



## Research paper

## *Trichostrongylus colubriformis* infection: Impact on digesta passage rate and lamb performance

Tairon Pannunzio Dias e Silva<sup>a,\*</sup>, Thiago Francisco Ventoso Bompadre<sup>a</sup>,  
Dinesh Kumar Danasekaran<sup>a</sup>, Gabriel Zanuto Sakita<sup>a</sup>, Adibe Luiz Abdalla Filho<sup>a</sup>,  
Carolina Rodrigues Jimenez<sup>a</sup>, Ana Carolina Brandão de Campos Fonseca Pinto<sup>b</sup>,  
Alessandro Francisco Talamini do Amarante<sup>c</sup>, Concepta McManus<sup>d</sup>, Helder Louvandini<sup>a,e</sup>

<sup>a</sup> Laboratório de Nutrição Animal, Centro de Energia nuclear na Agricultura (CENA), Universidade de São Paulo, SP, Brazil

<sup>b</sup> Departamento de Cirurgia da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, SP, Brazil

<sup>c</sup> Instituto de Biociências, Universidade Estadual Paulista (UNESP), Botucatu, SP, Brazil

<sup>d</sup> Universidade de Brasília/Instituto de Ciências Biológicas, Distrito Federal, Brasília, Brazil

<sup>e</sup> University of Florida, Institute of Food and Agricultural Sciences, Department of Animal Sciences, PO Box 110910, Gainesville, FL, 32611-0910, Brazil

## ARTICLE INFO

## Keywords:

Endoparasite

Feed intake

Digesta passage rate

Ruminants

## ABSTRACT

In this study we aimed to evaluate the effect of *Trichostrongylus colubriformis* infection on digesta passage rate, rumen fermentation and lamb performance. Eighteen three-month-old Santa Ines castrated male lambs ( $16.9 \pm 1.43$  kg of body weight) were randomly distributed in two experimental treatments: Infected with *T. colubriformis* (I,  $n = 9$ ) and Uninfected (U,  $n = 9$ ). The I lambs received a total of 45,000 L<sub>3</sub> larvae of *T. colubriformis* (5,000 infective larvae, three times per week for three weeks). Daily feed intake was assessed using the I lambs as a reference for their respective pairs on the U group (pair-fed). Weight, body condition score and faeces (stool) samples were obtained every 15 days for 75 days. In both treatments, faecal egg count (FEC), digesta passage rate, rumen fermentation parameters, protozoa count and short chain fatty acids (SCFA) were evaluated. The lambs presented moderate infection (FEC = 620). The retention time of the digesta in the rumen-reticulum segment was lower ( $P < 0.05$ ) in I lambs. The I lambs presented no inappetence, however, lower concentrations of total SCFA and butyrate, while higher acetate concentration were observed in these lambs ( $P < 0.05$ ). The present findings highlight that *T. colubriformis* infection decreased the retention time (solid and liquid content) of the digesta in the rumen-reticulum, as well as negatively affected lamb growth.

## 1. Introduction

*Trichostrongylus colubriformis* is a nematode of the Trichostrongylidae family which is frequently found in ruminants in many regions worldwide (O'Connor et al., 2006). In severe infections, *T. colubriformis* can cause enteritis with atrophy of large intestinal mucosa microvilli, formation of tunnels, and duodenal mucosa epithelium erosion (Taylor et al., 2010; Cardia et al., 2011), hyperplasia and crypt hypertrophy (Cardia et al., 2011), leukocyte infiltration and high intestinal lumen serum protein exudation (Taylor et al., 2010). As a consequence of this infection, the capacity for digestion and absorption of nutrients, including minerals, is compromised (Cantacessi et al., 2010), leading to a reduction of skeletal growth, bone density and mineralization (Fox, 1997), resulting in reduced animal performance.

The negative impact of *T. colubriformis* infection on the productive

performance of lambs may compromise the commercial production of sheep, especially when the infections are subclinical, since this may not be perceived by the farmer. Barker (1973), (1975) reported reduction in nutrient digestion and absorption efficiency, altering the flow of digestive content and impairing sheep productivity.

Digesta passage rate and the variations in fluid contents throughout the gastrointestinal tract, as well as the importance of retention time of the digesta for gut function in ruminants have been described (Clausen et al., 2016). However, few studies have been conducted investigating how *T. colubriformis* infection affect the mean retention time of the digesta [solid phase (ytterbium - concentrate and Chromium - Hay) and liquid phase (Co-EDTA)] within each gut compartment.

Due to subclinical infection characteristics and the fact that only few investigations were conducted with lambs submitted to serial infection with *T. colubriformis*, we hypothesized that the *T. colubriformis* infection

\* Corresponding author.

