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Gastrointestinal nematode control programs in yearling Nellore heifers: Analysis of fecal egg counts, weight gain and reproductive indices

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ABSTRACT

The objective of this study was to evaluate two different gastrointestinal nematode treatment regimens. Fecal egg counts (FECs), proportion of nematode genera, weight gain, body condition score and reproductive indices (estrous cyclicity, conception and pregnancy rates) were evaluated in yearling heifers after imposing two treatment regimens for gastrointestinal nematodes: T1 = 306 calves treated in May and November with 3.5 % doramectin (700 μg/kg) and August (Aug) with saline solution; and T2 = 307 calves treated in May with 3.5 % doramectin (700 μg/kg), in August with 1% moxidectin (200 μg/kg) and in November with 3.5 % doramectin (700 μg/kg). The animals were weighed, and feces were collected for conducting FECs and coproculture. There was imposing of three fixed-time artificial inseminations (TAIs), and estrous cyclic and pregnancy statuses were determined. *Cooperia* was the most frequent genus detected in both groups. Heifers of the T2, as compared to those in the T1 group, had fewer FECs in November ($P \leq 0.05$) and greater weight gain and average daily weight gain ($P \leq 0.05$) from August to November. There tended to be more heifers of the T2 than T1 group estrous cycling ($P = 0.07$) at the beginning of the breeding season as well as greater pregnancy rates ($P = 0.03$) and conception rates ($P = 0.03$) as a result of the second FTAI. The results indicate there is greater reproduction outcomes as a result of strategic control of gastrointestinal nematodes in yearling Nellore heifers using the T1 as compared with T2 treatment regimen.

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1. Introduction

Global human population growth places a high demand on the production of animal protein, particularly beef (FAO, 2018). Increased productivity in kilograms of meat produced per area occurs mainly in three ways: (i) increasing animal stocking rate per area (animals/hectare) (Itavo et al., 2007; Santo et al., 2009); (ii) supplemental feeding of females from weaning until first parturition, which occurs at an age of approximately 24 months, thereby advancing the production cycle of females by about 12 months, specifically for heifers of the Nellore breed (Baruselli et al., 2017; Cooke et al., 2018); or (iii) enhancing cattle genetic performance to address parasitic or infectious occurrences by pharmaceutical administration (Canton et al., 2018).

Gastrointestinal nematodes (GINs) constitute one of the largest problems for beef cattle productivity, especially in tropical and subtropical regions (Felippelli et al., 2014). The vast amount of literature regarding the detrimental effects of these parasites highlights the negative effects of parasitic infections on weight gain and feed conversion, as well as the death of animals with a large GIN burden, while the use of chemicals remains the most effective method of GIN control (Coles et al., 2006; Soutello et al., 2007; Stromberg et al., 2012). After evaluating different strategic treatment regimens for GINs in male beef cattle, Heckler et al. (2016) reported that treatments with macrocyclic lactones in May, August and November can result in increases in animal weight by as much as 20.1 kg in 12 months compared to the most commonly used GIN treatment regimens for beef cattle in Brazil (May and November).

To the best of our knowledge, however, there are no reports of research conducted where there were evaluations of female beef cattle from birth until first calving (F1) while assessing the effects of different treatment regimens for the control of helminths after weaning on fecal egg counts (FEC), body weight gain and reproductive variables. The current study, therefore, was conducted to determine if imposing treatment regimens for GINs in May (doramectin 700 μg/kg), August (moxidectin 200 μg/kg), and November (doramectin 700 μg/kg) is more efficacious than imposing of a treatment regimen in May (doramectin 700 μg/kg), and November (doramectin 700 μg/kg) by evaluating parasite FECs, body weight gain, body condition score and reproductive variables (estrous cyclicity, conception and pregnancy rates) in yearling Nellore heifers.

2. Material and methods

2.1. Ethical statement

There was approval of the procedures and methods used in this study from the Animal Use Ethics Committee of the Federal University of Goiás (certificate number: No. 104/16) in the city of Goiânia, Brazil, and the study was conducted in accordance with the ethical principles governing animal experimentation stipulated by the Brazilian National Animal Experimentation Council.

2.2. Study location, animals and feed

The present study was conducted from September 2016 to October 2018 at the São Marcelo ranch located in the municipality of Juruena, state of Mato Grosso, Brazil. Nellore females ($n = 613$) were evaluated from the time of birth (September and October 2016) until the birth of their first calves (F1) as yearling heifers, which occurred between August and October 2018.

