UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CAMPUS JABOTICABAL

GENETIC CHARACTERIZATION OF RESISTANCE TO *Haemonchus contortus* IN MORADA NOVA SHEEP

Marei Borsch von Haehling

Veterinarian

2020

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Thesis presented to the faculty of Agricultural and Veterinary Sciences – Unesp, campus of Jaboticabal, as part of the requirements for the acquisition of the title "Mestre" in Veterinary Medicine
(area: animal pathology)

2020

H133g

Haehling, Marei Borsch von

Genetic characterization of resistance to Haemonchus contortus in Morada Nova sheep / Marei Borsch von Haehling. -- Jaboticabal, 2020

76 p. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientadora: Ana Carolina de Souza Chagas

1. Genetic markers. 2. Genetic parameters. 3. Host resistance. 4. Resilience. 5. Parasite challenge. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: GENETIC CHARACTERIZATION OF RESISTANCE TO *Haemonchus contortus* IN MORADA NOVA SHEEP

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Jaboticabal, 05 de maio de 2020

CURRICULUM VITAE

Marei Borsch von Haehling (née Borsch) was born in Cologne, Germany, on November 25th 1986. From 2007 to 2013, she studied veterinary medicine at the Ludwig–Maximilians–University Munich, obtaining the veterinary licence in May 2013. From 2009 to 2011, she worked as a graduate assistant in the department for veterinary anatomy. Internships at the veterinary practice Dr. Morsy in Wershofen (March 2010), as part of the clinical rotation at the clinics of the Ludwig–Maximilians–University for ruminants, equines, swine, birds and small animals (March 2011 to March 2012), at the veterinary practice Dr. Miguel Arcanjo Valencise in Dourado, Brazil (May 2012), at the clinic for equines in Kottenforst (June to July, 2012), at the department for animal welfare, ethology, animal hygiene and husbandry of the Ludwig–Maximilians–University (July 2012) and at the clinic for swine and small ruminants of the University of Veterinary Medicine in Hannover (July to September 2012) were completed successfully. She worked as a veterinarian at the veterinary practice Dr. Schneider and Gilg in Eichstätt (June 2013 to May 2014) and Dr. Zauscher in Odelzhausen (June 2014 to November 2016). In March 2018, she joined the master's programme of the FCAV – Unesp of Jaboticabal, with execution of the experiments at the Embrapa Southeast Livestock Unit in São Carlos, SP, Brazil.

AGRADECIMENTOS (ACKNOWLEDGEMENTS)

Ao meu marido **Ricardo**, à minha sogra **Calau**, à minha cunhada **Veri,** à **Mariana** e à **Sandi**. Em cada minuto que eu me dediquei ao trabalho, eu sabia que vocês se dedicaram à minha filha, com muito amor e carinho.

À minha orientadora, **Drª Ana Carolina de Souza Chagas,** pela oportunidade e pelo apoio nos momentos difíceis.

À **Drª Simone Meio Niciura,** pelos ensinamentos no laboratório, na genética molecular e na arte de escrever um artigo, pelo sorriso e pela disposição extraordinária.

À **Drª Patrícia Tholon**, pela introdução na estatística e análise genética e por ter insistido no “R”.

Ao **Dr. Waldomiro Barióni Júnior**, pela ajuda na estatística e pelas histórias.

À **Giovanna,** à **Carolzinha,** ao **Giba** e à **Flávia,** pelo apoio no laboratório.

Ao **Dr. Sérgio de Novita Esteves** e aos funcionários de campo da Embrapa Pecuária Sudeste, pela manutenção dos animais e pela ajuda nas colheitas.

À equipe do laboratório de parasitologia, **Isabella**, **Amanda**, **Luciana**, **Alemán**, **João**, **Júnior**, **Leo** e **Rafa,** pela grande ajuda e pela companhia nas colheitas.

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CERTIFICATE OF THE ETHICS COMMITTEE FOR ANIMAL EXPERIMENTATION

Embrapa
Pecuária Sudeste

CERTIFICADO

PRT Nº 04/2017

Certificamos que o projeto de pesquisa intitulado: **CARACTERIZAÇÃO GENÉTICA E DE RESPOSTAS IMUNES ASSOCIADAS AO FENÓTIPO DE RESISTÊNCIA PARASITÁRIA EM REBANHO OVINO DA RAÇA MORADA NOVA**, registrado com o nº 04/2017 sob a responsabilidade do pesquisador científico Ana Carolina de Souza Chagas, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA EMBRAPA PECUÁRIA SUDESTE.

São Carlos,

Dra. Márcia Cristina de Sena Oliveira
Presidente da Comissão de Ética no Uso de Animais
Embrapa Pecuária Sudeste

Reunião de ___/___/____

Finalidade	Pesquisa Científica
Vigência da autorização	Abril/2017 a março 2019
Espécie/linhagem/raça	Ovinos Morada Nova
Nº de animais	494
Peso/Idade	15-40 Kg
Sexo	174 M e 320 F
Origem	CPPSE

GENETIC CHARACTERIZATION OF RESISTANCE TO *Haemonchus contortus* IN MORADA NOVA SHEEP

ABSTRACT – Gastrointestinal nematodes are a major constraint in sheep production. Breeding for resistance has proven to be an effective and feasible approach to address this problem. The use and investigation of genetic markers for resistance traits could accelerate genetic progress and lead to a better understanding of underlying molecular mechanisms. The aim of this study was to verify the possibility of selection for resistance and resilience traits in Morada Nova lambs, to estimate potential correlated responses and to evaluate if five single nucleotide polymorphisms (SNPs) (OAR2_14765360, OAR6_81718546, OAR11_62887032, OAR12_69606944 and OAR15_59871543) are associated with resistance and resilience traits. A total of 287 lambs and 131 ewes were submitted to two consecutive independent parasite challenges by oral infection with 4,000 infective larvae (L_3) of *Haemonchus contortus*. Faecal egg counts (FEC), packed cell volume (PVC) and body weight were measured every one or two weeks for 42 days in each trial. DNA samples from 287 lambs, 131 ewes and 4 rams were amplified by ARMS-PCR or PCR-RFLP and genotypes were determined. Estimates of genetic parameters were obtained for individual records as well as for overall traits with repeated measures, using mixed animal models. Analysis of variance (ANOVA) was used for association analyses between SNP genotypes and phenotypes. In case of significant association, the allele substitution effect was calculated based on a linear model. Heritability estimates of FEC in the first and second parasite challenge were, respectively, 0.25 ± 0.18 and 0.46 ± 0.19 for lambs, and 0.00 ± 0.09 and 0.20 ± 0.16 for ewes. For PCV, heritability estimates were 0.23 ± 0.14 and 0.32 ± 0.16 for lambs and 0.13 ± 0.11 and 0.37 ± 0.18 for ewes. The overall weight gain heritability estimate was 0.70 ± 0.21. Genetic correlations of FEC and PCV between lambs and ewes were 0.36 ± 0.08 and 0.42 ± 0.08, respectively. No significant genetic correlation was found between weight gain and the other traits, while there was a negative genetic correlation between FEC and PCV (-0.70 ± 0.03). OAR2_14765360 and OAR12_69606944 were associated with FEC, and OAR12_69606944 also had significant effects on PCV and weight gain, showing favourable associations of the CC genotype with all evaluated traits. Both OAR6_81718546 and OAR11_62887032 were associated with weight gain, and OAR6_81718546 had an additional effect on PCV. OAR15_59871543 was not polymorphic in the population. OAR6_81718546 and OAR12_69606944 presented significant allele substitution effects of -1.06 ± 0.52 kg for the T allele on final body weight and 0.74 ± 0.32 for the C allele in PCV of the same sampling date, respectively. Selection for low FEC and high PCV is possible in Morada Nova lambs. Selection for low FEC should have a correlated response on PCV (leading to higher PCV), while no correlated response is expected on weight gain. Selection for low FEC and high PCV in lambs should lead to low FEC and high PCV in ewes. An index consisting of overall weight gain and two records of FEC and PCV (taken 4 to 6 weeks after infection during the second parasite challenge) can be used as selection criterion in the Morada Nova lambs, allowing simultaneous selection for lower FEC, higher PCV and higher weight gain. Our findings support the existence of quantitative trait loci (QTL) for resistance and resilience in linkage disequilibrium with the polymorphic SNPs and suggest their future use for explorations of these traits in Morada Nova sheep.