E-mail address: [tairon.mvet@gmail.com](mailto:tairon.mvet@gmail.com) (T.P. Dias e Silva).

can impair digesta passage rate and lamb performance. Hence, the aim of this study was to evaluate the digestion passage rate and performance of lambs with and without *T. colubriformis* infection.

## 2. Material and methods

### 2.1. Study site

The experiment was carried out at the Laboratory of Animal Nutrition (LANA) of the Center of Nuclear Energy in Agriculture (CENA) – University of Sao Paulo (USP), Piracicaba, Brazil. All the procedures were in accordance with the Committee on Ethics in the Use of Animals (CEUA/CENA – Protocol ID: 004/2015).

### 2.2. Experimental groups, experimental period, diet, and lambs' inoculation

Eighteen three-month-old Santa Ines castrated male lambs were used, with mean initial body weight (BW) of  $16.9 \pm 1.43$  kg, kept in a collective pen from birth to weaning (60 days of age) to avoid a previous infection with endoparasites. After this period, the animals were placed in individual pens (1.0 x 1.5 m) provided with feeder, water fountain and rubber floor. Before starting the experimental period, lambs were dewormed, weighed to form pairs which would receive the same quantity of feed (pair-fed), and randomly distributed into two groups: Infected (I,  $n = 9$ ) and uninfected (U,  $n = 9$ ). The I lambs received a total of 45,000  $L_3$  *T. colubriformis* larvae (5,000 infective larvae, three times per week for three weeks). The lambs from group U remained without infection.

The experimental period lasted 90 days, and the lambs from the I group received water and diet *ad libitum*, composed of Tifton hay (*Cynodon* spp.) triturated with 3 cm particles, concentrate (30% soybean meal and 70% ground corn), and mineral mixture, as described in Table 1. The diet was formulated with a roughage:concentrate ratio of 70:30, being adjusted every 15 days. The mean initial amount supplied was  $390 \pm 92.5$  g and  $171 \pm 40.5$  g and final of  $690 \pm 110.6$  g and  $212 \pm 46.6$  g for roughage and concentrate, respectively. The samples of roughage and concentrate were analyzed for dry matter (DM) and crude protein (CP) content according to AOAC (2011). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined according to Van Soest et al. (1991) adapted by Mertens (2002).

### 2.3. Body weight, body condition score, faecal egg count, and worm burden

The lambs were weighed and blood and faeces samples collected on days 0, 15, 30, 45, 60, and 75 of the experiment. The body condition score was evaluated every 15 days after weighing the animals, according to the method described by Thompson and Meyer (2006). The lambs body live weight was used to calculate the average daily gain (ADG, g/animal/day) and total weight gain (TWG, kg/animal). Faecal egg count and total worms count were determined according to Gordon and Whitlock (1939) and Ueno and Goncalves (1998), respectively.

**Table 1**  
Chemical composition of ingredients on dry matter basis of experimental diets.

Ingredients	DM %	CP %	NDF %	ADF %	Ca %	P %
Concentrate	89.89	21.86	49.05	8.21	0.13	0.52
Tifton hay	90.07	6.20	79.86	42.85	0.27	0.17
<sup>1</sup> Mineral mixture	96.50	–	–	–	13.20	7.90

<sup>a</sup>DM- dry matter, CP- crude protein, NDF- neutral detergent fiber, ADF- acid detergent fiber, Ca- calcium, P- phosphorus, <sup>1</sup>mineral mixture: magnesium-10 g, sulfur-35 g, sodium-120 g, copper-0.756 g, manganese-2.180 g, zinc-2.8 g, iodine-0.056 g, cobalt-0.044 g, selenium-0.14 g, fluorine-0.85 g. q.s. 1000 g.

### 2.4. Feed intake

During the whole experimental period, before offering the diet, the leftovers from the previous day were collected and weighed to determine the amount to be fed to the respective pairs of the U group (pair-fed). The use of pair-feeding ensured that the U animals received the same daily amount of diet consumed by the I lambs on the previous day to better analyze the effect of *T. colubriformis* infection on the evaluated parameters. The collection and weighing of leftovers were used to determine the intakes of DM (DMI, g/animal/day), CP (CPI, g/animal/day) and total DM (TDMI, % BW). Feed conversion (FC) was calculated by the ratio between the TDMI (kg DM/animal) and the TWG of the animals in the period.