Throughout the study, the animals were maintained on pastures of *Brachiaria brizantha*. From the time of birth (September and October 2016) to the time of weaning (May 2017), the diet consisted of milk, water, and pasture *ad libitum*. After weaning (May 2017) until confirmation of the total number of pregnancies (June 2018) among these yearling heifers, the heifers were fed Diet 1 with supplement at 0.5 % of their body weight (80.3 % corn bran, 12 % soy bran 1% protein nitrogen, 1.7 % urea, 4.0 % protein composed of mineral macro- and microelements and 1% calcium carbonate). From July to September 2018 (confirmation of birth of F1 calves), heifers were fed Diet 2 with supplementation at 0.5 % of their body weight (77.1 % corn bran, 15.2 % cotton bran, 1% protein nitrogen, 1.7 % urea, 4% protein composed of mineral macro- and microelements and 1% calcium carbonate).

2.3. Allocation of animals to treatments, paddocks, and different helminth control treatment regimens

The 613 animals used in the study were assigned after weaning to be maintained in five paddocks with there being 100–129 animals in each paddock. The heifers were naturally infected with gastrointestinal nematodes and had no history of anthelmintic treatment. Because the objective of the study was to evaluate the effect of different antiparasitic treatment regimens on production and reproduction indices (weight gain and reproductive indices), the animals assigned to the different treatment groups were maintained in the same paddocks throughout the experimental period based on the methodologies for conducting experiments of this type that was used in previous studies (Sá Filho et al., 2014; Gouvêa et al., 2018; Neves et al., 2020).

The female calves within each paddock were subjected to two different strategic treatment regimens, which were imposed during 2017 and 2018: T1 = 306 animals treated in May and November (Nov) with 3.5 % doramectin at a dose of 700 μg/kg (DOR) (Treo ® ACE – Zoetis, Brazil) and August (Aug) with saline solution; and T2 = 307 animals treated in May with DOR (Treo® ACE – Zoetis, Brazil), Aug with 1% moxidectin at a dose of 200 μg/kg (MOX) (Cydectin® - Zoetis, Brazil) and Nov with DOR (Treo® ACE – Zoetis, Brazil). There were the same number of heifers on which each treatment regimen was imposed in each paddock. The average stocking rate for each paddock immediately after weaning was 1.76 animal unit per hectare (AU/ha). At the end of the study, the average stocking rate was 2.59 AU/ha.

The criteria for allocating calves to the respective treatments within each paddock were based on the FEC and body weight of each animal at the start of the experimental period (20 days after weaning in May 2017), and the average weight gain of the heifer calves from birth until the time of initiation of the experimental period (May 2017). In addition, to treatments to prevent a large infestation by *Haematobia irritans* after weaning, the animals were administered insecticide-containing ear tags containing diazinon 6 g (Top Tag® - Zoetis, Brazil), which were placed in the left ear of each animal. The ear tags were replaced with new tags every 6 months until the end of the study.

2.4. Fecal egg counts (FECs) and coproculture

Fecal samples were collected from each animal in May, Aug and Nov (start of reproduction season) of 2017, and May, Aug and Nov of 2018. A modified McMaster technique was performed for the parasite diagnosis based on FEC count (Gordon and Whitlock, 1939). Sensitivity was 25 eggs per gram (EPG) of feces. After the FEC counting was completed, about 3 g of fecal sample from each animal that was determined to be positive for parasite infection were pooled from each treatment group to conduct coproculture (Roberts and ÓSullivan, 1950). The taxonomic criteria described by Keith (1953) and Costa (1982) were used for the identification of larvae (L_3).

2.5. Weighing and body condition scoring of Nellore females

The animals were weighed individually at birth (September and October 2016), weaning (May 2017), 20 days after weaning in May 2017 (onset of treatment), Aug 2017, Nov 2017 (initiation of treatment regimen for conducting fixed timed artificial insemination -FTAI [first -FTAI]), and in May, Aug and Nov of 2018. wt gain and daily weight gain were calculated for each animal considering differences in body weight during the study. The scales used for weighing the animals were previously tested and assessed for accuracy. The animals were fasted (food and water) for 12 h prior to the days on which weighing occurred.

To determine the possible effects of body condition score (BCS) on reproduction, a classification scale from 1 (emaciated) to 5 (obese) was used, based on criteria described by Ayres et al. (2009), for each heifer at the time of the first and second fixed-TAI.

2.6. Reproductive evaluation

2.6.1. Estrous cyclicity induction and evaluation, FTAI treatment regimen, resynchronizations of ovulation times and gestational diagnosis of yearling Nellore heifers

There was imposing on all yearling heifers an ovulation induction treatment regimen using the methods described by Rodrigues et al. (2014). Estrous cyclicity was evaluated using transrectal ultrasonic procedures with a 7.5-MHz linear probe (Chison 8200VET, Kylumax) on Day 0 of the treatment regimen for synchronization of ovulation times among heifers. The occurrence of estrous cycles was determined by the presence of the corpus luteum (CL) and follicle (FL) $\geq$ 8 mm in diameter (Rodrigues et al., 2014).

