive statistical analyses were used to summarise the number of L3 recovered from pasture.

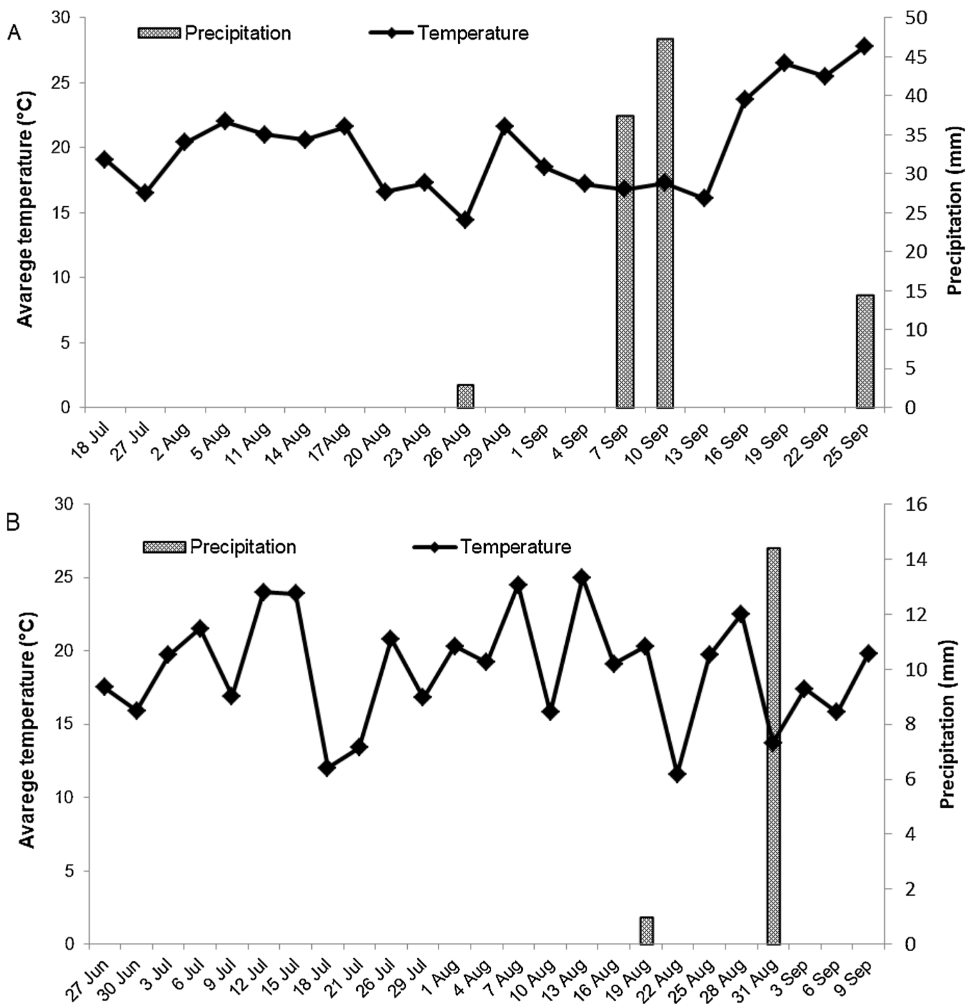

Fig. 2. Precipitation (mm) and mean daily temperature (°C), (A) in the period from July 18th to September 25th, 2015, (B) in the period from June 27th to September 9th, 2016, in the experimental area at Fazenda Lageado, Botucatu, São Paulo state, Brazil.

3. Results

The tracer lambs acquired no GIN infection during the grazing period in the experimental area. No eggs were detected on the faecal examination, and no L3 were recovered from their faecal cultures. Moreover, no nematode parasites were found in their gastrointestinal tracts. In addition, no L3 were found in the pasture samples collected before the animals began grazing in the area. Therefore, after 300 days (September 30, 2014–July 18, 2015) without infected sheep, the area became free of infective nematode larvae contamination.

During the trial in 2015 the overall average temperature was 20 °C, and the highest precipitation (47.4 mm) was recorded on September 10 (Fig. 2A). In 2016 the overall average temperature was 18 °C, and rains were recorded only at the end of the trial, with 1 mm on August 19 and 14.4 mm on August 31 (Fig. 2B).