### 2.5. Markers of digesta passage rate, administration of markers, and slaughter of lambs

The diet offered was previously prepared with indigestible markers to evaluate the digesta passage rate (DPR). All animals received three inert passage markers (external marker): cobalt[Co]-EDTA as a solute marker, chromium[Cr]-mordanted fiber and ytterbium[Yb]-labeled concentrate. The Yb-labeled concentrate was prepared according to de Vega et al. (1998). The particles were labeled with Yb by soaking the concentrate in acetate buffer (0.1 M acetic acid adjusted to pH 6.0 with  $\text{NH}_4\text{OH}$ ) for 3 h and then overnight in the same solution with an exposure of 17 g Yb acetate per kg DM. The labelled material was washed several times with distilled water and thereafter dried at 60 °C for 72 h. Co-EDTA and Cr-mordanted fiber were prepared according to Udén et al. (1980) as follows: 25 g of Cobalt was diluted in 200 mL of distilled water and then hydrogen peroxide (30%) was added. After 3 h, 300 mL of ethanol (95%) was added and the solution left to stand overnight. This was filtered with ethanol (80%) and dried in an oven at 100 °C. For the Cr-mordanted fiber, the cell walls were prepared from chopped hay dried at 60 °C, ground through a 1 mm mesh and treated with neutral detergent solution (Van Soest et al., 1991), placed in a cotton bag and washed several times. External markers were administered orally to all lambs during 5-d before slaughtering the animals, at 07:00, 13:00, 19:00, and 01:00 h, each day. Yb-labeled concentrate and Cr-mordanted fiber was offered in the same pellet at 3 g per dose, and the Co-EDTA solution was introduced with the aid of a 50 mL plastic syringe. The average dose of Yb-labeled concentrate, Cr-mordanted fiber and Co-EDTA was 2 g (0.034 mg of Yb), 1 g (0.056 g of Cr) and 30 mL (0.1425 g of Co), respectively. Digesta in each gastrointestinal segment was sampled and stored at  $-10$  °C until processing and determination of marker concentrations.

To determine digesta passage rate, different segments of the digestive tract were evacuated after the animals were slaughtered (approximately 2 h after feeding). The gastrointestinal tract (GIT) was removed and separated into rumen-reticulum, omasum, abomasum, small intestine, cecum, and colon. The compartments were weighed before and after emptying to determine the amount of digesta and the weight of tissues in each segment. Total GIT tissue weight and pool size were calculated from the sum of each tissue or pool size in the GIT. The digesta in each GIT segment was sampled and stored at  $-10$  °C until processing and chemical analysis. The content of external markers was determined by inductively coupled plasma mass spectrometry (ICP-MS).

### 2.6. Digesta passage rate

The retention time (RT) in the digestive compartments was calculated using Eq. (1) as described by Van Soest et al. (1992):

$$RT = Q/F, \quad (1)$$

Where, RT is measured in hours (h), Q is the marker quantity in g, and F

**Table 2**  
Intake and performance of lambs uninfected and infected with *Trichostrongylus colubriformis*.

Variables	Treatments		Effects		
	Uninfected (n = 9)	Infected (n = 9)	Treat	Time	Treat × time
Dry matter intake (g/lamb/day)	588.6 ± 5.23	620.7 ± 4.61	*	*	ns
Dry matter intake (BW <sup>0.75</sup> /lamb/day)	65.6 ± 0.30	71.4 ± 0.34	*	*	ns
Crude protein intake (g/lamb/day)	62.9 ± 0.51	66.2 ± 0.51	*	*	ns
Total dry matter intake (% PV)	3.1 ± 0.01	3.4 ± 0.01	*	*	ns
Body condition score	3.1 ± 0.07	2.7 ± 0.08	*	*	ns
Initial body weight (kg)	16.9 ± 1.42	16.8 ± 1.42	ns	—	—
Final body weight (kg)	21.2 ± 1.50	21.1 ± 1.50	ns	—	—
Average daily gain (g/lamb/day)	60.8 ± 4.32	50.3 ± 4.32	ns	—	—
Total weight gain (kg/lamb)	3.8 ± 0.30	3.3 ± 0.33	ns	—	—
Feed conversion	11.4 ± 1.23	14.7 ± 1.33	ns	—	—

\* Comparison of means by *t*-test at 5% probability; ns – not significant; Feed conversion - calculated by the ratio between the total intake (kg DM/lamb) and total weight gain; Treat – effect of treatment; Time – effect of time; Treat\**time* - Interaction between treatment and time.

is the marker administration rate in g/h. The feed intake during the last 6 d before slaughter, as well as amount of Yb, Cr and Co-EDTA administered in the same period, were used to determine F. The total GIT RT was calculated as the sum of RT in the rumen-reticulum, omasum, abomasum, small intestine, cecum, and colon (including rectum).

