

# Effect of alternate and simultaneous grazing on endoparasite infection in sheep and cattle

Efeitos do pastejo alternado e simultâneo sobre a infecção de endoparasitas em Ovinos e Bovinos

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## Abstract

This experiment was carried out on 8 ha of *Panicum maximum* cv. Tanzania pastures, with rotational grazing consisting of 7 days of occupation and 21 days of rest. Four treatments were evaluated: cattle grazing alone (BOV), sheep grazing alone (OVI), cattle and sheep grazing simultaneously (SIM) and cattle grazing followed by sheep (alternate - ALT). Twenty heifers and 30 male Santa Inês lambs were used. Fecal egg count (FEC) and fecal cultures were carried out. Blood was also collected to examine red and white cell series, total plasma protein (TPP), albumin and hemoglobin. FEC and estimated nematode pathogenicity index in sheep were lower in the SIM treatment. The *Haemonchus* spp. proportion was higher in isolated grazing systems. For sheep, mixed grazing was shown to reduce endoparasite infection, and SIM was better than ALT. For cattle, no difference between grazing systems was seen. Therefore, simultaneous grazing (sheep and cattle) may be a tool for reducing the need for anthelmintic treatments in sheep.

**Keywords:** Host specificity, fecal egg count, rotational pasture, alternative control method, Nematoda.

## Resumo

O experimento foi realizado em 8ha de pasto de *Panicum maximum* cv. Tanzania, com pastejo rotacionado de 7 dias de ocupação e 21 dias de descanso. Quatro tratamentos foram avaliados: bovinos pastejando isoladamente (BOV), ovinos pastejando isoladamente (OVI), bovinos e ovinos pastejando simultaneamente (SIM), e bovinos pastejando previamente aos ovinos (alternado – ALT). Vinte novilhas e 30 cordeiros Santa Inês foram utilizados. Contagem de ovos nas fezes (FEC) e coproculturas foram realizados. Sangue também foi colhido para examinar a série vermelha e branca, proteínas plasmáticas totais (TPP), albumina e hemoglobina. FEC e índice de patogenicidade estimada de nematoides nos ovinos foram menores no tratamento SIM. A proporção de *Haemonchus* spp. foi maior nos sistemas isolados de pastejo. Para os ovinos, os sistemas consorciados apresentaram redução na infecção endoparasitária, sendo SIM melhor que ALT. Para os bovinos, nenhuma diferença entre os sistemas de pastejo foi verificado. Entretanto, o pastejo simultâneo (ovinos e bovinos) pode ser uma ferramenta para reduzir a necessidade de tratamentos anti-helmínticos em ovinos.

**Palavras-chave:** Especificidade de hospedeiro, contagem de ovos nas fezes, pastejo rotacionado, método de controle alternativo, Nematoda.

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## Introduction

Infection by endoparasites is among the main factors affecting the performance of sheep raised on pasture and is one of the major problems that interfere with the development of livestock (LOUVANDINI et al., 2006). Infections cause reduction in animal growth, death and excessive management costs, thus resulting in herds with low productivity and high economic losses (ARAÚJO et al., 2004).

To control the negative effects of parasite infection, farmers worldwide depend heavily on the use of anthelmintic drugs. This situation has negative consequences in that it increases production costs by requiring more anthelmintic treatments and leads to production of carcasses with higher levels of chemical residues (YAMAMOTO et al., 2004).

In addition, many strains of nematodes have evolved and become resistant to anthelmintic drugs (JAMES et al., 2009). In Brazil, this phenomenon has been spreading throughout the regions where sheep are raised. The most common way of controlling helminths is through use of chemicals. However, indiscriminate and repetitive treatment regimens have led to selection of populations of helminths that are resistant to different chemical groups (SCZESNY-MORAES et al., 2010).

Finding ways to overcome this resistance and maintain effective parasite control is becoming increasingly important (MOLENTO; PRICHARD, 2001). Efforts have focused on finding new supplements or alternatives to chemical treatment. More judicious use of broad-spectrum anthelmintic drugs and bioactive forages (CENCI et al., 2007), pasture management, breeding for resistance or tolerance (AMARANTE et al., 2004) and biological control (LARSEN, 2008) are examples of options that can be used against a wide variety of nematode species.

While not sufficient to prevent parasitic infections in all situations, it is known that grazing management is an efficient aid for parasite control. Various forms of rational use are used to reduce the level of gastrointestinal parasite infection in ruminants. Their success depends on the husbandry conditions, climate, type of pasture, stocking rate and variations in the manner of conducting grassland management (such as number of rest days in rotational grazing) (CABARET et al., 2002).

The aim of this study was to investigate the effect of different grazing systems for cattle and sheep on parasite infections.

## Materials and Methods

This experiment was carried out over a 98-day period from January to April (rainy season) 2009 with a further 14-day adaption

period, in the Federal District, Brazil, at 15° 57' S and 47° 56' W. This location has a tropical seasonal (Aw) climate, according to the Koeppen classification. Data on the climatic conditions during the trial are shown in Table 1.

Pasture land comprising *Panicum maximum* cv. Tanzania grass was subdivided into 17 paddocks of 0.5 ha for four treatment groups in rotational grazing: cattle alone (BOV), 4 paddocks; sheep alone (OVI), 4 paddocks; simultaneous sheep and cattle grazing (SIM), 4 paddocks; and an alternate system with cattle grazing before sheep (ALT), 5 paddocks. Each paddock was grazed for 7 days and rested for 21 days, with the exception of ALT paddocks, which were grazed for 14 days (7 by cattle and 7 by sheep). This experimental area is part of a sheep management center and, prior its reformulation, it had only been used by sheep for many years. However, the experimental pasture was formed at the end of 2007 as a part of a pasture reformulation plan and in preparation exclusively for this experiment. Thus, it was left at rest for one year in order to become consolidated. Hence, no animals had been using this pasture before this experiment. However, grass samples from the experimental area were collected for recovery and identification of L<sub>3</sub>-larvae before the first grazing week, and the mean parasite load detected was 4.56 larvae/kg of dry matter (TORRES et al., 2009). Additional information about parasitic nematode load in this pasture can be found in TORRES et al. (2009).