Keywords: genetic markers, genetic parameters, host resistance, resilience, parasite challenge

CARACTERIZAÇÃO GENÉTICA DA RESISTÊNCIA A *Haemonchus contortus* EM OVINOS DA RAÇA MORADA NOVA

RESUMO – Nematódeos gastrintestinais são um grande obstáculo na produção de ovinos. A seleção para resistência tem sido uma abordagem eficiente e factível para lidar com esse problema. A utilização e investigação de marcadores genéticos para características de resistência poderiam acelerar o melhoramento genético e levar ao melhor entendimento dos mecanismos moleculares de resistência. O objetivo desse estudo foi verificar a possibilidade de seleção para características de resistência e resiliência em cordeiros da raça Morada Nova, estimar as potenciais respostas correlacionadas e avaliar se cinco polimorfismos de base única (SNPs, OAR2_14765360, OAR6_81718546, OAR11_62887032, OAR12_69606944 and OAR15_59871543) estão associados às características de resistência e resiliência. Um total de 287 cordeiros e 131 matrizes foram submetidas a dois desafios parasitários consecutivos e independentes por infecção oral com 4.000 larvas infectantes (L_3) de *Haemonchus contortus.* Contagem de ovos por grama de fezes (OPG), volume globular (VG) e peso corporal vivo (PV) foram monitorados a cada uma ou duas semanas para 42 dias em cada desafio. Amostras de DNA de 287 cordeiros, 131 matrizes e 4 reprodutores machos foram amplificados por ARMS-PCR ou PCR-RFLP e os genótipos foram determinados. Estimativas de parâmetros genéticos foram obtidas para dias individuais de coleta, e para a característica como um todo com medidas repetidas, utilizando modelos animais mistos. A análise de variância (ANOVA) foi utilizada para análises de associação entre genótipos dos SNPs e fenótipos. No caso de associação significativa, o efeito de substituição de alelo foi calculado baseado em um modelo linear. As estimativas de herdabilidade para OPG no primeiro e segundo desafio parasitário foram, respectivamente, 0,25 ± 0,18 e 0,46 ± 0,19 para cordeiros, e 0,00 ± 0,09 e 0,20 ± 0,16 para matrizes. Para VG, as estimativas de herdabilidade foram 0,23 ± 0,14 e 0,32 ± 0,16 para cordeiros e 0.13 ± 0.11 e 0.37 ± 0.18 para matrizes. A estimativa de herdabilidade do ganho de peso diário (GPD) total foi 0,70 ± 0,21. As correlações genéticas de OPG e VG entre cordeiros e matrizes foram 0,36 ± 0,08 e 0,42 ± 0,08, respectivamente. Não foi encontrada correlação genética significativa entre GPD e as demais características, enquanto ocorreu uma correlação genética negativa entre OPG e VG (-0.70 ± 0.03). OAR2_14765360 e OAR12_69606944 foram associados com OPG, e OAR12_69606944 também teve um efeito significativo no VG e GPD, mostrando associação favorável do genótipo CC com todas as características avaliadas. OAR6_81718546 e OAR11_62887032 foram associados ao GPD, e OAR6_81718546 teve um efeito adicional no VG. OAR15_59871543 não foi polimórfico na população. OAR6_81718546 e OAR12_69606944 apresentaram efeito de substituição de alelo significativo de -1.06 ± 0.52 kg para o alelo T no PV final e 0.74 ± 0.32 para o alelo C no VG do mesmo dia de desafio, respectivamente. A seleção para OPG baixo e VG alto é possível em cordeiros da raça Morada Nova. A seleção para OPG baixo deve ter uma resposta correlacionada no VG (levando a VG mais alto), enquanto não é esperada uma resposta correlacionada no GPD. A seleção para OPG baixo e VG alto em cordeiros deve levar a OPG baixo e VG alto em matrizes. Um índice contendo GPD total e dados de duas coletas de OPG e VG (conduzidas 4 a 6 semanas após a infecção durante o segundo desafio parasitário) pode ser utilizado como critério de seleção em cordeiros, permitindo seleção simultânea para OPG mais baixo, VG mais elevado e GPD mais elevado. Nossos resultados suportam a existência de locos de características quantitativas (QTL) para resistência e resiliência em desequilíbrio de ligação com os SNPs polimórficos e sugere o seu uso futuro para a exploração dessas características em ovinos Morada Nova.

Palavras-chave: marcadores genéticos, parâmetros genéticos, resistência do hospedeiro, resiliência, desafio parasitário

CHAPTER 1 – PRELIMINARY CONSIDERATIONS

1.1. INTRODUCTION

Gastrointestinal nematodes (GIN) are a major constraint in sheep production worldwide (Perry et al. 2002). These parasites cause high economic losses due to their direct detrimental effects on health and performance of animals, and due to the necessity of costly treatments. This is especially true for tropical environments, which favour the survival and development of free stage larvae of some gastrointestinal nematodes, including *Haemonchus contortus,* the most important GIN in Brazilian sheep flocks. Its high pathogenicity is mainly due to hematophagia of the adult worms (and fifth stage larvae), which can lead to severe anaemia and subsequent deaths of infected animals. Many flock holders are highly dependent on the use of anthelmintic drugs to maintain their productivity (Jackson et al., 2009). The efficiency of this approach, however, is limited due to an increasing prevalence of drug-resistant parasites (Veríssimo et al., 2012; Papadopoulos et al., 2012; Albuquerque et al., 2017). In São Paulo state of Brazil, there are reports of flock holders abandoning sheep production due to this problem (Amarante et al., 2004).

Several alternative strategies have been suggested in order to reduce the necessity of anthelmintic treatments and thus attenuate the development of anthelmintic resistance in parasites. Adequate nutrition and dietary supplementation, grazing management, targeted selective treatment (TST), biological control and the use of animals resistant or resilient to nematode infection are some examples, with the latter being one of the most promising approaches. The existence of variability in the ability of hosts to withstand GIN infections has been evidenced within and between sheep breeds in a number of studies (Baker and Gray, 2004; Aguerre et al., 2018; Snyman and Fisher, 2019). The feasibility and efficiency of genetic improvement of resistance is well-documented (Bisset et al., 2001; Eady et al., 2003; Kemper et al., 2010), and this trait has been included in commercial breeding programmes in Australia (Brown and Fogarty, 2017), New Zealand (Bisset et al., 2001) and Uruguay (Goldberg et al., 2012). Genetic parameters, especially correlations between resistance and performance traits, tend to vary across breeds and environments (Bishop and Stear, 2003), making it necessary to explore them separately for each situation. To obtain estimates of individual breeding values, it is also necessary to submit animals to either natural or artificial nematode infection, which can compromise performance and raise issues of comparability between environments and

generations (Hunt et al., 2008). Genetic markers for resistance and resilience could allow animals to be selected (in part or entirely) based on their genotypes. This could facilitate the selection process by reducing the need for phenotypic information and enhancing genetic prediction (Hunt et al., 2008; Rosa, 2011; Benavides et al., 2016).

