**Original Article** 

ISSN 1984-2961 (Electronic) www.cbpv.org.br/rbpv

# Gastrointestinal nematode infections do not hinder the development of Simmental X Nellore crossbred calves raised with a nutritionally enhanced diet

Infecções por nematódeos gastrintestinais não prejudicam o desenvolvimento de bezerros Simental x Nelore criados em plano nutricional elevado

José Henrique das Neves<sup>1</sup>\* <sup>(1)</sup>; Nadino Carvalho<sup>2</sup>; Alessandro Francisco Talamini do Amarante<sup>3</sup>

- ¹Setor de Produção Animal, Faculdade de Ensino Superior e Formação Integral FAEF, Garça, SP, Brasil
- <sup>2</sup>Departamento de Medicina Veterinária, Fundação Universidade Federal de Rondônia UNIR, Rolim de Moura, RO, Brasil

**How to cite:** Neves JH, Carvalho N, Amarante AFT. Gastrointestinal nematode infections do not hinder the development of Simmental X Nellore crossbred calves raised with a nutritionally enhanced diet. *Braz J Vet Parasitol* 2020; 29(1): e015819. http://dx.doi.org/10.1590/S1984-29612020006

#### **Abstract**

An evaluation was made of the effect of anthelmintic treatments on the performance of Simmental X Nellore crossbred calves before and after weaning. To this end, the calves were divided into three groups: (1) treated monthly with a low efficacy anthelmintic drug, ivermectin; (2) treated monthly with a highly effective anthelmintic drug, albendazole; and (3) untreated control group. All the groups in this experiment showed an average fecal egg count of less than 400 eggs per gram (EPG), and no clinical signs of parasitic gastroenteritis. The blood variables were within the normal range and no calf presented anemia. In most of the samplings, mean EPGs were significantly lower (P<0.05) in the group treated with albendazole. The calves received dietary supplementation before and after weaning, which enabled them to gain weight in every month of the experiment and reach a body weight of about 250 kg on the last sampling date, before turning one year old. The anthelmintic treatments did not affect body weight gain, leading to the conclusion that, when fed with suitable dietary supplements, Simmental X Nellore crossbred calves are not affected by gastrointestinal nematode parasites acquired by grazing.

Keywords: Haemonchus, nutrition, ivermectin, albendazole, cattle.

## Resumo

O objetivo do experimento foi avaliar o efeito de tratamentos anti-helmínticos no desempenho de bezerros Simental x Nelore antes e após o desmame. Os bezerros foram alocados em três grupos: (1) tratado mensalmente com anti-helmínticos de baixa eficácia, ivermectina; (2) tratado mensalmente com anti-helmíntico de alta eficácia, albendazol e (3) controle não tratado. A média das contagens de ovos de nematoides durante o experimento foi inferior a 400 ovos por grama (OPG) em todos os grupos sem manifestação clínica de gastroenterite parasitária. As variáveis sanguíneas mantiveram-se dentro dos limites de normalidade e nenhum bezerro apresentou anemia. Na maioria das coletas, as médias de OPG foram significativamente inferiores (P<0,05) no grupo tratado com albendazol. Os bezerros receberam suplementação antes e depois do desmame, o que lhes permitiu ganhar peso em todos os meses do experimento, atingindo peso corporal em torno de 250 kg, ao

Received September 2, 2019. Accepted December 16, 2019. Financial support: None.

\*Corresponding author: José Henrique das Neves. E-mail: nevesjhvet@hotmail.com



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

<sup>&</sup>lt;sup>3</sup> Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista – UNESP, Botucatu, SP, Brasil

final do experimento, antes de completarem um ano de idade. Não houve efeito dos tratamentos anti-helmínticos no ganho em peso, o que permitiu concluir que bezerros Nelore x Simental não são afetados pelo parasitismo por nematoides gastrintestinais sob condições de pastejo, quando devidamente suplementados com concentrado.

Palavras-chave: Haemonchus, nutrição, ivermectina, albendazol, bovinos.

## Introduction

Brazil has more than 214 million heads of cattle (ABIEC, 2019), placing the country among the leaders in the sector, with the world's second largest cattle inventory and the world's largest beef exporter. However, cattle productivity presents losses due to parasitism caused by flies, ticks and endoparasites, with annual losses of more than 13 billion US dollars (Grisi et al., 2014). The body weight gain of young cattle may be affected by the interaction of several factors, such as genetic background, sanitary conditions and environmental factors, especially those pertaining to nutritional quality (Pathak, 2017; Fachiolli et al., 2017).

Gastrointestinal nematode (GIN) infections play an important role in the losses attributed to parasitic diseases in cattle. In Central Brazil, pioneering investigations into losses due to GIN infections in young Zebu or Zebu X European crossbred cattle were conducted in the 1970s. In Mato Grosso, Melo & Bianchin (1977) showed that Zebu calves strategically treated four times per year (mid-May, mid-July, mid-September and mid-December) with broad-spectrum anthelmintics (tetramisole) gained significantly more weight (43.3±11 kg) than untreated animals. In contrast, in Minas Gerais, Costa et al. (1977) found no difference between the body weight gain of groups of 10-month-old calves treated with tetramisole twice (December and June) or four times a year (December, March, June and September) and that of a untreated control group.

