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Worm replacement with susceptible *Haemonchus contortus* benefits weight gain, reduces anthelmintic treatments and impacts sheep breeds differently

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ABSTRACT

This study evaluated the effect of partial and total replacement of *Haemonchus contortus* in sheep breeds. Pregnant ewes of White Dorper (DO), Santa Inês (SI) and Texel (TX) breeds were allocated into three groups: Control (C), Partial Replacement (PR) and Total Replacement (TR). PR and TR ewes received anthelmintics (AH), were artificially infected with *H. contortus*-susceptible isolate and grazed on resistant-infested or worm-free paddocks, respectively. Control animals were untreated and naturally infected. 106 lambs were born and kept in the paddocks of their respective mothers. Their egg count per gram of feces (FEC) and packed cell volume (PCV) were recorded every 21 days, until 189 days old. Fecal Egg Count Reduction Test revealed AH efficacy of 85 %, 92 % and 97 % in the C, PR and TR groups, respectively, for ewes and 60 %, 74 % and 98 %, respectively, for lambs at day 147. SI animals ($p < 0.001$) received fewer AH treatments and presented a higher PCV than DO and TX, reaching similar weight gain to the other breeds. Male lambs ($p < 0.001$) presented a higher FEC, lower PCV and lower frequency of Famacha score 1. Higher age at weaning resulted in a lower FEC ($p = 0.0073$), higher PCV ($p = 0.002$), and higher frequencies of Famacha 1 and body condition scores 3 and 4. AH treatment was more efficient after worm replacement, reducing FEC ($p < 0.001$) and favoring weight gain in the PR and TR groups. Avoiding early weaning, adopting selective AH treatments and using more resistant sheep breeds may delay the reestablishment of resistance after worm replacement.

1. Introduction

Gastrointestinal nematode infection poses a major threat to the health, welfare and productivity of sheep farms worldwide (Naeem et al., 2021; Chagas et al., 2022; Mohamed et al., 2024). *Haemonchus contortus* is the most prevalent species of gastrointestinal nematodes (GIN) and has a large negative impact on sheep and goat production in tropical and subtropical regions (Amarante, 2014). *H. contortus* is a hematophagous nematode that lodges in the abomasum, causing anemia, hypoproteinemia, submandibular edema, lethargy and death in susceptible hosts (Sanders et al., 2020).

The main strategy for parasite control in sheep is based on anthelmintic (AH) treatments. The widespread and frequent use of these AHs causes selection pressure favoring parasite resistance (Kaplan, 2004), especially when the AH is administered to all animals in the flock. The offspring of adult parasites that survive such treatments prevail and the population become more resistant as more treatments are applied, leading to treatment failure (Jackson and Coop, 2000; Besier, 2012; Falzon et al., 2014). The sustainability of small ruminant production systems is threatened by the expansion of resistance to multiple anthelmintics (Kaplan and Vidyashankar, 2012; Albuquerque et al., 2017; Soares et al., 2023; Bassetto et al., 2024).

To slow the development of anthelmintic resistance, Leathwick and Besier (2014) suggested producers to implement strategies based on preserving *refugia*, using combinations of highly effective anthelmintics and avoiding the introduction of resistant nematodes into the flock. *Refugia* are parasites that have not been exposed to AHs, including free-living stages in the environment and parasites in non-treated hosts.

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To increase the parasite population in *refugia*, targeted selective treatment (TST) of the most parasitized animals in the flock is an important and useful tool (Besier, 2012).

A strategy to restore AH efficacy in small ruminants is the replacement of parasite populations, called worm replacement (W*R*). Van Wyk and Van Schalkwyk (1990) developed a system to replace a resistant population with a susceptible one, by eliminating the resistant parasites and artificially introducing susceptible parasites. Studies conducted in different regions of the world, such as South Africa (Van Wyk and Van Schalkwyk, 1990), USA (Bird et al., 2001; George et al., 2021), Ethiopia (Sissay et al., 2006), France (Moussavou-Boussougou et al., 2007), and Argentina (Fiel et al., 2017; Muchiut et al., 2019; Muchiut et al., 2022), have produced promising results, encouraging the continuation of such research.

W*R* is based on the principle of dilution of anthelmintic resistance, restoring AH efficacy and, consequently, reducing the frequency of treatments. Despite being a sustainable pest control strategy, W*R* is still a challenging undertaking. As a consequence of the seriousness of anthelmintic resistance (Veríssimo et al., 2012; Bassetto et al., 2024), the control of helminthiasis based only on anthelmintic treatment has become impractical and may even make sheep production unfeasible (Kaplan and Vidyashankar, 2012; Chagas et al., 2022). As there is no fully effective alternative against GIN infection, farms still rely on the use of different control techniques that in combination may diminish the impact of GIN on sheep production. Thus, this study aimed to follow the effects of reverting anthelmintic resistance in different (White Dorper, Santa Inês and Texel) sheep breeds after W*R*. Since these breeds present different levels of haemonchosis resistance, our research may contribute to generate better understanding of their performance in the context of animal production under high parasitic pressure, typical of tropical countries.

2. Material and methods

2.1. Experimental design

The study was conducted at the experimental farm of Embrapa Pecuária Sudeste (CPPSE), located in São Carlos, SP, Brazil (21°57' S, 47°50' W, 860 m) from February 2022 to March 2023. The climate is classified as Cwa (Köppen) with two well-defined seasons: dry season (April to September) and rainy season (October to March).

For worm replacement, a susceptible *H. contortus* isolate with 100 % susceptibility to ivermectin and levamisole, 98.9 % sensitivity to albendazole (Echevarria et al., 1991) and no prior exposure to monepantel was used. This Echevarria susceptible isolate, preserved in liquid nitrogen, was thawed and used to infect sheep hosts kept in GIN-free pens to obtain infective larvae (L_3) for artificial infections.

Santa Inês (SI), Texel (TX) and White Dorper (DO) ewes from the flock were submitted to a Fecal Egg Count Reduction Test (FECRT) in Jan/2022 that indicated efficacy of 0 % for albendazole, 81 % for levamisole, 84 % for closantel, 40 % for ivermectin, 80 % for moxidectin, and 39 % for monepantel. This natural parasite population in the flock was named as CPPSE resistant GIN.

Ewes were mated with sires of the same breed and, in the third month of pregnancy, the ewes were homogeneously distributed into one of the following three groups considering mean egg count per gram of feces (FEC), weight, paternity and age: Control (C), Partial Replacement (PR) and Total Replacement (TR) (Fig. 1). Ewes from the PR and TR groups were dewormed with anthelmintic treatments for 3 consecutive days with a combination of Ripercol L-150F®, Zoetis (subcutaneous, 18.8 % levamisole, 9.4 mg/kg body weight - BW), Valbazen® 10 Cobalto, Zoetis (oral, albendazole, 20 mg/kg BW) and Zolvix®, Elanco (oral, monepantel, 2.5 mg/kg BW) (Albuquerque et al., 2022). After 28 days, then in the last third of pregnancy, the ewes from the PR and TR groups were artificially orally infected with 3000 L_3 of the susceptible *H. contortus* Echevarria isolate and allocated to their respective paddocks: the PR group was transferred to a pasture naturally infested with the CPPSE resistant GIN, while the TR group was moved to a reformed GIN-free paddock, which had been sealed for 10 months and checked for the absence of GINs by monitoring L_3 in the pasture and with tracer animals. The ewes from the control group (C) did not receive anthelmintic treatment nor artificial infection and were allocated into a pasture naturally infested with CPPSE resistant GIN.

A total of 106 lambs (31 SI, 50 TX and 25 DO) were born, remained in the original paddocks of their mothers and were evaluated until 189 days old (Fig. 1). All lambs were vaccinated against clostridiosis (2 mL/animal, subcutaneously, two doses at 30-day intervals; Excell-10®, Vencofarma) and received toltrazuril (15 mg/kg BW, oral, Isocox®,