### 2.7. Rumen fermentation parameters, protozoa count and short chain fatty acids

With the aid of a gastric tube, samples of rumen fluid from both groups were taken on the last day of the apparent nutrient digestibility assay, 3 h after feeding. Ruminal pH was measured immediately after collection using a pre-calibrated pH meter (Model TEC-2; Tecnal, Piracicaba, São Paulo, Brazil) and aliquots were separated to protozoa count and kept under refrigeration (–20 °C) for further analysis of short chain fatty acids (SCFA), ammoniacal-N (N-NH<sub>3</sub>). Short chain fatty acids were quantified by gas chromatography according to Palmquist and Conrad (1971) with modifications as described by Abdalla Filho et al. (2017). The net release of N-NH<sub>3</sub> was analysed using the Kjeldahl micro-method (Nocek et al., 1987; Preston, 1995) and for protozoa count, 2 mL of each sample was mixed with 4 mL of methyl green formalin (35% formaldehyde) saline solution (MFS) and preserved from light at room temperature until counting (Dehority et al., 1983).

### 2.8. Statistical analysis of data

The statistical analysis of the data was performed using SAS program version 9.3<sup>®</sup> (SAS Institute Inc., Cary, North Carolina, USA). Intake and performance were submitted to analysis of variance using the MIXED procedure, with measures repeated over time and unstructured covariance matrix according to the lower Akaike Information (AIC) and Schwarz Bayesian (SBC) criteria. The GLM procedure was used for analysis of digesta passage rate and rumen fermentation parameters variables. The means were compared by the *t*-test adopting 5% probability. In the variables with measures repeated over time, the effects of treatment, time and the interaction treatment × time were tested. The ADG was calculated by linear regression of BW as a function of the experimental day at the weighing dates (0, 15, 30, 45, 60 and 75) in each animal. The model of the regression equation corresponded to

$$Y_i = a_0 + b_1 X_i$$

Where,  $Y_i$  = body weight (kg) on the experimental day  $i$ ;  $a_0$  = regression intercept;  $b_1$  = average daily gain (kg/day);  $X_i$  = experimental day.

Correlation coefficients among worm counts, average daily gain and body condition score were estimated using the CORR procedure. For

statistical analysis, the eggs per gram data were transformed by log ( $x + 10$ ), being the results presented with the original values.

## 3. Results

### 3.1. Faecal egg count, parasitic load and clinical signs

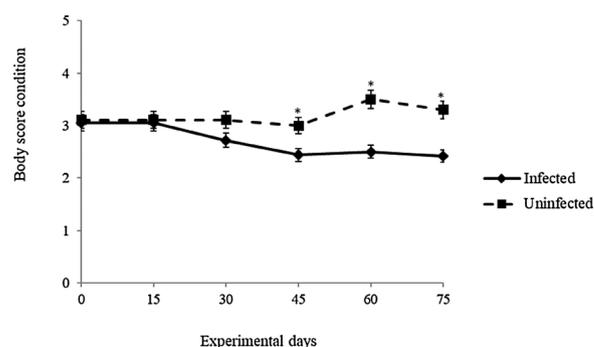
Infected lambs presented a moderate infection, eliminating a mean value of 620 eggs per gram of faeces (EPG). During the experiment, on days 30, 45, 60 and 75, the mean FEC was 1400, 1189, 572 and 561, respectively. The individual count ranged from 350 to 2400 EPG.

The lowest number of worms recovered was 4,280 corresponding to 9.5% of the total inoculated infective larvae. The mean number of worms recovered was 6,642 corresponding to 14.8% of the total inoculated and the highest number of worms recovered was 12,310, totalling 27.3% of inoculated infective larvae. Clinical signs such as semi pasty faeces (two lambs) and hair loss (one lamb) occurred after 45 days.

### 3.2. Intake and lamb performance

Infected lambs had higher DMI (g/lamb/day and BW<sup>0.75</sup>/lamb/day) compared to the uninfected lambs ( $P < 0.05$ ). There was a significant effect of time on DMI, however, there was no significant interaction between treatment × time (Table 2). There was no difference in ADG, TWG and FC between I and U lambs ( $P > 0.05$ ) and there was also no effect of parasitic infection on the final weight of the animals ( $P > 0.05$ ) (Table 2). However, I lambs had a decrease in the body condition score (Fig. 1) from the 30<sup>th</sup> day, differing significantly ( $P < 0.05$ ) from U animals at 45, 60 and 75 days after initial infection.