Twenty crossbreed heifers weighing on average 206.70 ± 20.79 kg and thirty Santa Ines male lambs weighing 22.70 ± 2.23 kg were used. For ALT and SIM, six cattle and ten lambs were used. For BOV, eight animals were used, and for OVI, 10 lambs and 15 adult sheep were used. Although these adult sheep were not part of the experiment, they were used to complete the established stocking rate of 2 animals units (AU) per hectare. The animals remained in the experimental area for 14 days of adaptation and 98 days in the experiment. The cattle remained inside the paddocks all the time, while the sheep were gathered into shelters every night. In addition to pasture, the animals were feed with concentrate (200 g/day for sheep and 2 kg/day for cattle) and received water and mineral salt *ad libitum*. For the sheep, the concentrate mixture was made up of 55% corn, 30% soya bean meal, 10% cotton meal and 5% wheat meal, with 88% dry matter (DM), 22% crude protein (CP), 72% total digestible nutrients (TDN) and 2.613 Mcal/kg of metabolizable energy (ME). For the cattle, the concentrate mixture consisted of 60% corn and 40% soya bean meal, with 88% DM, 23% CP, 78% TDN and 2.839 Mcal/kg of ME.

Prior to putting the animals into the experimental area, and with the aim of reaching FEC of zero, all the animals remained

**Table 1.** Climatic characterization of experimental field during the trial.

Month	Precipitation (mm) <sup>1</sup>		Days with rain <sup>2</sup>	RH (%) <sup>2</sup>	Temperature <sup>1</sup>		
	Mean*	Total			Mean*	Max	Min
January	9.6	297.4	22	81.1	21.8	27.4	16.1
February	9.5	266.7	18	80.2	21.8	27.4	16.2
March	8.3	257.6	21	79.2	21.4	27.1	15.7
April	6.4	191.8	16	83.5	21.5	28.1	14.9

<sup>1</sup>According to Santos et al. (2011); <sup>2</sup>According to INMET (2013). \*Mean based on all days of month; **Max**, maximum temperature; **Min**, minimum temperature; **RH**, relative humidity.

confined and received levamisole hydrochloride (Fort Dodge Ripercol-L® 5%), albendazole (Labovet Albendazole® 10%) and sulfaquinoxaline sodium (Ourofino Coccifin®), orally in accordance with the manufacturers' instructions.

Nematode fecal egg counts (FEC) and fecal cultures were used to calculate the estimated pathogenicity equivalence (PES) of the worm burden, as described by Ueno and Gonçalves (1998). These were carried out weekly on the sheep and fortnightly on the cattle. Fecal cultures were carried out on a pool of feces from all animals belonging to the same species and the same group, as described by Ueno and Gonçalves (1998).

Blood was collected fortnightly from all animals by venipuncture, using two vacuum tubes (with and without EDTA). The packed cell volume (PCV) was determined by means of microhematocrit tube centrifuge. Eosinophils were quantified in a Neubauer chamber after staining with Carpentier solution (DAWKINS et al., 1989) and were expressed as units/ $\mu$ L of blood. Hemoglobin were quantified using a commercial kit (LABTEST®) and spectrophotometer. Serum was used to quantify total plasma protein (TPP) using a refractometer, and albumin using a commercial kit (LABTEST®) and spectrophotometer.

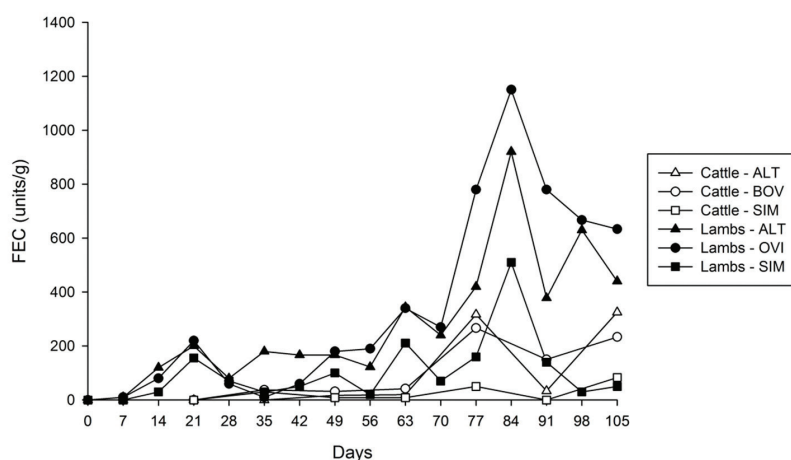
Statistical analyses were carried out using the SAS® package. The PROC MIXED procedure was used for the pasture cycle, treatment group, FEC, PES and proportion of third-stage larvae in fecal cultures. Means were compared using the Duncan test ( $P < 0.05$ ). Correlations (PROC CORR) and principal component analysis (PROC PRINCOMP) were also used to study relationships between the variables measured.

## Results

No anthelmintic treatment was carried out during the experiment, since FEC was under 4000 and PCV was greater than 21% (AMARANTE et al., 2004). The animals showed no clinical signs of parasitic gastroenteritis (such as diarrhea or submandibular edema).

FEC was higher ( $P = 0.033$ ) in sheep (mean of 253.74) than in cattle (mean of 65.60), and it increased over the course of the experimental period, reaching a peak between the 77<sup>th</sup> and 84<sup>th</sup> days in both ruminant species (Figure 1). In general, more *Haemonchus* spp. larvae were isolated from sheep samples while *Trichostrongylus* spp. were more prevalent in cattle. Although there were statistical differences between examination days, the genera maintained similar proportions over the study period.

Among the sheep, SIM treatment gave rise to lower FEC than in ALT and OVI respectively, while for the cattle, there were no significant differences in FEC between treatments (Table 2). Significant differences ( $P < 0.0001$ ) were found between treatments on sheep and cattle in relation to fecal cultures (Table 3): for sheep, ALT had the lowest proportion of *Haemonchus* spp., followed by SIM and OVI, with the highest proportion of this parasite. For the other parasite species (*Trichostrongylus*, *Cooperia* and *Oesophagostomum*), the proportions between treatments did not have any specific profile. In cattle, the larval proportion in fecal cultures for *Haemonchus* spp. was lower in the treatments with sheep (SIM and ALT). SIM treatment had the lowest proportion of *Haemonchus* spp., but on the other hand, this treatment showed the highest proportion of *Trichostrongylus* spp. (Table 3). An



**Figure 1.** Mean nematode fecal egg counts (FEC) of cattle and sheep grazing simultaneously (SIM), alternately (ALT) or singly (cattle, BOV; or sheep, OVI) during the experimental period.