Heifers were submitted to a P4/E2 based FTAI treatment regimen using a $PGF_{2\alpha}$ analogue treatment 2 days before CIDR withdrawal using methods described by Meneghetti et al. (2009) and Noronha et al. (2020). The date artificial insemination was performed, paddock of the animals, identification of the inseminator, sire and the lot of the semen used for AI were recorded for each heifer. The semen from each bull (eight Nellore bulls) were distributed in equal amounts among the different inseminators ($n = 6$) for insemination of equal numbers of heifers in each paddock ($n = 5$).

There were two regimens imposed for resynchronization of timing of ovulations among the yearling heifers diagnosed to not be pregnant 30 days after the first and second FTAI. The treatment regimen for the resynchronizing of the time of ovulations to conduct the second and third FTAI was the same at that used for the synchronizing the timing of ovulation among heifers at the first FTAI.

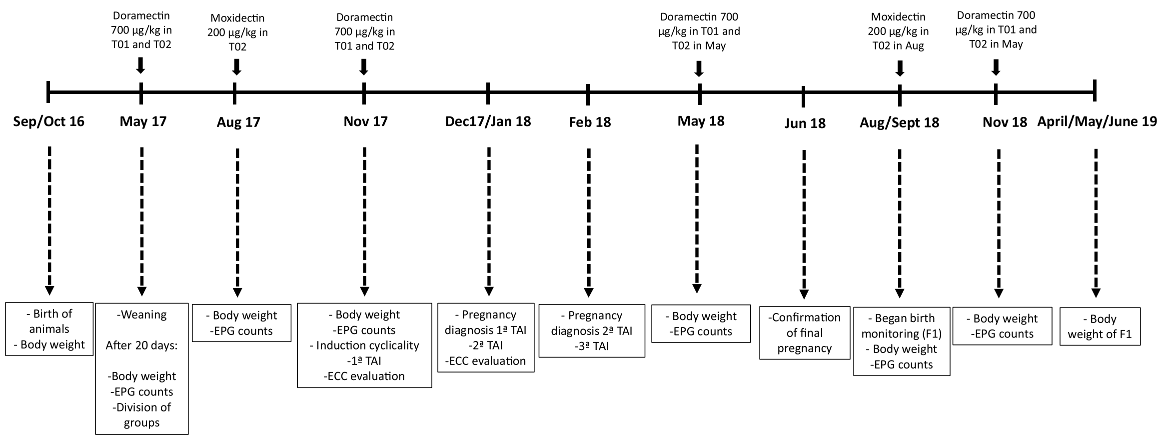

Fig. 1. Depictions of the primary procedures and methods used to conduct the study; Legend: T1: doramectin (700 µg/kg) in May and November.; T2: doramectin (700 µg/kg) in May, moxidectin (200 µg/kg) in August and doramectin (700 µg/kg) in November; EPG: eggs per gram; TAI: fixed-time artificial insemination; ECC: Body condition score.

Pregnancy was diagnosed using transrectal ultrasonic procedures with a 7.5-MHz linear probe (Chison 8200VET, Kylumax) 30 days after the after imposing the first FTAI treatment regimen (Day 41 of the experimental period) and the second FTAI (Day 82 of the experimental period) as well as 120 days after the third FTAI (Day 213 of the experimental period), the latter occurring in all animals.

The results of the pregnancy diagnosis made it possible to calculate the conception rate as a result of the first, second and third FTAI; gestational loss between the time of the first and second FTAI; and gestational loss during the experimental period and cumulative final pregnancy rates. In Fig. 1, there is depiction of the procedures conducted during the study. In Fig. 2, there is depiction of the methods used for imposing the reproductive control treatment regimens and pregnancy diagnoses.

2.7. Birth of F1 calves and body weight at weaning

The birth of F1 calves was determined during September, October and November of 2018, with calves being weighed at weaning in April, May and June of 2019.

2.8. Data analysis

2.8.1. FEC, body weight and weight gain of yearling Nellore heifers

The data for FECs were log transformed [ln (FEC + 1)] prior to analysis. Data were analyzed using a linear mixed model for repeated measures. The model included the fixed effects of treatment, day, and treatment compared with day interaction (Proc GLM, SAS Institute, 2016).

The individual weights of the animals were analyzed considering body weight, body weight gain and daily body weight gain that were determined before initiation of treatments (May 2017, 20 days after weaning) as covariates utilized to conduct the analysis of variance. Treatment means for both productive performance variables and FECs were compared using the F test (Proc GLM, SAS Institute, 2016). There were considered to be mean differences when there was a $P \leq 0.05$ and there was considered to be a trend for a mean difference when there was a $0.05 < P \leq 0.10$.