No significant ( $P > 0.05$ ) correlation was observed among the variables despite a tendency ( $P = 0.07$ ) of negative relation between



**Fig. 1.** Body condition score of uninfected and infected lambs during the experimental period.

**Table 3**  
Digesta passage rate in lambs uninfected and infected with *Trichostrongylus colubriformis*.

	Treatments		Prob
	Uninfected (n = 9)	Infected (n = 9)	
<b>Ytterbium (Solid Phase - Concentrate)</b>			
<b>Mean retention time (hour)</b>			
Rumen-reticulum	15.9 <sup>a</sup>	10.2 <sup>b</sup>	< 0.01
Omasum	0.79	0.59	ns
Abomasum	0.64	0.61	ns
Small intestine	0.16	0.16	ns
Caecum	3.59	3.33	ns
Colon	2.08	1.91	ns
Gastrointestinal tract	23.2 <sup>a</sup>	16.8 <sup>b</sup>	0.02
<b>Passage rate (%/hour)</b>			
Rumen-reticulum	6.7 <sup>b</sup>	10.2 <sup>a</sup>	0.01
Omasum	129.8	209.3	ns
Abomasum	214.5	176.3	ns
Small intestine	1067.7	670.1	ns
Caecum	286.8	357.6	ns
Colon	537.8	568.0	ns
Gastrointestinal tract	4.49 <sup>b</sup>	6.28 <sup>a</sup>	0.04
<b>Chromium (Solid Phase - Hay)</b>			
<b>Mean retention time (hour)</b>			
Rumen-reticulum	375.9 <sup>a</sup>	282.2 <sup>b</sup>	0.03
Omasum	15.9	12.6	ns
Abomasum	20.9	20.6	ns
Small intestine	1.98	3.02	ns
Caecum	56.8	59.7	ns
Colon	33.5	32.8	ns
Gastrointestinal tract	595.1	408.1	ns
<b>Passage rate (%/hour)</b>			
Rumen-reticulum	0.29	0.36	ns
Omasum	6.53	10.0	ns
Abomasum	6.14	5.67	ns
Small intestine	0.80	0.39	ns
Caecum	1.86	2.11	ns
Colon	3.33	3.35	ns
Gastrointestinal tract	0.002	0.003	ns
<b>Cobalt EDTA (Liquid phase)</b>			
<b>Mean retention time (hour)</b>			
Rumen-reticulum	3.51 <sup>a</sup>	2.57 <sup>b</sup>	0.03
Omasum	0.107	0.095	ns
Abomasum	0.087	0.091	ns
Small intestine	0.089	0.066	ns
Caecum	1.33	1.06	ns
Colon	0.948	0.826	ns
Gastrointestinal tract	6.31 <sup>a</sup>	4.67 <sup>b</sup>	0.02
<b>Passage rate (%/hour)</b>			
Rumen-reticulum	31.1	39.4	ns
Omasum	981.5	1307	ns
Abomasum	1403	1163	ns
Small intestine	1718.5	1634.1	ns
Caecum	82.6	110.7	ns
Colon	120.2	129.2	ns
Gastrointestinal tract	16.5 <sup>b</sup>	21.8 <sup>a</sup>	0.01

\*Comparison of means by *t*-test at 5% probability; ns - not significant; Prob-probability.

body condition score and worm counts.

### 3.3. Digesta passage rate

The mean retention time (h) of the digesta [solid phase (ytterbium - concentrate and Chromium - Hay) and liquid phase (Co-EDTA)] in the rumen-reticulum segment was higher ( $P < 0.05$ ) in U lambs (Table 3). In the gastrointestinal tract, the retention time (h) differed ( $P < 0.05$ ) for the solid (concentrate) and liquid phase of the digesta (Table 3).

**Table 4**  
Rumen fermentation parameters, protozoa count and short chain fatty acids in lambs uninfected and infected with *Trichostrongylus colubriformis*.

Variables	Treatments		Prob
	Uninfected (n = 9)	Infected (n = 9)	
pH (%)	6.29 ± 0.04	6.39 ± 0.04	ns
ammoniacal-N (mg/dL)	19.34 ± 1.54	17.35 ± 1.44	ns
Protozoa (x10 <sup>5</sup> /mL)	1.2 ± 3.14	1.2 ± 3.36	ns
<b>Short chain fatty acids (SCFA)</b>			
Total SCFA (mmol/L)	94.3 ± 2.38	87.3 ± 2.22	*
Acetate (%)	74.5 ± 0.65	76.3 ± 0.61	*
Propionate (%)	12.9 ± 0.33	12.8 ± 0.31	ns
Butyrate (%)	10.3 ± 0.45	8.5 ± 0.42	*
Valerate (%)	0.62 ± 0.02	0.58 ± 0.02	ns
Isobutyrate (%)	0.46 ± 0.02	0.49 ± 0.02	ns
Isovalerate (%)	1.06 ± 0.07	1.17 ± 0.06	ns
Acetate/propionate ratio (%)	5.8 ± 0.17	5.9 ± 0.16	ns

\*Comparison of means by *t*-test at 5% probability; ns - not significant; Prob-probability; Total SCFA = total of short chain fatty acids; Acetate/propionate ratio.