Third-stage larvae of *Haemonchus* were recovered from the pastures at the end of the second grazing cycle, on September 25, 2015, with averages of 448 L3/ kg DM, 3396 L3 / kg DM, 156 L3 / kg DM and 4364 L3 / kg DM in the ICL systems 1, 2, 3 and 4, respectively. *Trichostrongylus* L3 also only recovered on September 25, 2015 in the ICL systems 2 and 4 with 238 L3/ kg DM and 311 L3/ kg DM, respectively. The average temperature on this date was 25 °C, and it rained 15 mm (Fig. 2A). In 2016 no larvae were recovered from pasture.

In both years of the study, there was time effect ($P \leq 0.05$) on EPG, PCV, PPT and body weight, without any significant influence ($P \geq 0.05$) of the ICL system on these variables (Table 1). In the first year, at the beginning of the trial, the animals had a high EPG (6733 ± 7525) (Table 1). Six animals had FEC above 15,000 EPG (Fig. 3A). After treatment with closantel the EPG values decreased (58% in average) in the following sampling (July 31, 2015); however, the EPG values increased in the subsequent sampling (August 13, 2015) indicating poor efficacy of such drench (Fig. 3A). Nevertheless, with the progression of the trial, the FECs declined reaching the lowest values of EPG at the end of the experiment (Fig. 3A)). The progressive decrease in the degree of infection of the animals was associated with increase of the PCV values from averages below 24% in the beginning of the trial to averages above 29% in the last sampling (Table 1). Except for the initial TPP values of the sheep from the ICL3 and ICL4 systems, the TPP values were within normal limits for ovines (PPT ≥ 5 g/dL) (Table 1).

Lambs were in better shape at the beginning of the second-year trial, with mild GIN infections and normal PCV and TPP values (Table 1). The individual EPG values did not exceed 10,000 EPG during the trial (Fig. 3B and Table 1). The treatment with the closantel in the beginning of the trial had poor efficacy (27%), with negligible impact on FECs (Fig. 3B). As in the previous year, the FECs declined during the experiment. Thus, in the last sampling, most animals shed between 0 and 500 EPG (Fig. 3B).

The initial weights of the young sheep averaged 25 kg for both years (Table 1). The body weight gain was similar in the four ICLs ($P > 0.05$), in the first and second years, the DWGs were 0.192 ± 0.05 kg and 0.221 ± 0.06 kg, respectively (Table 1).

There was no significant correlation between the DWG and average

Table 1
Initial (I) and final (F) means (minimum and maximum values) followed by the means of the log transformed values of eggs per gram of faeces (EPG) and means ± standard deviation of packed cell volume (PCV), total plasma protein (TPP), body weight (BW) and daily weight gain (DWG) of the lambs in the first year (2015) and in the second year (2016) of the integrated crop-livestock systems (ICL). Details of the ICL are presented in Fig. 1.

Year	Variable	Group 1 (ICL 1)	Group 2 (ICL 2)	Group 3 (ICL 3)	Group 4 (ICL 4)
2015	I-EPG	8781 (0–40500), 3.3	7560 (0–25300), 3.4	5593 (0–20800), 3.1	5000 (900–16900), 3.4
	F-EPG	1838 (100–4300), 3.1	920 (0–3400), 2.6	1368 (0–4300), 2.7	1500 (300–4000), 3.1
	I-PCV (%)	21.4 ± 5.3	22.3 ± 4.4	21.8 ± 4.6	23.5 ± 5.5
	F-PCV (%)	29.4 ± 3.3	30.9 ± 1.8	31.1 ± 4.3	31.5 ± 2.8
	I-TPP (g/dL)	5.1 ± 0.82	5.1 ± 0.70	4.8 ± 0.69	4.9 ± 0.81
	F-TPP (g/dL)	6.1 ± 0.56	6.2 ± 0.41	6.3 ± 0.79	6.6 ± 0.68
	I-BW (kg)	25.96 ± 2.9	25.16 ± 2.9	25.96 ± 2.8	24.83 ± 3.5
	F-BW (kg)	39.94 ± 3.8	38.59 ± 3.8	39.99 ± 5.2	38.79 ± 5.9
	DWG (kg)	0.194 ± 0.04	0.186 ± 0.04	0.195 ± 0.05	0.194 ± 0.06
2016	I-EPG	2092 (0–8400), 2.7	617(0–1800), 1.9	608 (0–2600), 2.1	992 (0–6500), 2.0
	F-EPG	117 (0–700), 1.1	392 (0–3900), 0.9	150 (0–1100), 1.1	483 (0–3100), 1.5
	I-PCV (%)	33 ± 4.9	36 ± 2.9	34 ± 4.0	33 ± 4.6
	F-PCV (%)	31 ± 2.4	32 ± 2.7	33 ± 2.6	33 ± 2.9
	I-TPP (g/dL)	6.4 ± 0.49	6.6 ± 0.38	6.8 ± 0.59	7.0 ± 0.55
	F-TPP (g/dL)	6.0 ± 0.51	6.1 ± 0.66	6.5 ± 0.43	6.6 ± 0.63
	I-BW (kg)	26.25 ± 4.61	25.56 ± 2.9	24.51 ± 2.8	25.62 ± 3.84
	F-BW (kg)	40.60 ± 6.54	40.25 ± 6.5	40.98 ± 3.04	43.78 ± 4.4
	DWG (kg)	0.199 ± 0.07	0.204 ± 0.08	0.229 ± 0.03	0.252 ± 0.05