**Table 2.** Mean FEC (eggs per gram) of strongyles in cattle and sheep in different treatments.

Grazing system <sup>1</sup>	Cattle				Sheep			
	Mean	SD	Max	Min	Mean	SD	Max	Min
SIM	22.3	57.9	300	0	107.4 <sup>a</sup>	255.3	2400	0
ALT	89.7	304.6	1800	0	296.5 <sup>b</sup>	462.0	3400	0
BOV	85.4	152.3	600	0	-	-	-	-
OVI	-	-	-	-	358.1 <sup>b</sup>	628.7	4000	0

Means followed by different letters (a,b) in the same column were significantly different using Duncan test ( $P < 0.0001$ ). <sup>1</sup>SIM = simultaneous sheep and cattle; ALT = alternately cattle and then sheep; BOV = cattle alone; OVI = sheep alone.

interaction between treatment and time was observed in lambs for *Trichostrongylus*, *Cooperia* and *Oesophagostomum* FEC ( $P < 0.0001$ ) and their fecal cultures. However, this interaction did not present any specific profile over the course of time.

PES showed differences for sheep ( $P = 0.0001$ ), with SIM treatment showing lower means (Table 4). For cattle, in general, PES did not show differences. However, for *Haemonchus* spp., *Cooperia* spp. and *Oesophagostomum* spp., the means were lower for SIM treatment (Table 4).

Although there were no interactions between treatments and time in relation to blood parameters in this experiment, a consistent increase in eosinophil count in both ruminant species over the trial period was seen (Figure 2). In sheep, there was a peak in eosinophil count at the same time as the peak in FEC and an evident decrease in PCV values over the course of the experiment (Figure 2).

Among the blood parameters in sheep, only the eosinophils did not show any significant difference between treatments (Table 5). ALT had the lowest TPP and albumin, although these values did not differ from OVI and SIM treatments (Table 5). SIM had highest PCV, TPP and hemoglobin. In cattle, only PCV showed significant differences between treatments, and was lowest in SIM.

The correlations were mostly highly significant ( $P < 0.001$  or  $P < 0.01$ ) and had medium to high values (Table 6). FEC had medium negative correlations with blood parameters (Hb, PPT and PCV) and high correlations with total PES and with the PES for each genus, especially with *Haemonchus* spp. PES (0.98). Eosinophils were correlated positively with experimental week and with *Cooperia* spp., *Oesophagostomum* spp. and larval count in fecal cultures, but had a negative association with *Haemonchus* spp. larval proportion. Hb was negatively correlated with PES (total and for each genus studied) and with experimental week. PPT and PCV were negatively correlated with *Haemonchus* spp. larvae in fecal culture (−0.44 and −0.24, respectively) and *Haemonchus* spp. PES (−0.25 and −0.34, respectively).

In general, the PES for each genus was high, positive and highly significant in relation to other PES (Table 6 and Figure 3). *Trichostrongylus* spp. larval percentage presented high and positive correlations with *Cooperia* spp. and *Oesophagostomum* spp. larval percentage.

### Discussion

It is believed that infective larvae of sheep parasites are destroyed when ingested by cattle and vice versa (AMARANTE et al., 1997). This hypothesis, i.e. that certain parasite species common to several types of domestic animals have developed more specific relationships of parasitism with certain host species (AMARANTE et al., 1997; ROCHA et al., 2008; TORRES et al., 2009), was shown in the present study to be true, at least for the relationship between sheep and *Haemonchus* spp., given that lower larval counts in fecal cultures and lower FEC were seen when mixed grazing systems were used (Figure 1 and Table 2).

This is important, because *Haemonchus* is the most important internal parasite for sheep worldwide, and it presents increasing drug resistance and high pathogenicity. This pathogenicity, together with the high prolificacy of *Haemonchus* spp. (which present the highest oviposition rates (ROMERO; BOERO, 2001) among the genera found in this study) and the grazing habits of sheep (close to the ground and sometimes picking up their own feces, given that they are pelleted, unlike the habits of cattle, which do not have access to the grass under their pats and avoid the less appetizing grass near the pats (DUVAL, 1994)), makes sheep more susceptible to infections, thus contributing to the difference between cattle and sheep FEC. However, this difference in FEC between the two species would certainly be greater if the animals were not well nourished. In the present study, the animals received a considerable quantity of concentrate supplementation (especially regarding crude protein content), which probably provided body

**Table 3.** Mean percentages of larvae found in overall fecal cultures from sheep and cattle in different grazing systems\* in the Federal District, Brazil.

Host species	Grazing system*	<i>Haemonchus</i>	<i>Trichostrongylus</i>	<i>Cooperia</i>	<i>Oesophagostomum</i>
Sheep	SIM	89.50 <sup>b</sup>	5.71 <sup>b</sup>	1.36 <sup>b</sup>	3.43 <sup>a</sup>
	ALT	84.70 <sup>c</sup>	11.27 <sup>a</sup>	1.91 <sup>a</sup>	1.90 <sup>b</sup>
	OVI	96.43 <sup>a</sup>	2.36 <sup>c</sup>	0.29 <sup>c</sup>	0.93 <sup>c</sup>
Cattle	SIM	0.50 <sup>c</sup>	83.25 <sup>a</sup>	1.50 <sup>c</sup>	13.63 <sup>b</sup>
	ALT	2.50 <sup>b</sup>	66.75 <sup>b</sup>	16.00 <sup>a</sup>	13.00 <sup>c</sup>
	BOV	13.50 <sup>a</sup>	65.13 <sup>c</sup>	4.63 <sup>b</sup>	14.25 <sup>a</sup>

