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Association of β-globin polymorphisms and tolerance to haemonchosis in ewes and lambs of different sheep breeds

Rafaela Tami Ikeda Kapritchkoff [a,*], Cintia Hiromi Okino [b], Simone Cristina Méo Niciura [b], Hornblenda Joaquina Silva Bello [b], Renata Silva Matos [b], Glaucia Roberta Melito [b], Flavia Aline Bressani [b], Sérgio Novita Esteves [b], Ana Carolina de Souza Chagas [b]

[a] *Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Via de Acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, São Paulo 14884-900, Brazil*

[b] *Embrapa Pecuária Sudeste, Rodovia Washington Luiz, Km 234 s/n, Fazenda Canchim, P.O. Box 339, São Carlos, São Paulo 13560-970, Brazil*

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ABSTRACT

Gastrointestinal nematodes (GIN), especially *Haemonchus contortus*, represent a significant challenge for sheep production. Given the global concern about GIN anthelmintic resistance, alternative control methods able to reduce the dependence on these drugs are highly advisable. Since previous studies have shown that sheep carrying the Hb-A allele of β-globin are more resistant to *H. contortus*, this study aimed to investigate the relationship between the different haplotypes (Hb-AA, Hb-AB and Hb-BB) and phenotypes in Santa Inês (SI), Texel (TX) and White Dorper (DO) breeds infected with *H. contortus*. Blood samples were collected from 180 ewes and 123 lambs of the three breeds for DNA extraction followed by qPCR using a hydrolysis probe to identify the β-globin haplotypes. Phenotypic data, including fecal egg count (FEC), packed cell volume (PCV), FAMACHA score and body condition score for ewes and lambs, as well as weight gain for lambs, were collected. The genotypic frequencies of β-globin for ewes and lambs were, respectively: 21.7% and 21.4% Hb-AA, 50% and 50% Hb-AB and 28.3% and 28.6% Hb-BB in SI; 0% and 0% Hb-AA, 18.6% and 9.4% Hb-AB and 81.4% and 90.6% Hb-BB in TX; and 0% and 0% Hb-AA, 13.1% and 0% Hb-AB and 86.9% and 100% Hb-BB in DO. In ewes, mean PCV differed ($p<0.05$) between the three haplotypes, with higher PCV in Hb-AA animals, followed by Hb-AB and Hb-BB. When considering each breed separately, SI Hb-AA ewes presented higher PCV ($p<0.05$), highlighting that even in a breed already considered resistant, animals with Hb-AA haplotype showed superior performance. Lambs with the Hb-AA haplotype exhibited a higher ($p<0.05$) mean PCV compared to those with Hb-AB and Hb-BB. The same pattern was found in SI when analyzing each breed separately. No significant association was found between β-globin haplotypes and FEC, FAMACHA score, body condition score, or weight gain. Nevertheless, given that anemia is the major clinical sign of haemonchosis, our findings on PCV reinforce that sheep carrying the Hb-A allele of β-globin are more tolerant to haemonchosis. This study may support the development of a valuable tool, targeting genetic selection for GIN control, reducing the dependence on anthelmintics and boosting sheep production worldwide.

1. Introduction

Gastrointestinal nematodes (GIN) represent the primary bottleneck in sheep production (Mcrae et al., 2015; Chagas et al., 2022), with *Haemonchus contortus* being the most significant species affecting the productivity of sheep flocks in Brazil and worldwide (Waller, 2006; Chagas et al., 2008; Penago et al., 2021). Responsible for the disease haemonchosis, this parasite leads to a significant reduction in production in infected animals, including decreased growth in lambs, reduced milk production in lactating dams, decreased wool production and deaths, resulting in substantial economic losses (Fthenakis and Papadopoulos, 2018; Chagas et al., 2022). During more vulnerable periods, such as the peripartum period, ewes become even more susceptible to helminth infections (Basseto et al., 2018), with an increase in fecal egg counts (FEC) being common during this period (Pereira et al., 2020).

* Corresponding author:
E-mail address: rafaelakapri@gmail.com (R.T.I. Kapritchkoff).

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The most common practice for controlling GIN is the use of anthelmintics (AH). Unfortunately, their frequent and indiscriminate administration has led to the emergence of AH resistance in parasites to all drug classes, making them multidrug-resistant (Kotze and Prichard, 2016; Arsenopoulos et al., 2021). As a consequence, the annual cost of AH treatments is estimated at tens of billions of dollars worldwide (Ali et al., 2021), highlighting the need to seek support methods for *H. contortus* control.

The selection of sheep breeds naturally resistant to GIN is a tool to control *H. contortus* infections, based on phenotypic and genotypic characteristics (Cruz-Tamayo et al., 2021). The ability to resist GIN is strictly related to the existence of genetic variation among animals, making it possible, from the identification, to select the most resistant and resilient animals (Bishop, 2012). FEC is the most common method for the identification of resistant animals; however, there are other methods, such as the GIN worm count, the degree of anemia, the frequency of treatments and the immune response to parasitism (Salgado et al., 2022).