The digesta passage rate (%/h) along with the gastrointestinal tract differed ( $P < 0.05$ ) between the treatments, in the solid portion of the feed represented by the concentrate and in the liquid phase, with higher percentage of passage in I animals. The passage rate in the rumen-reticulum segment was higher in I lambs for both solid portions of the feed (hay and concentrate).

The intestinal segments omasum, abomasum, small intestine, caecum and colon did not differ ( $P > 0.05$ ) in rate of retention and passage between uninfected and infected lambs.

### 3.4. Rumen fermentation parameters, protozoa count and short chain fatty acids (SCFA)

The pH, ammoniacal-N, and protozoa count in the rumen fluid did not differ between U and I lambs ( $P > 0.05$ ). Uninfected lambs showed higher concentrations of total SCFA, acetate and butyrate ( $P < 0.05$ ). However, there was no significant difference between treatments in propionate production, as well as in the acetate: propionate ratio (Table 4).

## 4. Discussion

In the present study, *T. colubriformis* infection was moderate, since, according to Goncalves and Ueno (1998), mild infection is from 100 to 500, moderate from 500 to 2,000 and heavy above 2000 EPG for *T. colubriformis*. It is important to emphasize that the lambs were submitted to a chronic infection that consequently promotes a stimulus to the immune response, *i.e.* the immune response is potentialized when the larval challenge is continuous (Cardia et al., 2011). Thus, during the experimental period, a decrease in the elimination of eggs was observed, with stabilization after 60 days on experiment.

In most cases, trichostrongylosis infection is a subclinical pathology, and clinical symptomatology was observed only in three lambs of the present study, which presented agglomerated and semi-pasty stools and hair loss, previously reported in Silva et al. (2018). Some causal factors are the ruptures of the villi (Sykes and Coop, 1976; Sykes et al., 1979) and consequently exudation of liquids to the intestinal lumen (Taylor et al., 2010) and its excretion.

Infected lambs did not show any signs of anorexia or reduction of DMI. This may be justified by the moderate infection (Ueno and Goncalves, 1998), and in this study, infected consumed more feed than uninfected lambs. However, we have to consider that this could have occurred due to the use of pair-fed methodology, which ensured that the uninfected lambs received the same daily amount of feed that was consumed by its infected pair on the previous day. An important feature

in relation to the feeding habits of sheep is the high selectivity during feed intake. Both treatments consumed all of the offered concentrate, the daily difference in DMI being related to hay consumption. Infected lambs had *ad libitum* access to hay, and portions of low nutritional quality such as stalks and stems were discarded. This situation was also observed in uninfected lambs. However, as the amount of roughage offered was restricted to the amount consumed by the infected lambs, consequently the intake of the uninfected lambs was lower.

The ruminal parameters of both treatments were similar, wherein pH in the ruminal fluid, in the case of a diet with roughage:concentrate ratio of 70:30, was within 6–7, which is characteristic of a fibrous diet. Thus, the protozoa count, which in many cases is directly influenced by pH, did not differ between treatments. The values of ammoniacal-N were considered adequate for the fermentative activities, being superior to 10 mg/dL, which were values reported by Van Soest (1994) as minimum concentration for microbial growth. However, the changes imposed by the infection with *T. colubriformis* reduced the total SCFA production, while reducing butyrate and increasing acetate proportions. This suggests a lower microbiota fermentation process, providing less energy and protein to the host animal, because in this situation, the microbial protein synthesis and the amino acid supply to the ruminant can also be reduced (Steel, 1972). Even when considering that ruminal function is important for the availability of energy and metabolizable protein, it is still necessary to better understand the effects of gastrointestinal nematodes on ruminal physiology, so evaluating digesta flow in the gastrointestinal tract is important to better understand on the consequences of this infection.

This study presents new information on the evaluation of digesta flow through the gastrointestinal tract, in different segments (rumen, abomasum, small intestine and large intestine) using external markers in the different fractions of the diet (concentrate, hay and liquid phase). Infected lambs presented lower retention time of the solid (ytterbium [Yb]-labeled concentrate and chromium[Cr]-mordanted fiber) and liquid content (cobalt[Co]-EDTA) of the digesta in the rumen-reticulum and consequently lower production of SCFA, as when the time of permanence of the digesta in the rumen decreases, it also decreases its degradability. Our hypothesis is that the increased digesta flow in the rumen-reticulum compartment in infected lambs may be due to the greater demand for nutrients by the host. Based on this, it is clear that it is more important that the nutrients are readily available for digestion and absorption in the host than their permanence in the rumen for degradation and use by the microorganisms for multiplication and production of SCFA. We did not observe alterations in the flow of digesta in other segments (abomasum and intestines) with chronic infection.