Later, in Central Brazil, young treated cattle showed higher gains in body weight than untreated controls in the states of Mato Grosso do Sul (Bianchin et al., 1995, 2007; Borges et al., 2013; Catto et al., 2013; Heckler et al., 2016), Minas Gerais (Furlong et al., 1993; Guimarães et al., 2000; Lima et al., 1985; 1997), and São Paulo (Oliveira & Freitas, 1998; Soutello, 2001). In contrast, no difference was found between treated and untreated cattle in the Pantanal region of Mato Grosso do Sul (Catto & Furlong, 1982; Catto et al., 1993; Sereno et al., 2000) and in São Paulo (Santos et al., 1977). According to the above cited authors, the anthelmintics used in the experiments published before 1985 were benzimidazoles and imidazothiazoles (tetramisole and levamisole). The findings of the first investigation into the effect of a macrocyclic lactone (ivermectin) in young cattle were then published in 1985 (Lima et al., 1985). The use of these endectocides has become customary in the last few decades, and macrocyclic lactones, particularly ivermectin, are among the most widely sold drugs for the control of cattle parasites (Delgado et al., 2009; Neves et al., 2014). However, the continuous use of ivermectin has resulted in anthelmintic resistance, a widespread problem reported in the states of Minas Gerais (Rangel et al., 2005), Rio de Janeiro (Cardoso et al., 2008), Santa Catarina (Souza et al., 2008), Mato Grosso do Sul (Borges et al., 2013), São Paulo (Neves et al., 2014), Paraná (Holsback et al., 2015) and Rio Grande do Sul (Ramos et al., 2016).

Crossbreeding between Zebu and European cattle breeds has become a common practice aimed at improving beef cattle weight gain and carcass grade. The age group most susceptible to GIN infections is young cattle. Therefore, in this study we evaluated Simmental X Nellore crossbred calves before and after weaning. Our aim was to determine whether the animals were more likely to acquire severe infections before or after weaning, and to evaluate the effect of such infections on their body weight gain (BWG). One group was not treated with anthelmintic drugs (control group), while the other was treated monthly with a highly effective anthelmintic (albendazole) to keep it as free as possible of GIN infections. Because BWG reportedly benefits from the use of macrocyclic lactones with low or moderate efficacy due to anthelmintic resistance (Borges et al., 2013; Heckler et al., 2016), we also included a group treated monthly with an anthelmintic of low efficacy (ivermectin).

#### **Materials and Methods**

This study was carried out in accordance with the ethical guidelines of animal research and was approved by the local ethics committee on animal use (protocol 38/2014/CEUA-FMVZ).

# **Description of the experiment**

We evaluated young beef cattle on a farm located in the municipality of Piracicaba, state of São Paulo, Brazil. The cattle on this farm presented an ivermectin resistant worm population, which was susceptible to albendazole (Neves et al., 2014). The calves we evaluated had not been treated with anthelmintics prior to this experiment.

The animals, identified by numbered ear tags, were born between January 09 and March 05, 2015. At the beginning of this study, on April 15, the youngest calf was 41 days old and the oldest was 96 days old. The average age ( $\pm$  standard deviation) of the ivermectin, albendazole and untreated control groups was  $70\pm15.92$ ,  $72\pm10.67$  and  $69\pm13.96$  days (P> 0.05), respectively.

The unweaned Simmental X Nellore crossbred calves initially grazed with their mothers. They were divided into three groups, based on to sex, paternal genealogy and previous stratification according to their nematode fecal egg counts (FEC) in individual fecal samples taken two days before administering the first anthelmintic treatment. The groups were as follows: G1 (8 females and 6 males) - treated monthly with an ineffective anthelmintic, ivermectin 1% (0.2 mg/kg, Ivomec®, Merial), G2 (7 females and 10 males) - treated monthly with a highly effective anthelmintic, albendazole sulfoxide 10% (2.5 mg/kg; Albendathor®, Fabiani Animal Health), and G3 (8 females and 9 males) - untreated Control group.

All the grazing animals were kept on pastures (*Urochloa* spp.), at a stocking rate of approximately 1.4 livestock units per hectare, and had free access to a multimineral supplement (Fosbovi® 15, DSM Animal Nutrition). From April to June, all the calves had access to creep feeding with 310 g of concentrate/animal/day. The amount of concentrate was increased to 630 grams/animal/day in July, and to 950 grams/animal/day from August to the end of the experiment. The concentrate was produced on the farm and consisted of soybean meal (25%), corn (70%) and millet enriched with minerals (5%). All the calves were weaned on 22 September 2015, when males and females were placed in separate paddocks of similar size and pasture grasses to prevent undesired pregnancy, since the bull calves were not castrated.

All the animals were vaccinated against foot-and-mouth disease, according to the mandatory vaccination schedule. Females were vaccinated against brucellosis on 10 June 2015, and all the animals were vaccinated against clostridiosis and rabies on 25 August 2015.

A single dose of Fipronil (1 mg/kg/BW, TopLine®, Merial) was applied in September to control the ectoparasites *Rhipicephalus microplus* and *Haematobia irritans*.

# **Fecal testing**

Fecal samples were collected from each animal every 28 days. The samples were processed individually using the Flotac dual technique with a sensitivity of two eggs/g, using saturated sodium chloride (NaCl, specific gravity 1.2) and zinc sulfate solution (ZnSO $_4$ , specific gravity 1.35) (Cringoli et al., 2010). The ZnSO $_4$  solution was used to detect *Dictyocaulus viviparus* larvae in the fecal tests carried out from 30 July up to the end of the experiment.

On the above-mentioned days, composite fecal cultures from each group were prepared to obtain third-stage larvae and identify them according to nematode genus (Ueno & Gonçalves, 1998; van Wyk & Mayhew, 2013; Amarante, 2011). In the case of *Haemonchus* larvae, the distance between the tip of the larval tail and the end of the sheath tail was measured. Larvae with lengths of approximately 99.2 µm were identified as *Haemonchus placei*, while those with lengths of about 57.3 µm were identified as *Haemonchus similis* (Amarante, 2011).

On two occasions during the experiment (April and October), additional individual fecal samples were taken 10 days post-treatment for a fecal egg count reduction (FECR) test to evaluate the effectiveness of albendazole sulfoxide and ivermectin. The FECR was calculated as described by Torgerson et al. (2014).