Studies have progressively focused on investigating molecular polymorphisms that can be used in strategies for selecting resistant animals. In this context, two different chromosomal arrangements of β-globin have given rise to two distinct haplotypes in domestic sheep: Hb-A and Hb-B (Garner and Lingrel, 1989). In Hb-A, the β-globin locus consists of 12 genes, organized as a set of four genes that are triplicated and expressed during development (fetal β^F, juvenile β^C and adult β^A), while in Hb-B, this arrangement of four genes is duplicated (fetal β^F and adult β^B) (Pieragostini et al., 2010). Sheep with Hb-A synthesize β^C (juvenile), which has a higher oxygen affinity not only at birth but also in adulthood under anemic conditions. In contrast, sheep with Hb-B, as they do not synthesize β^C, continue to produce β^B (adult) even in anemic circumstances (Pieragostini et al., 2010). Since *H. contortus* is a hematophagous GIN, consuming blood directly from the hosts' abomasum and leading to anemia (Andrews, 1942), studies have shown that animals with the β^A allele, including the Hb-AA and Hb-AB haplotypes, especially the former, are more resistant to infection by this GIN (Evans et al., 1963; Jilek and Bradley, 1969; Allonby and Urquhart, 1976; Preston and Allonby, 1979; Okino et al., 2021a; Okino et al., 2021b; Okino et al., 2023).

Therefore, the main purpose of this study was to investigate the relationship between different β-globin haplotypes (Hb-AA, Hb-AB and Hb-BB) and phenotypes related to resistance to haemonchosis in ewes and lambs of the Santa Inês (SI), Texel (TX) and White Dorper (DO) breeds. We aimed to provide an alternative approach for the control of *H. contortus* through genetic selection, using this polymorphism with the intention of reducing dependency on the frequent use of AH agents.

2. Material and methods

2.1. Experimental design

The study was performed 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). The average temperatures, humidity, and rainfall were 19.6°C, 66.5%, and 44.4 mm, respectively, during the dry season and 22.2°C, 81.1%, and 249.3 mm during the rainy season. These data were collected at the CPPSE meteorological station.

A total of 180 ewes, with an average age of 5 years, were utilized. Among them, 60 belonged to the Santa Inês (SI) breed, with an average weight of 62.7 kg, 59 were Texel (TX), with an average weight of 66.7 kg, and 61 were White Dorper (DO), with an average weight of 71.2 kg.

The natural mating season took place between March 23 and May 6, 2022. In brief, there were three rams for each breed. Thus, the ratio was one ram mating with approximately 20 ewes of the same breed, in separate and controlled paddocks, until all 60 SI, 59 TX, and 61 DO were covered. On ultrasound examinations, 133 (40 SI, 54 TX and 39 DO) out of 180 ewes were identified as pregnant. The lambing season occurred between August 21 and October 13, 2022, resulting in a total of 144 live-born lambs, comprising 50 SI, 57 TX and 37 DO. Of these, 126 lambs (42 SI, 54 TX and 30 DO) were followed in the current experiment.

The ewes were kept throughout the experimental period on pasture (*Panicum maximum* cv. Tanzânia, *ad libtum*), and during the dry period, they received supplementation with corn silage (4 kg/ewe/day) and concentrated feed (0.4 kg/ewe/day) in the morning. All animals received mineral salt and water *ad libitum*. During the pre-partum (30 days) and early lactation (45 days) periods, the ewes were supplemented similarly. The lambs were kept with their mothers on pasture and supplemented with concentrated feed (up to 0.3 kg/animal/day) in the morning. The weaning began in November 2022, when the lambs were about 84 days old. Some conditions, such as mastitis, insufficient milk production, rejection by the dam or weak lambs, impaired natural suckling. Thus, 16 lambs (2SI AA, 4 SI AB, 3 SI BB, 3 TX BB, and 4 DO BB) were transferred to stalls and subjected to artificial feeding, including sheep colostrum administration at birth, followed by cow milk and concentrated feed (from the 10th day of life) until weaning, when they were moved to pasture (average age of 42 days).

The ewes and lambs were monitored daily by a veterinarian, who also assessed the clinical results of the tests. In necessary cases, the animals received medication or vitamin supplementation to accomplish their needs.

2.2. Genotyping for identification of β-globin haplotypes in ewes and lambs

The blood was collected through jugular venipuncture into an EDTA vacutainer tube and stored at −20ºC until processing. DNA extraction was performed with the Easy DNA kit (Invitrogen, Cat. K180001), following the manufacturer's recommendations. The concentration and purity of the isolated DNA were estimated by absorbance at 260 nm and the 260/280 nm ratio in spectrophotometer (Nanodrop | 2000, Thermo Scientific). DNA was stored at −20ºC until processing and individually adjusted to a final concentration of 2.5 ng/μL.

qPCR assays for the identification of the ovine β-globin haplotype were performed as described by Okino et al. (2021a). The reactions contained 5 ng DNA, 1x Mastermix Solution (Solis Biodyne, Tartu, Estonia), 5 pmol forward and reverse primers and 2 pmol of each probe, in a final volume of 10 μL.

