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Research paper

New approach for the strategic control of gastrointestinal nematodes in grazed beef cattle during the growing phase in central Brazil

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ABSTRACT

We evaluated the effect of different treatment protocols against gastrointestinal nematodes in Nelore beef cattle during the growing phase in the municipality of Terenos, MS, in central Brazil from May 2013 to April 2014 and from May 2014 to April 2015. Ninety-six Nelore calves were kept on *Brachiaria brizantha* grass during each trial period and were distributed into six experimental groups (replicate paddocks for each group) based on live weight and the number of eggs per gram of feces (EPG): T1 (control)—treated in May, July and September with a saline solution; T2—treated in May and November with 700 μg/kg doramectin; T3—treated in May (doramectin), July (4.7 mg/kg levamisole phosphate) and September (doramectin); T4—treated in May (doramectin), July (200 μg/kg moxidectin) and September (doramectin); T5—treated in May (doramectin), August (levamisole phosphate) and November (doramectin) and T6—treated in May (doramectin), August (moxidectin) and November (doramectin). The calves were weighed and feces were collected (for faecal culture and EPG counts) from calves every 28 days, concomitantly with the collection of forage samples. The efficacies of doramectin, moxidectin and levamisole were low, at 69.2, 65.9 and 69.4% in the first and 13.8, 92.6, and 76.5% in the second experimental periods, respectively, but only the untreated animals lost weight during the dry season. Final weight gains did not differ significantly ($p > 0.05$) among the animals in T2 (120.8 kg), T3 (131.4 kg), T4 (131.2 kg) and T5 (134.4 kg). T6 was the only group with a significantly higher final weight gain (140.9 kg) compared to the protocol with two annual dosages (T2). The weight gain was 31.9% higher in T6 than in the untreated animals (T1). None of the protocols affected the number of larvae on the pasture. Body weight was significantly and negatively ($r = -0.65$) correlated with EPG counts, which were significantly lower in June (T2, T3, T4 and T6), August (T3), September (T5 and T6), October (T5) and November (T5 and T6). *Haemonchus*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum* were identified. Treatments in May and November, the most common practice in Brazil, did not increase the final weight gain, so an additional and intermediate treatment during the dry season (August) is recommended.

1. Introduction

Gastrointestinal nematodes (GINs) are a major disease in beef cattle around the world, and decreased production is its main consequence (Vercruysse and Claerebout, 2001; Knox et al., 2012; Van der Voort et al., 2013; Charlier et al., 2014). The consequences of cattle parasitism are generally more severe in tropical/subtropical than temperate regions due to the combination of high temperatures and rainfall (Waller, 1997), which promote the survival and maintenance of parasites throughout the year. GIN is controlled in Brazil almost exclusively by the use of anthelmintic agents, which can provide higher weight gains of 11.85–53 kg/head when properly administered (Pinheiro et al., 2000; Soutello et al., 2002; Bianchin et al., 2007; Borges et al. 2013). The incorrect application of anthelmintics will have little or no effect on parasite populations or the rising cost of production, and the insufficient selection pressure will increase the rate of development of anthelmintic resistance (Lanusse et al., 2014).

Eighty percent of farmers in Brazil, however, still use inappropriate anthelmintic dosages and treat animals at epidemiologically inappropriate times to coordinate handling with other activities,

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especially the compulsory vaccination against foot and mouth disease (FMD) (Bianchin, 1991; Soutello et al., 2007), which is usually in May and November in most of Brazil. The development of long-acting anthelmintics (e.g. avermectins and milbemycins) has thus greatly contributed to the control of GIN, because the long period of protection against reinfection favors a longer interval between treatments and decreases the stress from handling (Borges et al., 2013) compared to anthelmintics with short residual periods (e.g. benzimidazoles and imidazoles). The inappropriate use of long-acting anthelmintics, however, may have little or no impact on the parasite populations (Stromberg and Averbeck, 1999) and may aggravate the development of resistance, which is among the largest obstacles in cattle production in many countries around the world (Kaplan and Vidyashankar, 2012; Martínez-Valladares et al., 2015).

The reduced effectiveness of anthelmintics, the lack of perspective for introducing new molecules to the market (Lanusse et al., 2014) and the lack of practical implementation of selective treatments in large herds in tropical conditions (Höglund et al., 2009) has necessitated studies of the strategic, rational and sustainable use of anthelmintics for reducing the negative impact of nematodiasis on the productivity of beef cattle raised on pasture.

The strategic control of GIN in beef cattle during the growing phase in central Brazil currently consists of treating animals from weaning up to 18–24 months of age. This age group (from weaning up to 18–24 months of age) has a higher susceptibility to parasitism and consequently represents a higher financial loss. Animals should be treated at the beginning, middle and end of the dry season. The conditions at this time are unfavorable for both larval development in the environment, due to reductions in temperature and humidity, and for the cattle, due to the reduced quantity and quality of available forage for consumption. Anthelmintic treatments should therefore be concentrated in May, July and September (Bianchin et al., 1996).