Morada Nova is a naturalized Brazilian hair sheep breed that is very well adapted to tropical conditions and has shown to be highly resistant and resilient to GIN infections (Facó et al., 2008; Issakowicz et al., 2016; Toscano et al., 2019). Furthermore, the Morada Nova's aptitude as a maternal breed in meat production has been evidenced by Issakowicz et al. (2016), making this breed an important resource for efficient and sustainable production in tropical, highly parasitised environments.

The aim of the present study is the genetic characterization of resistance and resilience to *H. contortus* in a Morada Nova population with the purpose of evaluating the feasibility of genetic selection and investigating underlying biological mechanisms. Genetic parameters for faecal egg counts (FEC), packed cell volume (PCV) and body weight, including phenotypic and genetic correlations between these traits, were estimated. In addition, five single nucleotide polymorphisms, formerly associated to FEC in a Red Maasai and Dorper packcross population (Benavides et al., 2015) were evaluated as for their association to FEC, PCV and body weight.

1.2. LITERATURE REVIEW

1.2.1. Relevance of gastrointestinal nematodes

Gastrointestinal parasitism was estimated to be the most important animal health constraint based on a worldwide ranking elaborated by Perry et al. (2002), considering their wide geographical distribution, wide range of host species and their high economic impact on farms. The economic damage due to internal parasites was estimated to be 436 million $ AUD per year in the Australian sheep industry (Brown and Fogarty, 2017), with the greater proportion probably being caused by *H. contortus* (Emery et al., 2016). *H. contortus* is the most prevalent GIN in small ruminant flocks of Brazil (Chagas et al., 2013), including the state of São Paulo (Amarante et al., 2004). Apart from anaemia (which is due to the hematophagia of *H. contortus*), clinical signs of haemonchosis are oedemas (typically submandibular) caused by loss of plasmatic protein, anorexia, or, in severe cases, hemorrhagic gastritis. Even in cases of subclinical infections, performance is often constrained in terms of retarded growth, weight loss or low carcass quality.

H. contortus possesses a direct life cycle. The hosts are infected at grazing, by third stage larvae (L_3) present on pastures. After shedding of their cuticle in the rumen, L_3 larvae settle in apposition to the gastric glands of the abomasum, where they undergo the transformation to the fourth (L_4) and fifth (L_5) larval stage. L_5 larvae develop an oral lancet that enables them to obtain blood from the mucosal vessels, briefly before their final transformation into adults. Eggs are eliminated with the faeces onto the pastures where the first stage (L_1) larvae hatch within a few hours. Under favourable conditions, L_3 may develop in 5 days, allowing the conclusion of a full life cycle within 20 days, the shortest of all GIN (Emery et al., 2016). This fact, together with an establishment rate of 60% and a daily egg output of 5000 to 15,000, guarantees *H. contortus*'s high ability of genetic adaptation, including acquisition of resistance to anthelmintic drugs (Emery et al., 2016).

Counting of worm eggs in the animal's faeces – faecal egg count (FEC) – is the measure most commonly used to access infection levels. It is a good indicator of worm burden of *H. contortus* (unless the worm burden is very high, which decreases fecundity) (Bishop et al., 2012b). FEC can range from zero to several thousand egg counts per gram of faeces. Within flocks, the majority of the animals have relatively low FEC, while a few animals shed a much higher number of eggs (Amarante et al., 1998). Therefore, the distribution of FEC is almost inevitably right-skewed, which is why most studies use some kind of transformation before performing analyses on FEC (Bishop et al., 2012b).

Due to the hematophagia of *H. contortus,* packed cell volume (PCV) can be used to access the effect of infection on the health status of individual animals. PCV is a blood parameter that needs to be maintained within its physiological scope. With rising levels of *H. contortus*-infection, an increasing amount of blood is removed by the parasites, eventually causing anaemia. A negative correlation between FEC and PCV has consequently been related in a number of studies (Albers et al., 1987; Baker et al., 2003; Lôbo et al., 2009).

The efficiency of anthelmintic treatments as a control measure for GIN is threatened by the increasing prevalence of drug resistant parasites, with multi-drug resistance against different classes of anthelmintics being frequently reported (Jackson et al., 2009; Zvinorova et al., 2016). Field studies have even revealed resistance of GIN to Monepantel, the most recent active component launched in 2009 (Scott et al., 2013; Mederos et al., 2014; Sales and Love, 2016). In São Paulo state, reduced efficiency of albendazole and ivermectin were found in 100%, of moxidectin in 96.6%, of closantel in 92.9% and of levamisole in 53.6% of the sheep flocks investigated by Veríssimo et al. (2012). As a result of this situation, mortality rates can rise despite anthelmintic treatments, causing the abandonment of sheep production (Amarante et al., 2004). The

implementation of alternative strategies for nematode control could reestablish the productivity of these production systems and attenuate the development of drug resistance in parasites.

1.2.2. Characteristics and potential of the Morada Nova breed

Morada Nova is a naturalized hair sheep breed from the Northeast of Brazil that is very well adapted to the hot, semi-arid climate of this region. The first description of Morada Nova sheep was by Octávio Domingues, who first encountered sheep of this breed in 1937, in the municipality “Morada Nova” (Ceará), the supposed geographical centre of origin (Arandas, 2017). In the Northeast, Morada Nova sheep are an important resource for traditional, low-input production of meat and skin (Arandas et al., 2017). There are no records of the genetic origin of the Morada Nova breed, but contribution of both Mediterranean and African sheep have been suggested (Facó et al., 2008). Results of two studies using genome-wide genotyping of single nucleotide polimorphism (SNP) revealed a high genetic relatedness between Morada Nova and African breeds (Kijas et al., 2012; Toledo, 2014).

Until 2006, the flock holder’s preference for larger sheep and indiscriminate crossbreeding with exotic breeds had caused a continuous decrease in the number of Morada Nova sheep. Since then, programmes with the aim of preservation, characterization and development of this breed have been implemented (McManus et al., 2019). In 2010, about 800 animals were registered by ARCO (Brazilian Association of sheep breeders), compared to about 400 animals in 2004. The Northeastern region of Brazil possesses the highest amount of Morada Nova sheep, but numbers are growing in the state of São Paulo. Between 350 and 400 of the 800 animals registered in 2010 were from Ceará, while both São Paulo and Paraiba accounted for 100 to 150 animals. Since 2012, São Paulo has surpassed the Northeastern states in numbers of registered animals, although the overall numbers have dropped considerably from 2010 to 2014, the last year of registration considered in this study (McManus et al., 2019). Due to the authors, this decline in numbers of registered animals is possibly related to costs for registration and a long drought in the Northeast, that began in 2011.