Amplification was performed in a CFX 96 (BioRad) thermocycler including a pre-incubation step at 95ºC for 5 minutes, followed by 40 cycles of 95ºC for 15 seconds and 60ºC for 30 seconds. In each run, a negative control and three positive controls (samples of Hb-AA, Hb-BB and Hb-AB of β-globin haplotypes; confirmed by DNA sequencing) were included.

Hardy-Weinberg equilibrium of allele frequencies was investigated on https://gene-calc.pl/hardy-weinberg-page at a significance level of 0.05.

2.3. Phenotypic data

In ewes, fecal samples were collected directly from the rectal ampoule in the months preceding parturition (February, March, April, May and July) and during the peripartum period (August, September and October). Individual fecal egg count (FEC) x 50 (adapted from Gordon and Whitlock, 1939) and fecal culture in an incubator (± 27°C, RH >80%) for 7 days, followed by infective larvae (L_3) identification (Van Wyk et al., 2004), were performed. In February (the beginning of the experiment), March (the rainy season) and July (after mating), the packed cell volume (PCV) was determined by micro-hematocrit; in February, March, September, October and November, the score of ocular conjunctiva FAMACHA was measured (Van Wyk and Bath, 2002), while in February body score was estimated (Russel, 1984).

The phenotypic data of the lambs were collected every 21 days, from D0 (birth) to D189. To minimize age differences, the lambs were evaluated in two lots (Lot 1 - lambs born from 18 Aug to 7 Sep and Lot 2 – lambs born from 8 Sep and to 10 Oct) and the collection dates were adjusted according to the average age. On D0 and D42, only weight was measured, while on D63, weighing, FEC and PCV were performed. From D84 (average weaning age) to D189, complete phenotyping was performed, including body and FAMACHA scores. Weight gain was calculated and adjusted for the interval of 21 days between collections.

The animals were individually evaluated, and selective treatment with AH was administered according to the assessment of the responsible veterinarian indicating the need.

2.4. Statistical analysis

Prior to the analysis, data from one Texel lamb (deceased before D189) and two Dorper lambs (the only DO with the AB β-globin haplotype and the only offspring of a sire) were removed. Thus, a database containing 180 ewes and 123 lambs was used.

Analyses were performed in R (version 4.2.3). For normalization, FEC data were transformed by cubic root (tFEC). The quantitative phenotypic data (tFEC, PCV and weight gain) were analyzed in a mixed linear model with the lmer() function of the lme4 package (Bates et al., 2015) and maximum likelihood optimization. Pearson's correlation among quantitative variables was estimated using the chart.Correlation function of the PerformanceAnalytics package (Peterson and Carl, 2020). Categorical data (FAMACHA score and body condition score) were analyzed in a mixed multinomial regression model with the mclogit package (Elff, 2023).

Mixed models considered repeated measures (collection dates) and nested variables (sire within dam) as random effects. Breed and β-globin haplotype were tested as a fixed effect for both ewes and lambs. Additionally for the lambs, group (from another ongoing study on parasitic replacement; manuscript under development), average environmental temperature, humidity and precipitation, AH treatment 21 days before collection, sex (male or female), type of birth (single or twin), birth weight, maternal age (in years), weaning's age (in days), artificial feeding (yes = orphan lambs and no = lambs that stayed with their mothers) and lot (1 and 2) were investigated as covariates. For ewes, condition (0 = not pregnant, 1 = pregnant and raised the lamb and 2 = pregnant, but the lamb was subjected to artificial feeding) and prolificacy (single or twin pregnancy) were tested as fixed effects. The analysis was performed initially for the entire dataset comprised of all three sheep breeds and subsequently, due to the lack of all β-globin haplotypes in all breeds, data was analyzed separately by breed.

After inclusion, one by one, of random and fixed effects and test of all relevant first order interactions between fixed effects, the best fitted model was selected based on the Akaike Information Criterion (AIC) and non-significant ($P>0.05$) terms were removed. The LMERConvenienceFunctions package (Tremblay et al., 2020) was employed for model assumption assessment, outlier removal, analysis of variance and post-hoc tests. The Mumin package (Bartoń, 2023) extracted the R^2 of the models.

3. Results

Descriptive statistics of all phenotypes are presented for ewes (Table 1) and lambs (Table 2) grouped by breed and β-globin haplotype.

In fecal cultures, *Haemonchus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. were identified, but *H. contortus* was the predominant genus in all collection dates from ewes (92–96%) and lambs (92–99%).

Blood samples from all ewes (60 SI, 59 TX and 61 DO) and lambs (42 SI, 54 TX and 30 DO) were genotyped for β-globin, and allelic and genotypic frequencies were calculated (Table 3). Only SI presented the Hb-AA haplotype, and the Hb-AB haplotype was the most frequent in SI, while Hb-BB was the most common in TX and DO. All β-globin haplotype frequencies were under Hardy-Weinberg equilibrium, indicating that the β-globin alleles were not under selective pressure in the experimental animals.