#### **Blood tests**

Every four months, blood samples were drawn from the jugular vein into tubes containing EDTA (Vacutainer,  $BD^{\circ}$ , USA) in order to determine the packed cell volume (PCV) by microcentrifugation and total plasma protein (TPP) by refractometry. The eosinophil count in peripheral blood was performed in a Neubauer chamber after staining with Carpentier's solution (Dawkins et al., 1989), and the counts were expressed as the number of cells per  $\mu$ L of blood.

# Statistical analysis

Data were analyzed by one-way analysis of variance (one-way ANOVA) in the case of variables measured only once (BWG and daily BWG) and by ANOVA with repeated measures in the case of variables measured at several time points (FEC, body weight, PCV, TPP and eosinophils), using SAS® version 9.4 software. Group and sex were the classes evaluated. Means were compared by Tukey's test at a 5% level of significance, and only significant interactions at the 5% significance level are reported in the results. The EPG count and eosinophil-related data were analyzed under log transformation (log<sub>10</sub> (x +1)).

## **Results**

The effectiveness of the anthelmintics was evaluated 10 days post treatment, in April and October. Albendazole proved to be highly effective, with a FECR of 99.2% (confidence interval: 100 – 99). In contrast, ivermectin effectiveness was only 45.3% (51; 40) in April and zero in October, with the following nematode larvae in cultures of this group: *Cooperia* spp. (96%) and *H. placei* (4%) in April; and *Cooperia* spp. (97%), *H. placei* (1%) and *Oesophagostomum radiatum* (2%) in October.

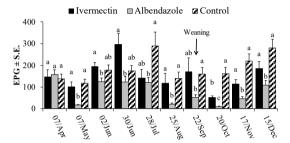
Throughout the experiment, the most prevalent parasite in the control group was *Cooperia* spp., followed, in decreasing order, by *Haemonchus placei*, *Haemonchus similis*, *Oesophagostomum radiatum* and *Trichostrongylus* spp. (Table 1). The group treated with ivermectin showed similar percentages of genera, except for *H. similis* and *Trichostrongylus*, which were never identified in this group (Table 1).

**Table 1.** Percentage of third stage larvae ( $L_3$ ) of *Haemonchus placei* (HP), *Haemonchus similis* (HS), *Cooperia* spp. (COO), *Trichostrongylus* sp. (TRI) and *Oesophagostomum radiatum* (OES) from fecal cultures of Simmental X Nellore crossbred calves treated every 28 days with ivermectin, albendazole sulfoxide, or not treated (Control).

Campling Date	Ivermectin				Albendazole				Control						
Sampling Date	HP	HS	coo	OES	TRI	HP	HS	coo	OES	TRI	HP	HS	coo	OES	TRI
07 Apr	0	0	100	0	0	11	0	89	0	0	0	1	98	1	0
05 May	4	0	96	0	0	0	0	100	0	0	13	2	85	0	0
02 Jun	5	0	95	0	0	0	0	100	0	0	1	2	97	0	0
30 Jun	5	0	95	0	0	0	0	100	0	0	3	3	93	1	0
28 Jul	14	0	86	0	0	0	0	100	0	0	6	4	89	1	0
25 Aug	4	0	96	0	0	0	0	100	0	0	8	14	69	8	1
22 Sep	10	0	90	0	0	0	0	100	0	0	12	16	65	7	0
20 Oct	25	0	75	0	0	0	0	100	0	0	6	9	83	2	0
17 Nov	20	0	79	1	0	0	0	100	0	0	8	2	82	8	0
15 Dec	64	0	33	3	0	0	0	100	0	0	44	5	44	6	1

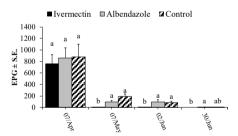
Calves were born between 09 Jan and 05 Mar and were weaned on 22 Sep 2015.

On the day of the first anthelmintic treatment (7 Apr), the mean EPG of strongylids were similar among groups: overall mean = 147.7 EPG with minimum and maximum values of zero and 518 EPG, respectively. The FECs of the control group remained relatively stable throughout the experiment, with means ranging from 117 to 289 EPG. On seven occasions, the average EPGs of the calves treated with albendazole were significantly lower than those of the ivermectin and control groups (Figure 1). Nevertheless, calves in the albendazole treated group shed eggs in feces. The identification of infective larvae from fecal cultures indicated that these eggs were from *Cooperia* spp. (Table 1). In only one of the 10 samplings (on 20 Oct), the mean FEC of ivermectin treated calves was significantly lower than that of the control. Sex was found to have an influence on FECs only in the last sampling, when females presented lower mean EPG (logEPG = 1.30) than males (logEPG = 2.07).



**Figure 1.** Mean number (± standard error) of strongyle eggs per gram of feces (EPG) from crossbred Simmental X Nellore calves treated every 28 days with ivermectin, albendazole sulfoxide or untreated (Control). On each sampling date, mean values with different letters differ significantly according to Tukey's test (P<0.05). Bars represent standard error.

At the beginning of the experiment, the three groups showed high FECs of *Strongyloides* sp. (Figure 2), with maximum individual values of 2,304 EPG and 3,504 EPG, respectively, in a female and in a male calf. Thereafter, the number of *Strongyloides* eggs gradually decreased until they were no longer detected after June (Figure 2). In the samples collected on 07 May and 02 June, the mean EPG of the group treated with ivermectin was significantly lower than those of the other groups (P<0.05).



**Figure 2.** Mean number (± standard error) of *Strongyloides* spp. eggs per gram of feces (EPG) from crossbred Simmental X Nellore calves treated every 28 days with ivermectin, albendazole sulfoxide or untreated (Control). On each sampling date, mean values with different letters differ significantly according to Tukey's test (P<0.05). Bars represent standard error.