Arandas et al. (2012) investigated the population structure of 36 Morada Nova herds, located in Ceará, Pernambuco, Paraíba, Rio Grande do Norte and São Paulo. Estimates of the effective population size (N_e), inbreeding rate (ΔF) and panmixia value (PAM) were calculated for each herd, obtaining a mean N_e of 12.88, ΔF of 0.08 and PAM

of 0.92, considering all flocks investigated. For the herds in São Paulo, means of 6.66 (N_e), 0.092 (ΔF) and 0.908 (PAM) were estimated. The authors concluded that the variability within the flocks of Morada Nova sheep was in danger, and suggest the implementation of a management plan for its maintenance. The relatively high heterozigosity, indicated by the panmixia value, might be a result of crossbreeding, rather than a within-breed phenomenon, due to the authors (Arandas et al., 2012). McManus et al. (2019) performed a pedigree analysis on 10,015 registered Morada Nova sheep, born between 1973 and 2014. Effective herd sizes ranged from 10 to 30 between 1983 and 2004. From 2004 to 2009, there was an increase in effective herd size, with a peak of over 70 N_e for animals born between 2006 and 2009, and a subsequent slight decrease to a value of about 60 when considering animals born between 2008 and 2011. Genetic variability was found to be availble within the breed, given that the ratio between the effective number of ancestors (fa) and the effective number of founders (fe) was approximately 1. In the Santa Inês breed, this ratio was found to be 1.35, indicating a reduction in variability. The authors concluded that the genetic diversity of the Morada Nova breed is not immediately endangered (McManus et al., 2019).

This breed's characteristics are small size, high adaptation to tropical climate, high litter size, non-existent reproductive seasonality, good maternal ability and excellent pelt quality, but also low weight gain and carcass quality (Facó et al., 2008; Lôbo et al., 2011). The Morada Nova's low adult body weight is considered advantageous by several authors (Facó et al., 2008; Lôbo et al., 2011; Issakowicz et al., 2016; McManus et al., 2019), because it results in higher stocking rates per hectare and higher production of kg lamb per kg feed. The latter is highly relevant insofar that feeding of the ewes constitutes the most important expense factor in lamb production (Lôbo et al., 2011).

Economic values were estimated for a Morada Nova flock situated in a semi-arid environment of the Northeastern region of Brazil. This study showed that meat production was profitable under these circumstances and that improvement in carcass quality, slaughter weight, survival and some reproductive traits (like litter size) could increase the lucrativeness of the system. No significant economic value was found for the number of anthelmintic treatments in this study. A possible reason for this could be low parasite challenge (the very short rainy seasons during the experiment did not favour GIN development on pastures) or the natural resistance of Morada Nova sheep (Lôbo et al., 2011).

In a study conducted by Issakowicz et al. (2016), ewes of the Santa Inês and Morada Nova breed showed low GIN infection level under natural parasite challenge as well as a high level of resilience. Even during the periparturient period – a phase in which

FEC usually increases due to a decrease in immunity (Kahn, 2003) – the ewe's health was not affected. The Morada Nova ewes had lower FEC than Santa Inês ewes at 30 days *post partum* (mean values of 1114 and 2005, respectively). Also, they presented significantly higher PCV at 56 days of gestation as well as 30 and 60 days *post partum* (30.6 ± 1.1, 32.5 ± 1.5 and 33.5 ± 1.3%, respectively, for Morada Nova and 28.0 ± 1.1, 28.8 ± 1.5 and 30.9 ± 1.3% for Santa Inês ewes). Both breeds showed similarly high reproductive performance, with 1.5 lambs per ewe, a conception rate of 91 and 93 % and multiple births of 57 and 53% for Morada Nova and Santa Inês ewes, respectively. In addition, Morada Nova and Santa Inês ewes were mated either to rams of the same breed or to Dorper rams and measures of productivity were calculated for the pure-breed and cross-breed lambs. Santa Inês ewes are heavier than Morada Nova ewes (51.8 ± 7.07 kg and 33.1 ± 4.98, respectively), and they produced lambs with higher body weight at birth (6.20 ± 0.4 kg and 4.27 ± 0.8 kg, respectively) and at weaning (26.9 ± 2.0 kg and 18.9 ± 1.9 kg, respectively). However, when crossed with Dorper rams, the Morada Nova ewes produced significantly higher amounts of litter weight at weaning per kg body weight of dam (0.62 ± 0.03 kg) compared to Santa Inês X Dorper (0.47 ± 0.03 kg), Santa Inês X Santa Inês (0.42 ± 0.03 kg) and Morada Nova X Morada Nova (0.43 ± 0.03 kg), showing its potential as a maternal breed in meat production (Issakowicz et al., 2016).

In a study that compared Strongylida FEC of several breeds and crosses under natural infection, animals of the Morada Nova breed had the lowest FEC (mean FEC of 8.65), compared to Bergamasca (193.99), Santa Inês (347.88), Ile de France (484.62), Ile de France X Santa Inês (482.26) and Texel X Santa Inês (279.50) (McManus et al., 2009). These results are consistent with the observations made in the Morada Nova flock at the Embrapa Southeast Livestock Unit (CPPSE) in Brazil, that showed these animals to be extremely resistant to GIN, evidenced by a low number of anthelmintic treatments required. In monthly samplings, Morada Nova sheep consistently showed higher PCV than animals of the other breeds kept under equal management conditions (36.3% for Morada Nova, 29.5% Santa Inês, 25.9% Dorper and 26.9% Texel) (Chagas et al., 2015). Resistance and resilience to nematodes are abilities of great interest to sheep production systems in Brazil, given the economic relevance of GIN in the tropics and the problem of drug resistant parasites. The Morada Nova breed could be a tool to increase efficiency and sustainability of sheep production.

1.2.3. The host's response to parasites

Resistance is defined by Bishop and Stear (2003) as the "ability of the host to

resist infection or to control the parasite life cycle". Measures to quantify resistance can be worm burden, worm size, fecundity and FEC (Bishop, 2012b), with the latter being the most commonly used.

Apart from resistance, other aspects of the host's response to parasites are of interest. Especially for parasites with high prevalence (where a high percentage of the flock is infected) the performance of a flock will depend on the ability of the individuals to minimize the damage that the infection causes on their health and performance status. Tolerance is defined as the "net impact on performance of a given level of infection" (Bishop 2012b). In other words: an animal that is resistant to parasites is able to decrease the level of infection, while a tolerant animal is able to decrease the detrimental effect of infection on its performance. Tolerance is difficult to estimate on the individual animal level and for this purpose, the infection status (measured by FEC) has to be taken into account. Breeding values for tolerance can be estimated when related animals graze pastures of different (and well-defined) contamination levels (Bishop, 2012b), although no empirical example of this was found for tolerance of nematodes in sheep.

Resilience, the productivity of an animal in the face of infection (Bishop,2012b), is closely related to tolerance and both terms are used synonymously by some authors. Bishop and Stear (2003) stated that resilience, often measured as a performance trait under parasite challenge conditions, is a combination of resistance, tolerance and performance. Tolerance could thus be considered resilience in the narrow sense. Examples for performance traits quantified under parasite challenge as a measure of resilience are body weight or weight gain (Albers et al., 1987), but the necessity of anthelmintic treatments (Bisset et al., 2001; Morris et al., 2010) or indicators of anaemia in the case of *H. contortus* (Baker et al., 2003; Aguerre et al., 2018; Oliveira et al., 2018) have also been treated as resilience traits.