Regardless of the breed, the genotypic frequencies for the β-globin were: 7.2% Hb-AA (13/180), 27.2% Hb-AB (49/180) and 65.6% Hb-BB (118/180) in ewes; and 7% Hb-AA (9/123), 21% Hb-AB (26/123) and 72% Hb-BB (88/123) in lambs.

In lambs, tFEC was negatively correlated ($p<0.05$) to both PCV (-0.50) and weight gain (-0.16), and PCV was positively correlated ($p<0.05$) to weight gain (0.38).

An association ($p<0.05$) of β-globin haplotypes to mean PCV was detected in both ewes and lambs. In ewes, considering the three breeds altogether, the best fitted model (AIC=2080.1 and R^2=0.27) was significant ($p<0.05$) for collection date as a random effect and breed and β-globin haplotypes as fixed effects. Higher ($p<0.05$) PCV was detected in SI and in the Hb-AA β-globin haplotype, which was followed by Hb-AB and Hb-BB (Fig. 1).

When considering ewes of each breed separately, collection date as a random effect and β-globin haplotypes and condition as fixed effects improved the model adjustment for SI (AIC=600.8 and R^2=0.38), with higher ($p<0.05$) PCV in animals Hb-AA and in conditions 0 and 2 (Fig. 2). No significant effects were observed for PCV in TX, and β-globin haplotypes were not associated ($p>0.05$) with PCV in DO.

In lambs, random effects of sire/dam and collection date and fixed effects of β-globin haplotype, breed, sex, maternal age, mean precipitation, AH treatment, artificial feeding and lot, and interactions between group and AH treatment and between maternal age and artificial feeding improved the model adjustment (AIC=4823.0 and R^2=0.57). For β-globin, lambs with the Hb-AA haplotype showed higher ($p<0.05$) PCV than Hb-AB and Hb-BB, and for breed, SI presented higher ($p<0.05$) PCV than DO and TX (Fig. 3).

For lambs, when considering each breed individually, significant effect of the β-globin haplotype on PCV was detected only in SI, where Hb-AA animals and lambs that stayed with their mothers showed higher ($p>0.05$) PCV (Fig. 4). For SI, random effects of dam and collection date, and fixed effects of β-globin haplotype, artificial feeding and mean

Table 1

Overall mean (± standard deviation) of fecal egg count (FEC) (February, March, April, May, July, August, September and October) and packed cell volume (PCV) (February, March and July), and frequency of FAMACHA scores (February, March, September, October and November) and body condition scores (February, March and August) in ewes, according to the combination of sheep breeds (Dorper (DO), Santa Inês (SI) and Texel (TX)) and β-globin haplotypes (Hb-AA, Hb-AB and Hb-BB).

	n	FEC	PCV	Famacha				Body condition			
			(%)	1	2	3	4	1	2	3	4
DO_AB	8	1225 ± 2044	35.0 ± 5.5	91%	9%	0%	0%	0%	0%	80%	20%
DO_BB	53	1155 ± 1715	33.0 ± 4.6	73%	22%	3%	2%	0%	9%	76%	15%
SI_AA	13	1253 ± 2300	39.2 ± 3.9	72%	28%	0%	0%	0%	11%	67%	22%
SI_AB	30	1172 ± 1971	36.8 ± 3.9	82%	15%	3%	0%	0%	11%	89%	0%
SI_BB	17	830 ± 1882	35.2 ± 3.9	77%	23%	0%	0%	0%	11%	78%	11%
TX_AB	11	1218 ± 3280	33.5 ± 3.0	76%	24%	0%	0%	0%	0%	100%	0%
TX_BB	48	1646 ± 4348	32.7 ± 4.1	69%	27%	4%	0%	0%	12%	81%	7%

Table 2
Overall mean (± standard deviation) of fecal egg count (FEC) and packed cell volume (PCV) (collections on D63, D84, D105, D126, D147, D168, and D189) and weight gain (collections on D0, D21, D42, D63, D84, D105, D126, D147, D168, and D189), and frequency of FAMACHA and body condition score (collections on D84, D105, D126, D147, D168, and D189) in lambs, according to the combination of sheep breeds (Dorper (DO), Santa Inês (SI), and Texel (TX)) and β-globin haplotypes (Hb-AA, Hb-AB, and Hb-BB).

	n	FEC	PCV	Weight gain*	Famacha			Body condition			
			(%)	(kg)	1	2	3	1	2	3	4
DO_BB	28	5901 ± 7494	29.1 ± 5.92	2.90 ± 2.21	66%	23%	11%	1%	30%	68%	1%
SI_AA	9	4030 ± 5139	34.8 ± 4.19	3.37 ± 1.96	74%	24%	2%	2%	57%	39%	2%
SI_AB	21	3153 ± 4119	32.7 ± 5.66	2.75 ± 1.89	67%	29%	4%	7%	68%	25%	0%
SI_BB	12	1854 ± 2130	32.9 ± 4.81	2.79 ± 1.87	67%	25%	8%	7%	58%	33%	2%
TX_AB	5	3221 ± 3686	31.6 ± 3.97	3.32 ± 2.43	70%	23%	7%	1%	36%	61%	2%
TX_BB	48	5272 ± 9126	29.6 ± 5.60	3.10 ± 2.30	64%	25%	11%	0%	33%	67%	0%

*At a 21-day interval.