Other parasites were found sporadically in some animals (Table 2). In the first sampling, two animals of the ivermectin group presented *Toxocara vitulorum* eggs, which were absent after this anthelmintic was administered. *Trichuris* spp. and *Moniezia* spp. eggs were detected on a few occasions, while *Eimeria* spp. oocysts were found in most of the samples, albeit in small numbers. *Dictyocaulus viviparus* was found intermittently in some animals (Table 2).

**Table 2.** Means (± standard error) of *Trichuris* spp. eggs per gram (EPG), *Toxocara vitulorum* EPG, and *Dictyocaulus viviparus* larvae per gram of feces; presence of *Moniezia* spp. eggs and *Eimeria* spp. oocysts in Simmental X Nellore calves of the groups treated with ivermectin (n=14), with albendazole (n=18), and in the untreated control group (n=17).

Parasite	Group	07 Apr	05 May	02 Jun	30 Jun	28 Jul	25 Aug	22 Sep	20 Oct	17 Nov	15 Dec
	Ivermectin	0	0	0	0	0	0.1±0.1 (1)	0	0	0.1±0.1(1)	0
Trichuris	Albendazole	0	0	0	0	0.1±0.1 (1)	0.4±0.2 (4)	0.7±0.4 (3)	0.1±0.1 (1)	0.2±0.2 (2)	0.2±0.2 (2)
	Control	0	0	0	0	0	0.4±0.2 (3)	0.4±0.2 (3)	0.2±0.2 (1)	0.5±0.4 (2)	0.5±0.4 (2)
	Ivermectin	93.1±72.6 (2)	3.9±3.7 (1)	0	0	0	0	0	0	0	0
T. vitulorum	Albendazole	42.0±29.1 (2)	0	0	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0	0	0	0
	Ivermectin	NE	NE	NE	0	0	0	0	0.1±0.1 (1)	0	0
D. viviparus	Albendazole	NE	NE	NE	0	0	0	0	0	0	0
	Control	NE	NE	NE	0	0	0.6±0.6 (1)	1.3±0.6 (5)	0.7±0.3 (4)	0.2±0.2 (2)	0
	Ivermectin	No	No	Yes (1)	Yes (4)	Yes (3)	Yes (1)	No	No	No	No
Moniezia	Albendazole	No	No	No	Yes (2)	Yes (6)	Yes (4)	Yes (1)	Yes (1)	No	No
	Control	No	Yes (2)	Yes (1)	Yes (2)	Yes (7)	No	No	No	No	No
	Ivermectin	Yes (14)	Yes (14)	Yes (14)	Yes (14)	Yes (14)	Yes (6)	Yes (10)	Yes (8)	Yes (14)	Yes (13)
Eimeria	Albendazole	Yes (18)	Yes (17)	Yes (18)	Yes (18)	Yes (18)	Yes (6)	Yes (12)	Yes (8)	Yes (12)	Yes (16)
	Control	Yes (16)	Yes (16)	Yes (17)	Yes (17)	Yes (17)	Yes (7)	Yes (13)	Yes (11)	Yes (16)	Yes (17)

Values in parenthesis indicate the number of animals in each group that presented the diagnosed parasite. NE (Not evaluated).

The anthelmintic treatments had no significant effect (P>0.05) on PCV, TPP and blood eosinophil counts (Table 3). As for TPP, a significant group\*sex interaction was identified on 28 Jul, when the albendazole-female group and male-control group showed mean values higher than 7 g/dL, while the other groups presented values below 6.7 g/dL, albeit without significant differences between group means (P>0.05). In the case of eosinophil counts, the influence of sex was detected on 7 Apr and 28 Jul, when females presented higher overall means than males.

**Table 3.** Means ( $\pm$  standard error) of packed cell volume (PCV, %), total plasma protein (TPP, g/dL) and blood eosinophils (EOS, cells/ $\mu$ L) of crossbred Simmental X Nellore calves naturally infected with gastrointestinal nematodes. Groups were treated monthly with albendazole, ivermectin or untreated (Control).

Variable	Date	lverm	ectin	Albeno	dazole	Con	Effects P-value			
		Females (n=8)	Males (n=6)	Females (n=7)	Males (n=10)	Females (n=8)	Males (n=9)	Group	Sex	Group x Sex
	07 Apr	34.7±1.50	34.1±1.73	38.3±1.58	37.3±1.28	36.4±2.00	36.2±1.41	0.08	0.89	0.91
PCV	28 Jul	35.0±2.14	37.0±1.40	37.5±0.91	36.4±1.13	37.4±1.34	35.7±1.62	0.82	0.82	0.45
	17 Nov	32.4±1.71	33.6±0.96	35.8±1.00	33.4±1.22	33.4±0.73	34.0±1.12	0.43	0.86	0.26
	07 Apr	6.3±0.17	6.2±0.13	6.6±0.12	6.5±0.21	6.5±0.19	6.6±0.12	0.18	0.86	0.73
TPP	28 Jul	6.6±0.23	6.6±0.19	7.1±0.16	6.7±0.12	6.6±0.18	7.1±0.19	0.23	0.83	0.04
	17 Nov	7.2±0.17	7.2±0.19	7.5±0.12	7.5±0.13	7.5±0.17	7.6±0.14	0.07	0.98	0.93
	07 Apr	152.1±61.78	28.1±7.38	243.8±80.66	43.8±9.36	259.4±96.38	127.8±40.71	0.69	0.03	0.52
EOS	28 Jul	1097.9±379.08	504.7±261.82	1010.9±336.99	412.5±133.22	948.4±300.09	620.8±103.79	0.40	0.00	0.36
	17 Nov	339.6±111.87	628.1±220.68	751.6±175.61	487.5±90.75	469.9±170.17	487.5±103.04	0.27	0.83	0.23

PCV (Time: P<0.001; Time\*Group: P>0.05; Time\*Sex: P>0.05; Time\*Group\*Sex: P>0.05), TPP (Time: P<0,001; Time\*Group: P>0.05; Time\*Group\*Sex: P>0.05; Time\*Group\*Sex: P>0.05; Time\*Group\*Sex: P>0.05; Time\*Group\*Sex: P>0.05).