There were no significant differences between group means ($P > 0.05$).

EPG ($r = -0.030$ and degree of freedom (DF) = 58 in 2015 and $r = -0.058$ and DF = 46 in 2016; $P > 0.05$) (Fig. 4) or between the IgG level against the *H. contortus* L3 antigens and the final EPG ($r = -0.119$ and DF = 58 in 2015 and $r = -0.219$ and DF = 46 in 2016; $P > 0.05$) (Fig. 5).

Haemonchus, *Trichostrongylus* and *Cooperia* were the genera found in the faecal cultures (Table 2). In addition, *Trichuris* spp. and *Nematodirus* spp. eggs were detected in a few of the faecal examinations.

4. Discussion

In the ICL system, intercropping enables maize production for grain or silage in the summer, followed by pasture formation in the winter/spring for livestock use in the same field. In addition, we demonstrated that a period of several months without animals to manage the area for pasture desiccation, ploughing, sowing and harvesting, decontaminated the pasture. At Rio Grande do Sul, Echevarria et al. (1993) reported a similar efficiency of the ICL system in the decontamination of pastures by GIN larvae. The same was not observed by other researchers that evaluated the integrated livestock-forest systems (ILF) (Faria et al., 2016; Oliveira et al., 2017a). Faria et al. (2016), when evaluating the pasture contamination in the ILF system, found a higher number of GIN infective larvae of sheep in the ILF system compared to the conventional system. Certainly, there are differences between the ICL and ILF regarding the contamination of pastures by GIN L3. The microclimate created by the shading of the trees on the pasture could be more favourable to the development of the early nematode stages (from egg to infective larvae) and the survival of infective larvae.

The animals shed large number of eggs in their faeces, which, however, did not result in heavy contamination of the pasture by infective larvae. An interaction of several factors might be involved in low pasture contamination during the trial. In our conditions, during the dry season, the lack of rain may be the most important limiting factor for developing early nematode stages, because moisture is required for eggs to develop into the infective larval stage (O'Connor et al., 2006). L3 migration and survival in the environment is also greatly influenced by the climatic conditions and the pasture's microclimate (Silva et al., 2008; Santos et al., 2012; Rocha et al., 2014). Thus, L3 were only recovered when the environmental conditions favoured L3 development and survival in the pastures, which coincided with rainfall at the beginning of spring. Faria et al. (2016) also recovered a higher quantity of L3 in the spring when evaluating the pasture contamination in the ILF system.

The systems that had pigeon pea, because of the fixation of nitrogen in the soil, could have greater soil cover by herbage favouring the development and survival of L3 in the pasture. But we observed that, independently of the ICL system, the pasture was relatively short, allowing direct solar radiation on the faeces, which could rapidly dry and destroy the free-living stages, preventing L3 from migrating from the faecal pellets to the grass (Carneiro and Amarante, 2008; Van Dijk et al., 2009; Besier et al., 2016). Carneiro and Amarante (2008) observed that larval recovery was much lower when sheep faecal samples with GIN eggs were deposited on short grasses (5 cm height) compared to tall grasses (30 cm height).