The strategic program of deworming currently recommended, however, can decrease parasitic load and environmental contamination and is cost effective (Bianchin, 1991), even with proven efficiency in increased weight gain, but handling and labor can be inconvenient, because the frequency of anthelmintic dosage does not coincide with the handling times for other operations, except for May, on most of the farms in central Brazil. A new protocol for the treatment of GIN is thus needed for the growing phase, with dosages in May and November (time of vaccination against FMD) and an additional intermediate dosing to maintain the current recommendation of three treatments concentrated in the dry season.

The current scenario of anthelmintic resistance in beef cattle in Brazil, with almost complete ineffectiveness of most of the anthelmintic formulations used in the field, especially macrocyclic lactones (Borges et al., 2015), has necessitated a re-evaluation of these drugs for controlling gastrointestinal nematodes and avoiding their negative impact on the productive performance of the animals.

The goal of this study was thus to evaluate three general protocols for treatment against parasitic gastrointestinal nematodes in beef cattle, given the current scenario of anthelmintic resistance: the current recommended treatment in May, July and September; the commonly used treatment in May and November and a new protocol for treatment in May, August and November.

2. Material and methods

2.1. Experimental location

The experiment was conducted at the Farm School of the Federal University of Mato Grosso do Sul (FAMEZ/UFMS) in Terenos, Mato Grosso do Sul, Brazil (20°26′32″S, 54°51′37″W). The region is characterized by a tropical savannah climate, with hot, humid summers and cold, dry winters. The state's annual rainfall is approximately 1500 mm. The state of Mato Grosso do Sul is at the confluence of the main atmospheric systems in South America and thus has more than one type of rainfall regime, some areas with a "central Brazil" regime and others with a "southern Brazil" regime (Zavattini, 2009).

2.2. Animals

The study used a total of 192 male Nelore cattle with initial ages of 8–10 months, naturally infected with gastrointestinal nematodes and no history of anthelmintic treatment.

2.3. Experimental design

The experiment was conducted in two trial periods, the first from May 2013 to April 2014 and the second from May 2014 to April 2015. The animals for the first and second periods came from properties with natural mating and fixed times of artificial insemination, respectively.

The study used a randomized-block design, in which each block (i.e., trial period) had two area replicates for each treatment. Each experimental group thus consisted of eight animals (192 animals ÷ 2 trial periods ÷ 6 treatment protocols ÷ 2 replicates = 8 animals) and was kept in its own paddock throughout the trial period.

The following treatment protocols, each with two area replicates per trial period, were evaluated: T1 (control)—animals treated in May, July and September with a saline solution; T2—animals treated in May and November with doramectin; T3—animals treated in May (doramectin), July (levamisole phosphate) and September (doramectin); T4—animals treated in May (doramectin), July (moxidectin) and September (doramectin); T5—animals treated in May (doramectin), August (levamisole phosphate) and November (doramectin) and T6—animals treated in May (doramectin), August (moxidectin) and November (doramectin).

We used 3.5% doramectin (Treo® Ace, Zoetis Brasil) at a dose of 700 μg/kg (1 ml/50 kg), levamisole phosphate (Ripercol®L 150F, Zoetis Brasil) at a dose of 4.7 mg/kg (1 ml/40 kg), 1% moxidectin (Cydectin, Zoetis Brasil) at a dose of 200 μg/kg (1 ml/50 kg) and a 0.9% saline solution (Isofarma Industrial Farmacêutica Ltda.) at a dose of 1 ml/50 kg. All formulations were administered according to manufacturer's label recommendations, on the left side of the animal.

2.4. Grazing

Each experimental group (eight animals) was maintained in a separate 4-ha paddock containing the grass *Brachiaria brizantha* cv. Marandu. The same treatment protocols were assigned to the same paddocks for the two trial periods. The initial capacity rates were 0.68 and 0.84 AU/ha (1 AU (animal unit) = 450 kg body weight) in the first and second trial periods, respectively. This is an initial stocking rate below the commonly used for these same conditions; however, this was a strategy necessary to ensure forage availability by the end of each cycle, when stocking rates were 1.2 and 1:47 for the first and second cycle respectively.

2.5. Handling

The animals were allowed to recover from the stress of transportation and to adapt to their new surroundings for a pre-trial period of 20 days for each of the two trial periods. All cattle had access to mineral supplementation (Zoorecria 60), protein supplementation (Suplemax 45 R, daily consumption of 100 g for each

Table 1
Mean live weight gain (kg) on each weighing day since the previous weighing day, final live weight gain since the day of treatment (day 0) (LWG1) and the difference between the Mean weight gain for each treatment protocol and the control group (LWG2) in Nelore cattle naturally infected with gastrointestinal nematodes submitted to the treatment protocols (T1–6).