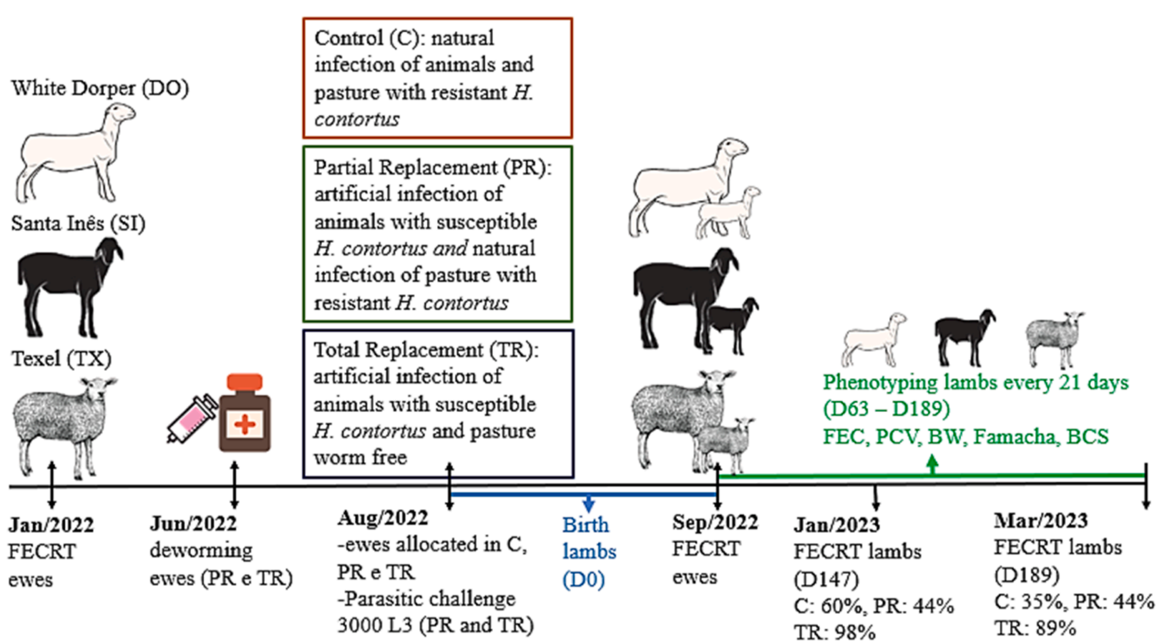

Fig. 1. Phenotyping scheme to evaluate the effects of total and partial worm replacement with *H. contortus* susceptible strain in White Dorper (DO), Santa Inês (SI) and Texel (TX) breeds.

Ourofino) to prevent coccidiosis.

Sheep were kept on pasture (*Panicum maximum* cv. Tanzania, *ad libitum*) throughout the experiment. All animals received mineral salt and water *ad libitum*. Lambs were born and kept within the paddocks of the respective ewe groups (C, PR and TR) and were supplemented with concentrated feed (up to 0.3 kg/animal/day) by creep feeding. Weaning occurred when the lambs were 84 days old, on average.

The three experimental groups were located geographically near to each other, but each one had a completely separate infrastructure (handling shed, scale, feeder and drinking trough) to avoid contact among animals and/or feces of the different groups. At the entrance of each paddock a container with disinfectant was kept for cleaning the team's shoes as they moved in and out of the experimental area. The order of entry to each animal management group was TR, then PR and C.

2.2. Phenotyping data

The lambs were the main subject of this study. In total 106 lambs of eight sires (2 DO, 3 SI and 3 TX) and 87 ewes (19 DO, 27 SI and 41 TX) were born in the C (12 DO, 12 SI and 15 TX), PR (7 DO, 10 SI and 18 TX) and TR (7 DO, 9 SI and 16 TX) groups. The effects of group (39 C, 35 PR and 32 TR), breed (25 DO, 31 SI and 50 TX), sex (56 F and 50 M) and type of birth (50 single and 56 twin) were considered.

Lamb phenotypic evaluations and sample collections occurred every 21 days, at 63, 84, 105, 126, 147, 168 and 189 days of age (D63, D84, D105, D126, D147, D168 and D189, respectively), which covered the period from November 2022 to March 2023. This period was specifically chosen because lambs are more vulnerable to gastrointestinal nematode parasitism during this stage, in which the negative impact of the infection will be noticeable in the production system, with significant commercial consequences.

Fecal samples were collected directly from the rectal ampoule for counting eggs per gram of feces (FEC), using the modified McMaster method, which uses 2 g of feces and 28 mL of saturated NaCl solution (d=1.2), with a correction factor 1 egg = 50 eggs per gram. Fecal cultures were performed for each group every 21 days, and 100 larvae from each culture were examined by microscope for morphological larval differentiation (Ueno and Gonçalves, 1998).

Blood samples from all animals were collected from the jugular vein using Vacutainer® tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant for packed cell volume (PCV) evaluation through centrifugation of capillary tubes (6 min/12,298 xg, Hettich - HEMATOKRIT 200®).

Additionally, at each collection date, lambs were individually weighed and evaluated for body condition score that range from 1 to 5 (Russel, 1984) and score of eye mucosa coloration according to the FAMACHA method that range from 5 to 1 (Van Wyk and Bath, 2002).

To assure animal welfare, lambs with FEC ≥ 4000 in D63 were treated. To preserve *refugia*, the criteria for subsequent treatments was adjusted to FEC $\geq 10{,}000$ associated with PCV ≤ 25 % in D84, PCV ≤ 26 % in D105 and D126, and PCV ≤ 24 % in D147, D168 and D189. Based on the susceptibility of the Echevaria *H. contortus* isolate (100 % susceptible to levamisole and 98.9 % to albendazole; Echevarria, 1991), the combination of Ripercol L-150F® and Valbazen® 10 Cobalto was used for AH treatment. The combination of drugs was adopted to further improve the efficacy of treatment, especially for the C group.

FECRTs were performed in lambs on D147 and D189 according to standardized guidelines produced by WAAVP using pre- and post-treatment FECs, and at least 7 animals per group with FEC > 200. Animals were selected to have a similar mean FEC among groups (Coles et al., 2006). Then, the anthelmintics' efficacy was calculated comparing FEC from 14 days after treatment (D14) with that from the day of treatment (D0) (Kaplan et al., 2023), using the formula: FECRT = 100 * (1 – (D14/D0)), and adopting the 95 % confidence limits (CL) (Denwood et al., 2023).

2.3. Statistical analysis

The analyses were performed using the R software (version 4.4.0) in RStudio (version 2024–04–24). Comparisons were performed at a significance level of 5 %.

For data normalization, FEC was transformed by cubic root (tFEC), leading to an approximation of a normal distribution, while the bestNormalize package, which tests many techniques, selected the orderNorm transformation as the best one for normalizing PCV and weight gain (tPCV and tGain, respectively). The quantitative phenotypic data (tFEC, tPCV and tGain) were analyzed in a linear mixed model with the lmer() function of the lme4 package (Bates et al., 2015), which fits a linear mixed-effect model with nested or crossed grouping factors for the random effects, and optimization by maximum likelihood. The Famacha and body condition scores, which are categorical, were analyzed in a mixed model of multinomial logistic regression using the mclogit package (Elff, 2023), which fits conditional logit models with or without random effects. For the number of anthelmintic treatments, a Poisson distribution was considered using the glm() function of the stats package, which fits generalized linear models that can analyze count data.

The mixed models considered repeated measurements (collection dates) and nested variables (sire/ewe) as random effects. Breed, group, mean temperature, humidity and precipitation, AH treatment 21 days before collection, sex (male or female), type of birth (single or twin), weight at birth, maternal age (years), weaning age (days), and lot (1 =animals with higher mean age and 2 =lot with lower mean age) were tested as fixed effects for all response variables.

After one-by-one inclusion of random and fixed effects and testing all first order relevant interactions between fixed effects, the best fitted model was selected based on the Akaike Information Criterion (AIC) and non-significant terms ($P > 0.05$) were removed. The LMERConvenienceFunctions package (Tremblay et al., 2020), which performs backward selection of fixed effects, forward fitting of the random effects, and post-hoc analyses was used for model assumption evaluation, outlier removal, variance analysis and post-hoc tests. The MuMIn package (Bartoń, 2023) extracted R2 from the models.

3. Results

During the experimental period, from 1st August 2022–31 st March 2023, relative humidity ranged from 59 % to 90 %, temperature from 8 to 33°C and precipitation from 35 to 516 mm^3, with the highest precipitation observed in December (516 mm) of 2022 and January (307 mm) of 2023 (Fig. 2).

The multiple resistance status of the CPPSE resistant GIN population was confirmed with a FECRT performed in the ewes before the start of the experiment (efficacy of 0 % for albendazole and 81 % for levamisole). In lambs of the C, PR and TR groups, the FECRT (confidence intervals) for the treatment combination of levamisole and albendazole demonstrated an efficacy of 60 % (42 – 77 %), 74 % (53 – 95.7 %) and 98 % (95.1 – 100 %), respectively, in D147, and 35 % (15 – 56 %), 44 % (13 – 74 %) and 89 % (81 – 97 %), respectively, in D189.

In fecal cultures, *Haemonchus* spp. was the predominant genus, ranging between 85–100 %, at all collection dates. In the TR group, 1 % of the nematodes identified in the first collection were *Trichostrongylus* spp. (Table 1).