However, once the injuries caused by *T. colubriformis* in the small intestine (mainly in the duodenum - site of greater absorption of nutrients), which are described by several authors (Coop et al., 1982; Coop and Kyriazakis, 1999; Cardia et al., 2011; Silva et al., 2018) promote negative impact on the absorptive mucosa. Also, *T. colubriformis* competing for nutrients, increasing the nutritional requirements to eliminate the infection, the retention time in the other segments of the gastrointestinal tract were similar, allowing the compensatory absorption in the infected lambs (Leng, 1981), mainly in segments not primarily affected by the worm burden. Furthermore, a repartitioning of nutrients probably occurred to repair functions of the gastrointestinal tract, such as mucus secretion and/or plasma and blood replacement (Jones and Symons, 1982; MacRae, 1993), which may have compromised the productive functions such as muscle, bone and wool growth of infected lambs. Moreover, the liquid retention time here was lower in infected lambs and probably affected the resorption process, causing increased excretion of fluids, nutrients and electrolytes via faeces (Roseby and Leng, 1974), emphasizing the phosphorus excretion described by Silva et al. (2018), causing faeces with semi-pasty consistency seen in these lambs.

There was no difference in BW nor ADG between uninfected and

infected lambs. However, the uninfected lambs had constant body condition score over time (Fig. 1), while the infected lambs showed significant decrease in body condition score, consistent with the early elimination of eggs. Despite not being evaluated here, this could be explained by the occurrence of intestinal inflammation that occurs in sheep infected with helminths, causing alteration in vascular permeability, with diffusion of liquids into the abdominal cavity, especially in cases of chronic infections, electrolyte imbalance and protein loss (Kyriazakis et al., 1996; Silva, 2014).

## 5. Conclusions

Chronic infection by *T. colubriformis* showed negative impact on lamb production, affecting digesta passage rate in rumen-reticulum (solid and liquid phases) and short chain fatty acids.

## Declaration of Competing Interest

The authors declare that there were no conflicting interests that could have influenced the conduct and reporting of this study.

## Acknowledgments

The authors would like to thank the National Council of Technological and Scientific Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES) and São Paulo Research Foundation (FAPESP) for providing financial support (process number 2014/05023-1 and process number 2018/06191-6).

## References

- Abdalla Filho, A.L., Corrêa, P.S., Lemos, L.N., Dineshkumar, D., Issakowicz, J., Ieda, E.H., Louvandini, H., 2017. Diets based on plants from Brazilian Caatinga altering ruminal parameters, microbial community and meat fatty acids of Santa Inês lambs. *Small Rumin. Res.* 154, 70–77. <https://doi.org/10.1016/j.smallrumres.2017.07.005>.
- Association of Official Analytical Chemists – AOAC, 2011. *Official Methods of Analysis*, 18th ed. AOAC International, Arlington.
- Barker, I.K., 1973. A study of pathogenesis of *Trichostrongylus colubriformis* infection in lambs with observations on the contribution of gastrointestinal plasma loss. *Int. J. Parasitol.* 3, 743–757.
- Barker, I.K., 1975. Intestinal pathology associated with *Trichostrongylus colubriformis* infection in sheep: vascular permeability and ultrastructure of the mucosa. *Parasitology* 70, 173–180.
- Cantacessi, C., Mitreva, M., Campbell, B.E., Hall, R.S., Young, N.D., Jex, A.R., Ranganathan, S., Gasser, R.B., 2010. First transcriptomic analysis of the economically important parasitic nematode, *Trichostrongylus colubriformis*, using a next-generation sequencing approach. *Infect. Genet. Evol.* 10, 1199–1207.
- Cardia, D.F.F., Rocha-Oliveira, R.A., Tsunemi, M.H., Amarante, A.F., 2011. Immune response and performance of growing Santa Inês lambs to artificial *Trichostrongylus colubriformis* infections. *Vet. Parasitol.* 182, 248–258.
- Clauss, M., Stewart, M., Price, E., Peilon, A., Savage, T., Van Ekris, I., Munn, A., 2016. The effect of feed intake on digesta passage, digestive organ fill and mass, and digesta dry matter content in sheep (*Ovis aries*): flexibility in digestion but not in water reabsorption. *Small Rumin. Res.* 138, 12–19.
- Coop, R.L., Kyriazakis, I., 1999. Nutrition–parasite interaction. *Vet. Parasitol.* 84, 187–204.
- Coop, R.L., Sykes, A.R., Angus, K.W., 1982. The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. *J. Agric. Sci.* 98, 247–255.
- Dehority, B.A., Damron, W.S., McLaren, J.B., 1983. Occurrence of the rumen ciliate *Oligoisotricha bubali* in domestic cattle (*Bos taurus*). *Appl. Environ. Microbiol.* 45, 1394–1397.
- Fox, M.T., 1997. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Vet. Parasitol.* 72, 285–308.
- Gordon, H.M., Whitlock, H.V.A., 1939. New technique for counting nematode eggs in sheep faeces. *J. Coun. Sci. Ind. Res.* 12, 50–52.
- Jones, W.O., Symons, L.E.A., 1982. Protein synthesis in the whole body, liver, skeletal muscle and kidney cortex of lambs infected by the nematode *Trichostrongylus colubriformis*. *Int. J. Parasitol.* 12, 295–301.
- Kyriazakis, I., Anderson, D.H., Oldham, J.D., Coop, R.L., Jackson, F., 1996. Long-term subclinical infection with *Trichostrongylus colubriformis*: effects on food intake, diet selection and performance of growing lambs. *Vet. Parasitol.* 61, 297–313.
- Leng, R.A., 1981. Nutrition and metabolism of parasitized and non-parasitized ruminants. Some approaches for studying the mode of action of parasites. *Isotopes and Radiation in Parasitology* 4. International Atomic Energy Agency, Vienna, pp. 191–206.
- MacRae, J.C., 1993. Metabolic consequences of intestinal parasitism. *Proc. Nutr. Soc.* 52,