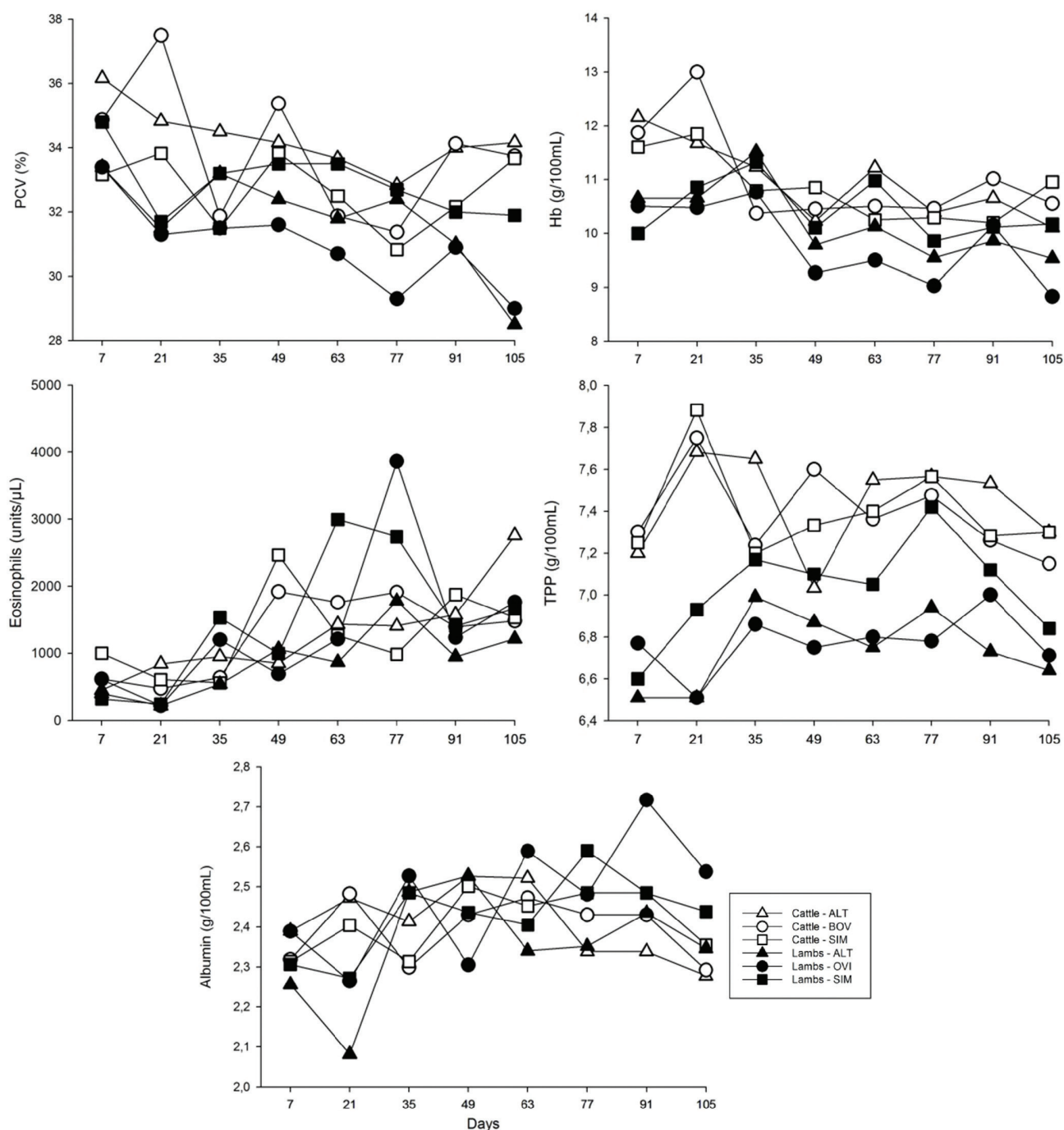
Means followed by different letters (a, b, c) in the same column for each ruminant species were significantly different using Duncan test ( $P < 0.05$ ). \*SIM = simultaneous sheep and cattle; ALT = alternately cattle and then sheep; BOV = cattle alone; OVI = sheep alone.

**Table 4.** Overall estimated pathogenicity for sheep and cattle in different grazing systems in the Federal District, Brazil.

Host species	Grazing system*	<i>Haemonchus</i>	<i>Trichostrongylus</i>	<i>Cooperia</i> (x10 <sup>-1</sup> )	<i>Oesophagostomum</i>	Total
Sheep	SIM	0.11 <sup>b</sup>	0.01 <sup>b</sup>	0.08 <sup>b</sup>	0.02 <sup>c</sup>	0.14 <sup>b</sup>
	ALT	0.30 <sup>a</sup>	0.06 <sup>a</sup>	0.31 <sup>a</sup>	0.10 <sup>a</sup>	0.44 <sup>a</sup>
	OVI	0.38 <sup>a</sup>	0.04 <sup>a</sup>	0.07 <sup>b</sup>	0.05 <sup>b</sup>	0.45 <sup>a</sup>
Cattle	SIM	0.00 <sup>b</sup>	0.59 <sup>a</sup>	0.23 <sup>b</sup>	0.23 <sup>b</sup>	0.82 <sup>a</sup>
	ALT	0.01 <sup>b</sup>	1.72 <sup>a</sup>	4.94 <sup>a</sup>	1.00 <sup>ab</sup>	2.62 <sup>a</sup>
	BOV	0.12 <sup>a</sup>	1.64 <sup>a</sup>	0.84 <sup>b</sup>	1.37 <sup>a</sup>	2.77 <sup>a</sup>

Means followed by different letters (a, b, c) in the same column for each ruminant species were significantly different using Duncan test ( $P < 0.05$ ). \*SIM = simultaneous sheep and cattle; ALT = alternately cattle and then sheep; BOV = cattle alone; OVI = sheep alone.





**Figure 2.** Blood parameters of cattle and sheep under different grazing systems: (SIM) simultaneous, (ALT) alternate, (BOV) cattle alone and (OVI) sheep alone.