Day/month	Weight gain (kg)											
	T1		T2		T3		T4		T5		T6	
	1C	2C	1C	2C	1C	2C	1C	2C	1C	2C	1C	2C
28/Jun	3.06	2.57	4.00	3.81	5.50	4.97	4.50	2.28	5.09	4.31	7.09	5.59
56/Jul	1.84	11.88	4.69	10.19	2.88	10.41	3.53	7.16	1.38	13.63	1.63	13.19
84/Aug	3.38	−0.59	6.53	1.66	8.97	3.31	8.67	4.69	13.11	2.56	5.91	3.75
112/Sep	−1.13	1.22	0.50	4.00	2.00	6.34	3.91	6.19	1.97	7.94	2.53	5.72
140/Oct	4.28	2.97	4.69	4.34	6.53	8.09	5.53	6.66	4.27	3.97	6.88	6.66
168/Nov	5.28	5.91	6.13	5.84	8.19	8.63	7.77	12.30	5.10	11.16	11.59	10.53
196/Dec	11.81	13.06	17.06	12.59	15.31	12.88	13.47	15.00	19.10	9.00	14.44	18.94
224/Jan	10.25	10.38	13.41	13.25	9.44	11.59	12.07	11.47	13.30	19.61	11.72	14.16
252/Jan	13.47	12.19	12.66	13.13	14.28	15.31	12.23	14.30	15.00	12.71	17.16	14.97
280/Feb	12.94	22.34	12.78	23.13	15.50	19.87	16.70	22.04	13.64	24.93	13.78	22.75
308/Mar	20.03	16.78	19.59	18.69	18.56	23.73	20.07	22.83	19.50	14.50	20.06	18.06
336/Apr	11.91	18.06	11.34	17.56	14.25	16.34	12.80	17.01	12.07	22.23	13.28	21.38
LWG1	97.13	116.76	113.38	128.19	121.41	141.47	121.33	141.91	123.18	146.55	126.06	155.69
LWG2	–	–	16.25	11.43	24.28	24.71	24.21	25.15	26.05	29.79	28.94	38.93

T1, dosages in May, July and September with a saline solution; T2, dosages in May and November with doramectin; T3, dosages in May (doramectin), July (levamisole) and September (doramectin); T4, dosages in May (doramectin), July (moxidectin) and September (doramectin); T5, dosages in May (doramectin), August (levamisole) and November (doramectin); T6, dosages in May (doramectin), August (moxidectin) and November (doramectin); 1C, first trial period, 2013–2014; 2C, second trial period, 2014–2015.

100 kg of live weight) with 45% crude protein during the driest periods of the year (July to October and August to December during the first and second trial periods, respectively) and drinking water *ad libitum* throughout the study period. The animals received a single topical treatment with fluazuron in July of the first trial period to control an infestation of the tick *Rhipicephalus* (*B.*) *microplus*.

This study was approved by the FAMEZ/UFMS Ethics Committee for use in animals identified under the protocol 437/2012.

2.6. Determination of weight and parasitic load

The animals were deprived of food and water for 12 h and then weighed on an electronic scale on days 0, 28, 56, 84, 112, 140, 168, 196, 224, 252, 280, 308 and 336. Faecal samples were collected every day directly from the rectum of all animals, weighed in labelled plastic bags and transported refrigerated to the UFMS/FAMEZ Parasitic Diseases Laboratory. The parasitic load was determined by the count of eggs per gram of feces (EPG) using a modified McMaster technique (Gordon and Whitlock, 1939) in a magnesium sulfate medium (Specific gravity = 1.200), with a sensitivity of 25 EPG. Fecal cultures were prepared, by experimental group, for the identification of the genera of gastrointestinal nematodes (Roberts and O'Sullivan, 1950).

2.7. Determination of the population of gastrointestinal nematodes on the pasture

Forage subsamples approximately 2 cm in height were collected at ground level at eight points in each paddock using a 0.5-m^2 quadrat and combined to obtain one representative sample per paddock. Samples were collected randomly every 28 days following a zigzag pattern (Taylor, 1939). The samples were immersed in buckets containing water for 24 h for sedimentation. Detergent was added to each bucket to decrease the surface tension and facilitate the detachment of third-stage larvae (L3's) from the forage. All forage in each bucket was then washed with water, and the bucket was kept for an additional 24 h at rest. The supernatant was removed, and the sediments were transferred to a sedimentation apparatus consisting of a 15-ml Falcon tube attached to a funnel. The total number of L3 was estimated from the count in the volume of the last stage of the dropping funnel and was expressed as L3's/m^2 of sampled area.