Table 3
Frequency of β-globin haplotypes (AA, AB, and BB) and alleles (βA and βB) according to sheep breed and animal category.

	Santa Inês					Texel				White Dorper			
Category(n)	**Hb-AA**	**Hb-AB**	**Hb-BB**	**βA**	**βB**	**Hb-AB**	**Hb-BB**	**βA**	**βB**	**Hb-AB**	**Hb-BB**	**βA**	**βB**
Ewes (n=180)	21.67%	50%	28.33%	0.38	0.62	18.64%	81.36%	0.09	0.91	13.11%	86.89%	0.07	0.93
Lambs (n=126)	21.43%	50%	28.57%	0.38	0.62	9.26%	90.74%	0.05	0.95	3.33%	96.67%	0.02	0.98

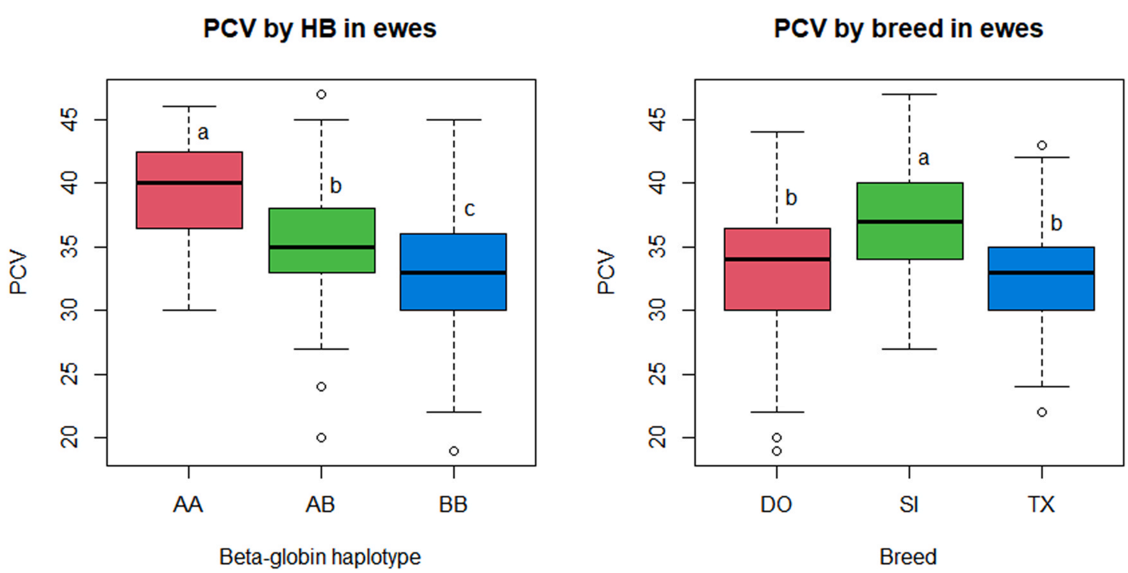

Fig. 1. Effect of β-globin haplotypes (Hb-AA, Hb-AB, and Hb-BB) and sheep breeds (Dorper (DO), Santa Inês (SI), and Texel (TX)) on packed cell volume (PCV) in ewes. $_{abc}$Different letters indicate significant differences (p<05). Box plots showing median (central line), first and third quartiles (box), minimum and maximum values (whiskers), and outliers (circles) of PCV data collected on February, March and July.

precipitation and interaction between mean precipitation and mean humidity contributed to the model fitting (AIC=1510.1 and R^2=0.60).

There was no significant association (p>0.05) of β-globin haplotypes to the other parasite resistance phenotypes (FEC, weight gain, FAMACHA score and body composition score) evaluated in ewes and lambs from all three sheep breeds.

4. Discussion

SI was the only breed with Hb-AA individuals, whereas the TX and DO breeds presented a higher frequency of Hb-BB individuals and no Hb-AA. Jiang et al. (2015) also characterized the different β-globin alleles in 70 domestic sheep of 42 breeds and the Hb-B haplotype also presented the highest allele frequency (71.4%). In addition, as in the present study, Jiang et al. (2015) did not find Hb-AA in TX and DO breeds, but it was detected in SI. Based on the sample size used in the present research, we hypothesized that the allelic frequency of β-globin in sheep is dependent on the breed and, consequently, on its origin and evolutionary processes through natural selection. SI possibly descends from a sheep breed submitted to natural selection for a long period, until they become tolerant to *H. contortus* infections (Amarante et al., 1997). Evans et al. (1958) reported that Hb-A has a selective advantage in sheep at higher altitudes, constituting the most common allele in land breeds with high altitudes, as in England and Scotland. Thus, the population sampled in this study has a higher frequency for Hb-BB, suggesting its adjustment to lower altitudes, as is the case for the geographic location of Brazil, where the samples were derived. Additionally, for this study, it was challenging to find Hb-AA breeding stock available for purchase.