**Table 5.** Overall blood components in sheep and cattle in different grazing systems in the Federal District, Brazil.

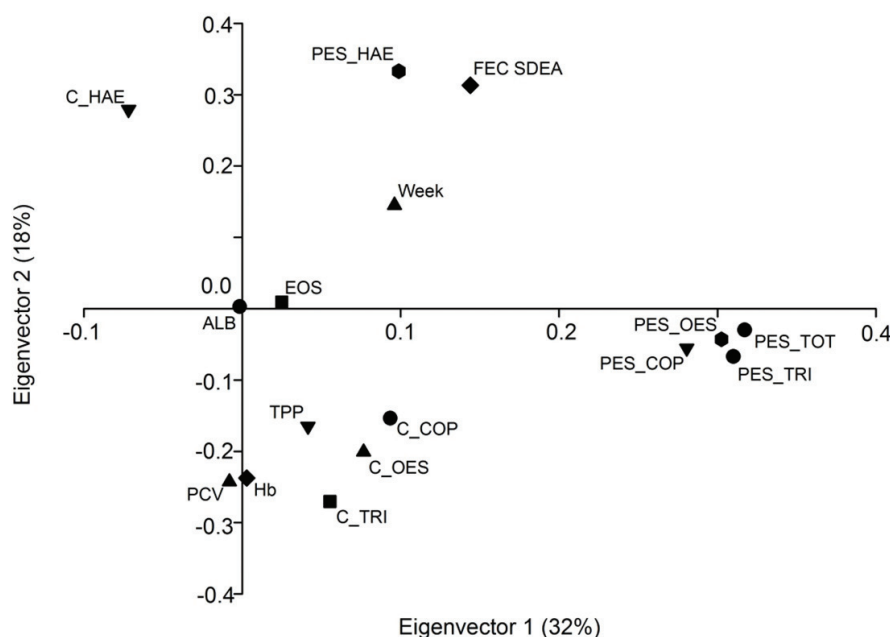
Host species	Grazing system*	Eosinophils (units/mL)	Hemoglobin (g/100 mL)	TPP (g/100 mL)	PCV (%)	Albumin (g/100 mL)
Sheep	SIM	1486.9 <sup>a</sup>	10.48 <sup>a</sup>	7.03 <sup>a</sup>	32.91 <sup>a</sup>	2.43 <sup>ab</sup>
	ALT	878.1 <sup>a</sup>	10.21 <sup>b</sup>	6.74 <sup>b</sup>	31.77 <sup>b</sup>	2.35 <sup>b</sup>
	OVI	1343.0 <sup>a</sup>	9.83 <sup>c</sup>	6.77 <sup>b</sup>	30.99 <sup>b</sup>	2.47 <sup>a</sup>
Cattle	SIM	1286.5 <sup>a</sup>	10.85 <sup>a</sup>	7.40 <sup>a</sup>	32.69 <sup>b</sup>	2.42 <sup>a</sup>
	ALT	1283.3 <sup>a</sup>	10.96 <sup>a</sup>	7.44 <sup>a</sup>	34.29 <sup>a</sup>	2.41 <sup>a</sup>
	BOV	1274.2 <sup>a</sup>	11.03 <sup>a</sup>	7.39 <sup>a</sup>	33.84 <sup>a</sup>	2.39 <sup>a</sup>

Means followed by different letters (a, b, c) in the same column for each ruminant species were significantly different using Duncan test ( $P < 0.05$ ). \*SIM = simultaneous sheep and cattle; ALT = alternately cattle and then sheep; BOV = cattle alone; OVI = sheep alone.

Table 6. Correlations between blood parameters, fecal parameters and fecal culture variables in sheep and cattle.

	FEC Sdea	Eosino- phils	Hb	PPT	PCV	Albumin	Copro Tri	Copro Hae	Copro Coop	Copro Oes	PES Hae	PES Tri	PES Coop	PES Oes	Total PES
Eosinophils	0.06														
Hb	ns														
	-0.33	-0.12													
TPP	***	*													
	-0.23	0.18	0.33												
	***	***	***												
PCV	-0.34	-0.03	0.68	0.29											
	***	ns	***	***											
Albumin	0.05	0.15	0.06	0.16	0.13										
	ns	**	ns	**	**										
Copro Tri	-0.21	0.10	0.24	0.43	0.27	-0.06									
	***	*	***	***	***	ns									
Copro Hae	0.17	-0.16	-0.21	-0.44	-0.24	0.06	-0.95								
	***	**	***	***	***	ns	***								
Copro Coop	0.02	0.26	0.06	0.37	0.14	0.05	0.46	-0.60							
	ns	***	ns	***	**	ns	***	***							
Copro Oes	-0.07	0.21	0.15	0.40	0.20	-0.03	0.75	-0.84	0.60						
	ns	***	**	***	***	ns	***	***	***						
PES Hae	0.98	0.05	-0.34	-0.25	-0.34	0.07	-0.24	0.21	<-0.01	-0.08					
	***	ns	***	***	***	ns	***	***	ns	ns					
PES Tri	0.80	0.22	-0.25	-0.02	-0.20	0.07	0.16	-0.18	0.25	0.21	0.75				
	***	***	***	ns	***	ns	***	***	***	***	***				
PES Coop	0.60	0.24	-0.18	0.01	-0.13	0.03	0.13	-0.25	0.54	0.31	0.57	0.77			
	***	***	***	ns	*	ns	**	***	***	***	***	***			
PES Oes	0.73	0.25	-0.23	<-0.01	-0.20	0.09	0.16	-0.23	0.33	0.35	0.70	0.90	0.82		
	***	***	***	ns	***	ns	***	***	***	***	***	***	***		
Total PES	0.90	0.10	-0.24	-0.12	-0.25	0.05	-0.08	0.04	0.11	0.05	0.88	0.91	0.74	0.87	
	***	*	***	*	***	ns	ns	ns	**	ns	***	***	***	***	
Week	0.38	0.47	-0.35	0.06	-0.24	0.16	0.07	-0.18	0.39	0.30	0.38	0.48	0.50	0.55	0.43
	***	***	***	ns	***	**	ns	***	***	***	***	***	***	***	***

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns = not significant; **FEC** = fecal egg count; **Hb** = hemoglobin; **TPP** = total plasma protein; **PCV** = packed cell volume; **Copro Tri** = proportion of *Trichostrongylus* spp. in fecal culture; **Copro Hae** = proportion of *Haemonchus* spp. in fecal culture; **Copro Coop** = proportion of *Cooperia* spp. in fecal culture; **Copro Oes** = proportion of *Oesophagostomum* spp. in fecal culture; **PES Hae** = estimated pathogenicity of *Haemonchus* spp.; **PES Tri** = estimated pathogenicity of *Trichostrongylus* spp.; **PES Coop** = estimated pathogenicity of *Cooperia* spp.; **PES Oes** = estimated pathogenicity of *Oesophagostomum* spp.; **Total PES** = total estimated pathogenicity.