Table 2 shows the means of all measured parameters in lambs, from D63 to D189 days of age. Additionally, descriptive statistic and confidence intervals, as a measure of precision, were included in the Supplementary Table 1. Regarding FEC, no statistical differences ($p > 0.05$) were detected among worm replacement groups or sheep breeds. However, anthelmintic (AH) treatment 21 days before collection decreased ($p < 0.001$) mean FEC (4640 in untreated *versus* 3070 in treated animals; Fig. 3A). A significant interaction between AH treatment and group was detected, resulting in lower FEC after treatment in the PR (1780 in treated *versus* 4354 in untreated; $p = 0.012$) and TR

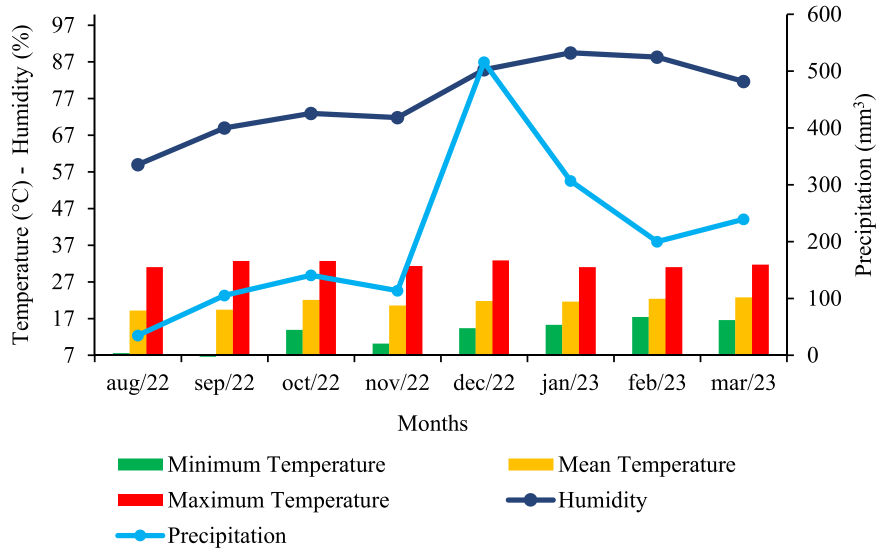

Fig. 2. Climatic data comprising mean, maximum and minimum temperatures (°C), average relative humidity (%) and mean precipitation (mm^3) from August 2022 to March 2023.

Table 1
Frequency of GIN genera (*Haemonchus* spp. and *Trichostrongylus* spp.) in the lambs of the Control (C), Partial Replacement (PR) and Total Replacement (TR) groups, on each day of collection.

	C		PR		TR	
Days	*Haem*	*Trich*	*Haem*	*Trich*	*Haem*	*Trich*
D 63	85 %	15 %	91 %	9 %	99 %	1 %
D 84	96 %	4 %	98 %	2 %	100 %	0 %
D 105	99 %	1 %	100 %	0 %	100 %	0 %
D 126	100 %	0 %	100 %	0 %	100 %	0 %
D 147	99 %	1 %	100 %	0 %	100 %	0 %
D 168	85 %	15 %	100 %	0 %	100 %	0 %
D 189	99 %	1 %	95 %	5 %	100 %	0 %
Mean	94.7 %	5.3 %	97.7 %	2.3 %	99.9 %	0.1 %

Table 2
Average (± standard error) number of eggs per gram of feces (FEC), packed cell volume (PCV), weight gain (WG) and number of anthelmintic treatments (AH), and frequency of each Famacha score and body condition score (BCS), from D63 to D189, in the Control (C), Partial Replacement (PR) and Total Replacement (TR) groups, in Dorper (DO), Santa Inês (SI) and Texel (TX) lambs.

	FEC	PVC (%)	WG* (kg)	AH	Famacha (%)			BCS (%)			
Group					1	2	3	1	2	3	4
C	5171 (7131) a	30.8 (6.0) a	19.5 (8.4) c	1.81 (1.4) a	52	29	19	1	52	46	0
PR	4160 (5646) a	30.7 (5.6) a	22.0 (9.1) b	1.44 (1.3) a	77	20	3	4	46	50	0
TR	5239 (9156) a	32.1 (5.2) a	25.0 (10.4) a	1.09 (1.2) a	78	21	1	3	32	61	4
Breed											
DO	6197 (7106) a	29.5 (5.7) b	21.4 (8.7) a	2.36 (1.3) b	67	23	10	1	30	68	1
SI	3450 (4251) a	34.4 (4.7) a	21.5 (8.4) a	0.39 (0.7) a	68	27	5	6	63	30	1
TX	5081 (8822) a	30.0 (5.3) b	22.5 (10.3) a	1.75 (1.5) b	65	25	10	1	36	61	3

a,b,c Values followed by different lower case letters in the same column indicate statistical difference ($P < 0.05$). * from D42 to D189.

(1238 in treated *versus* 4936 in untreated; $p < 0.001$) groups, but not in the C group (4829 in treated and 4643 in untreated; $p = 0.706$) (Fig. 3D). Males (4409) presented higher ($p = 0.037$) mean FEC than females (4308) (Fig. 3B). In addition, lambs weaned older showed lower ($p = 0.007$) FEC, as observed in mean FEC for the quartiles of age at weaning: 5120 from 59 to 84 days, 4229 from 85 to 89 days, 3624 from 90 to 92 days, and 4142 from 92 to 97 days (Fig. 3C).

The number of AH treatments each lamb received varied from 0 to 5; no significant differences were detected among C, PR and TR groups (1.81, 1.44 and 1.09 AH treatment means, respectively) (Table 2), but animals receiving 4 or 5 AH treatments were only observed in the C and PR groups. The breed influenced the number of AH treatments (Fig. 4A), and SI animals received fewer ($p < 0.001$) AH treatments (0.39) than DO and TX (2.36 and 1.75, respectively) (Table 2). Among the breeds, 23 SI, 12 TX and 2 DO lambs were never treated, while only DO and TX lambs were treated 3 times or more. In relation to the sire (Fig. 4B), many offspring of SI666 and SI667 sires, followed by SI665 and TX365, were not treated at all, while lambs from DO398, DO399, TX363 and TX364 sires were treated 4 or 5 times. In the TX breed, offspring of the TX365 sire were treated less ($p = 0.038$) than those from the TX364 sire (Fig. 4B).

The mean PCV was 30.8 %, 30.7 % and 32.1 % for C, PR and TR, respectively, and 29.5 %, 34.4 % and 30.0 % for DO, SI and TX, respectively (Table 2). Mean PCV was influenced by breed, with higher

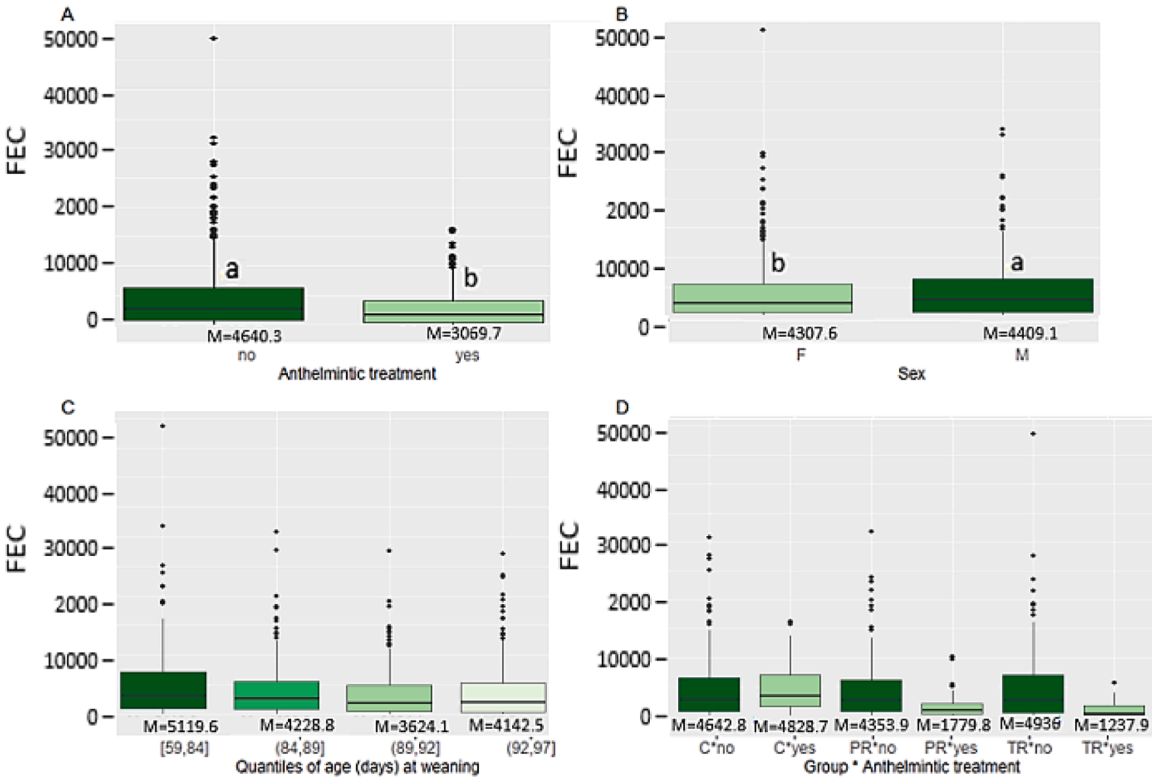

Fig. 3. Effects of anthelmintic treatment (A), sex (B), age at weaning (C), and anthelmintic treatment and group interaction (D) on cubic root transformed mean number of eggs per gram of feces (tFEC) in lambs. A) Untreated (no) versus anthelmintic treated (yes) animals. B) Females (F) and males (M). C) Quartiles of ages at weaning ranging from 59 to 97 days. D) Interaction among control (C), partial replacement (PR) and total replacement (TR) groups and anthelmintic treatment.