In the present study, *H. contortus* infection impaired the performance of the lambs. The stronger negative correlation showed that increased tFEC resulted in a reduction in PCV, while the weaker correlation suggested a decrease in weight gain with increased tFEC. On the other hand, the positive correlation between PCV and weight gain indicated that the increase in PCV also led to higher weight gain. Sheep highly infected

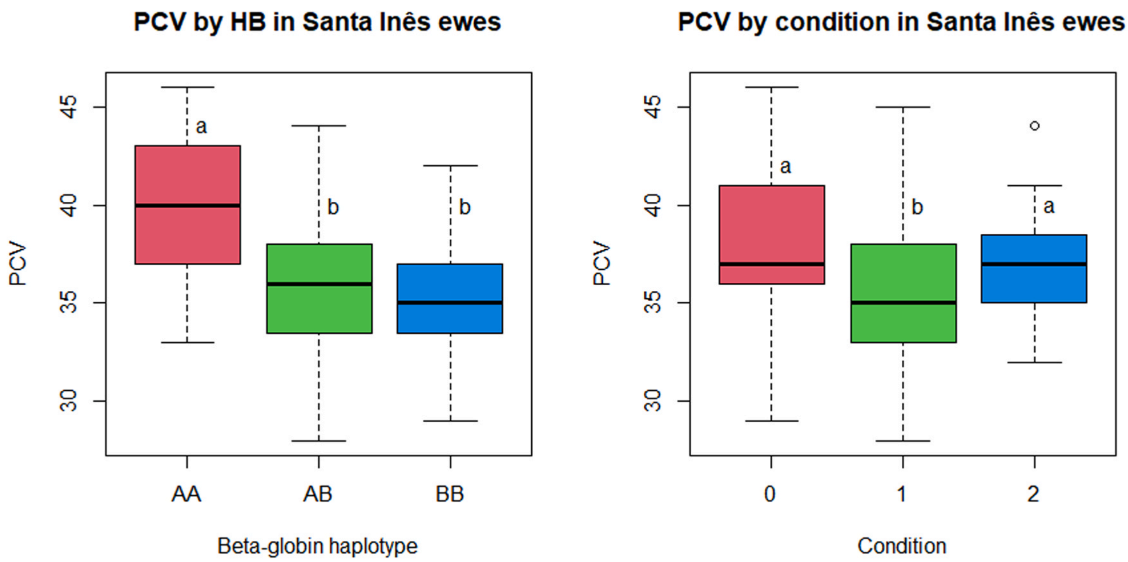

Fig. 2. Effect of β-globin haplotypes (Hb-AA, Hb-AB, and Hb-BB) and condition (0 = not pregnant, 1 = pregnant and raised the lamb and 2 = pregnant, but the lamb was subjected to artificial feeding) on packed cell volume (PCV) in Santa Inês ewes. $_{abc}$Different letters indicate significant differences (p<05). Box plots showing median (central line), first and third quartiles (box), minimum and maximum values (whiskers), and outliers (circles) of PCV data collected on February, March and July.