**Figure 3.** Eigenvectors of fecal and fecal culture parameters and blood parameters during the experimental period. **FEC SDEA**, strongyle fecal egg count; **ALB**, albumin; **EOS**, eosinophils; **Hb**, hemoglobin; **TPP**, total plasma protein; **PCV**, packed cell volume; **C\_TRI**, proportion of *Trichostrongylus* spp. in fecal culture; **C\_HAE**, proportion of *Haemonchus* spp. in fecal culture; **C\_COP**, proportion of *Cooperia* spp. in fecal culture; **C\_OES**, proportion of *Oesophagostomum* spp. in fecal culture; **PES\_HAE**, estimated pathogenicity of *Haemonchus* spp.; **PES\_TRI**, estimated pathogenicity of *Trichostrongylus* spp.; **PES\_COP**, estimated pathogenicity of *Cooperia* spp.; **PES\_OES**, estimated pathogenicity of *Oesophagostomum* spp.; **PES\_TOT**, total estimated pathogenicity.

and immunity conditions that allowed them to withstand the entire trial period in a healthy condition. Dietetic protein was shown to increase resilience and resistance to natural infection by endoparasites (LOUVANDINI et al., 2006).

The prepatent period for *Haemonchus* spp. is from 17 to 21 days (ZAJAC, 2006), and it is 21 days for *Trichostrongylus* spp. (BOWMAN et al., 2009). These cycles, as well as the animals' return to pastures that had already been grazed (the animals returned to the original pasture every 21 days) probably led to the high FEC from days 77 to 84. The peak occurred between the 14<sup>th</sup> and 21<sup>st</sup> days in the third cycle.

The high correlation (0.94) between FEC and *Haemonchus* spp. PES indicated that this FEC was mainly due to *Haemonchus* spp. This high presence of *Haemonchus* spp., added to the fact that the cattle did not show significant differences in FEC between treatments helps to explain why OVI had higher FEC means. Fernandes et al. (2004) also found that sheep grazing alone had higher FEC than did mixed species grazing. The reduction in parasite infection in the pasture with mixed grazing (ROCHA et al., 2008; TORRES et al., 2009) was probably due to the different grazing habits of cattle, as previously described.

However, grazing habits are not enough to explain why SIM treatment presented better results than ALT treatment. Actually, although grazing together or separately could influence the grazing habits, this was not within the scope of our investigation and therefore was not measured. Nevertheless, the results from Torres et al. (2009), who studied the parasitic nematode load on the same paddocks during this trial, corroborates our results: they

observed that ALT paddocks were more heavily infested (for overall parasites and especially for *Haemonchus* spp.) than SIM paddocks. In addition, Santos et al. (2011), who studied the structural and dietary characteristics of the pasture used in this experiment, observed differences in leaf and stem proportions between pastures grazed simultaneously or alternately, with a higher proportion of leaf in the SIM treatment. Thus, it is possible that the height of the grass could help to explain why SIM treatment showed better results in controlling parasites than ALT.

The greater proportions of *Haemonchus* spp. in sheep and *Trichostrongylus* spp. in cattle probably occurred due to parasite host specificity. The predominance of *Haemonchus* spp. in sheep was also noted by Vieira et al. (2008) in Rio Grande do Sul, as well as by other authors in Brazil (ROCHA et al., 2008; MCMANUS et al., 2009; SCZESNY-MORAES et al., 2010). However, in comparing the grazing systems, the proportion of *Haemonchus* spp. was greater and *Trichostrongylus* spp. was smaller in systems of grazing alone (OVI and BOV), thus showing that mixed grazing increases the proportion of other less pathogenic species (UENO; GONÇALVES, 1998). Giudici et al. (1999) and Amarante et al. (1997) described similar results. Greater diversity of parasite species is desirable, since this increases the competition between different parasite species and thus reduces the need for drug application, with consequent reduction in the drug resistance process. However, this does not mean that the overall pathogenicity is reduced, because the total worm burden may be high, even with this competition process.

The specificity of *Trichostrongylus* spp. for cattle and the influence of mixed grazing on decontamination of pastures, especially with regard to *Haemonchus*, may explain the high proportion of *Trichostrongylus* spp. larvae in the cattle fecal cultures and lower *Haemonchus* spp. larval count in the mixed treatments.

The proportion of *Haemonchus* spp. larvae in fecal cultures had a negative correlation with all other genera, which may be explained by the predominance and prolificacy of these species. Because the larval identification results are expressed as percentages, an increase in one genus leads to a reduction in others. Correlations between the other genera were positive. Amarante et al. (2004) also observed that most correlations between different genera of nematodes were positive, thus showing that animals with higher levels of one species tended to have higher overall infestation. This corroborates the idea that the variance of host resistance to gastrointestinal nematodes has a genetic basis with a mechanism that is not completely species-specific (SRÉTER et al., 1994).

The degree of infection and the quantity of parasites are relatively easy to measure, but the relationship between parasitic load, its pathogenic impact and the clinical signs is difficult to analyze (UENO; GONÇALVES, 1998) because of other factors such as nutrition, age, pregnancy, stress and the synergism or competitive interactions between parasite species. These factors influence the likelihood of successful transmission to other hosts and increase or decrease the overall pathogenic impact (PETNEY; ANDREWS, 1998). Thus, to estimate the pathogenic impact of multiple parasite infection and compare infections themselves, PES is used. The highest PES was in BOV, but there was no significant difference between treatments. The greater presence of *Oesophagostomum* spp. in cattle, together with the high pathogenicity of this parasite – according Ueno and Gonçalves (1998) *Oesophagostomum* is 5 times more pathogenic than *Haemonchus* – justifies this observation. The higher PES in ALT than in OVI may have been caused by inclusion of data from the cattle infestation in the PES calculation; thus, with more *Oesophagostomum* spp., PES increases. Low FEC and lower percentage of *Haemonchus* spp. in the sheep with SIM treatment meant that this group had the lowest PES. Therefore, it can be hypothesized that lower FEC and lower PES mean lower need for anthelmintic treatments. Indeed, Fernandes et al. (2004) observed that animals (cattle and sheep) sharing the same pasture presented lower parasitic infection, and this could be converted into lower frequency of anthelmintic treatments.

The peak in eosinophils observed near the peak in FEC was also observed by Amarante et al. (1999), 21 days after inducement of artificial infection with *H. contortus*. The relationship between eosinophil count and parasite load is controversial: some authors have suggested that eosinophils are important elements in the inflammatory response against helminth parasites (BUDDLE et al., 1992). Eosinophilia in blood and tissue has been correlated with expression of greater resistance to nematodes (ROTWELL et al., 1988; DAWKINS et al., 1989). On the other hand, Salman and Duncan (1984) considered that these blood cells were only responsive to the degree of stimulation by the parasite or parasite load and not to greater expression of resistance. Eosinophilia has also been interpreted as a general response to infection (STEAR et al., 1995), which is monitored as it increases during parasite infections (HUNTLEY et al., 1995), as seen here.