2.8. Statistical analysis

The data for animal weights, EPG counts and L3's for the experimental groups were first analyzed for variability, skewness and kurtosis and then for normality using the Shapiro-Wilk test. To meet the assumptions of parametric analyses, the EPG and L3 data were converted to the base-10 logarithm of (x + 1). The results were expressed as geometric means (GM) using the formula: GM = antilog {Mean [log 10 (x + 1)]} − 1.

The variabilities of the weight and transformed EPG and L3 data were analyzed using Duncan's tests for comparing the Means among the groups. The strength and direction of correlations between weight and EPG were evaluated using Pearson correlation coefficients. The transformed EPG data were analyzed using Student's *t*-tests. Regression models were estimated from the weight data and tested by an orthogonal polynomial analysis. Student's *t*-tests compared the models of each treatment protocol.

The 5% significance level was used for all analyses. All statistical analyses were based on those used by Zar (2010) and were performed using the Statistical Analysis System (SAS Institute, 2002). The anthelmintic effectiveness of each formulation was calculated as described by Torgerson et al. (2014). EPG counts pre- (day 0) and post- (day 28) treatments were compared using the R package "eggCount" version 2.0 (web interface to the R package eggCounts http://www.math.uzh.ch/as/index.php?id=254), which took into account the random sampling errors, the degree of aggregation between individuals within each group and the 95% confidence interval.

3. Results

The anthelmintic efficacies of doramectin, moxidectin and levamisole were 69.2, 65.9 and 69.4% in the first and 13.8, 92.6 and 76.5% in the second trial periods, respectively. The Mean weights of the animals at the beginning of the first and second trial periods were 153.3 and 191.5 kg, respectively. The Mean weight gains per experimental group relative to day 0 and to the immediately preceding weighing day are presented in Table 1. Only the untreated

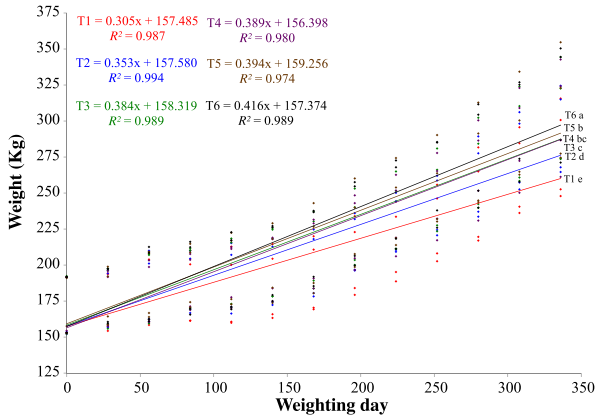

Fig. 1. Linear regressions of the effects of the treatment protocols for verminosis for the entire study period on the live weight in Nelore cattle naturally infected with gastrointestinal nematodes. Each dot represents an individual weight. Lines followed by the same letter in the same row do not differ significantly ($p < 0.05$).

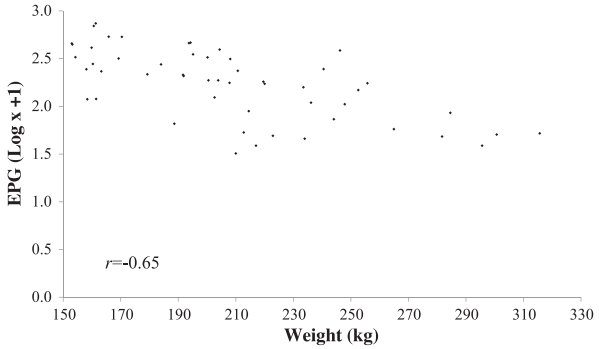

Fig. 2. Correlation between live weight (kg) and EPG (Log x + 1) for both trial periods in untreated (T1) Nelore cattle naturally infected with gastrointestinal nematodes.

animals lost weight during the dry season, specifically in September (mean −1.12 kg) and August (mean −0.59 kg) in the first and second trial periods, respectively. GIN caused no mortality or other clinical signs.

The effect of the first anthelmintic treatments (day 0) on live weight was not immediate, but performance increased ($p < 0.05$) from August for T3, T5 and T6 and from September for T2 and T4. All protocols with three annual dosages (T3, T4, T5 and T6) significantly increased live weights ($p < 0.05$) compared to the control group until the end of the study. Live weights did not increase in T2 (dosages in May and November) compared to the control group until March (day 308) (Table 2). All proposed treatment protocols except T2 thus provided higher final weights compared to the control group (T1).

The relationship between weight and weighing day for each treatment protocol is illustrated by regression lines in Fig. 1. Growth (live weight) was higher ($p < 0.05$) for the treated than the untreated animals, especially those treated in May (doramectin), August (moxidectin) and November (doramectin) (T6), indicated by higher slopes of the lines. The coefficients of determination (R^2) were >97%, indicating good fits of the proposed models describing the variations in weight among the treatment protocols.