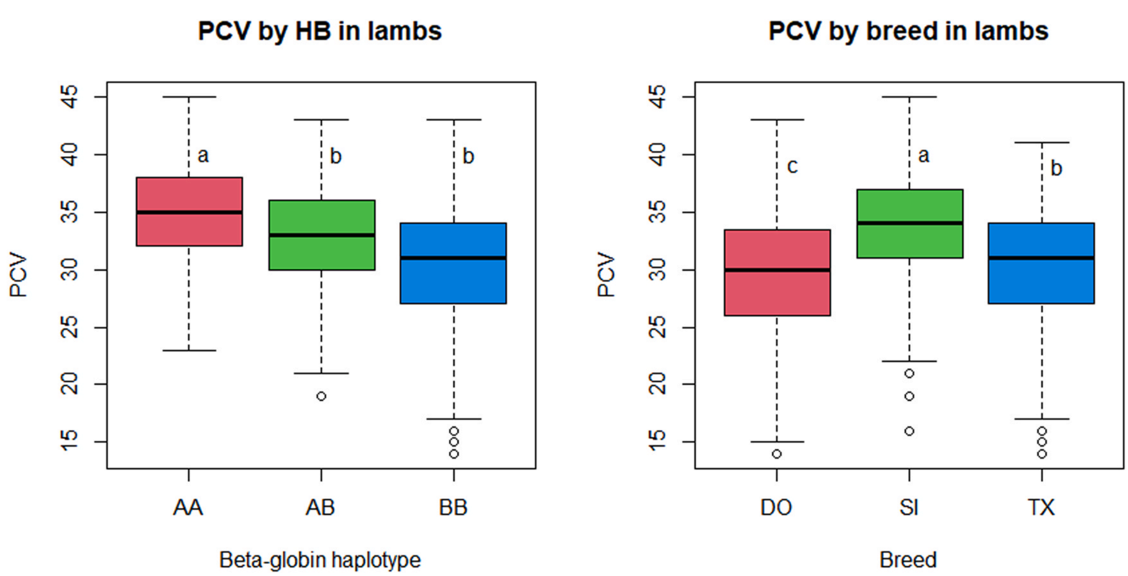

Fig. 3. Effects of β-globin haplotypes (Hb-AA, Hb-AB, and Hb-BB) and sheep breeds (Dorper (DO), Santa Inês (SI), and Texel (TX)) on packed cell volume (PCV) in lambs. $_{abc}$Different letters indicate significant differences (p<0.05). Box plots showing median (central line), first and third quartiles (box), minimum and maximum values (whiskers), and outliers (circles) of PCV data collected on D63, D84, D105, D126, D147, D168, and D189.

with GIN have lower PCV compared to less infected animals (Coop et al., 1977). In addition, dewormed animals can have weight gain approximately twice as high as untreated ones (Keegan et al., 2018).

The ewes and lambs of the Hb-AA haplotype presented significantly better PCV in relation to the Hb-AB and Hb-BB haplotypes. Even when comparing within the same breed, as was the case with SI, a breed considered resistant (Chagas et al., 2013) and the only one that had animals of all three haplotypes, it was observed that Hb-AA ewes and lambs had a higher PCV than Hb-AB and HB-BB. This highlights the hypothesis that animals carrying the A allele (especially Hb-AA) are more tolerant to haemonchosis, as the main clinical sign of this disease is anemia (Bertagnon et al., 2019). This is due to the fact that Hb-A allele animals have the coding gene for β^{C}-globin, which has a greater affinity for oxygen, being expressed in situations of anemia or hypoxia as a compensatory mechanism (Hammerberg et al., 1974), while there is a deletion in the gene encoding β^{C} globin in Hb-BB (Garner and Lingrel, 1988), causing a lower resistance of these animals to anemia (Huisman and Kitchens, 1968). We believe that this mechanism has the potential to mitigate disease severity, as sheep infected with *H. contortus* can lose up to 30 μL of blood per day per adult worm (Emery et al., 2016). Since FEC levels were elevated and similar in the three breeds and haplotypes, we can infer that individuals of Hb-AA haplotype of the ovine β-globin demonstrate tolerance to haemonchosis. This is due to the fact that the tolerance of an animal is intrinsically related to its ability to maintain performance and remain asymptomatic, even when they harbor a substantial quantity of L_3 (Bishop, 2012; Hine et al., 2022). On the other hand, animals carrying the Hb-BB haplotype may be more susceptible to haemonchosis, since, according to Freitas et al. (2023), susceptible sheep are those that house GIN and manifest severe clinical signs, such as anemia, and whose production is affected.

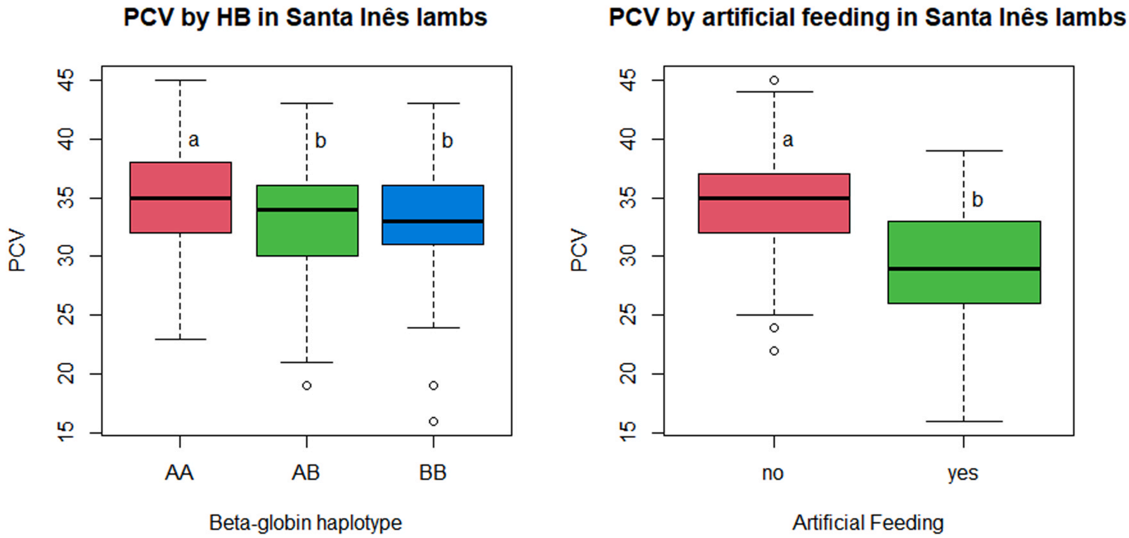

Fig. 4. Effect of β-globin haplotypes (Hb-AA, Hb-AB, and Hb-BB) and artificial feeding (yes = orphan lambs and no = lambs that stayed with their mothers) on packed cell volume (PCV) in Santa Inês lambs. $_{abc}$Different letters indicate significant differences (p<05). Box plots showing median (central line), first and third quartiles (box), minimum and maximum values (whiskers), and outliers (circles) of PCV data collected on D63, D84, D105, D126, D147, D168, and D189.