- 121–130.
- Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. *J. AOAC Int.* 85, 1217–1240.
- Nocek, J.E., Hart, S.P., Polan, C.E., 1987. Rumen ammonia concentrations as influenced by storage time, freezing and thawing, acid preservative and method of ammonia determination. *J. Dairy Sci.* 70, 601–607.
- O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Vet. Parasitol.* 142, 1–15.
- Palmquist, D., Conrad, H., 1971. Origin of plasma fatty acids in lactating cows fed high fat diets. *J. Dairy Sci.* 54, 1025–1033.
- Preston, T.R., 1995. *Tropical Animal Feeding: a Manual for Research Workers*. FAO, Rome 126 p.
- Roseby, F.B., Leng, R.A., 1974. Effects of *Trichostrongylus colubriformis* (Nematoda) on sheep. 11. Metabolism of urea. *Aust. J. Agric. Res.* 25, 363–367.
- Silva, H.M., 2014. Nematodioses gastrintestinais de caprinos: uma revisão. *Rev. Ciênc. Agrovet.* 13, 199–208.
- Silva, T.P.D., Jimenez, C.R., Ieda, E.H., Abdalla, A.L., Louvandini, H., 2018. Phosphorus kinetics in lambs experimentally infected with *Trichostrongylus colubriformis* with the use of <sup>32</sup>P. *Exp. Parasitol.* 188, 13–20.
- Steel, W.J., 1972. Effects of the intestinal nematode *Trichostrongylus colubriformis* on ruminal acetate metabolism in young sheep. *Proc. Aust. Soc. Anim. Prod.* 9, 402.
- Sykes, A.R., Coop, R.L., 1976. Intake and utilization of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformis* larvae. *J. Agric. Sci.* 86, 507–515.
- Sykes, A.R., Coop, R.L., Angus, K.W., 1979. Chronic infection with *Trichostrongylus vitrinus* in sheep. Some effects on food utilization, skeletal growth and certain serum constituents. *Res. Vet. Sci.* 26, 372–377.
- Taylor, M.A., Wall, R.L., Coop, R.L., 2010. *Parasitologia Veterinária*, 3rd ed. Guanabara Koogan, Rio de Janeiro 742 p.
- Thompson, J., Meyer, H., 2006. Body condition scoring of sheep. *Biennial Spooner Sheep Day*, 52. 2006. Proceedings... Madison, Wisconsin: University of Wisconsin, Madison, Madiosn, Wisconsin, pp. 28–31.
- Udén, P., Colucci, P.E., Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31, 625–632.
- Ueno, H., Gonçalves, P.C., 1998. *Manual Para Diagnóstico Das Helminthoses De Ruminantes*. 4th ed. Japan International Cooperation Agency, Tokyo 143 p.
- Van Soest, P.J., 1994. *Nutrition Ecology of the Ruminants*. Cornell University Press, Ithaca 476 p.
- Van Soest, P.J., France, J., Siddons, R.C., 1992. On the steady state turnover of compartments in the ruminant gastrointestinal tract. *J. Theor. Biol.* 159, 135–145.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Vega, A., Gasa, J., Castrillo, C., Guada, J.A., 1998. Passage through the rumen and the large intestine of sheep estimated from faecal marker excretion curves and slaughter trials. *Br. J. Nutr.* 80, 381–389.