Resistance, resilience and tolerance are results of defense mechanisms – primarily the immune response – but also of hemostasis, hematopoesis and other mechanisms linked to maintenance and recovery of the integrity of tissues. These mechanisms require resources and energy, and they are highly dependent on the environment (particularly nutrition) and physiological status of the animal (Bishop and Stear, 2003). Ewes usually undergo a considerable increase in GIN infection during the periparturient period (Miller et al. 1998; Matika et al., 2003; Williams et al., 2010), turning it the most significant phase in pasture contamination (Williams et al., 2010). This effect is caused by a temporary decrease in immunity, which is probably due to an increased requirement for energy and nutrients (essentially protein), with the partial priority of the gut immune system for protein supply being reduced in favour of the maintenance of

pregnancy and lactation (Kahn, 2003). GIN infections decrease feed intake, efficiency of feed use and protein levels, with protein being lost into the gastrointestinal tract and increased requirement of protein for repair of affected tissues, maintenance of plasma protein levels and mucoprotein production (Coop and Holmes, 1996). Thus, protein supplementation attenuates the effects of GIN infection and favours an effective immune response (Coop and Holmes, 1996; Kahn, 2003). Regarding susceptibility to GIN infections, an important factor is age, with lambs consistently showing higher infection levels than adults of the same breed (Woolaston and Piper, 1996; Vanimisetti et al., 2004; Goldberg et al., 2012). Apart from the fact that younger animals have less previous contact with parasites and thus had less time to mount an effective immune response against them, immunological hyporesponsiveness in young animals has also been discussed (Colditz et al., 1996). Another factor with great importance for susceptibility to GIN is sex: male animals are frequently reported to have higher FEC in the face of GIN infection, and lower PCV in the case of *H. contortus* (Eady et al., 2003; Gauly et al., 2006; Snyman and Fisher, 2019), and a positive correlation between testosterone levels and worm burden was related by Gauly et al. (2006).

1.2.4. Epidemiological factors of resistance to GIN

Within sheep flocks, highly susceptible animals comprise a large amount of parasites, being responsible for a disproportionately high fraction of larval pasture contamination (Amarante et al., 1998). Resistant animals, on the contrary, eliminate less parasite eggs, which leads to low levels of pasture contamination and decreases the infection pressure within the flock (Bishop and Stear, 2003). This effect was first evidenced in Romney lambs by Bisset et al. (1997), who showed that on pastures grazed by animals of a high-FEC selection line (susceptible), contamination with *Trichostrongylus* larvae was 5 to 6-fold compared to pastures grazed by animals selected for low FEC (resistant), leading to higher weight gains in the resistant group. Morris et al. (2005) observed that a superior performance of susceptible animals in weight gain and wool traits compared to resistant animals was levelled when these two groups were managed in different pastures. Williams et al., (2010) found similar results in periparturient Australian Merino ewes, showing lower infection levels and reduction of pasture contamination with *T. colubriformis* larvae in a group selected for resistance compared to an unselected control group. Consequently, lambs of resistant ewes have the advantage of an environment with low contamination levels, promoting performance (Williams et al., 2010).

In a mathematical model implemented by Bishop and Stear (1999), increase in

weight gain after selection for low FEC was twice as high as would have been expected based on additive genetic effects, suggesting that a considerable part of the increase was due to epidemiological benefits (reduced contamination of pastures by resistant animals). Using a similar model, Laurenson et al. (2012) estimated that susceptible lambs can benefit from contemporary grazing with resistant animals, reaching higher body weights (BW) than when grazed separately, while the resistant lamb's performance is not impaired.

1.2.5. Basic concepts of animal breeding

Animal breeding has the aim of improving the performance of future generations, which is achieved by selecting and mating animals that are considered superior in one or several traits, in comparison to the average population. One precondition for selection is the existence of variation: animals have to differ in their performance, or else it would not be possible to select the "best" animals. If individuals differ in their body weight, for example, it is possible to select and mate only the heaviest animals. However, to achieve genetic merit, the respective trait needs to be – at least to some extent – heritable. Essentially, heritability is the correlation between phenotypic and genetic values of a trait. If the heritability is high, the phenotype of an animal is a good indicator of its genetic merit. In this case, animals of high phenotypic values for a trait tend to have progeny that also (in average) present high phenotypic values for this trait. Heritability estimates can take values between 0 and 1. Traits with heritability estimates of 0 to 0.2 are considered lowly, those of 0.2 to 0.4 moderately, and above 0.4 highly heritable (Bourdon, 2000).

1.2.6. Selection of animals resistant to GIN infection

Because of its easy application, FEC is the measure most commonly used for GIN resistance quantification in sheep. For animals to express their genetic potential for resistance, a certain level of infection is indispensable. Therefore, animals have to be submitted to either natural or artificial infection in order to permit genetic selection (at least when selection is based on phenotypes). The existence of genetic variation in FEC has been evidenced by many studies, both after natural (Gowane et al., 2019 Brown and Fogarty, 2017; Snyman and Fisher, 2019) and artificial (Woolaston and Piper, 1996; Morris et al., 2005; Aguerre et al., 2018) parasite challenge and in a number of sheep breeds, including Australian Merino (Brown and Fogarty, 2017), Perendale (Morris et al., 2005), Dohne Merino (Snyman and Fisher, 2019), Avakalin and Malpura sheep (Gowane et al.,

2019), Blond-faced Manech (Aguerre et al., 2018), Scottish Blackface (Bishop et al., 1996), Texel (Bishop et al., 2004) and Santa Inês (Oliveira et al., 2018). Most studies related moderate heritability of FEC, although estimates differed considerably between genetic groups and environments.

The feasibility of selection for FEC is well-documented. Lines of resistant animals were successfully implemented by means of selection based on artificial challenge with *H. contortus* in Merino (Woolaston and Piper,1996) and based on natural mixed infection in Perendale (Morris et al., 2005) and Rylington Merino (Karlsson and Greeff, 2006) sheep. Moderate heritabilities and high phenotypic variation of FEC permit noticeable genetic progress of this trait (Eady et al., 2003; Kemper et al., 2010; Brown and Fogarty, 2017). For the Rylington Merino selection line, Kemper et al. (2010) found that FEC of resistant sheep was approximately 82% lower compared to unselected animals, after 15 years of selection. Eady et al. (2003) detected a 69% reduction of FEC in animals of the selected Merino line described by Woolaston and Piper (1996), compared to an unselected group. These authors also related that FEC was lower in animals that were not drenched, but selected for resistance than in unselected animals under a regular drenching regime (Eady et al., 2003).

Selection is often based on FEC records in lambs (Woolaston and Piper,1996; Morris et al., 2005; Karlsson and Greeff, 2006). However, given that the periparturient period is the most significant phase in larval pasture contamination (due to the temporary decrease in ewe's immunity described earlier), higher resistance in periparturient ewes is a desirable goal, leading to reduced larval challenge for lambs (Williams et al., 2010). The genetic correlation between FEC in lambs and FEC in periparturient ewes under natural infection was estimated to be 0.81 ± 0.11 in Uruguayan Merino sheep (Goldberg et al., 2012) and Williams et al. (2010) related that animals from the Rylington Merino selection line (Karlsson and Greeff, 2006), selected for low FEC as lambs, remained low infection levels later in life, during the periparturient period. These authors concluded that, in the respective sheep populations they investigated, FEC in lambs can be used as a criterion to select for more resistant ewes, avoiding sampling during the periparturient period, which is often stressful for the animals. On the other hand, Vanimisetti et al. (2004) did not detect a significant genetic correlation between FEC in lambs and FEC in ewes in a Dorset X Rambouillet X Finnsheep population after artificial infection with *H. contortus.*

Another approach to indirectly access resistance in ewes was described by Aguerre et al. (2018): instead of selecting lambs, these authors suggested the selection of rams after artificial challenge with *H. contortus*. The genetic correlation of 0.56 ± 0.01 to 0.71 ± 0.01 between FEC in artificially infected rams and FEC in ewes under natural infection on

pasture confirmed that selection for low FEC in rams (which is more easily implemented in this production system than selection of ewes) should, in the future, increase resistance of ewes (Aguerre et al., 2018).