The duration of time in the experiment had a positive correlation with eosinophils (0.32), while with hemoglobin and PCV it showed medium negative correlations (–0.34 and –0.26, respectively). Progression of the infection with time led to a reduction in red blood cells and increase in white cells.

According to Amarante et al. (2009), higher FEC tended to result in lower PCV. In this experiment, sheep with higher *Haemonchus* spp. percentages had lower PCV, and this parameter progressively decreased as FEC increased over the experimental period. The PCV was compatible with FEC for *Haemonchus* spp., as described above, with a correlation of –0.24. Daily blood loss in the gastrointestinal tract caused by *Haemonchus* spp. can reach 250 mL per day in cases of massive infection (ROWE et al., 1988), which did not occur in any animal of the present study. The large proportion of *Haemonchus* spp. observed in sheep fecal cultures and the increasing FEC are in accordance with blood counts. The oscillations observed in protein levels may have been due to alterations in permeability of the mucosa and absorption of protein components caused by nematodes (BOWMAN et al., 2009).

In this experiment, the effects of gastrointestinal parasites on the blood system were more clearly seen in sheep than in cattle. This may have been due, in part, to their greater susceptibility and higher *Haemonchus* spp. infestation.

The principal components analysis showed that the pathogenicity of parasite infection increased over time, while blood parameters showed few modifications over the trial period (first eigenvector, Figure 2). In addition, as FEC increased, *Haemonchus* spp. PES also increased, thus showing that the largest proportion of the eggs counted belonged to this species. On the other hand, as *Haemonchus* spp. parameters increased, the other parasite parameters decreased. Blood parameters (especially PCV and Hb) became lower as FEC and *Haemonchus* spp. PES increased.

The blood values were affected by parasite infection and became more intensely affected as the infection increased over time. The mixed-species grazing system was able to reduce the parasite load, especially for sheep, and can be used as a tool for reducing the level of nematode gastrointestinal infections, thus reducing the need for anthelmintic treatments. This was an initial and pioneering study in the Federal District region that raised a lot of questions, and further studies will be necessary to clarify these issues.

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## References

- Amarante AFT, Bricarello PA, Rocha RA, Gennari SM. Resistance of Santa Ines, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. *Vet Parasitol* 2004; 120(1-2): 91-106. PMID:15019147. <http://dx.doi.org/10.1016/j.vetpar.2003.12.004>
- Amarante AFT, Craig TM, Ramsey WS, El-Sayed NM, Desouki AY, Bazer FW. Comparison of naturally acquired parasite burdens among Florida Native, Rambouillet and crossbreed ewes. *Vet Parasitol* 1999; 85(1): 61-69. [http://dx.doi.org/10.1016/S0304-4017\(99\)00103-X](http://dx.doi.org/10.1016/S0304-4017(99)00103-X)