In addition to the low L3 contamination in the area, other factors may have influenced the progressively reduced degree of infection (mean EPG) in the animals, among them, the nutritional quality. The animals were finished in semi-confinement, where they were supplemented with silage and concentrate. Quality nutrition increases the animals' ability to develop an efficient immune response and withstand the adversities of parasitism (Bricarello et al., 2005; Melo et al., 2017). Melo et al. (2017) evaluated the performance and GIN infection in grazing lambs supplemented or not supplemented with concentrate. The authors observed a high proportion of non-supplemented animals with EPG > 1000, while most of the supplemented animals had EPG < 1000. In the second-year animals had higher IgG anti-L3 than the first-year animals, indicating that they likely had a more efficient immunological response and therefore lower EPG counts throughout the experimental period.

In both years the reduction in the EPG values after the treatment with closantel was followed by an increase in EPG means in the next sampling demonstrating low efficacy of such anthelmintic. Resistance to closantel is widespread in Rio Grande do Sul, where of the 22 sheep flocks evaluated only in one closantel presented efficacy above 95% (Oliveira et al., 2017b).

Haemonchus and *Trichostrongylus* were the predominant genera in the composite faecal cultures, confirming that these are the most frequent gastrointestinal nematodes in sheep in Brazil (Amarante, 2014; Wilmsen et al., 2014). GIN mixed infections can cause considerable losses in sheep production, including decreased daily weight gain in young animals (Besier et al., 2016) which apparently was not the case in the sheep in the present study. Independent of the ICL system, all animals reached the expected body weight gain, considering their breed, age and the nutrition offered.

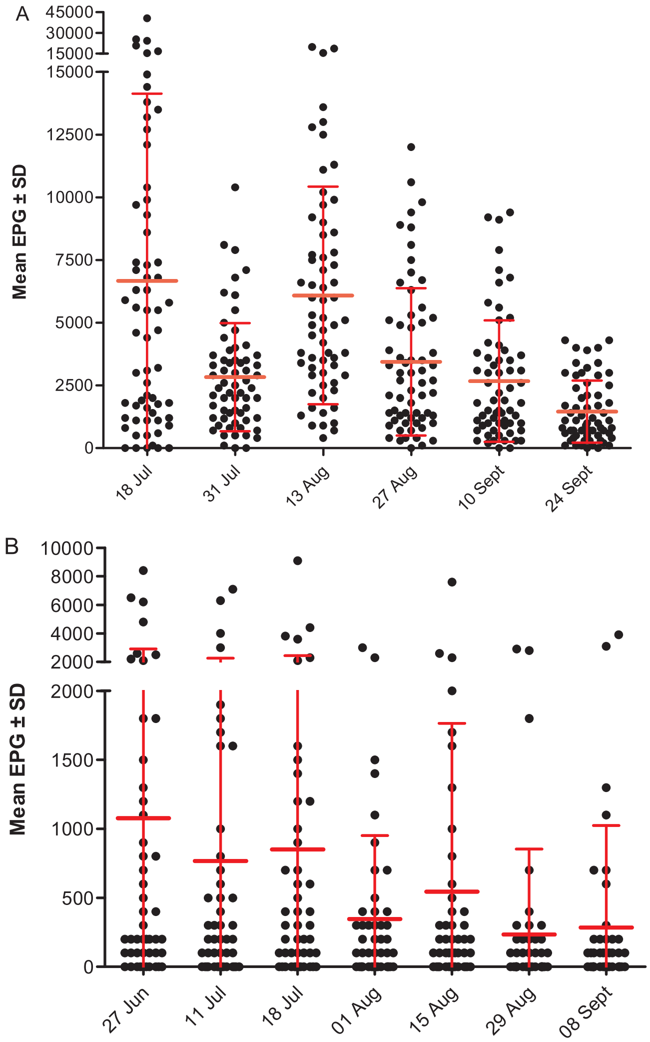

Fig. 3. Eggs per gram of faeces (EPG) of the lambs finishing in the integrated crop-livestock systems (ICL): (A) first year (2015) and (B) second year (2016). In July 18, 2015 and June 27, 2016 the animals were treated with closantel. The largest horizontal bar is the mean and the vertical bar is the standard deviation.