2.8.2. Reproductive evaluation of yearling Nellore heifers

Data were analyzed using the GLIMMIX procedure described in the SAS Enterprise Guide (Statistical Analysis System®, version 5.1, Inst. Inc., Cary, NC, 2016). A regression model was utilized determine whether there were associations between value for different variables and variables considered to be fixed effects, such as paddock, presence of CL, sire, AI technician, treatment and/or the interactions, were sequentially removed from the initial model based on Wald's criterion (P-value was > 0.20) and Akaike's information criterion (smallest value is best). There were considered to be differences when there was a $P < 0.05$ and there was considered to be a tendency for differences when there was $0.05 < P \leq 0.10$.

2.8.2.1. Estrous cyclicity and pregnancy rate at times of ultrasonography (P/AI - US) of yearling Nellore heifers. Estrous cyclicity rate was calculated as the number of heifers with a CL divided by the total number of heifers (N° of heifers with a CL/total N° of heifers). Proportion of heifers pregnant as a result of the first FTAI was calculated as the number of pregnant heifers divided by the total number heifers inseminated at the time of the first FTAI (N^{O} of pregnant heifers/N heifers inseminated at first FTAI). Proportion of heifers pregnant as a result of the second FTAI was calculated as the number of pregnant heifers divided by the total number of heifers inseminated at the time of the second FTAI (N of pregnant heifers/N heifers inseminated at the time of the second FTAI). Proportion of heifers pregnant as a result of the third FTAI was calculated as the number of pregnant heifers divided by the total number of heifers inseminated at the third FTAI (N of pregnant heifers/N heifers inseminated at third FTAI). Cumulative proportion of heifers pregnant was calculated as the sum of pregnant heifers as a result of all three FTAI divided by the total number of heifers included in the study (N° of pregnant heifers as a result of the first, second and third FTAI/total N° of heifers in the experimental group).

2.8.2.2. Pregnancy losses between ultrasonic assessments/end of breeding season and final cumulative pregnancy. Pregnancy losses when there was conception as a result of the first and second FTAI were determined based on the number of heifers that were pregnant at the time of the first or second ultrasonic diagnoses that were subsequently diagnosed as not being pregnant at end of the breeding season (BS). Overall pregnancy losses were determined as the sum of all pregnancy losses at end of BS (sum of non-pregnant heifers at the time of the third ultrasonic diagnosis for pregnancy that had previously been diagnosed pregnant as a result of the first or second FTAI).

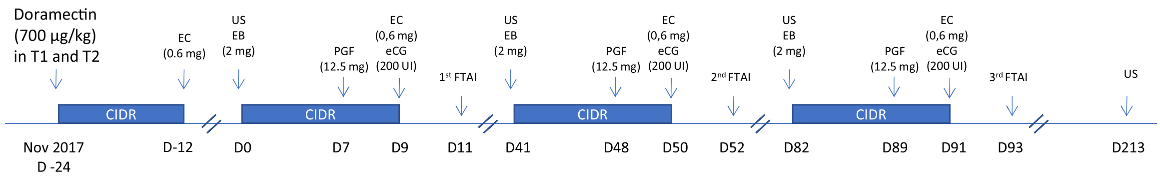

Fig. 2. Depictions of the treatment regimen for synchronization of the timing of ovulation among in yearling Nellore heifers; Legend: T1: doramectin (700 µg/kg) in May and November.; T2: doramectin (700 µg/kg) in May, moxidectin (200 µg/kg) in August and doramectin (700 µg/kg) in November; CIDR: T-shaped silicone P_4 intra-vaginal device containing 1.9 g of progesterone; EB: estradiol benzoate; EC: estradiol cypionate; eCG: equine chorionic gonadotrophin; FTAI: fixed-time artificial insemination; $PGF_{2\alpha}$: dinoprost tromethamine; US: transrectal ultrasonography.

Final cumulative proportion of heifers pregnant was determined based on the number of heifers that were still pregnant at the end of BS, on D 230.

3. Results

3.1. FEC, coproculture, body weight and weight gain of yearling Nellore heifers

Data for results of FECs performed on the 613 animals during the study are reported in Table 1. There were no differences ($P >$ 0.05) between treatment groups (T1 = DOR-May and DOR-Nov; T2 = DOR-May, MOX-Aug and DOR-Nov) for mean FECs conducted in May and August 2017. In Nov 2017, however, the heifers of the T2 group had a lesser mean FEC than those of the T1 group ($P \leq 0.05$). There were similar results during the year of 2018 (Table 1).