The final weight gain (difference between the first and last day of weighing) did not differ significantly ($p > 0.05$) between the groups treated in May and November (T2, 120.8 kg); May, July and September (T3, 131.4 kg; T4, 131.2 kg) and May (doramectin), August (levamisole) and November (doramectin) (T5, 134.4 kg) (Table 2). The final weight gain for the treatment protocol with dosages in May (doramectin), August (moxidectin) and November (doramectin) (T6, 140.9 kg), however, was significantly higher ($p < 0.05$) than that for the treatment protocol with two annual dosages (T2).

The effect of the treatments on Mean EPG counts in the treatment protocols are shown in Table 3. The protocol with doramectin treatments in May and November (T2) decreased ($p < 0.05$) mean EPG only in June, 28 days after the first treatment, compared to the control group (T1). Mean EPG decreased ($p < 0.05$) in T3 and T4 compared to T1 in June, 28 days after treatment with doramectin, and in August, 28 days after treatment with levamizole (T3) and moxidectin (T4). T5 had a significant effect on Mean EPG only in September, 28 days after treatment with levamizole. EPG decreased significantly ($p < 0.05$) in June after treatment with doramectin in May and in September and November after treatment with moxidectin in August. None of the treatments administered in September decreased mean EPG significantly compared to the untreated animals.

T2, T3, T4 and T6 significantly decreased ($p < 0.05$) parasitic loads compared to the control group by 28 days after the first treatment with doramectin and in August for T3 after the second dosage with levamisole. Treatment with doramectin in May (day 0) in T5 did not significantly decrease ($p > 0.05$) the parasitic load in June (day 28), but the second dosage with levamisole in August significantly decreased the EPG counts in September, October and November. EPG decreased significantly in T6 ($p < 0.05$) in June after treatment with doramectin and in September after treatment with moxidectin and again in November. EPG counts did not differ significantly within each protocol from December (day 196) to the last day of assessment (day 336) (Table 3).

Live weight was negatively ($r = -0.65$) and significantly ($p < 0.05$) correlated with parasitic load (EPG) (Fig. 2). The faecal cultures contained *Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus* (Fig. 3). The number of recovered L3's on the pasture varied greatly over time, within and between the experimental groups, but did not differ significantly ($p > 0.05$) between the treatment protocols (Fig. 4), indicating that none of the proposed treatment models were able to significantly decrease the populations of parasites on the pasture.

4. Discussion

This study presented a practical approach for solving one of the major barriers in the strategic control of GIN in intensive beef

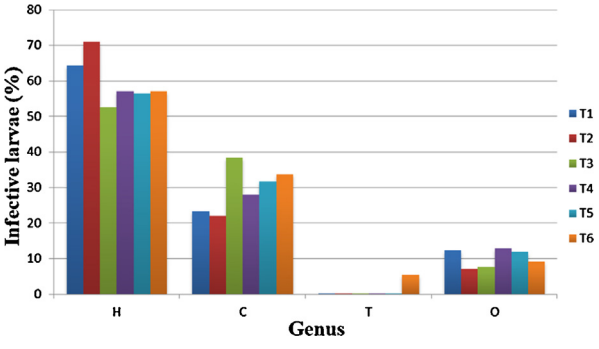

Fig. 3. Mean percentages of infective larvae for each treatment protocol in the stool cultures for animals naturally infected by gastrointestinal nematodes. H, *Haemonchus* sp.; C, *Cooperia* sp.; T, *Trichostrongylus* sp.; O, *Oesophagostomum* sp. T1, dosages in May, July and September with a saline solution; T2, dosages in May and November with doramectin; T3, dosages in May (doramectin), July (levamisole) and September (doramectin); T4, dosages in May (doramectin), July (moxidectin) and September (doramectin); T5, dosages in May (doramectin), August (levamisole) and November (doramectin); T6, dosages in May (doramectin), August (moxidectin) and November (doramectin).

Table 2
Effects of the six treatment protocols on the mean live weight (MLW) and final weight gain (WG) for both trial periods in Nelore cattle naturally infected with gastrointestinal nematodes.