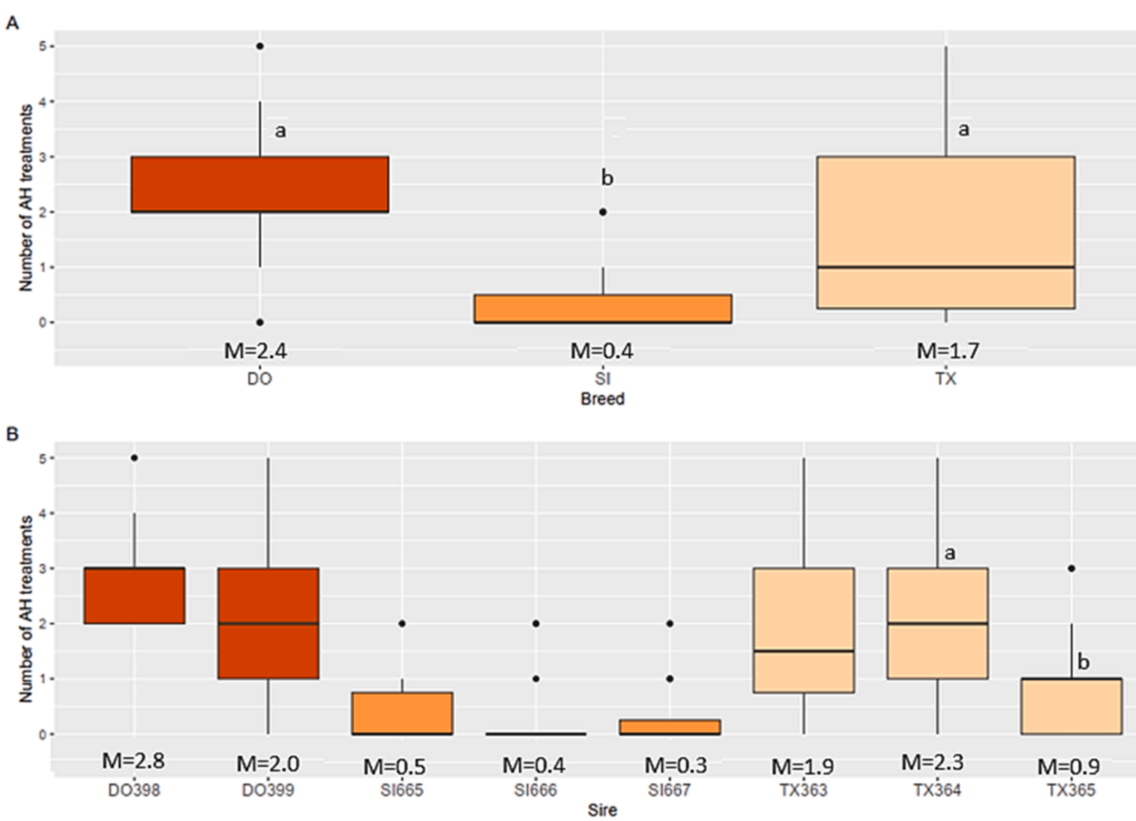

Fig. 4. Effect of breed (A) and sire (B) on the number of anthelmintic treatments (AH) in Dorper (DO), Santa Inês (SI) and Texel (TX) lambs.

($p < 0.001$) PCV in SI compared with TX and DO (Fig. 5A); by sex, with lower ($p < 0.001$) PCV in males (30.7 %) than in females (31.6 %) (Fig. 5B); and by mother's age ($p = 0.002$), with lower PCV in lambs from 7 to 8 year-old mothers (28.0 %) and higher PCV in lambs from 1 to 2 year-old mothers (31.5 %) (Fig. 5C). An interaction between group and AH treatment ($p < 0.001$) resulted in lower PCV in the C group treated with AH (28.1 %) compared with untreated animals (31.5 %), but no significant differences were detected between treated and untreated animals from the PR and TR groups (Fig. 5D).

The average weight gain in 147 days (from D42 to D189) was 19.5 kg, 22.0 kg and 25.0 kg for C, PR and TR groups, respectively, and 21.4 kg, 21.5 kg and 22.5 kg for DO, SI and TX breeds, respectively (Table 2). Mean weight gain in the 21-day interval was higher in the TR group compared with PR and C ($p < 0.001$), and in PR compared with C ($p = 0.005$) (Fig. 6A). Weight gain was affected by sex ($p < 0.001$), with greater gain in males (3.33 kg) than in females (2.94 kg) (Fig. 6B); and by the type of birth ($p = 0.036$), with higher gain in single lambs (3.25 kg) compared to twins (3.00 kg) (Fig. 6C). Average air relative humidity ($p < 0.001$) and mean precipitation ($p = 0.039$) affected weight gain: the higher the humidity (87.5 – 91.3 %), the lower the weight gain (1.50 kg)(Fig. 6D); and the lower the precipitation (3.12 – 5.33 mm), the higher the weight gain (5.20 kg) (Fig. 6E).

Group affected ($p < 0.05$) Famacha scores, with a higher frequency of score 1 in PR and TR and of score 3 in C (Table 2 and Fig. 7A). Higher humidity reduced ($p < 0.05$) Famacha score 1 and increased score 3 (Fig. 7B); and higher precipitation increased ($p < 0.05$) Famacha score 3 (Fig. 7C). Famacha score 1 frequency was higher ($p < 0.05$) in females (Fig. 7E) and lower ($p < 0.05$) in lambs weaned under 84 days of age (Fig. 7D).

Body condition scores 2 and 3, respectively, were the most frequent in all groups (52.1 % and 46.3 % in C, 45.5 % and 50.0 % in PR and 32.5 % and 60.8 % in TR) and breeds (29.9 % and 68.4 % in DO, 63.1 % and 30.2 % in SI and 35.7 % and 60.9 % in TX) (Table 2), and no lambs presented score 5. Lambs with higher birth weight (4.8 – 7.8 kg) showed higher ($p = 0.006$) frequency of body condition scores 3 and 4 (Fig. 8A), and lambs weaned at older ages (92–97 days) showed higher ($p = 0.042$) frequency of body condition score 4 (Fig. 8B).

4. Discussion

The present study evaluated the effect of replacing a *H. contortus* population resistant to multiple anthelmintics with a susceptible isolate in Santa Inês, White Dorper and Texel sheep breeds. Artificial infection was only done once in the ewes in the final third of pregnancy, and the target of the study was the lambs naturally infected with larvae from the pasture where they were born.

The degree of parasitism of animals measured by FEC was similar between groups, demonstrating that the susceptible isolate was viable and infective after thawing, multiplication in host lambs, artificial infection of the ewes and contamination of pastures. The biotic potential and pathogenicity of this *H. contortus* isolate was previously confirmed by its comparison with a resistant field isolate in Brazil (Chocobar et al., 2023).

Mean FEC values were statistically similar among breeds and groups, indicating that the animal distribution was homogenous and the infection levels were similar. As expected, anthelmintic treatment promoted reduction in FEC 21 days later. However, based on the significant interaction between group and anthelmintic treatment, this was mainly due to the higher efficacy of AHs in the PR and TR groups, which were parasitized by a susceptible *H. contortus* population.

WR resulted in higher anthelmintic efficacy in addition to the reduction in the number of anthelmintic treatments, but this effect was diluted over the time (from D63 to D189). However, any increase in AH efficacy or reduction in the number of treatments can contribute to the *refugia* maintenance (Muchiut et al., 2019), especially when associated with TST. The implementation of TST, in which treatments with AH are