The calves were all healthy during the experiment, with no clinical signs of parasitic diseases. Their development was satisfactory throughout the experiment and the anthelmintic treatments did not affect (P>0.05) their body weight gain (Table 4).

**Table 4.** Mean (± standard error) body weight (BW), BW gain and daily BW gain (kg) of crossbred Simmental X Nellore calves naturally infected with gastrointestinal nematodes, treated every 28 days with ivermectin, albendazole sulfoxide or untreated (Control).

Date	Albendazol	e Sulfoxide	lverm	ectin	Conti	Effects (P-value)			
	Females (n=8)	Males (n=10)	Females (n=6)	Males (n=8)	Females (n=8)	Males (n=9)	Group	Sex	Group x Sex
7 April	85±2.40	87±6.09	89±8.80	81±7.54	81±4.86	86±2.88	0.72	0.73	0.54
7 May	113±3.55	114±7.11	115±9.92	104±8.25	107±5.35	109±4.48	0.68	0.68	0.60
2 June	127±4.63	127±7.20	133±9.57	119±9.89	120±6.21	123±5.27	0.72	0.55	0.48
30 June	144±5.57	148±8.22	152±10.42	139±0.38	138±5.87	142±6.06	0.69	0.85	0.50
28 July	161±5.97	166±8.12	169±9.60	156±11.28	155±6.45	158±7.22	0.65	0.81	0.55
25 August	183±5.62	185±9.05	189±8.83	175±11.99	176±7.13	179±6.98	0.73	0.72	0.60
22 September	203±7.12	208±9.31	211±8.57	196±11.95	193±7.33	201±7.84	0.61	0.96	0.42
20 October	228±7.57	228±10.19	234±8.63	216±12.66	215±8.92	223±7.74	0.61	0.63	0.42
17 November	233±8.42	237±9.65	241±8.07	225±12.84	218±8.20	229±9.13	0.45	0.96	0.37
15 December	250±8.33	258±10.21	259±10.69	244±13.26	234±8.66	249±8.29	0.44	0.76	0.35
BW gain	165±22.48	170±26.50	169±14.49	163±19.79	153±14.58	166±21.93	0.53	0.49	0.50
Daily BW gain	0.611±0.08	0.631±0.10	0.624±0.05	0.604±0.07	0.568±0.05	0.615±0.08	0.53	0.49	0.50

Calves were born between 09 Jan 9 and 05 Mar and were weaned on 22 Sep 2015. Weight gain (Time: P<0.001; Time\*Group: P>0.05; Time\*Sex: P>0.05; Time\*Group\*Sex: P>0.05).

## **Discussion**

The mean FECs during the experiment were lower than 400 EPG in all the groups, which presented no clinical signs of parasitic gastroenteritis. The blood variables fell within the normal range and no calf showed signs of anemia. Mean EPGs were significantly higher in the control and ivermectin group than in the albendazole group. However, these differences in the degree of infection between groups did not have a statistically significant effect on body weight gain, which was similar in the three groups. This result was unexpected and differed from findings reported in other experiments conducted in Brazil, where anthelmintic treatments usually result in greater body weight gain among young cattle (Ramos et al., 2018), sometimes even when they are treated with drugs that are ineffective due to anthelmintic resistance (Borges et al., 2013; Heckler et al., 2016).

The calves involved in this experiment had been infected from an early age with gastrointestinal nematodes, beginning with *Strongyloides* spp., eliminated naturally through the development of immune response. Then, as expected, the parasitic fauna consisted predominantly of the strongylids (*Cooperia* spp., *Haemonchus* spp. and *Oesophagostomum* spp.). The absence of clinical signs of parasitic gastroenteritis and the good performance of the calves in the control group suggest that the host-parasite interaction reached equilibrium under the continuous parasite challenge that occurred in this experiment.

The fecal cultures revealed the presence, in decreasing order of occurrence, of *Cooperia* spp., *Haemonchus* spp. and *Oesophagostomum* spp. This finding is consistent with other studies, which also found that the parasite with the highest infection intensity was *Cooperia punctata*, followed, in decreasing order, by *Haemonchus* (*H. placei* and/or *H. similis*) and *Oesophagostomum radiatum* (Lima, 1998; Pimentel Neto & Fonseca, 2002; Bresciani et al.,

2001). These three genera were detected in the group treated with ivermectin, which confirms their resistance to avermectins and is in agreement with a previous report of drug resistance on this farm (Neves et al., 2014). The nematode population was susceptible to albendazole, and only *Cooperia* was detected after treatment with this drug. This finding can be explained by the short prepatent period of this parasite (11-14 days), while *H. placei* and *O. radiatum* present a longer prepatent period of 26-28 and 35-41 days, respectively (Wood et al., 1995). Therefore, it can be deduced that calves in the albendazole group become reinfected during the interval between drenches. In Minas Gerais, the FECs of treated calves were similar to those of the untreated control calves six weeks post-treatment with tetramisole or fenbendazole, indicating the rapid reestablishment of the parasite population (Lima et al., 1983). Taken together, these results indicate that such treatments may not have a meaningful impact on the epidemiology of GIN infections.