Treatment	Cycle	0/May	28/Jun	56/Jul	84/Aug	112/Sep	14/Oct	168/Nov	196/Dec	224/Jan	252/Jan	280/Feb	308/Mar	336/Apr	Final WG
T1	1C	153.09	156.16	159.34	161.38	160.25	164.53	169.81	181.63	191.88	205.34	218.28	238.31	250.22	97.13
	2C	191.75	193.97	205.84	205.25	206.47	209.44	215.34	228.41	238.78	250.97	273.31	290.09	308.16	116.41
MLW		172.42^{a}	175.06^{a}	182.59^{a}	183.31^{b}	183.36^{c}	186.98^{c}	192.58^{c}	205.02^{c}	215.33^{c}	228.16^{c}	245.80^{c}	264.20^{c}	279.19^{c}	106.77^{c}
T2	1C	152.84	156.84	161.53	168.06	168.56	173.25	179.38	196.44	209.84	222.50	235.28	254.88	266.22	113.38
	2C	191.47	195.31	205.50	207.16	211.16	215.50	221.34	233.94	247.19	260.31	283.44	302.13	319.69	128.22
MLW		172.16^{a}	176.08^{a}	183.52^{a}	187.61^{ab}	189.86^{b}	194.38^{b}	200.36^{b}	215.19^{b}	228.52^{b}	241.41^{b}	259.36^{b}	278.50^{b}	292.95^{bc}	120.80^{bc}
T3	1C	152.41	157.34	160.78	169.75	171.75	178.28	186.47	201.78	211.22	225.50	241.00	259.56	273.81	121.41
	2C	191.97	196.94	207.34	210.66	217.00	225.09	233.72	246.59	258.19	273.50	293.23	317.09	333.44	141.47
MLW		172.19^{a}	177.14^{a}	184.06^{a}	190.20^{a}	194.38^{ab}	201.69^{a}	210.09^{a}	224.19^{ab}	234.70^{ab}	249.50^{ab}	267.12^{ab}	288.33^{ab}	303.63^{ab}	131.44^{ab}
T4	1C	153.50	157.96	161.43	170.05	173.88	179.37	187.20	200.63	212.54	224.70	241.21	261.23	273.92	120.42
	2C	191.50	193.78	200.94	205.63	211.81	218.47	230.22	245.66	257.19	271.53	293.44	315.97	333.41	141.91
MLW		172.50^{a}	175.87^{a}	181.18^{a}	187.84^{ab}	192.85^{ab}	198.92^{ab}	208.71^{a}	223.14^{ab}	234.86^{ab}	248.12^{ab}	267.32^{ab}	288.60^{ab}	303.66^{ab}	131.17^{ab}
T5	1C	155.16	161.36	165.33	172.39	174.49	178.99	183.97	203.13	216.29	231.08	244.26	263.73	275.96	120.80
	2C	191.19	195.50	209.13	211.69	219.63	223.59	234.75	250.00	264.05	276.95	302.05	317.29	338.79	147.97
MLW		173.17^{a}	178.43^{a}	187.23^{a}	192.04^{a}	197.06^{a}	201.29^{a}	209.36^{a}	226.57^{a}	240.17^{a}	254.01^{a}	273.16^{a}	290.51^{ab}	307.38^{ab}	134.39^{ab}
T6	1C	152.97	160.06	161.69	167.59	170.13	177.00	188.59	203.03	214.75	231.91	245.69	265.75	279.03	126.06
	2C	191.72	197.31	210.50	214.25	219.97	226.63	237.16	256.09	270.25	285.22	307.97	326.03	347.41	155.69
MLW		172.34^{a}	178.69^{a}	186.09^{a}	190.92^{s}	195.05^{ab}	201.81^{a}	212.88^{a}	229.56^{a}	242.50^{a}	258.56^{a}	276.83^{a}	295.89^{a}	313.22^{a}	140.88^{a}

T1, dosages in May, July and September with a saline solution; T2, dosages in May and November with doramectin; T3, dosages in May (doramectin), July (levamisole) and September (doramectin); T4, dosages in May (doramectin), July (moxidectin) and September (doramectin); T5, dosages in May (doramectin), August (levamisole) and November (doramectin); T6, dosages in May (doramectin), August (moxidectin) and November (doramectin). Mean live weights (kg) followed by the same letter in the same row do not differ significantly in a Duncan's test ($p < 0.05$). CV, coefficient of variation; 1C, first trial period, 2013–2014; 2C, second trial period, 2014–2015.

cattle production. Previous recommendations indicated that three anthelmintic dosages annually (May, July and September) may be enough to efficiently control this disease (Bianchin, 1991). The anthelmintic treatment of animals at times that do not coincide with normal handling at rural properties in central Brazil, such as in July and September, however, confer operational limitations and reduced productivity due to increased stress caused by additional handling (Borges et al., 2015).

The reduced efficacy of anthelmintic formulations used in beef cattle in the current scenario of anthelmintic resistance worldwide has become a limiting factor to the adequate control of parasitic gastrointestinal nematodes and to an increase in productive performance. Fazzio et al. (2014) in Argentina and Sutherland and Leathwick (2011) in New Zealand have shown that the failure of anthelmintic treatment, especially due to the ineffectiveness of the formulation used, may cause the loss of up to 10 and 14 kg per animal, respectively, even when *Cooperia* is the primary genus. Nevertheless, our study found significant increases in animal performance (weigh gain) even with the use of anthelmintics with reduced efficacy (<95%, Coles et al., 1992). These results corroborate those by Borges et al. (2013) who reported an increase in performance (11.85 kg) of Nelore calves after treatment with an anthelmintic with 84% efficacy (2.25% ivermectin + 1.25% abamectin). They also reported, however, that the use of anthelmintic formulations with efficacies below 50% (3.15% ivermectin, 3.5% doramectin and 1% doramectin) did not increase performance.