Results from conducting coprocutlure procedures for the heifers in the T1 group (DOR-May and DOR-Nov) indicated there were the following mean frequencies of nematode genera: 64 % *Cooperia*, 21 % *Haemonchus*, 2.3 % *Trichostrongylus* and 12.7 % *Oesophagostomum*. Results from conducting of coproculture procedures for the heifers of the T2 group (DOR-May, MOX-Aug and DOR-Nov) resulted in the following mean frequencies: 63 % *Cooperia*, 25.3 % *Haemonchus*, 1% *Trichostrongylus* and 10.7 % *Oesophagostomum*.

There were no differences ($P > 0.05$) throughout the study in average body weight of heifers of the T1 and T2 groups. From Aug 2017 (date at which T2 received MOX) to Nov 2017 (First FTAI), however, the heifers of the T2 group (DOR-May, MOX-Aug and DOR-Nov) had a greater average weight gain and average daily weight gain ($P \leq 0.05$) than those of the T1 group. Furthermore, from May 2017 (allocation of heifer calves to treatments) to Nov 2017 (First FTAI), the heifers in the T2 group tended ($P = 0.06$) to have a greater average weight gain and average daily weight gain than those of the T1 group (Table 2).

3.2. Reproductive evaluation

Data for mean age at the beginning of the study/reproductive season, body weight at the onset of treatment and BCS for yearling heifers at the time of the first and second FTAI are summarized in Table 2. There were no differences ($P > 0.05$) in values for these variables between treatment groups.

There tended ($P = 0.07$) to be an effect of treatment regimen on heifer estrous cyclicity at the beginning of the experimental period. There were more yearling heifers of the T2 group (85.7 %, 263/307) that were estrous cycling at the beginning of the breeding season than those of the T1 group (81.4 %, 249/306; Table 3). The heifers of the T2 group (DOR-May, MOX-Aug and DOR-Nov) had a greater conception rate (35.7 %, 61/171) than those of the T1 group (DOR-May and DOR-Nov: 26.4 %, 46/174) as a result of the second FTAI ($P = 0.03$), whereas there was no effect of the type of treatment regimen on conception rate at the first FTAI (T1 = 43.1 %, 132/306; T2 = 44.3 %, 136/307; $P = 0.50$) or third FTAI (T1 = 25.0 %, 32/128 and T2 = 23.6 %, 26/110; $P = 0.90$) (Table 3).

Treatment regimen also had an effect on pregnancy loss rate ($P = 0.03$) after there was conception as a result of the second FTAI (T1 = 20.1 %, 35/174; T2 = 30.4 %, 52/171); however, there was no treatment regimen effect when there was conception as a result of the first FTAI (T1 = 32.4 %, 99/306; T2 = 33.9 %, 104/307). Considering only yearling heifers that were pregnant at end of breeding season, the overall proportion of heifers pregnant was 56.8 % with a tendency ($P = 0.08$) toward more pregnant yearling heifers in the T2 (DOR-May, MOX-Aug and DOR-Nov; 59.3 %, 182/307) compared to the T1 (DOR-May and DOR-Nov; 54.2 %, 166/306) group. There, however was not this tendency for a treatment regimen effect on overall pregnancy losses ($P = 0.14$; Table 3). There was no effect ($P > 0.05$) of paddock in which heifers were maintained, BCS, inseminator, sire or semen batch on the results.

3.3. Number of births and body weight of F1 calves at weaning

During the study, 166 calves were born from yearling Nellore heifers of the T1 (DOR-May and DOR-Nov) group and 182 calves were born from heifers of the T2 (DOR-May, MOX-Aug and DOR-Nov) group. There was no difference ($P > 0.05$) in body weight of F1 calves at the time of weaning from cows in which the two treatments were imposed on their dams when dams were heifers (Table 3).

Table 1
Means (± SD) of EPG (eggs per gram) counts of yearling Nellore heifers on which there was imposed two different treatment regimens for control helminth infection.

Study stage	Experimental treatments /Mean[1] EPG counts ± Standard deviation								Coefficient of variation	Value of P
	T1*				T2**					
May 2017	111.58	±	141.30	a	109.20	±	159.01	a	80.62	0.1399
August 2017	91.37	±	132.98	a	89.55	±	132.06	a	103.97	0.6864
November 2017	111.31	±	155.74	a	60.58	±	15.29	b	104.91	0.00001
May 2018	72.47	±	116.87	a	75.69	±	149.04	a	98.32	0.9457
August 2018	69.23	±	97.67	a	61.23	±	95.56	a	102.54	0.7845
November 2018	78.78	±	126.17	a	25.53	±	19.29	b	134.02	0.00001

* T01: doramectin (700 μg/kg) in May and November; and August (Aug) with saline solution.
** T02: doramectin (700 μg/kg) in May, moxidectin (200 μg/kg) in August and doramectin (700 μg/kg) in November.
[1] Mean values followed by the same letter on the same line do not differ as a $P > 0.05$.