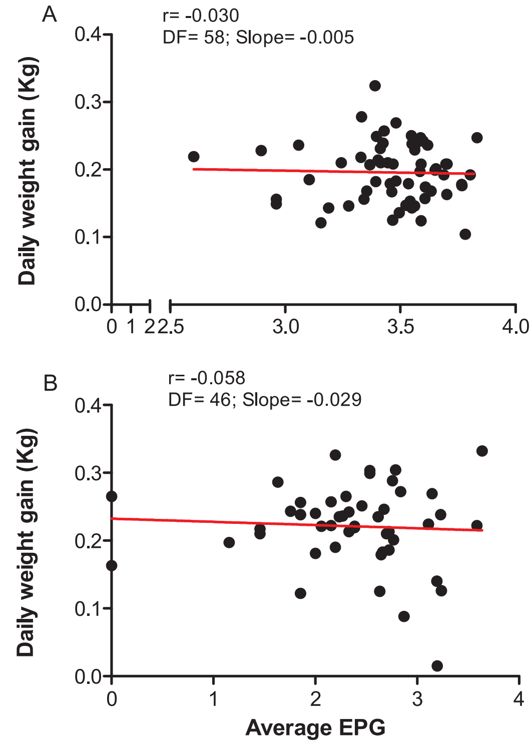

Fig. 4. Relationship between the daily weight gain and log transformed values of eggs per gram of faeces (EPG) of the lambs finishing in the integrated crop-livestock systems (ICL). (A) First year (2015) and (B) second year (2016). *r*, Pearson correlation coefficient; DF, degree of freedom.

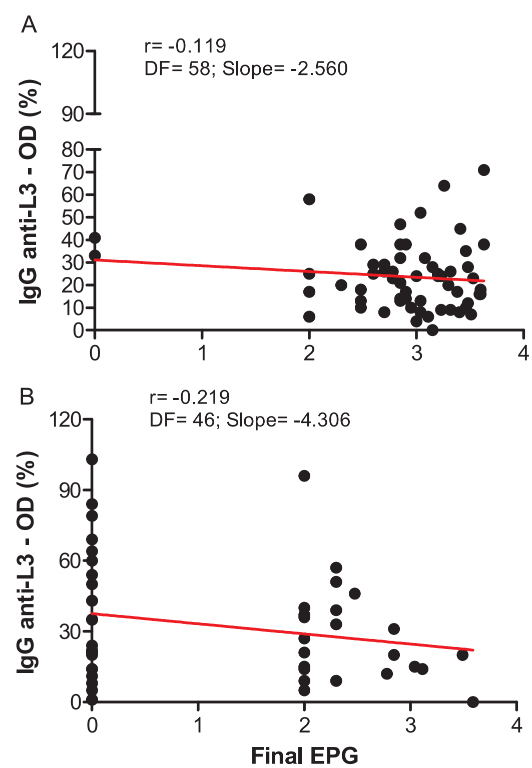

Fig. 5. Relationship between the IgG against third stage larvae (L3) antigens of *Haemonchus contortus* and log transformed values of eggs per gram of faeces (EPG) in the last collection. (A) First year (2015) and (B) second year (2016). *r*, Pearson correlation coefficient; DF, degree of freedom.

In conclusion, in our climate, the different ICL systems efficiently produced clean pastures. The use of such pastures associated with a good nutrition plan resulted in progressively declining degrees of GIN infections and satisfactory performance of young sheep finished during the winter in São Paulo state. The benefits of the GIN infection prophylaxis are of great importance, reducing the major sanitary problems in sheep. In such a system, the need for antiparasitic treatments may be reduced, alleviating costs and anthelmintic resistance.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Table 2
Mean percentage of infective gastrointestinal nematode larvae in the faecal cultures made with samples from the lambs finished in the different integrated crop-livestock systems (ICL), in the first year (2015) and in the second year (2016). Details of the ICL are presented in Fig. 1.

Year	Genera	Group 1 (ICL 1)	Group 2 (ICL 2)	Group 3 (ICL 3)	Group 4 (ICL 4)
2015	*Haemonchus* (%)	89	88	91	86
	Trichostrongylus (%)	10	11	8	13
	Cooperia (%)	1	1	1	1
2016	*Haemonchus* (%)	77	74	77	89
	Trichostrongylus (%)	20	24	19	10
	Cooperia (%)	3	2	4	1

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