The protocol schedule with treatments in May and November, which is used by 80% of rural properties in Brazil (Bianchin, 1991), did not increase productivity compared to the other protocols. This result thus demonstrated the need for intermediate and additional dosages to promote growth in animals during the growing phase and to provide a longer period of protection against re-infection. The use of a long-term (moxidectin) and short-term (levamisole) residual molecule, however, did not influence the final weight gain; the live weights of all animals receiving three anthelmintic treatments during the year did not differ significantly at the end of the study ($p > 0.05$).

Although the animals of all treatment protocols, except T5 (dosages in May, August and November), presented significantly

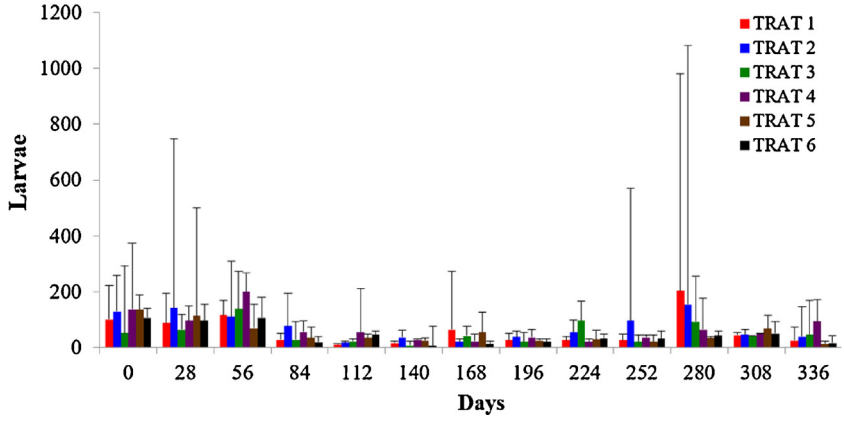

Fig. 4. Effect of the treatment protocols for gastrointestinal verminosis in Nelore cattle naturally infected with gastrointestinal nematodes on environmental contamination with the free-living stages on the pasture (Log x + 1).
T1, dosages in May, July and September with a saline solution; T2, dosages in May and November with doramectin; T3, dosages in May (doramectin), July (levamisole) and September (doramectin); T4, dosages in May (doramectin), July (moxidectin) and September (doramectin); T5, dosages in May (doramectin), August (levamisole) and November (doramectin); T6, dosages in May (doramectin), August (moxidectin) and November (doramectin). Mean number of larvae/m^2 on the pasture did not differ significantly by Duncan's test ($p > 0.05$).

Table 3
Effect of the treatment protocols on Mean EPG counts obtained during the two trial periods (2013–2015) in Nelore cattle naturally infected with gastrointestinal nematodes.

Day/month	EPG counts (Mean)						
	T1	T2	T3	T4	T5	T6	CV
0/May	307.52^{a}	240.10^{a}	254.70^{a}	309.75^{a}	326.84^{a}	227.00^{a}	3.967
28/Jun	361.19^{a}	162.08^{b}	131.66^{b}	136.58^{b}	242.25^{ab}	173.45^{b}	8.150
56/Jul	180.67^{a}	146.50^{a}	195.87^{a}	163.81^{a}	179.47^{a}	180.47^{a}	11.093
84/Aug	150.94^{ab}	236.92^{a}	15.43^{c}	52.73^{b}	235.77^{a}	188.84^{a}	16.979
112/Sep	264.60^{ab}	318.46^{a}	85.59^{abc}	60.88^{bc}	12.19^{d}	29.28^{dc}	22.522
140/Oct	256.45^{a}	303.93^{a}	137.12^{ab}	87.40^{ab}	49.62^{b}	94.80^{ab}	16.646
168/Nov	287.33^{a}	203.81^{ab}	144.20^{abc}	173.74^{abc}	118.75^{bc}	79.58^{c}	9.984
196/Dec	106.67^{a}	101.13^{a}	134.55^{a}	58.83^{a}	63.08^{a}	69.85^{a}	9.381
224/Jan	126.88^{a}	72.67^{a}	64.72^{a}	84.73^{a}	42.62^{a}	34.78^{a}	16.366
252/Jan	225.26^{a}	112.48^{a}	90.43^{a}	154.51^{a}	84.80^{a}	64.47^{a}	16.852
280/Feb	65.51^{a}	38.99^{a}	62.39^{a}	53.39^{a}	29.94^{a}	22.32^{a}	27.483
308/Mar	96.14^{a}	65.71^{a}	72.53^{a}	60.09^{a}	31.77^{a}	39.43^{a}	20.946
336/Apr	79.16^{a}	67.48^{a}	40.32^{a}	29.10^{a}	52.67^{a}	20.68^{a}	20.999