Table 2
Mean (±SD) for body weight and reproduction variables evaluated in yearling Nellore heifers on which there were two different treatment regimens imposed to control helminth infection.

Study stage	Variable	Experimental treatments/Mean[1]								$P > F$[2]	CV[3]
		T1*				T2**					
May 2017 - Allocation of animals to groups		264.8		±	18.3	265.0		±	17.7	–	–
Estrous cyclicality induction	Age in days	409.3		±	19.5	409.8		±	23.6	–	–
1st TAI[4]		433.5		±	19.6	432.5		±	25.3	–	–
September to October 2016 – birth		30.4	a	±	4.0	30.3	a	±	4.0	0.758	13.10
May 2017 – Weaning		190.5	a	±	19.4	190.5	a	±	19.4	0.834	10.19
May 2017 - Allocation of animals to groups	Body weight (kg)	201.6	a	±	20.1	201.6	a	±	20.1	0.988	9.97
August 2017		248.0	a	±	24.2	247.0	a	±	24.2	0.599	9.77
November 2017–1st TAI		271.4	a	±	24.3	273.7	a	±	23.5	0.251	8.78
May 2018		351.0	a	±	31.6	354.0	a	±	30.3	0.162	7.65
August 2018 (beginning of births)		340.1	a	±	33.5	344.4	a	±	38.1	0.241	11.23
November 2018 (end of births)		354.8	a	±	47.0	358.9	a	±	51.5	0.624	6.62
Birth (October and November 2016) until the allocation of animals to groups (May 2017)		171.2	a	±	18.7	171.3	a	±	18.9	0.935	10.95
Birth (October and November 2016) the 1st TAI (November 2017)		241.1	a	±	23.5	243.4	a	±	23.0	0.217	9.60
May 2017 until August 2017		46.4	a	±	10.9	45.4	a	±	11.2	0.240	24.11
August 2017 until November 2017–1st TAI	Weight gain (kg)	23.4	b	±	10.1	26.7	a	±	10.1	<0.001	40.28
May 2017 until November 2017–1st TAI		69.9	a	±	14.5	72.1	a	±	14.7	0.063	20.58
Novermber 2017 until May 2018		79.3	a	±	16.5	80.3	a	±	16.8	0.354	27.56
May 2018 until August 2018		−10.9	a	±	46.8	−9.6	a	±	52.4	0.421	35.24
August 2018 until November 2018		14.7	a	±	16.8	14.5	a	±	18.6	0.465	12.35
May 2017 until November 2018		153.16	a	±	28.1	157.28	a	±	22.8	0.101	32.35
Birth (October and November 2016) until the allocation of animals to groups (May 2017)		0.81	a	±	0.082	0.81	a	±	0.083	0.935	10.95
May 2017 until August 2017		0.52	a	±	0.122	0.50	a	±	0.124	0.240	24.11
August 2017 until November 2017–1st TAI	Daily weight gain (kg)	0.26	b	±	0.112	0.30	a	±	0.113	<0.001	40.28
May 2017 until November 2017–1st TAI		0.39	a	±	0.081	0.40	a	±	0.082	0.063	20.58
Novermber 2017 until May 2018		0.44	a	±	0.021	0.44	a	±	0.032	0.354	27.56
May 2018 until August 2018		−0.12	a	±	0.046	−0.10	a	±	0.063	0.421	35.24
August 2018 until November 2018		0.15	a	±	0.021	0.16	a	±	0.041	0.465	12.35
May 2017 until november 2018		0.27	a	±	0.014	0.28	a	±	0.032	0.101	32.35
November 2017–1st FTAI	Body condition	3.40	a	±	0.161	3.44	a	±	0.121	0.975	9.88
January 2018 – 2nd FTAI	score (ECC)	3.47	a	±	0.116	3.34	a	±	0.269	0.962	7.12
Total births (F1) between August and October 2018	Total of births	166				182				–	
Body weight of births (F1) in the weaning – May 2019	Body weight (kg)	165.3	a	±	26.4	166.2	a	±	27.2	0.901	8.88

* T01: doramectin (700 μg/kg) in May and November; and August (Aug) with saline solution.
** T02: doramectin (700 μg/kg) in May, moxidectin (200 μg/kg) in August and doramectin (700 μg/kg) in November.
1 Means values followed by the same letter on the same line, for each parameter, do not differ significantly at a 95 % reliability level.
2 Test significance probability.
3 Coefficient of variation.
4 FTAI: fixed-time artificial insemination.

Table 3
Mean percentage for pregnancy variables for yearling Nellore heifers on which there were two different treatment regimens imposed to control helminth infection.