Another issue is the question of whether or not resistance to different GIN species is correlated. In a study conducted on Texel lambs, Bishop et al. (2004) found that the genetic correlation between *Nematodirus* FEC and Strongyle FEC (including the genera *Oesophagostomum, Chabertia, Bunostomum, Trichostrongylus, Cooperia, Ostertagia, Teladorsagia* and *Haemonchus*) ranged between 0.38 and 0.95 on different sampling dates. Gruner et al. (2004) compared resistance to *Trichostrongylus colubriformis* and *H. contortus* by artificially infecting two groups of animals with both parasite species in subsequent challenges. One group was first infected with *H. contortus,* then drenched, and subsequently infected with *T. colubriformis.* The other group was also infected with both species, but in reverse order. Genetic correlations between the two groups for FEC of different species were predominantly close to 1. These results suggest that resistance against GIN is not species-specific. Selection for resistance to one species should therefore lead to genetic progress in resistance to other GIN species (Gruner et al., 2004).

1.2.7. Selection of animals resilient to GIN infection

The level of anaemia that an animal shows when submitted to hematophagous *H. contortus* depends on its resistance: highly resistant animals have lower FEC and worm burdens, with less blood being removed by parasites. On the other hand, some animals are able to maintain blood parameters within the physiological scope even when heavily parasitized, which is why most authors consider measures of anaemia to be resilience traits (Baker et al., 2003; Bishop, 2012; Aguerre et al., 2018). The existence of genetic variance of PCV after *H. contortus* infection in sheep has been evidenced in several studies (Baker et al., 2003; Lôbo et al., 2009; Oliveira et al., 2018). Baker et al. (2003) estimated the heritability of PCV to be 0.14 ± 0.05 in a population consisting of 6 – 8 month old Red Maasai, Dorper and Red Maasai X Dorper lambs after natural mixed infection, with *H. contortus* being predominant (73%). A heritability of 0.39 was estimated for PCV in lambs and 0.15 in ewes after a single artificial infection with *H. contortus* (Vanimisetti et al., 2004). For the Santa Inês breed, Oliveira et al. (2018) found a heritability estimate of 0.30 ± 0.06 under natural challenge, while Lôbo et al. (2009) conducted two subsequent artificial infections with *H. contortus* in lambs of the same breed, with a heritability peak of 0.31 in the first and 0.12 in the second parasite challenge.

A widely-used measure for anaemia in small ruminants is the Famacha© score, which allows the assessment of the degree of anaemia by comparison of the colour of the animal's eye mucosa to the Famacha© chart. This method has been successfully implemented as a criterion for targeted selective treatment (TST) in a number of tropical sheep farming systems (Burke et al., 2007), although there are studies that claim it is not suitable for some breeds (Moors et al., 2009; Bishop 2012a). Also, Cintra et al. (2018) highlighted that the sensitivity of this method was low in lambs (30.8 % when Famacha© 3-5 and PCV of ≤ 18% were considered anaemic). Heritability estimates for the Famacha© score were 0,29 ± 0.05 in a Dohne Merino flock (Snyman and Fisher, 2019), and 0.21 ± 0.04 for Santa Inês lambs (Oliveira et al., 2018).

Measures of anaemia, like PCV and Famacha©, consistently have favourable phenotypic correlations with FEC (that is, PCV is negatively and Famacha© positively correlated with FEC) (Baker et al., 2003; Lôbo et al., 2009; Snyman and Fisher, 2019). While Lôbo et al. (2009) and Aguerre et al., (2018) found no significant genetic correlation between FEC and PCV, other studies related genetic correlations that ranged from – 0.63 ± 0.58 to – 0.98 ± 0.24 (Albers et al. 1987; Baker et al., 2003). Genetic correlations of the Famacha© score with FEC were estimated to be favourable in Santa Inês (0.28 ± 0.03) (Oliveira et al., 2018), as well as in the Dohne Merino Flock (0.62 ± 0.08) (Snyman and Fisher, 2019).

Body weight and weight gain in the face of GIN infection are appealing breeding goals, given that animals are continually exposed to parasites in many production systems (Bishop, 2012a; Bishop, 2012b). Albers et al. (1987) compared growth rates of both infected and uninfected animals, and related that heritability estimates for weight gain (WG) in infected animals and WG depression under infection were low, with high standard error (0.15 ± 0.08 and 0.09 ± 0.07, respectively). The same was true for genetic correlations between WG depression and resistance (0.36 ± 0.34 and 0.31 ± 0.36) (Albers et al., 1987). Another approach to assess resilience was described by Morris et al. (2010). These authors defined resilience as the age at which an anthelmintic treatment was necessary to maintain acceptable growth rates under natural parasite challenge. Animals were drenched based on their weight gain compared to a control group of uninfected animals. The heritability estimate of the described trait was 0.13 ± 0.02. After 13 years of selection, the age at first drench was increased by 23.6 days in animals of the resilience selection line compared to an unselected control group and resulted in a 4.5 kg increase in 6-month life weight. The genetic correlation between resilience and FEC (resistance) was not significantly different from zero. Consequently, the FEC in the resilience line did not differ from that of the control line, suggesting that resistance and resilience are based on

different biological mechanisms (Morris et al., 2010).

Selection based on resilience traits is a controversial issue: If animals were selected solely based on resilience traits similar to that described by Morris et al. (2010), resistance would not be affected (Albers et al., 1987; Bisset et al., 2001). In this case, animals would shed the same amount of eggs and resilience mechanisms could break down in phases of high larval contamination levels, leading to high morbidity. On the other hand, resilience is a mechanism that does not interfere with the parasite life-cycle, and thus imposes low selective pressure on the parasite, lowering the risk of an eventual overcoming of the host's response mechanisms. High resilience is frequently described in breeds that have adapted to a high challenge environment (Bishop and Stear, 2003). Baker et al. (2003) affirmed that in Red Maasai sheep, which are highly resistant, improvement of resilience (measured as PCV) should be given more weight than resistance, while in the Dorper breed, which is more susceptible, both traits should be selected for. However, the resilience trait that is cited here – PCV – has a moderate negative correlation with FEC (-0.34), which would lead to a correlated response in resistance, even if it is not directly selected for (Baker et al., 2003). Baker and Gray (2004) also suggested to select the heaviest rams under parasite challenge or to use the Famacha© score as a culling criterion in Red Maasai sheep, as a simple breeding programme for small flock holders who do not have resources for FEC or PCV sampling. Bishop (2012a) considers selection indices which include resistance, performance under parasite challenge and measures of anaemia to be a sensible approach for sheep production in the tropics. In summary, the usefulness of selection towards higher resilience probably depends on the resilience trait in question, whether other traits are also being selected for, as well as on the breed and on environmental factors.

1.2.8. Genetic correlations between resistance and performance

Because of the importance of estimating correlated responses prior to selection for resistance traits, the genetic relationship between FEC and weight or weight gain has been studied in a variety of environments and breeds. There has been little consistency across studies: negative genetic correlations were related by Bishop et al. (1996) (-0.63 – -1.00), Snyman and Fisher (2019) (-0.39 ± 0.22), Oliveira et al. (2018) (-0.27 ± 0.03) and Benavides et al. (2016) (-0.05 ± 0.00), while Lôbo et al. (2009) and Brown and Fogarty (2017) found no significant correlations, and positive correlations were detected by Morris et al. (1997) (0.05 ± 0.04 and 0.07 ± 0.05) and Gowane et al. (2019) (0.35 ± 0.16). A mathematical model, implemented by Bishop and Stear (1999), that simulated different

scenarios of host-parasite interaction in a sheep population, considering breed, diet, parasite species, pathogenicity and epidemiological factors, genetic correlations between FEC and growth traits increased from -0.02 to -0.46, simultaneously with an increase in disease severity. According to Bishop and Stear (2003) and Bishop (2012), the relationship between resistance and performance traits is highly dependent on the environment: in heavily parasitized environments, the effort of building and maintaining an effective immune response is beneficial as it leads to lower FEC and subsequent better performance of resistant animals. Under low challenge conditions, performance may not be compromised by infection, which makes the mounting of an immune response an unnecessary waste of energy and resources, leading to poorer performance of resistant animals.