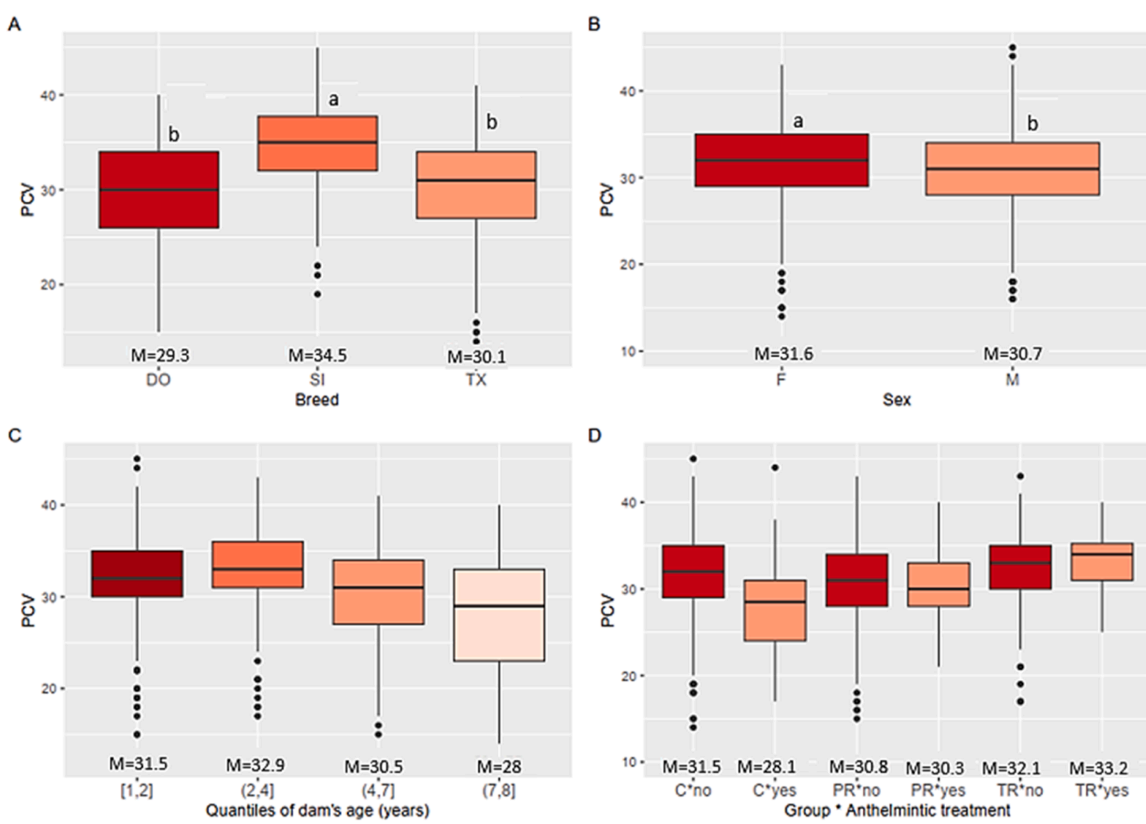

Fig. 5. Effect of breed (A), sex (B), age of the ewe at delivery (C), ranging from 1 to 8 years, and interaction between anthelmintic treatment and group (D) on mean packed cell volume (PCV) in Dorper (DO), Santa Inês (SI) and Texel (TX) lambs from control (C), partial replacement (PR) and total replacement (TR) groups.

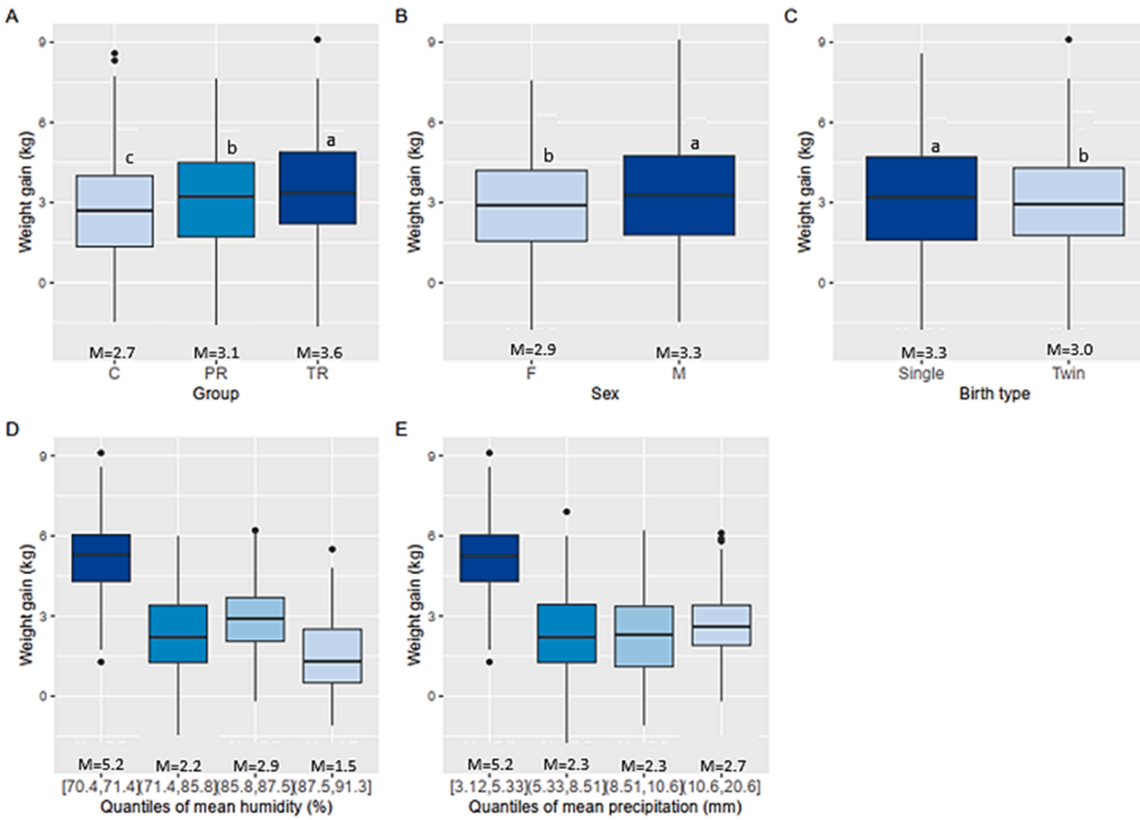

Fig. 6. Effect of group (A), sex (B), type of birth (C), mean relative humidity, ranging from 70.4 % to 91.3 % (D) and precipitation, ranging from 3.12 to 20.6 mm^3 (E) on mean weight gain at 21-day intervals in lambs from the control (C), partial replacement (PR) and total replacement (TR) groups.

restricted to animals that need treatment, may be based on several phenotypic parameters, such as body weight gain, anemia, body condition score, FEC and PCV (Kenyon et al., 2009). These parameters can help to identify animals that achieve the same performance with fewer AH treatments, as well as those that should be discarded due to unsatisfactory performance and the requirement of a higher number of treatments. In the present study, TST was based on both FEC and PCV.

The SI lambs presented a higher PCV and received fewer AH treatments throughout the experiment than DO and TX breeds. In addition, there was an influence of the sire, illustrating the natural genetic resistance to parasites, which can be targeted by breeding programs and animal selection, but equally may reflect resilience whereby the animals are better able to cope with challenge. SI is a native breed adapted to tropical regions and presents marked resistance against haemonchosis in different age categories, when compared with TX and DO (Rocha et al., 2004; Amarante et al., 2009; Albuquerque et al., 2019).

Males exhibited a higher FEC, lower PCV, and a lower frequency of Famacha score 1, but greater weight gain compared with females. Establishment and larval development are regulated by the immune response, which in turn is modulated by host sex hormones (Wesołowska, 2022). The presence of testosterone has an inhibitory effect on the differentiation of Th2 cells, which secrete cytokines that activate other cells to act against the establishment of endo and ectoparasites before or after puberty, increasing the susceptibility to parasitism in males (Barger, 1993; Gauly et al., 2002; Wesołowska, 2022). On the other hand, estrogens present in females have the opposite effect, stimulating both cellular and humoral immune responses, thus increasing resistance to parasitic diseases (Barger, 1993; Wesołowska, 2022). The influence of gender on weight can be explained by differences in body condition, since adipose tissue development occurs earlier in females than in males (Furusho-Garcia et al., 2004; Rodríguez et al., 2011).

Lambs weaned earlier had a higher FEC than those that stayed with the ewes for longer periods. Watson and Gill (1991) observed that lambs weaned at 8 weeks had a higher FEC than lambs kept with their mothers for 16 weeks, since they graze less, ingesting fewer GIN (Campbell et al., 2017). More importantly, feeding on milk will provide a higher supply of protein to replace endogenous N losses and develop the lambs' immunity (Ansia et al., 2020). In addition, early weaning leads to an acute response to stress, which raises cortisol levels, resulting in increased susceptibility to disease and infection (Högberg et al., 2023).

The age of the ewe influenced the PCV of the lambs. Progeny of ewe over 7 years old had a lower mean PCV than younger ewes. These data are contrary to what was described by Babar et al. (2004), who grouped the ewe into young (<3.5), mature (3.5–5.5 years) and old (>5.5 years) and observed a lower PCV in lambs from young mothers. The authors suggested that younger mothers use part of their energy for the development and improvement of their own reproductive system, while in older mothers the reproductive system is fully developed, with less competition for energy resources.