Reproductive variable analyzed	T1*	T2**	Value of P
Estrous cyclicity	81.4 % (249/306)	85.7 % (263/307)	0.07
Pregnancy US[1] 1st FTAI[2]	43.1 % (132/306)	44.3 % (136/307)	0.50
Final pregnancy 1st FTAI	32.4 % (99/306)	33.9 % (104/307)	0.79
Pregnancy loss 1st FTAI	25.0 % (33/132)	25.3 % (32/136)	0.78
Pregnancy US 2nd FTAI	26.4 % (46/174)	35.7 % (61/171)	0.03
Final pregnancy 2nd FTAI	20.1 % (35/174)	30.4 % (52/171)	0.03
Pregnancy loss 2nd FTAI	23.9 % (11/46)	14.8 % (9/61)	0.12
Pregnancy US 3rd FTAI	25.0 % (32/128)	23.6 % (26/110)	0.90
Cumulative pregnancy US	68.6 % (210/306)	72.6 % (223/307)	0.14
Final accumulated pregnancy	54.2 % (166/306)	59.3 % (182/307)	0.08
General pregnancy loss	24.7 % (44/178)	20.8 % (41/197)	0.14

* T1: doramectin (700 μg/kg) in May and November; and August (Aug) with saline solution.
** T2: doramectin (700 μg/kg) in May, moxidectin (200 μg/kg) in August and doramectin (700 μg/kg) in November.
1 US: Transrectal ultrasonograph.
2 FTAI: fixed-time artificial insemination.

4. Discussion

For 30 years, the T1 treatment has been used on approximately 80 % of the cattle properties in Brazil (Bianchin, 1991). The results from the present study, however, as well as from other studies (Bianchin et al., 1996, 2007; Heckler et al., 2016), indicate there is a lack of efficacy of this treatment (*i.e.*, T1 treatment regimen in the present study) compared with the T2 treatment used in the present study which was more efficacious. It is important to highlight that, in the current study, the treatment regimen that included an additional annual administration of MOX in Aug, in addition to DOR in May and Nov (T2), resulted in a greater heifer weight gain and reproductive indices than heifers that were only administered DOR in May and Nov (T1). It, however, is not possible to ascertain whether the beneficial effects, mainly for weight gain and reproductive variables, resulted in Nellore heifers on which there were three annual treatments (DOR-May, MOX-Aug and DOR-Nov) were due to (i) the additional treatment in Aug, (ii) the action of moxidectin, or (iii) of both factors simultaneously. Future studies must be conducted to ascertain the reason for the results in the present study. Results from other studies have indicated that the mode of action of moxidectin is similar to that of the avermectins, however, moxidectin has a greater efficacy against GINs in cattle (Prichard et al., 2012. Lopes et al., 2014). The relatively lesser efficacy of moxidectin (200 μg/kg) has only been reported for *Oesophagostomum radiatum* (Condi et al., 2009; Lopes et al., 2014).

Helminth resistance to anthelmintic drugs is associated with several factors, mainly the frequency and length of time that a particular formulation is used on a specific property for treatment of cattle (Borges et al., 2008; Prichard et al., 2012). It, therefore, is possible that helminth resistance to moxidectin occurs more quickly when there are three treatments annually with this compound. Other questions, however, need to be answered regarding helminth resistance to moxidectin: (i) How many years can the compound be used and have optimal efficacy, considering that it will be administered to the animals once a year and only in animals between 7 and 16–20 months of age? (ii) Would helminths in cattle from the same property that were not treated with moxidectin maintain susceptibility to moxidectin while "in refugia"? (iii) Should the treatment regimen for strategic control of helminths be avoided considering that moxidectin will be administered to cattle in some age groups (weaning to 20 months of age) and only once a year? (iv) How long will moxidectin formulation be effective, even though it has already been classified as being ineffective in international publications? (Wood et al., 1995; MAPA 1997; Vercruysse et al., 2001; VICH, Guideline 12, 2021: Bovine; Vercruysse et al., 2011). Considering these questions, field studies are needed to ascertain how quickly this additional treatment with moxidectin can contribute to development of helminth resistant populations to moxidectin.

Results from previous studies indicate there is greater body weight gain during the post-weaning period when there are endocrine and physiological changes that result in maturation of the reproductive processes that result in pubertal onset (Lammers et al., 1999; Quintans et al., 2004; Gasser et al., 2006a,b). Likewise, an improvement in nutritional status contributes to increased concentrations of IGF-1, glucose and leptin as well as greater weight, and the increased likelihood of females being pubertal with resulting greater conception rates as compared with heifers having dietary intakes of lesser quality (Cardoso et al., 2014; Samadi et al., 2014; Freitas, 2015). In the present study, the additional treatment with MOX in Aug led to greater body weight gains, which could explain the increase in reproductive indices and lesser gestational losses. Diaz-Torga et al. (2001) reported that when there was treatment with ivermectin (200 μg/kg) every 14 days until heifers weighed 150 kg, followed by administration of an intraruminal bolus of 1.72 g every 4 months resulted in an earlier age at puberty in heifers that were 29.3–39.7 weeks of age. It is important to highlight that the body weight and weight gain that occurred in the present study for Nellore heifers after heifers were pubertal (Nov 2018) should be interpreted with caution, because these body weight indices were affected by stage of gestation at the time weights were determined.