In this experiment, we evaluated only the effect of GIN infections, since a single dose of fipronil prevented losses caused by ectoparasites. It is therefore difficult to compare our results with those of studies that used products also active against ectoparasites (Bianchin et al., 2007; Catto et al., 2009; Soutello, 2001). For instance, Catto et al. (2013) reported that Brangus heifers treated with moxidectin and an acaricide showed lower FECs and tick infestations than untreated animals or animals treated with alternative drugs, which resulted in heifers 22 to 30 kg heavier in the treated group. Hence, in those experiments, the benefits in body weight gain may have resulted from the control of both endo- and ectoparasites.

The calves in our study were fed dietary supplements before and after weaning, resulting in weight gains throughout the experiment and enabling them to reach a body weight of about 250 kg on the last sampling date, before turning one year old. This nutritional quality enabled the infected animals, especially those of the untreated control and ivermectin groups, to become resilient to GIN infections. Melo (1977) had already pointed out that the effect of anthelmintic drugs on BWG appeared to be related to environmental conditions, especially nutrition, confirming our findings. The good level of nutrition employed in this experiment may not correspond to the reality of cattle production in Brazil, especially in the past, when cattle normally failed to receive proper nutrition throughout the year, leading to low body weight gain in the rainy season and body weight loss in the dry season (Melo, 1977). It should be noted that tropical grasses have a high potential for mass production, but low crude protein content. The benefits of anthelmintic treatments may be greater under such stressful dietary conditions. To exemplify this statement, Lima et al. (1985) conducted a study of Holstein X Nellore and Chianina X Nellore crossbred cattle in Minas Gerais and reported that the animals' performance improved in response to different ivermectin treatment strategies. In their study, the cattle grazed on Guinea grass (Panicum maximum) and their initial mean body weight (BW) varied from 104 kg to 134 kg at 6-8 months of age. In our experiment, the animals weaned at a similar age had a BW of about 200 kg, which indicates that the animals in our study were in much better nutritional condition than those of the experiment described by Lima et al. (1985). This may explain the discrepant results of the two studies, i.e., Lima et al. (1985) reported a significant improvement in the BWG of treated animals over that of the controls, but this was not the case of the well-fed animals in our study. Therefore, the results of our experiment suggest that calves that are given proper dietary supplementation are more likely to develop normally, without significant losses due to GIN infections. Studies that include poorly managed nutrition may not reflect the current reality of the beef cattle industry, which is increasingly adopting techniques aimed at improving the animals' nutritional status.

Grisi et al. (2014), who reassessed the potential economic impact of cattle parasites in Brazil, suggested that gastrointestinal nematode infections cause annual losses of US\$ 7.11 billion. This estimate was based on two studies, which found that cattle up to two years of age – the age group most affected by gastrointestinal nematodes – present a

reduction in weight gain ranging from 41 kg (Bianchin et al., 1995) to 67 kg (Pinheiro, 1983) compared to animals treated with anthelmintics. These losses, which were recorded in studies conducted more than two decades ago, may be outdated and no longer represent the reality of today's cattle industry, which has shown significant technological advances in recent years, especially in terms of nutritional management and crossbreeding strategies. Obviously, extrapolating our observations to other production systems would lead to inaccurate findings. However, considering the rapid development of the cattle industry, our results clearly indicate the need for further studies in different regions of Brazil in order to accurately estimate losses resulting from GIN infections.

There is also a need for the development of new control strategies less dependent on anthelmintics, given that the control methods recommended by Bianchin et al. (1995) and Pinheiro (1983), which involve frequent drenches, are no longer sustainable due to the widespread problem of anthelmintic resistance. In addition, the excessive use of avermectins leaves behind residues that are harmful to the environment. The widespread use of ivermectin disrupted the ecosystem function by affecting species richness, abundance and biomass, resulting in significant changes in soil functionality (Verdú et al., 2018). Therefore, improved nutrition appears to be an excellent option to optimize prophylaxis, minimizing the need for anthelmintics.

In conclusion, our results indicate that Simmental X Nellore crossbred calves properly fed with suitable dietary supplementation were not affected by gastrointestinal nematode parasitism under grazing conditions.

## References

- Amarante AFT. Why is it important to correctly identify *Haemonchus* species? *Rev Bras Parasitol Vet* 2011; 20(4): 263-268. http://dx.doi.org/10.1590/S1984-29612011000400002. PMid:22166378.
- Associação Brasileira das Indústrias Exportadoras de Carne ABIEC [online]. São Paulo: ABIEC; 2019 [cited 2019 Jul 9]. Available from: http://www.abiec.com.br/Sumario2019.aspx
- Bianchin I, Catto BJ, Kichel NA, Torres AA, Honer MR. The effect of the control of endo- and ectoparasites on weight gains in crossbred cattle (*Bos taurus taurus* × *Bos taurus indicus*) in the central region of Brazil. *Trop Anim Health Prod* 2007; 39(4): 287-296. http://dx.doi.org/10.1007/s11250-007-9017-1. PMid:17847824.
- Bianchin I, Honer MR, Nunes S, Nascimento YA. Effect of stocking rates and anthelminthic treatments on weight gains in weaned Nellore cattle on improved pasture in the Brazilian cerrado. *Trop Anim Health Prod* 1995; 27(1): 1-8. http://dx.doi.org/10.1007/BF02236326. PMid:7770946.
- Borges FA, Almeida GD, Heckler RP, Lemes RT, Onizuka MKV, Borges DGL. Anthelmintic resistance impact on tropical beef cattle productivity: Effect on weight gain of weaned calves. *Trop Anim Health Prod* 2013; 45(3): 723-727. http://dx.doi.org/10.1007/s11250-012-0280-4. PMid:23076819.
- Bresciani KDS, Nascimento AA, Costa AJ, Amarante AFT, Perri SHV, Lima LGF. Frequência e intensidade parasitária de helmintos gastrintestinais em bovinos abatidos em frigorífico da região noroeste do Estado de São Paulo, SP, Brasil. *Semina: Ciênc Agrár* 2001; 22(1): 93-97. http://dx.doi.org/10.5433/1679-0359.2001v22n1p93.
- Cardoso JMS, Martins IVF, Sant'anna FB, Correia TR, Tancredi IP, Coumendouros K, et al. Identification of ivermectin and doramectin-resistant *Cooperia punctata* (Linstow, 1907) in a dairy herd in the State of Rio de Janeiro, Brazil. *Arq Bras Med Vet Zootec* 2008; 45(Suppl.): 75-81. http://dx.doi.org/10.11606/S1413-95962008000700010.
- Catto JB, Barros ATM, Costa CAF. Efeito de tratamentos anti-helmínticos no ganho de peso de bezerros desmamados, criados em pastagens nativas, no pantanal mato-grossense, Brasil. *Rev Bras Parasitol Vet* 1993; 2(2): 127-132.
- Catto JB, Bianchin I, Feijó GLD, Araújo FR, Ramos CAN, Castelão ABC. Weight gain and control of endo- and ectoparasites of beef heifers treated with allopathic, herbal and homeopathic drugs. *Rev Bras Parasitol Vet* 2013; 22(4): 502-510. http://dx.doi.org/10.1590/S1984-29612013000400009. PMid:24473874.