The reestablishment of AH efficacy through W*R* positively impacted weight gain by animals from D42 to D189, as a significantly higher average weight gain was observed in the TR (25 kg) followed by the PR group (22 kg), and both gained more weight than animals in the C group (19.5 kg). Starling et al. (2019) compared GIN infected DO lambs supplemented or not and non-infected supplemented animals and detected 5 kg more gain in non-infected and supplemented animals than in infected animals supplemented or not. In the present study, the experiment covered the entire rainy season, when the development of GIN is favored by climatic conditions, there is a high rate of contamination of pastures by helminth eggs and the parasitic challenge is greater. In contrast, at the same time, there is better availability of pasture. Therefore, although there was no impact on the reduction of FEC, lambs that grew up ingesting pasture contaminated by the susceptible isolate

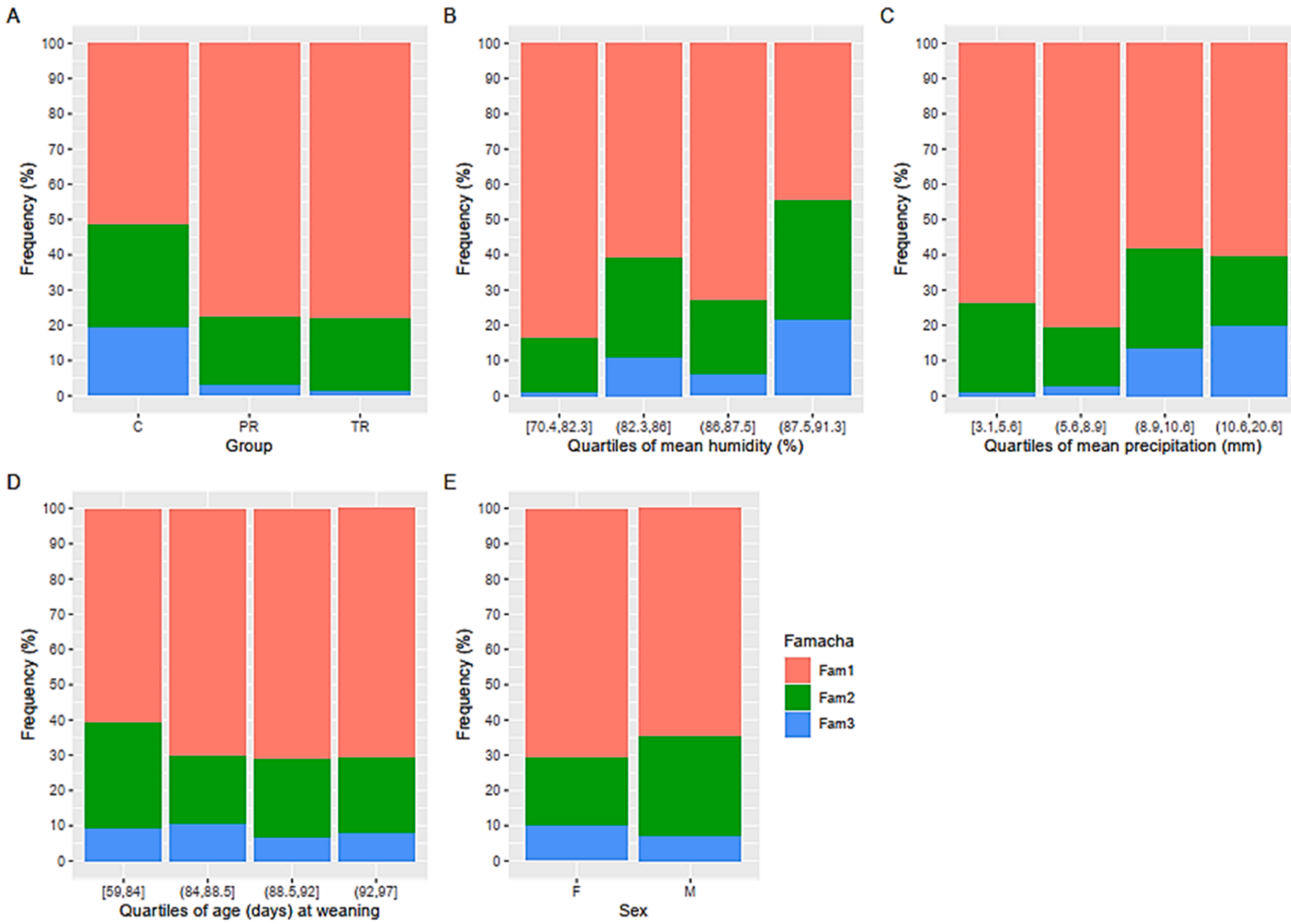

Fig. 7. Effects of group (A), mean humidity, ranging from 70.4 % to 91.3 % (B), mean precipitation, ranging from 3.12 to 20.6 mm³ (C), age at weaning, ranging from 59 to 97 days (D) and sex (E) on the frequency of Famacha scores (1−3) in lambs from the control (C), partial replacement (PR) and total replacement (TR) groups.

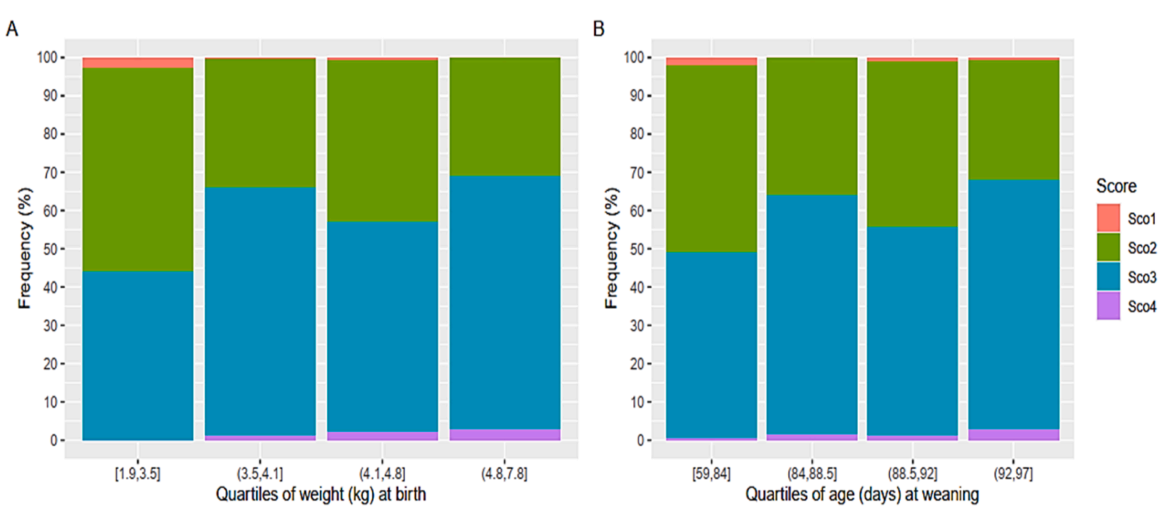

Fig. 8. Effect of birth weight, ranging from 1.9 to 7.8 kg (A) and age at weaning, ranging from 59 to 97 days (B) on the frequency of body condition scores (1−4) in lambs.

responded better to AH treatments. In addition, it was observed that as average precipitation and relative humidity increased, weight gain was lower, Famacha score of 1 was less frequent and Famacha score of 3 was more frequent. Moisture favors the exit of L_3 from the dung and, therefore, increases contamination of the pasture and infection of animals (Pegoraro et al., 2008), impacting on weight gain and degree of anemia, especially in lambs in the growth phase.

The replacement of the resistant parasite population by the susceptible one was successfully carried out in the present study. It was proven by the FECRT performed in lambs, which were naturally infected with larvae from the susceptible isolate eliminated through feces from infected ewes. In the PR group, where there was no pasture decontamination, W*R* increased the efficacy of AH, but it did not reach 95 %. In addition, 8 months after introduction of the susceptible isolate into the flock, the population in TR was resistant based on the FECRT of D189. However, the W*R* groups still showed far greater efficacy. Muchiut et al. (2019) observed the reestablishment of fenbendazole efficacy against *H. contortus* 16 months after the introduction of a susceptible population. George et al. (2021), on the other hand, succeeded in replacing the population of resistant *H. contortus* with a susceptible population in 7 weeks, but observed reappearance of albendazole resistance after 1.5 years. The establishment of a new susceptible parasitic population in the flock, as well as the rate of development of resistance, certainly depends on several well-known factors such as sheep breed, climate, sheep stocking rates, *refugia* and nutrition (Van Wyk and Van Schalkwyk, 1990; Bird et al., 2001; Moussavou-Boussougou et al., 2007; Muchiut et al., 2022). Overall, it can be said that the success of W*R* will greatly vary depending on a wide range of factors, which deserve further investigation for better understanding of the basis in which the anthelmintic resistance mechanisms are orchestrated. W*R* increased AH efficacy in the PR and TR groups, since the initial resistant GIN population was completely removed from the ewes. The effect of substitution was initially potentiated in animals that remained in the TR group, where the pasture was also decontaminated.

W*R* was effective but resistance developed in the short term. The rapid reversion to resistance may be due to the fact that the susceptible isolate has the same biotic potential as resistant GIN populations, presenting ability to infect lambs, similar FEC, and the infective larvae production is similar *in vitro* and in the field (Chocobar et al., 2023). W*R* is not an easy technique to implement in commercial flocks due to the challenge of eliminating the GIN infection of animals and pasture prior to subsequent artificial infection with a susceptible isolate. However, faced with an extreme scenario of anthelmintic resistance (Bassetto et al., 2024) and few alternatives for nematode control, W*R* could complement other techniques in the process of dilution of parasite resistance, such as genetic selection of resistant animals and breeds. Notably, despite SI being a breed that usually gains less weight than the other breeds evaluated (Brandão et al., 2024), there was no statistical difference for this parameter, indicating that SI was the breed that benefited most from W*R*, reaching the same productive performance as DO and TX.