The greater conception and pregnancy rates as a result of the first FTAI compared with these rates as a result of the second and third FTAIs have been previously reported in yearling heifers as well as primiparous and multiparous females (Baruselli et al., 2017; Colli et al., 2017). A possible explanation for this may be related to the fact that females that have the capacity to conceive as a result of the first FTAI (beginning of the breeding season) are generally physiologically more mature, even when in a moderate body condition. In contrast, animals that conceive as a result of the second or third FTAI are those that matured physiologically and develop the capacity to reproduce after the time when the first FTAI occurred. Byerly and Staigmiller (1987) reported that physiological maturity of heifers resulted in greater pregnancy rates and that this increase likely resulted from a lesser negative feedback of 17β-estradiol (E_2) at the hypothalamus leading to an increase in GnRH/LH secretion resulting in greater ovarian development (Anderson et al., 1996). In 11-month-old taurine beef heifers treated with implants containing norgestomet, the hypothalamic sensitivity to E_2 in inhibiting release of GnRH is greater than in heifers aged $\geq$ 11 months, resulting in heifers remaining prepubertal (Hall et al., 1997). These findings may help explain the results from the present study, in which the greater conception and pregnancy rates occurred as a result of the second FTAI in heifers treated with DOR-May, MOX-Aug and DOR-Nov treatment regimen. It is possible that imposing this treatment regimen resulted in a greater physiologic development of heifers that were less mature at the beginning of the breeding season (first FTAI). The outcomes from imposing treatment regimens that effect the growth pattern of prepubertal heifers on reproductive efficiency of yearling Nellore heifers is still poorly understood and further studies are certainly needed to gain further insights in these regards.

Both cumulative conception and pregnancy rates in the present study were similar to values reported previously when there was imposing of two FTAI treatment regimens ($\approx$ 70 % for accumulated conception rate and $\approx$ 60 % for the cumulative pregnancy rate) (Rodrigues et al., 2014; Marques et al., 2015; Colli et al., 2017). Furthermore, the cumulative pregnancy loss rate that occurred in the present study (22.8 %) was greater than the rate for ($\approx$ 3.0 %) for primiparous and multiparous cows (Rhinehart et al., 2009; Aono et al., 2013). This was possibly due to the present study being conducted with yearling Nellore heifers). The greater value for pregnancy losses in the present study than most previous studies could be associated with the relatively later timing of pregnancy diagnosis as compared with that in most previous studies for heifers conceiving at the first and second FTAI. The timing of these pregnancy loss detections at day 230 of the present experiment, therefore, may have resulted in there being the greater pregnancy loss value in the present experiment similar to what has been previously reported (Reese et al., 2020). Freitas (2015) reported that the gestational loss rate in yearling Nellore heifers is greater than that for primiparous and multiparous cows and ranges from 12.5% to 22.8%, similar to that in the present study.

5. Conclusions

The imposing of the helminth control treatment regimen that included administrations of DOR-May, MOX-Aug and DOR-Nov to yearling Nellore heifers led to greater growth rate and reproductive performance than when there was administration of DOR in both May and Nov. The most common nematodes in the animals were of the genus *Cooperia*. The heifers on which the DOR-May, MOX-Aug and DOR-Nov annual treatment regimens were imposed had lesser FECs, greater body weight gain and average daily weight gain when there was treatment with moxidectin (200 μg/kg) in August, until the time of initiation of the breeding season. Furthermore, yearling Nellore females treated with moxidectin had an earlier estrous cyclicity and greater conception and pregnancy rates at the second FTAI as well as a greater pregnancy rate at the end of the breeding season and, consequently, there were more F1 calves produced by these females. Future studies should be conducted to determine whether the differences in responses as a result of the treatments were due to the additional moxidectin treatment conducted in Aug or due to the combination of treatments imposed.

Author agreement form

This Form should be signed by all authors OR by the corresponding (or senior) author who can vouch for all co-authors. A scanned copy of the completed Form may be submitted online. The authors confirm the following statements: 1 that there has been no duplicate publication or submission elsewhere of this work 2 that all authors have read and approved the manuscript, are aware of the submission for publication and agree to be listed as co-authors

Declaration of Competing Interest

The authors declare no conflict of interest.

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