- Catto JB, Bianchin I, Santurio JM, Feijó GLD, Kichel AN, Silva JM. Grazing systems, rotenone and parasites control in crossbred calves: effect on live weight gain and on parasites burdens. *Rev Bras Parasitol Vet* 2009; 18(4): 37-43. http://dx.doi.org/10.4322/rbpv.01804007. PMid:20040207.
- Catto JB, Furlong J. Desenvolvimento de bovinos criados extensivamente, submetidos a vários esquemas de tratamentos anti-helmínticos, no Pantanal Matogrossense. *Pesq Agropec Bras* 1982; 17(1): 131-136.
- Costa JO, Costa HMA, Guimarães MP, Freitas MG. Efeito de tratamentos anti-helmínticos sobre o OPG e o desenvolvimento ponderal de bezerros. *Arg Esc Vet UFMG* 1977; 29(2): 171-178.
- Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat Protoc* 2010; 5(3): 503-515. http://dx.doi.org/10.1038/nprot.2009.235. PMid:20203667.
- 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. PMid:2722393.
- Delgado FEF, Lima WS, Cunha AP, Bello ACPP, Domingues LN, Wanderley RPB, et al. Verminoses dos bovinos: percepção de pecuaristas em Minas Gerais, Brasil. *Rev Bras Parasitol Vet* 2009; 18(3): 29-33. http://dx.doi.org/10.4322/rbpv.01803005. PMid:19772773.
- Fachiolli DF, Saes IL, Dellaqua JVT, Souza OA, Pinto LD, Santi PF, et al. Anthelmintic treatment and supplementation in Nellore calves performance in the post-weaning period. *Semina: Ciênc Agrár* 2017; 38(3): 1551-1560. http://dx.doi.org/10.5433/1679-0359.2017v38n3p1541.
- Furlong J, Silva AM, Verneque RS, Gardner AL, Brockington NR. Análise bio-econômica do uso de anti-helmíntico em bezerros na zona da mata de Minas Gerais. *Rev Bras Parasitol Vet* 1993; 2(2): 119-126.
- Grisi L, Leite RC, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, et al. Reassessment of the potential economic impact of cattle parasites in Brazil. *Rev Bras Parasitol Vet* 2014; 23(2): 150-156. http://dx.doi.org/10.1590/S1984-29612014042. PMid:25054492.
- Guimarães MP, Ribeiro MFB, Facuri-Filho EJ, Lima WS. Strategic control of gastrointestinal nematodes in dairy calves in Florestal, Minas Gerais, Brazil. *Vet Res Commun* 2000; 24(1): 31-38. http://dx.doi.org/10.1023/A:1006373221169. PMid:10703752.
- Heckler RP, Borges DGL, Vieira MC, Conde MH, Green M, Amorim ML, et al. New approach for the strategic control of gastrointestinal nematodes in grazed beef cattle during the growing phase in central Brazil. *Vet Parasitol* 2016; 221: 123-129. http://dx.doi.org/10.1016/j.vetpar.2016.03.010. PMid:27084483.
- Holsback L, Silva MA, Patelli THC, Jesus AP, Sanches JR. Resistance of *Haemonchus, Cooperia, Trichostrongylus*, and *Oesophagostomum* to ivermectin in dairy cattle in Paraná. *Semina: Ciênc Agrár* 2015; 36(3): 2031-2036. http://dx.doi.org/10.5433/1679-0359.2015v36n3Supl1p2031.
- Lima WS, Costa HMA, Guimarães MP, Costa JO, Leite ACR. Reinfecção de bezerros após tratamentos anti-helmínticos. *Arq Bras Med Vet Zootec* 1983; 35(1): 101-112.
- Lima WS, Fakuri E, Guimarães MP, Malacco MA. Dinâmica das helmintoses de bovinos de leite na região Metalúrgica de Minas Gerais. *Rev Bras Parasitol Vet* 1997; 6(2): 97-103.
- Lima WS, Guimarães MP, Leite ACR. Custo-benefício de diferentes dosificações anti-helmínticas em relação ao ganho de peso de bezerros de corte. *Pesq Agropec Bras* 1985; 20(11): 1333-1335.
- Lima WS. Seasonal infection pattern of gastrointestinal nematodes of beef cattle in Minas Gerais State- Brazil. *Vet Parasitol* 1998; 74(2-4): 203-214. http://dx.doi.org/10.1016/S0304-4017(97)00164-7. PMid:9561708.
- Melo HJH, Bianchin I. Estudos epidemiológicos de infecções por nematódeos gastrintestinais de bovinos de corte em zona de cerrado de Mato Grosso. *Pesa Agropec Bras* 1977; 12: 205-216.
- Melo HJH. Efeito de diferentes esquemas de tratamento anti-helmíntico no ganho de peso de bezerros nelore desmamados e criados extensivamente em pastagens de Jaraguá (*Hyparrhenia rufa* (Nees Staft). *Arq Esc Vet UFMG* 1977; 29(3): 269-277.
- Neves JH, Carvalho N, Rinaldi L, Cringoli G, Amarante AFT. Diagnosis of anthelmintic resistance in cattle in Brazil: A comparison of different methodologies. *Vet Parasitol* 2014; 206(3-4): 216-226. http://dx.doi.org/10.1016/j.vetpar.2014.10.015. PMid:25468021.