1.2.9. Variation between breeds and genotype by environment interactions

Variation of resistance and resilience has not only been found within populations, but it has frequently been reported between sheep breeds as well. (Baker and Gray, 2004). Resistant and resilient sheep breeds are typically native or naturalized breeds that have evolved in tropical environments, under high levels of parasite challenge, like Red Maasai (Baker et al., 2004), Sabi (Matika et al., 2003), Gulf Coast Native (Miller et al., 1998), Santa Inês (Amarante et al., 2004) and Morada Nova (Issakowicz et al., 2016). These breeds are usually characterized by low performance, which is probably due to low levels (or absence) of artificial selection and not necessarily to their lack of potential (Bishop and Stear, 2003). Flock holders in tropical countries often choose high performing commercial breeds. However, in some situations, these breeds do not express their potential for performance because they lack environmental adaptation. One example is the genotype by environment interaction documented by Baker et al. (2004) in Red Maasai and Dorper sheep: The Dorper breed, developed under semi-arid conditions in South Africa, performed equally well or better compared to the red Maasai breed when kept under semi-arid conditions. When both breeds were kept on a farm in a region of sub-humid climate, however, Red Maasai sheep showed superior performance. Apart from the higher resistance and resilience to GIN (evidenced by lower FEC and higher PCV values compared to the Dorper sheep), higher tolerance of heat and humidity, and the ability to utilize poor quality feed could also be of importance for the higher performance of Red Maasai sheep (Baker et al., 2004).

1.2.10. Genetic markers

Quantitative trait loci (QTL) are chromosomic regions contributing to variation in phenotypic traits. Information on QTL can provide an understanding of molecular mechanisms that lead to variation in traits (Benavides et al., 2016; Ali et al., 2019) and it can be used for prediction of genetic merit (Hunt et al., 2008; Rosa 2013). Direct genetic markers are known polymorphisms in the DNA sequence within QTLs that have a direct causative effect on the trait under investigation (functional polymorphism). “LD-markers“, on the other hand, are known polymorphisms which are not themselves causative but are located in close proximity to the causative mutation and are therefore very likely to be simultaneously passed on to the next generation (Rosa, 2013). If two loci are likely to be inherited together, they are in so-called linkage disequilibrium – LD with each other, which means that a crossing over (the recombination of loci between homologous chromosomes during meiosis) between these loci is unlikely. Once implemented in a population, both marker types provide information on a trait: based on the genotype of an animal and at least a part of the phenotypic variation or genetic merit of this animal can be predicted. The utilization of genetic markers for selection (marker-assisted selection – MAS) is especially advantageous in traits of low heritability, that are difficult to measure, that are expressed late or only once in the animal’s life (like carcass quality) or that are expressed in only one sex (like milk yield) (Rosa, 2013).

In simple-inherited traits or in the case of genes with a major effect on a trait, selecting individuals with a favourable marker genotype is straightforward. For litter size in sheep, a mutation in a major gene (Bone Morphogenetic Protein IB Receptor (BMPR1B)) was identified in highly prolific Boorola sheep (Wilson et al., 2001). Given the importance of this gene’s effect, selection for increased litter size can be based on a single marker information, as described by Chen et al. (2015). For most traits, however, MAS is more complex. If several combined markers account for a considerable proportion of phenotypic variance, information on animal’s genotypes for these markers can be used as an addition to breeding values (EBV) estimated in quantitative proceedings, either by including marker information into the calculation of EBVs, or by implementing a separate culling threshold, culling animals with undesirable genotypes and then applying selection on the remaining animals based on EBVs (Hunt et al., 2008).

Another approach for marker-assisted selection is the utilization of a large number of markers spread throughout the genome (Genome-wide Marker-Assisted Selection (GWMAS), or Genomic Selection (GS)). For this purpose, it is common to use indirect markers provided by precast marker chips. Indirect markers are not necessarily in LD with

causative mutations and therefore, are not associated to the trait of interest. However, when marker distribution is sufficiently dense throughout the genome, the pedigree structure of the population can be derived from the marker information. This pedigree structure can then be integrated into EBV calculation in order to enhance prediction of genetic merit (Genomic Selection) (Rosa, 2013).

There are different methods to detect LD or direct markers (although it is far less likely to detect a direct marker than an LD marker). Variants of genes that are known or supposed to be involved in biological processes associated to the respective trait can be tested for their aptitude as genetic markers (candidate gene approach; Schwaiger et al., 1995; Coltman et al., 2001; Ali et al., 2019). Genome wide association studies do not make any prior assumptions of relevant genes or regions, but perform a search of the whole genome, based on SNP markers, to identify regions with association to the respective trait (QTLs) (Kemper et al., 2011; Benavides et al., 2015; Berton et al., 2017).

1.2.11. Genetic markers for resistance to GIN in sheep

Results on QTL for resistance to GIN in sheep are not always consistent throughout studies. According to Zvinorova et al. (2016), this is due to diverging methods of QTL detection as well as to the very variable circumstances under which the studies are conducted. Different parasite species have been investigated in various breeds and at different ages, using animals of only one or both sexes and of varying physiological status, which makes comparison of study results problematic (Zvinorova et al., 2016). Another issue is the reconstruction of causative genes in LD to significant markers, given that annotation of the sheep genome is still in progress (Benavides et al., 2016).

The regions most consistently associated to nematode resistance are those containing the major histocompatibility complex II (MHC II) gene on chromosome 20 (Schwaiger et al., 1994; Janssen et al., 2002; Ali et al., 2019) and the interferon γ (IFNγ) gene on chromosome 3 (Coltman et al., 2001; Sayers et al., 2005). Both proteins are involved in the immune response, with MHC II being an antigen-presenting receptor and IFNγ being an inhibitor of the Th2 response, favouring the Th1 pathway (Coltman et al., 2001). The Th1 response was shown to be associated to *H. contortus* susceptibility in sheep (Zaros et al., 2010). In a review by Benavides et al. (2016), the Th2 response has also been suggested as relevant for GIN resistance, given that regions containing genes of the Th2 response were associated to FEC in several studies, including genes of the eosinophilia, mastocytosis and immunoglobulin E (IgE) pathways. Abomasal mucus

production is a part of the innate immune response against gastrointestinal parasites and genetic markers with proximity to genes involved in the glykosylation of mucins (the main component of mucus) have been associated to parasite resistance in several studies (Benavides et al., 2016). A third mechanism that is suggested to be important by these authors is hemostasis, with regions containing genes of hemostasis pathways being frequently reported as relevant. In a GWAS performed by Berton et al. (2017) on Santa Inês sheep, regions associated to FEC contained genes of immune response pathways, including the *IL15*, a known activator of the Th2 response (Toscano, 2019). Results of the same study also pointed to iron transportation and construction pathways (Berton et al., 2017).

Benavides et al. (2015) conducted a GWAS study on animals of extreme phenotypes for resistance, selected from a Red Maasai and Dorper backcross population. Associations with FEC, PCV and body weight under parasite challenge were evaluated. The five most relevant SNPs associated to FEC, located on the chromosomes 2, 6, 11, 12 and 15 (OAR2_14765360, OAR6_81718546, OAR11_62887032, OAR12_69606944 e OAR15_59871543), were responsible for 2,33 % of phenotypic variation. None of these SNPs was associated to PCV or body weight.