Although W*R* is a complex and labor-intensive strategy, our findings indicate that it may still be a viable approach. The results suggest that W*R* has the potential to contribute for delaying or diluting the development of anthelmintic resistance. However, its implementation requires careful evaluation by specialists, who must consider the specific context, the technique's complexity, and the available resources.

5. Conclusion

In this study the W*R* approach reduced anthelmintic use and increased the weight gain of lambs in the PR and TR groups. It could be a useful technique in the dilution of resistance mainly when associated with decontamination of pasture (TR group). However, results will greatly vary depending on the farm context, taking into account its complexity and the availability of resources. To manage parasites in sheep flocks, we suggest the adoption of later weaning, selective treatment and more resistant animals and breeds, which can delay the development of resistance and improve the sustainability of parasite control by anthelmintics. Our findings may shed light on the underlying mechanisms impacting most on the performance of different sheep breeds in an environment that favors survival and reproduction of *H. contortus*, easily unbalancing the *refugia*-resistant parasite relationship.

CRediT authorship contribution statement

Chagas Ana Carolina de Souza: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Santos Juliana de Carvalho:** Methodology, Investigation, Data curation. **Kapritchkoff Rafaela Tami Ikeda:** Methodology, Investigation, Data curation. **Bello Hornblenda Joaquina Silva:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bressani Flavia Aline:** Investigation, Data curation. **Esteves Sérgio Novita:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Niciura Simone Cristina Méo:** Writing – review & editing, Formal analysis, Conceptualization. **Melito Glaucia Roberta:** Methodology, Investigation, Data curation.

Ethics approval

All procedures were approved by the Embrapa Pecuária Sudeste Ethical Committee for Animal Experimentation (Process nº 02/2022).

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2025.110490.

References

Albuquerque, A.C.A., Bassetto, C.C., Almeida, F.A., Amarante, A.F.T., 2017. Development of *Haemonchus contortus* resistance in sheep under suppressive or targeted selective treatment with monepantel. Vet. Parasitol. 246, 112–117. https://doi.org/10.1016/j.vetpar.2017.09.010.

Albuquerque, A.C.A., Bassetto, C.C., Almeida, F.A., Hildersley, K.A., Mcneilly, T.N., Britton, C., Amarante, A.F.T., 2019. Differences in immune responses to *Haemonchus contortus* infection in the susceptible Ile de France and the resistant Santa Ines sheep under different anthelmintic treatments regimens. Vet. Res. 50, 1–12. https://doi.org/10.1186/s13567-019-0722-3.

Albuquerque, A.C.A., Almeida, F.A., Bassetto, C.C., Lins, J.G.G., Amarante, A.F.T., 2022. Influence of breed and parasite challenge on the immune response to naturally acquired intestinal nematode infection in sheep. J. Helminthol. 96, e27. https://doi.org/10.1017/S0022149X21000821.

Amarante, A.F.T., 2014. Os parasitas de ovinos. Editora UNESP, São Paulo. https://doi.org/10.7476/9788568334423.

Amarante, A.F.T., Susin, I., Rocha, R.A., Silva, M.B., Mendes, C.Q., PIRES, A.V., 2009. Resistance of Santa Ines and crossbred ewes to naturally acquired gastrointestinal nematode infections. Vet. Parasitol. 165, 273–280. https://doi.org/10.1016/j.vetpar.2009.07.009.

Ansia, I., Stein, H.H., Vermeire, D.A., Brøkner, C., Drackley, J.K., 2020. Ileal digestibility and endogenous protein losses of milk replacers based on whey proteins alone or with an enzyme-treated soybean meal in young dairy calves. J. Dairy Sci. 103, 4390–4407. https://doi.org/10.3168/jds.2019-17699.

Babar, M.E., Ahmad, Z., Nadeem, A., Yaqoob, M., 2004. Environmental factors affecting birth weight in Lohi sheep. Pak. Vet. J. 24, 5–8. ⟨https://doi/full/10.5555/20043015518⟩.

Barger, I.A., 1993. Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. Int. J. Parasitol. Drugs Drug Resist 23, 463–469. https://doi.org/10.1016/0020-7519(93)90034-V.

Bartoń, K., 2023. _MuMIn: Multi-Model Inference_. R package version 1.47.5, <https://CRAN.R-project.org/package=MuMIn>.

Bassetto, C.C., Albuquerque, A.C.A., Lins, J.G.G., Marinho-Silva, N.M., Chocobar, M.L., Bello, H.J., Mena, M.O., Niciura, S.C.M., Amarante, A.F.T., Chagas, A.C.S., 2024. Revisiting anthelmintic resistance in sheep flocks from Sao Paulo State, Brazil. Int. J. Parasitol. Drugs Drug Resist 24, 100527. https://doi.org/10.1016/j.ijpddr.2024.100527.

Bates, D., Bolker, M.M.B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. ⟨https://www.jstatsoft.org/⟩. article/view/v067i01/.

Besier, R.B., 2012. Refugia-based strategies for sustainable worm control: factors affecting the acceptability to sheep and goat owners. Vet. Parasitol. 186 (2-9). https://doi.org/10.1016/j.vetpar.2011.11.057.

Bird, J., Shulaw, W.P., Pope, W.F., Bremer, C.A., 2001. Control of anthelmintic resistant endoparasites in a commercial sheep flock through parasite community replacement. Vet. Parasitol. 97, 221–227. https://doi.org/10.1016/S0304-4017(01)00406-X.

Brandão, J.C.A.B., Cartaxo, F.Q., Pinto, M.S.C., Targino, L.C., Gomes, R.N., Souza, D.D. R., Cardoso, A.F.M., Morais, L.K.C., Farias, C.A., Ramos, J.P.F., 2024. Comparative analysis of Santa Inês and Dorper crossbred sheep: productive and reproductive efficacy in the semi-arid. Ciênc. Anim. Bras. 25, 75817E. https://doi.org/10.1590/1809-6891v25e-75817E.

Campbell, B.J., Pullin, A.N., Pairis-Garcia, M.D., McCutcheon, J.S., Lowe, G.D., Campler, M.R., Fluharty, F.L., 2017. The effects of alternative weaning strategies on lamb health and performance. Small Rumin. Res. 156, 57–65. https://doi.org/10.1016/j.smallrumres.2017.09.006.

Chagas, A.C.S., Tupy, O., Santos, I.B.D., Esteves, S.N., 2022. Economic impact of gastrointestinal nematodes in Morada Nova sheep in Brazil. Rev. Bras. Parasitol. Vet. 31, e008722. https://doi.org/10.1590/S1984-29612022044.

Chocobar, M.L.E., Bello, H.J.S., Bassetto, C.C., Silva-Marinho, N.M., Sato, L.M.N., Sperb, C., Chagas, A.C.S., Amarante, A.F.T., 2023. Biotic potential and pathogenicity of a *Haemonchus contortus* susceptible laboratory isolate compared to a resistant field isolate in Brazil. Small Rumin. Res. 227, 107063. https://doi.org/10.1016/j.smallrumres.2023.107063.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet., Parasitol. 136, 167–185. https://doi.org/10.1016/j.vetpar.2005.11.019.

Denwood, M.J., Kaplan, R.M., McKendrick, I.J., Thamsborg, S.M., Nielsen, M.K., Levecke, B., 2023. A statistical framework for calculating prospective sample sizes and classifying efficacy results for faecal egg count reduction tests in ruminants, horses and swine. Vet. Parasitol. 314, 109867. https://doi.org/10.1016/j.vetpar.2022.109867. Epub 2022 Dec 23. PMID: 36621042.

Echevarria, F.A.M., Armour, J.L., Duncan, J.L., 1991. Efficacy of some anthelmintics on an ivermectin-resistant strain of *Haemonchus contortus* in sheep. Vet. Parasitol. 39, 279–284. https://doi.org/10.1016/0304-4017(91)90044-v.

Elff, M., 2023. _mclogit: Multinomial Logit Models, with or without Random Effects or Overdispersion_. http://mclogit.elff.eu,https://github.com/melff/mclogit/.

Falzon, L.C., O'neill, T.J., Menzies, P.I., Peregrine, A.S., Jones-Bitton, A., Mederos, A., 2014. A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. Prev. Vet. Med. 117, 388–402. https://doi.org/10.1016/j.prevetmed.2014.07.003.

Fiel, C.A., Steffan, P.E., Muchiut, S.M., Fernandez, A.S., Bernat, G., Riva, E., Lloberas, M. M., Almada, A., Homer, D., 2017. An attempt to replace an ivermectin-resistant *Cooperia* spp. population by a susceptible one on grazing pastures based on epidemiological principles and refugia management. Vet. Parasitol. 246, 53–59. https://doi.org/10.1016/j.vetpar.2017.08.026.