Previous studies conducted by our research group, involving Morada Nova lambs, corroborated the current results regarding PCV (Okino et al., 2021a, 2021b; Okino et al., 2023). In the first study, 286 lambs were artificially infected with 4000 third-stage larvae of *H. contortus*, where, in addition to the Hb-AA animals showing significantly higher PCV values ($p<0.05$), they also exhibited lower FEC and higher birth weight (Okino et al., 2021a, 2021b). In the second study, which shared a similar sample size with the current study and involved natural infection, no significant differences were observed ($p>0.07$) for FEC, which is in line with the results of this study. Okino et al. (2023), explained that the reduced sample size, the high variability of FEC and its susceptibility to climatic and nutritional influences may contribute to the less pronounced differences between the haplotypes. Furthermore, in natural infection, the parasite loads ingested by each animal vary and there is no control over the amount ingested by each one, unlike artificial infection, where standardized parasite loads are administered to each individual. Okino et al. (2023) observed an improved abomasum local response in Hb-AA lambs, with an increase in the Th2 response, mucin and lectin, demonstrating that animals of this haplotype are able to develop an improved response in the primary site of *H. contortus* infection.

In the current study, orphan lambs subjected to artificial feeding exhibited lower PCV compared to those under natural suckling. Further investigations are needed; however, it is suspected that this may be due to nutritional deficiency, rather than infection by *H. contortus*, since FEC and weight gain were lower (data not shown). Cow's milk may not adequately meet the nutritional needs of lambs (Kanwal et al., 2004) and artificial feeding, even with fresh sheep's milk, may impair the growth, health and emotional wellbeing of lambs (Mialon et al., 2021). Male lambs also exhibited lower PCV compared to females. This disparity can be attributed to greater parasitic susceptibility associated with androgen hormones in males, while females show the stimulatory effects of estrogen on immune responses (Urquhart et al., 1996). Additionally, in males, compared to females, there is increased competition for dietary protein to meet both growth and immune function requirements (Houdjik et al., 2001). Furthermore, lambs treated with AH showed higher PCV, as AH treatment tends to decrease FEC, consequently increasing PCV.

The body condition score of 3 emerged as the most prevalent category, both among ewes and lambs, revealing remarkable uniformity, with the absence of statistically significant differences between different haplotypes. This homogeneity can be attributed to the practice of dietary supplementation with concentrated feed, which was equally provided to all animals. As noted by Nechifor et al. (2022), dietary supplementation, particularly in reproductive ewes, represents an effective means to enhance the body condition score of animals.

The results of FAMACHA with the prevalence of degree 1 (indicating the absence of anemia), did not corroborate with those of PCV, showing no significant differences between the haplotypes. Given that this diagnostic method is categorical (with only five degrees, ranging from 1 to 5) and was performed by different evaluators in the context of this study, the observations became more susceptible to subjectivity. Dahourou et al. (2021) investigated the accuracy of the FAMACHA test and also identified a low agreement between the FAMACHA scores and PCV. A study conducted by Cintra et al. (2018) found that the FAMACHA method has low sensitivity in young lambs, indicating that it should not be employed alone for the control of haemonchosis in these animals.

Genetic strategies aiming to promote tolerance/resistance in sheep of different breeds and ages have the potential not only to increase the profitability of producers by reducing the need for AH treatment and animal mortality, but also to contribute to the mitigation of environmental impacts resulting from contamination caused by the use of these agents. In this sense, increasing the population of individuals of haplotype Hb-AA in a flock, through genetic selection, may become an effective support tool for the control of *H. contortus* and haemonchosis in the future, since strong evidence suggests that *H. contortus* does not adapt to long-term exposure to sheep that are genetically resistant to GIN infections (Amarante et al., 2009).

5. Conclusion

Since the reduction of PCV is the main consequence of haemonchosis, our results suggest that ewes and lambs of Hb-AA haplotype may be more tolerant to haemonchosis, presenting milder clinical signs of anemia despite a predominant infection with *H. contortus*. Further studies are still required, but the selection of β-globin A animals could be applied for genetic improvement, to select animals that are naturally more resilient to *H. contortus*.

Declaration of Interest Statement

The authors declare no conflicts of interest.

CRediT authorship contribution statement

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

Declaration of Competing Interest

The authors report no declarations of competing interest.

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Animal welfare statement

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

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