- Oliveira GP, Freitas AR. Doramectin e levamizole no controle dos helmintos de bovinos no início da estação seca. *Cienc Rural* 1998; 28(2): 277-282. http://dx.doi.org/10.1590/S0103-84781998000200016.
- Pathak AK. Nutritional Bases to Control Gastrointestinal Parasites of Livestock. *J Dairy Vet Sci* 2017; 4(1): 555632.
- Pimentel Neto M, Fonseca AH. Epidemiologia das helmintoses pulmonares e gastrintestinais de bezerros em região de baixada do Estado do Rio de Janeiro. *Pesq Vet Bras* 2002; 22(4): 148-152. http://dx.doi.org/10.1590/S0100-736X2002000400004.
- Pinheiro AC. *Programa integrado de controle de verminose de bovinos de corte.* 2nd ed. Bagé: EMBRAPA-UEPAE; 1983.
- Ramos F, Marques CB, Reginato CZ, Rodrigues FS, Sangioni LA, Vogel FSF, et al. Economic viability of anthelmintic treatment in naturally infected beef cattle under different nutritional strategies after weaning. *Parasitol Res* 2018; 117(12): 3993-4002. http://dx.doi.org/10.1007/s00436-018-6108-z. PMid:30302586.
- Ramos F, Portella LP, Rodrigues FS, Reginato CZ, Potter L, Cezar AS, et al. Anthelmintic resistance in gastrointestinal nematodes of beef catlle in the state of Rio Grande do Sul, Brazil. *Int J Parasitol Drugs Drug Resist* 2016; 6(1): 93-101. http://dx.doi.org/10.1016/j.ijpddr.2016.02.002. PMid:27054068.
- Rangel VB, Leite RC, Oliveira PR, Santos EJ Jr. Resistência de *Cooperia* spp. e *Haemonchus* spp. às avermectinas em bovinos de corte. *Arq Bras Med Vet Zootec* 2005; 57(2): 186-190. http://dx.doi.org/10.1590/S0102-09352005000200008.
- Santos LE, Mendes MFM, Fontenello D, Miguel O, Paulo MS, Santos OL. Avaliação de tratamentos antihelmínticos no ganho de peso, no nível de hemoglobina e na infestação parasitária de novilhas "Guzerá" na região de Sertãozinho, S. Paulo, Brasil. *Rev Fac Med Vet Zootec Univ S Paulo* 1977; 14(1): 149-169. http://dx.doi.org/10.11606/issn.2318-3659.v14i1p149-169.
- Sereno JRB, Catto JB, Silva MP, Sereno FTPS. Veda e vermifugação como alternativas de manejo para desmama de bezerros nelore em pastagem nativa do Pantanal. *Pesq Agropec Bras* 2000; 35(10): 1099-2105. http://dx.doi.org/10.1590/S0100-204X2000001000023.
- Soutello RVG. *Influência do parasitismo e da suplementação no desenvolvimento ponderal de novilhos mestiços Angus-Nelore e da raça Guzerá* [dissertação]. Ilha Solteira: Faculdade de Engenharia, Universidade do Estado de São Paulo; 2001.
- Souza AP, Ramos CI, Bellato V, Sartor AA, Schelbauer CA. Resistência de helmintos gastrintestinais de bovinos a anti-helmínticos no Planalto Catarinense. *Cienc Rural* 2008; 38(5): 1363-1367. http://dx.doi.org/10.1590/S0103-84782008000500026.
- Torgerson PR, Paul M, Furrer R. Evaluating faecal egg count reduction using a specifically designed package "eggCounts" in R and a user friendly web interface. *Int J Parasitol* 2014; 44(5): 299-303. http://dx.doi.org/10.1016/j.ijpara.2014.01.005. PMid:24556564.
- Ueno H, Gonçalves PC. *Manual para diagnóstico das Helmintoses de ruminantes*. Tokyo: Japan International Cooperation Agency; 1998.
- van Wyk J, Mayhew E. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort J Vet Res* 2013; 80(1): 539. http://dx.doi.org/10.4102/ojvr.v80i1.539. PMid:23718204.
- Verdú JR, Lobo JM, Sánchez-Piñero F, Gallego B, Numa C, Lumaret JP, et al. Ivermectin residues disrupt dung beetle diversity, soil properties and ecosystem functioning: an interdisciplinary field study. *Sci Total Environ* 2018; 618: 219-228. http://dx.doi.org/10.1016/j.scitotenv.2017.10.331. PMid:29128770.
- Wood IB, Amaral NK, Bairden K, Duncan JL, Kassai T, Malone JB Jr, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet Parasitol* 1995; 58(3):181-213. https://doi.org/10.1016/0304-4017(95)00806-2