Furusho-Garcia, I.F., Perez, J.R.O., Bonagurio, S., Assis, R.D.M., Pedreira, B.C., Souza, X. R.D., 2004. Performance of Santa Inês lambs and its crosses with Texel, Ile de France e Bergamacia lambs. R. Bras. Zootec. 33, 1591–1603. https://doi.org/10.1590/S1516-35982004000600027.

Gauly, M., Kraus, M., Vervelde, L., Van Leeuwen, M.A.W., Erhardt, G., 2002. Estimating genetic differences in natural resistance in Rhön and Merinoland sheep following experimental *Haemonchus contortus* infection. Vet. Parasitol. 106, 55–67. https://doi.org/10.1016/S0304-4017(02)00028-6.

George, M.M., Vatta, A.F., Howell, S.B., Storey, B.E., McCoy, C.J., Wolstenholme, A.J., Kaplan, R.M., 2021. Evaluation of changes in drug susceptibility and population genetic structure in *Haemonchus contortus* following worm replacement as a means to reverse the impact of multiple-anthelmintic resistance on a sheep farm. Int. J. Parasitol. Drugs Drug Resist 15, 134–143. https://doi.org/10.1016/j.ijpddr.2021.02.004.

Högberg, N., Hessle, A., Lidfors, L., Höglund, J., 2023. The effect of weaning age on animal performance in lambs exposed to naturally acquired nematode infections. Vet. Parasitol. 316, 109900. https://doi.org/10.1016/j.vetpar.2023.109900.

Jackson, F., Coop, R.L., 2000. The development of anthelmintic resistance in sheep nematodes. Parasitol 120, 95–107. https://doi.org/10.1017/S0031182099005740.

Kaplan, R.M., Denwood, M.J., Nielsen, M.K., Thamsborg, S.M., Torgerson, P.R., Gilleard, J.S., Dobson, R.J., Vercruysse, J., Levecke, B., 2023. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses, and swine. Vet. Parasitol. 318, 109936. https://doi.org/10.1016/j.vetpar.2023.109936.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481. https://doi.org/10.1016/j.pt.2004.08.001.

Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: global worming and anthelmintic resistance. Vet. Parasitol. 186, 70–78. https://doi.org/10.1016/j.vetpar.2011.11.048.

Kenyon, F., Greer, A.W., Coles, G.C., Cringoli, G., Papadopoulos, E., Cabaret, J., Jackson, F., 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Vet. Parasitol. 164, 3–11. https://doi.org/10.1016/j.vetpar.2009.04.015.

Leathwick, D.M., Besier, R.B., 2014. The management of anthelmintic resistance in grazing ruminants in Australasia–strategies and experiences. Vet. Parasitol. 204, 44–54. https://doi.org/10.1016/j.vetpar.2013.12.022.

Mohamed, S.A.A., Dyab, A.K., Raya-Álvarez, E., Abdel-Aziz, F.M., Osman, F., Gareh, A., Elmahallawy, E.K., 2024. Molecular identification of *Haemonchus contortus* in sheep from Upper Egypt. Front. Vet. Sci. 10, 1327424. https://doi.org/10.3389/fvets.2023.1327424.

Moussavou-Boussougou, M.N., Silvestre, A., Cortet, J., Sauve, C., Cabaret, J., 2007. Substitution of benzimidazole-resistant nematodes for susceptible nematodes in grazing lambs. Parasitol 134, 553–560. https://doi.org/10.1017/S0031182006001697.

Muchiut, S.M., Fernandez, A.S., Lloberas, M., Steffan, P.E., Luque, S.E., Cardozo, P.A., Bernat, G.A., Riva, E., Fiel, C.A., 2019. Recovery of fenbendazole efficacy on resistant *Haemonchus contortus* by management of parasite refugia and population replacement. Vet. Parasitol. 271, 31–37. ⟨https://doi.org/10.1016/j.vetpar.2019.06.003⟩.

Muchiut, S., Fiel, C., Liron, ´J.P., Lloberas, M., Ceriani, C., Lorenzo, R., Riva, E., Bernat, G., Cardozo, P., Fern´andez, S., Steffan, P., 2022. Population replacement of benzimidazole-resistant *Haemonchus contortus* with susceptible strains: evidence of changes in the resistance status. Parasitol. Res. 121, 2623–2632. https://doi.org/10.1007/s00436-022-07582-9.

Naeem, M., Iqbal, Z., Roohi, N., 2021. Ovine haemonchosis: a review. Trop. Anim. Health Prod. 53, 19. ⟨https://doi.org/10.1007/s11250-020-02439-8⟩.

Pegoraro, E.J., Poli, C.H.E.C., Carvalho, P.C.F., Gomes, M.J.T.M., Fischer, V., 2008. Italian ryegrass management, pasture larval contamination and parasitic infection in sheep. Pesq. Agropec. Bras. 43, 1397–1403. ⟨https://doi.org/10.1590/S0100-204X2008001000019⟩.

Rocha, R.A., Amarante, A.F.T., Bricarello, P.A., 2004. Comparison of the susceptibility of Santa Inês and Ile de France ewes to nematode parasitism around parturition and during lactation. Small Rumin. Res. 55, 65–75. ⟨https://doi.org/10.1016/j.smallrumres.2003.12.004⟩.

Rodríguez, A.B., Bodas, R., Landa, R., López-Campos, Ó., Mantecón, A.R., Giráldez, F.J., 2011. Animal performance, carcass traits and meat characteristics of Assaf and Merino × Assaf growing lambs. Livest. Sci. 138, 13–19. ⟨https://doi.org/10.1016/j.livsci.2010.11.020⟩.

Russel, A., 1984. Body condition scoring of sheep. Pract 6, 91–93.

Sanders, J., Xie, Y., Gazzola, D., Li, H., Abraham, A., Flanagan, K., Aroian, R.V., 2020. A new paraprobiotic-based treatment for control of *Haemonchus contortus* in sheep. Int. J. Parasitol. Drugs Drug Res. 14, 230–236. https://doi.org/10.1016/j.ijpddr.2020.11.004.

Sissay, M.M., Asefa, A., Uggla, A., Waller, P.J., 2006. Anthelmintic resistance of nematode parasites of small ruminants in eastern Ethiopia: exploitation of refugia to restore anthelmintic efficacy. Vet. Parasitol. 135, 337–346. https://doi.org/10.1016/j.vetpar.2005.09.005.

Soares, S.C.P., Reis, A.C., Castro, R.L.P., Pires Filho, P.C.S., Cabral, C.S.M., Diniz Júnior, D.O., Brito, D.R.B., 2023. Resistência de nematoides gastrintestinais de caprinos e ovinos aos anti-helmínticos levamisol, ivermectina e albendazol. e-75316E Ciênc. Anim. Bras. 24. https://doi.org/10.1590/1809-6891v24e-75316E.

Starling, R.Z.C., Almeida, F.A.D., Viana, M.V.G., Castilhos, A.M.D., Amarante, A.F.T., 2019. Losses caused by gastrointestinal nematode infections in Dorper lambs under two nutritional status. Rev. Bras. Parasitol. Vet. 28, 652–660. https://doi.org/10.1590/S1984-29612019084.

Tremblay A., Canada S., Ransijn, J., Copenhagen U.O., 2020. LMERConvenienceFunctions: Model Selection and Post-Hoc Analysis for (G)LMER Models_. R package version 3.0, https://CRAN.R-project.org/package=LMERConvenienceFunctions.

Ueno, H., Gonçalves, P.C., 1998. Manual para diagnóstico das helmintoses de ruminantes. Japan International Cooperation Agency.

Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529. https://doi.org/10.1051/vetres:2002036.

Van Wyk, J.A., Van Schalkwyk, P.C., 1990. A novel approach to the control of anthelmintic-resistant *Haemonchus contortus* in sheep. Vet. Parasitol. 35, 61–69. https://doi.org/10.1016/0304-4017(90)90116-S.

Veríssimo, C.J., Niciura, S.C.M., Alberti, A.L.L., Rodrigues, C.F.C., Barbosa, C.M.P., Chiebao, D.P., Cardoso, D., Silva, G.S., Pereira, J.R., Margatho, L.F.F., Costa, R.L.D., Nadon, R.F., Ueno, T.E.H., Curci, V.C.L.M., Molento, M.B., 2012. Multidrug and multispecies resistance in sheep flocks from São Paulo state, Brazil. Vet. Parasitol. 187 (1-2), 209–216. https://doi.org/10.1016/j.vetpar.2012.01.013.

Watson, D.L., Gill, H.S., 1991. Effect of weaning on antibody responses and nematode parasitism in Merino lambs. Res. Vet. Sci. 128–132. https://doi.org/10.1016/0034-5288(91)90002-6.

Wesołowska, A., 2022. Sex—the most underappreciated variable in research: insights from helminth-infected hosts. Vet. Res. 53 (1), 1–17. https://doi.org/10.1186/s13567-022-01103-3.