T1, dosages in May, July and September with a saline solution; T2, dosages in May and November with doramectin; T3, dosages in May (doramectin), July (levamisole) and September (doramectin); T4, dosages in May (doramectin), July (moxidectin) and September (doramectin); T5, dosages in May (doramectin), August (levamisole) and November (doramectin); T6, dosages in May (doramectin), August (moxidectin) and November (doramectin). Mean EPG counts followed by the same letter in the same row do not differ significantly in a Duncan's test ($p < 0.05$). CV, coefficient of variation.

reduction in parasitic loads in June (day 28), there was no increase in live weight in any treated groups in the first three months compared to the control group, perhaps because the animals were still adapting to their new surroundings, were stressed from weaning or because the beginning of the trial coincided with the dry season, a period with lower quantity and quality of forage. These factors, combined with the intense and constant parasitism suffered by untreated cattle (T1), may have been the cause of the weight loss in September (−1.12 kg) and August (−0.59 kg) of the first and second trial periods, respectively, which reinforces the importance of the proper use of anthelmintics, especially during the dry season.

The higher weight gains of the treated calves compared to the untreated calves were similar to those reported by Pinheiro et al. (2000), who found that weight gains in calves treated for GIN could reach 50 kg/head more than untreated animals. Miller et al. (1992) reported that the use of anthelmintics in beef cattle may increase weight gains by 29.5 kg. Bianchin et al. (2007), Catto et al. (2009) and Cleale et al. (2004) reported increases of 33 and 23 kg in live weights and 0.59 kg/day (23%), respectively, in treated compared to untreated animals.

Melo and Bianchin (1977) showed that animals strategically treated four times during the year (mid-May, mid-July, mid-September and mid-December) with broad-spectrum anthelmintics gained significantly more weight (43.3 ± 11 kg) than untreated animals, representing a 20% difference in live weight. Bianchin et al. (1996) reported similar results, an increase of 41 kg in live weight in Nelore cattle in paddocks containing *B. brizantha* compared to control animals, reinforcing the current strategic recommendation of three treatments annually.

Live weight in our study was significantly negatively correlated ($r = -0.65$) with EPG counts, i.e. the higher the parasitic load, the lower the live weight. This result corroborated those by Borges et al. (2013) who also reported a weak ($r = -0.22$) negative and significant correlation between live weight and EPG. These correlations, however, can also be influenced by a variety of factors associated with the species of nematode or host or with the technique used to measure parasitic load (Coles et al., 2006). The results of several studies have thus differed from ours, finding no (Jorgensen et al., 1978; Nicolau et al., 2002) or a weak (Henriksen et al., 1976) correlation between EPG and weight. Furthermore, the correlation between parasitic loads and fecal egg counts can often be weak (Taylor et al., 2002), null (Fenerich et al., 1987) or even significantly positive (0.652) ($p < 0.05$) (Condi et al., 2009). Further studies of the interaction between fecal egg counts and worm load in the cattle are still needed, because these variations have not yet been fully clarified (Coles et al., 2006). The weight gain of infected calves may be an additional criterion for evaluating the patterns of control of GIN, but controlled field studies still need to be validated (Höglund et al., 2009, 2013).

We chose to use L3's/m^2 on the pasture, regardless of the intake of forage by the cattle and the L3's in the soil. This measure has the advantage of estimating only the L3's present in the forage and therefore the actual environmental challenge, without inferring what is or is not available for animal consumption, which measures of L3's/kg dry matter (DM) cannot when forage is collected at random (Taylor, 1939). L3/kg DM cannot properly express the number of infective L3's on the pasture (challenge), because it depends directly on forage quality (% DM), estimates of animal consumption (between 2 and 3% of their live weight in DM) and especially the technique used for collecting the forage, because animals do not follow a random pattern of consumption and may prefer certain species of forage in the same area, which can also influence the height of grazing (Bryan and Kerr, 1988).

This study demonstrated that the use of anthelmintics, even those with low efficacy, can, when used appropriately, increase the weight gain in Nelore calves during the growing phase compared to untreated animals. The treatment protocol with anthelmintic dosages in May, August and November using long-acting anthelmintics can increase weight gains by up to 34.1 kg (31.9%) compared to untreated animals. We also demonstrated that treatment with doramectin only during the same handling periods for vaccination against FMD in May and November did not increase final weight gains. An additional intermediate dosage during the dry season is thus necessary to obtain high levels of productivity (weight gain). None of the evaluated control protocols had any effect on the number of gastrointestinal nematode L3's on the pasture. The results of this study may improve the practical recommendations for the control of GIN in beef cattle raised in tropical and subtropical areas under conditions similar to those in this study, with treatments concentrated in May, August and November, compared to the current technical recommendation (May, July and September) and to the usual protocol used by farmers in the field (May and November).

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