- Amarante AFT, Susin I, Rocha RA, Silva MB, Mendes CQ, Pires AV. Resistance of Santa Ines and crossbred ewes to naturally acquired gastrointestinal nematode infections. *Vet Parasitol* 2009; 165(3-4): 273-280. PMID:19656629. <http://dx.doi.org/10.1016/j.vetpar.2009.07.009>
- Amarante AFT, Bagnola J Jr, Amarante MRV, Barbosa MA. Host specificity of sheep and cattle nematodes in São Paulo state, Brazil. *Vet Parasitol* 1997; 73(1-2): 89-104. [http://dx.doi.org/10.1016/S0304-4017\(97\)00036-8](http://dx.doi.org/10.1016/S0304-4017(97)00036-8)
- Araújo JV, Guimarães MP, Campos AK, Sá NC, Sarti P, Assis RCL. Control of bovine gastrointestinal nematode parasites using pellets of the nematode-trapping fungus *Monacrosporium thaumasium*. *Cienc Rural* 2004; 34(2): 457-463. <http://dx.doi.org/10.1590/S0103-84782004000200019>
- Bowman DD, Georgi JR, Lynn RC. Helminths. In: Bowman DD, editor. *Georgis' Parasitology for Veterinarians*. St. Louis:Saunders Publishing Company; 2009. p. 115-239.
- Buddle BM, Jowett G, Green RS, Douch PGC, Risdon PL. Association of blood eosinophilia with the expression of resistance in Romney lambs to nematodes. *Int J Parasitol* 1992; 22(7): 955-960. [http://dx.doi.org/10.1016/0020-7519\(92\)90053-N](http://dx.doi.org/10.1016/0020-7519(92)90053-N)
- Cabaret J, Bouilhol M, Mage C. Managing helminthes of ruminants in organic farming. *Vet Res* 2002; 33(5): 625-640. PMID:12387494. <http://dx.doi.org/10.1051/vetres:2002043>
- Cenci FB, Louvandini H, McManus CM, Dell'Porto A, Costa DM, Araújo SC, et al. Effects of condensed tannin from *Acacia mearnsii* on sheep infected naturally with gastrointestinal helminthes. *Vet Parasitol* 2007; 144(1-2): 132-137. PMID:17067741. <http://dx.doi.org/10.1016/j.vetpar.2006.09.021>
- Dawkins HJS, Windon RG, Eagleson GK. Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus colubriformis*. *Int J Parasitol* 1989; 19(2): 199-205. [http://dx.doi.org/10.1016/0020-7519\(89\)90008-8](http://dx.doi.org/10.1016/0020-7519(89)90008-8)
- Duval J. *The control of internal parasites in ruminants. Ecological Agriculture Projects*. [on line]. 1994 [cited 2012 Dec 06]. Available from: <<http://eap.mcgill.ca/agrobio/ab370-04e.htm>>.
- Fernandes LH, Seno MCZ, Amarante AFT, Souza H, Belluzzo CEC. Efeito do pastejo rotacionado e alternado com bovinos adultos no controle da verminose em ovelhas. *Arq Bras Med Vet Zootec* 2004; 56(6):733-740. <http://dx.doi.org/10.1590/S0102-09352004000600006>
- Giudici C, Aumont G, Mahieu M, Saulai M, Cabaret J. Changes in gastro-intestinal helminth species diversity in lambs under mixed grazing on irrigated pastures in the tropics (French West Indies). *Vet Res* 1999; 30(6): 573-581. PMID:10596405.
- Huntley JF, Patterson M, Mackellar A, Jackson F, Stevenson LM, Coop RL. A comparison of the mast cell and eosinophil responses of sheep and goats to gastrointestinal nematode infection. *Res Vet Sci* 1995; 58(1): 5-10. [http://dx.doi.org/10.1016/0034-5288\(95\)90080-2](http://dx.doi.org/10.1016/0034-5288(95)90080-2)
- Instituto Nacional de Meteorologia. Banco de Dados Meteorológicos para Ensino e Pesquisa – INMET [on line]. 2013. Available from: <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>.
- James CE, Hudson AL, Davey MW. Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends Parasitol* 2009; 25(7): 328-335. PMID:19541539. <http://dx.doi.org/10.1016/j.pt.2009.04.004>
- Larsen M. Biological control of nematode parasites in sheep. *J Anim Sci* 2008; 84(Suppl): E133.
- 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. PMID:16495016. <http://dx.doi.org/10.1016/j.vetpar.2006.01.004>
- McManus C, Louvandini H, Paiva SR, Oliveira AA, Azevedo HC, Melo CB. Genetic factors of sheep affecting gastrointestinal parasite infections in the Distrito Federal, Brazil. *Vet Parasitol* 2009; 166(3-4): 308-313. PMID:19837513. <http://dx.doi.org/10.1016/j.vetpar.2009.09.037>
- Molento MB, Prichard RK. Effect of multidrug resistance modulators on the activity of ivermectin and moxidectin against selected strains of *Haemonchus contortus* infective larvae. *Pesq Vet Bras* 2001; 21(3): 117-121. <http://dx.doi.org/10.1590/S0100-736X2001000300004>
- Petney TN, Andrews RH. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *Int J Parasitol* 1998; 28(3): 377-393. [http://dx.doi.org/10.1016/S0020-7519\(97\)00189-6](http://dx.doi.org/10.1016/S0020-7519(97)00189-6)
- Rocha RA, Bresciani KDS, Barros TFM, Fernandes LH, Silva MB, Amarante AFT. Sheep and cattle grazing alternately: Nematode parasitism and pasture decontamination. *Small Rum Res* 2008; 75(2-3): 135-143. <http://dx.doi.org/10.1016/j.smallrumres.2007.09.001>
- Romero JR, Boero CA. Epidemiología de la gastroenteritis verminosa de los ovinos em las regiones templadas y cálidas de la Argentina. *Analecta Vet* 2001; 21(1): 21-37.
- Rotwell TLW, Abeydeera LR, Geczy AF. Relationship between basophils and eosinophils in cutaneous basophil hypersensitivity reactions in guinea pigs and susceptibility to *Trichostrongylus colubriformis* infection. *Int J Parasitol* 1988; 18(3): 347-351. [http://dx.doi.org/10.1016/0020-7519\(88\)90144-0](http://dx.doi.org/10.1016/0020-7519(88)90144-0)
- Rowe JB, Nolan JV, Chaneet G, Teleni E, Holmes PH. The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. *Br J Nutr* 1988; 59(1): 125-139. PMID:3257884. <http://dx.doi.org/10.1079/BJN19880016>
- Salman SK, Duncan JL. The abomasal histology of worm-free sheep given primary and challenge infection of *Haemonchus contortus*. *Vet Parasitol* 1984; 16(1-2): 43-54. [http://dx.doi.org/10.1016/0304-4017\(84\)90007-4](http://dx.doi.org/10.1016/0304-4017(84)90007-4)
- Santos VRV, Louvandini H, Pimentel CMM, Brito DL. Características estruturais e bromatológicas do capim Tanzânia sob pastejo isolado, simultâneo e alternado de ovinos com bovinos. *Ci Anim Bras* 2011; 12(4): 670-680. <http://dx.doi.org/10.5216/cab.v12i4.9946>
- Sczesny-Moraes EA, Bianchin I, Silva KF, Catto JB, Horner MR, Paiva F. Resistência anti-helmíntica de nematóides gastrintestinais em ovinos, Mato Grosso do Sul. *Pesq Vet Bras* 2010; 30(3): 229-236. <http://dx.doi.org/10.1590/S0100-736X2010000300007>
- Sréter T, Molnár V, Kassai T. The distribution of nematode egg counts and larval counts in grazing sheep and their implications for parasite control. *Int J Parasitol* 1994; 24(1): 103-108. [http://dx.doi.org/10.1016/0020-7519\(94\)90063-9](http://dx.doi.org/10.1016/0020-7519(94)90063-9)
- Stear MJ, Bishop SC, Duncan JL, McKellar QA, Murray M. The repeatability of fecal egg counts, peripheral eosinophil counts, and plasma pepsinogen concentrations during deliberate infections with *Ostertagia circumcincta*. *Int J Parasitol* 1995; 25(3): 375-380. [http://dx.doi.org/10.1016/0020-7519\(94\)00136-C](http://dx.doi.org/10.1016/0020-7519(94)00136-C)
- Torres SEF, McManus C, Amarante AFT, Verdolin V, Louvandini H. Nematódeos de ruminantes em pastagem com diferentes sistemas de

pastejo com ovinos e bovinos. *Pesq Agropec Bras* 2009; 44(9): 1191-1197. <http://dx.doi.org/10.1590/S0100-204X2009000900018>

Ueno H, Gonçalves PC. *Manual para o diagnóstico das helmintoses de ruminantes*. Salvador: Japan International Cooperation Agency; 1998.

Vieira MIB, Rocha HC, Ractz LAB, Nadal R, Moraes RB, Oliveira IS. Comparação de dois métodos de controle de nematódeos gastrintestinais em borregas e ovelhas de corte. *Semina: Ciênc Agrár* 2008; 29(4): 853-860. <http://dx.doi.org/10.5433/1679-0359.2008v29n4p853>

Yamamoto SM, Macedo FAF, Grande PA, Martins EN, Zundt M, Mexia AA, et al. Produção e contaminação por helmintos parasitos de ovinos, em forrageiras de diferentes hábitos de crescimento. *Acta Sci Anim Sci* 2004; 26(3): 379-384. <http://dx.doi.org/10.4025/actascianimsci.v26i3.1824>

Zajac AM. Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. *Vet Clin North Am Food Anim Pract* 2006; 22(3): 529-541. PMID:17071351. <http://dx.doi.org/10.1016/j.cvfa.2006.07.006>