The following two chapters will present the experimental design, statistical methods, results and discussion of genetic parameters (chapter 2) and association analyses of five SNPs (chapter 3) for FEC, PCV and weight gain in Morada Nova sheep under *H. contortus* infection.

1.3. REFERENCES

Aguerre S, Jacquiet P, Brodier H, Bournazel JP, Grisez C, Prévot F, Michot L, Fidelle F, Astruc JM, Moreno CR (2018) Resistance to gastrointestinal nematodes in dairy sheep: Genetic variability and relevance of artificial infection of nucleus rams to select for resistant ewes on farms. **Veterinary Parasitology** 256:16-23.

Albers GAA, Gray GD, Piper LR, Barker JSF, Le Jambre LF, Barger IA (1987) The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. **International Journal for Parasitology** 17:1355-1363.

Albuquerque ACA, Bassetto CC, Almeida FA, Amarante AFT (2017) Development of *Haemonchus contortus* resistance in sheep under suppressive or targeted selective treatment with monepantel. **Veterinary Parasitology** 246:112-117.

Ali AOA, Murphy L, Stear A, Fairlie-Clarke K, Brujeni GN, Donskow-Łysoniewska K, Groth D, Buitkamp J, Stear MJ (2019) Association of MHC class II haplotypes with reduced faecal nematode egg count and IgA activity in British Texel sheep. **Parasite Immunology** https://doi.org/10.1111/pim.12626

Amarante AFT, Godoy WAC, Barbosa MA (1998) Nematode egg counts, packed cell volume and body weight as parameters to identify sheep resistant and susceptible to infections by gastrointestinal nematodes. **Ars Veterinaria** 14:331-339.

Amarante AFT, Bricarello PA, Rocha RA, Gennari SM (2004) Resistance of Santa Inês, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. **Veterinary Parasitology** 120:91-106.

Arandas JKG (2017) **Etnozootecnia da raça ovina Morada Nova em seu centro de origem: história, critérios de seleção e sistema de produção.** 137 pp. Thesis (PhD of Animal Science) – Federal Rural University of Pernambuco

Arandas JKG, Ribeiro MN, Pimenta Filho EC, da Silva RCB, Facó O, Esteves SN (2012) Estrutura populacional de ovinos da raça Morada Nova. In: SIMPÓSIO BRASILEIRO DE MELHORAMENTO ANIMAL, 9., 2012, João Pessoa. **Anais...** João Pessoa: SBMA, 2012. 3 f. 1 CD-ROM.

Baker RL, Nagda S, Rodriguez-Zas SL, Southey BR, Audho JO, Aduda EO, Thorpe W (2003) Resistance and resilience to gastro-intestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai × Dorper crossbred lambs in the sub-humid tropics. **Animal Science** 76:119-136.

Baker RL, Gray GD (2004) Appropriate breeds and breeding schemes for sheep and goats in the tropics. In,: Sani RA, Gray GD, Baker RL (Eds.) **Worm Control for Small Ruminants in Tropical Asia.** Canberra, Australia: ACIAR Monograph 113, pp. 63-95.

Baker RL, Mugambi JM, Audho JO, Carles AB, Thorpe W (2004) Genotype by environment interactions for productivity and resistance to gastro-intestinal nematode parasites in Red Maasai and Dorper sheep. **Animal Science** 79:343-353.

Benavides MV, Sonstegard TS, Kemp S, Mugambi JM, Gibson JP, Baker RL, Hanotte O, Marshall K, Tassel C (2015) Identification of Novel Loci Associated with Gastrointestinal Parasite esistance in a Red Maasai x Dorper Backcross Population. **PLOS one** 10:1-20.

Benavides MV, Sonstegard TS, Van Tassell C (2016) Genomic regions associated with sheep resistance to gastrointestinal nematodes. **Trends in Parasitology** 32:470–480.

Berton MP, de Oliveira Silva RM, Peripolli E, Stafuzza NB, Martin JF, Álvarez MS, Gavinã BV, Toro MA, Banchero G, Oliveira PS, Eler, JP (2017) Genomic regions and pathways associated with gastrointestinal parasites resistance in Santa Inês breed adapted to tropical climate. **Journal of Animal Science and Biotechnology** 8:73.

Bishop SC (2012a) Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. **Animal** 6:741-747.

Bishop SC (2012b) A consideration of resistance and tolerance for ruminant nematode infections. **Frontiers in Genetics** 3:168/1-7.

Bishop SC, Jackson F, Coop RL, Stear MJ (2004) Genetic parameters for resistance to nematode infections in Texel lambs and their utility in breeding programmes. **Animal Science** 78:185-194.

Bishop SC, Stear MJ (2003) Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections **Veterinary Parasitology** 115:147-166.

Bishop SC, Morris CA (2007) Genetics of disease resistance in sheep and goats. **Small Ruminant Research** 70:48-59.

Bisset SA, Vlassof A, West CJ, Morrison L (1997) Epidemiology of nematodosis in Romney lambs selectively bred for resistance or susceptibility to nematode infection. **Veterinary Parasitology** 70:255-269.

Bisset SA, Morris CA, McEwan JC, Vlassof A (2001) Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. **New Zealand Veterinary Journal** 49:236-246.

Bourdon, R.M. (2000) **Understanding animal breeding.** Upper Saddle River, NJ: Prentice Hall. 418 pp

Brown DJ, Fogarty NM (2017) Genetic relationships between internal parasite resistance and production traits in Merino sheep. **Animal Production Science** 57:209-215.

Chagas ACS, Katiki, LM, Silva, IC, Giglioti R, Esteves SN, Oliveira MCS Barioni-Júnior W, (2013) Haemonchus contortus: A multiple-resistant Brazilian isolate and the costs for its characterization and maintenance for research use. **Parasitology International** 62:1–6.

Chagas ACS, Zaia MG, Domingues LF, Rabelo MD, Politi FAZ, Anibal FF, Chagas JR (2015) Desparasitación racional: estudio comparativo de técnicas para la detección de la anemia causada por nemátodos gastrointestinales en pequeños rumiantes. In: VII Congreso Argentino de Parasitología, **Libro de Resúmenes…** La Plata: Associación Parasitológica Argentina, p. 109.

Chen X, Sun H, Tian S, Xiang H, Zhou L, Dun W, Zhao X (2015) Increasing litter size in a sheep breed by marker-assisted selection of BMPR1B A746G mutation. **Journal of genetics** 94:139-142.

Colditz IG, Watson DL, Gray GD, Eady SJ (1996) Some relationships between age, immune responsiveness and resistance to parasites in ruminants. **International Journal for Parasitology** 26:869-877.

Coltman DW, Wilson K, Pilkington JG, Stear MJ, Pemberton JM (2001) A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. **Veterinary Parasitology** 122:571-582.

Coop and Holmes (1996) Nutrition and Parasite Interaction. **International Journal for Parasitology** 26:951-962.

Eady SJ, Woolaston RR, Barger IA (2003) Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. **Livestock Production Science** 81:11-23.

Emery DL, Hunt PW, Le Jambre LF (2016) Haemonchus contortus: the then and now, and where to from here?. **International Journal for Parasitology**. 46(12):755-69.

Facó O, Paiva SR, Alves LRN, Lôbo RNB, Villela LCV (2008) Raça Morada Nova: Origem, Características e Perspectivas. **Embrapa** Documentos 75 ISSN 1676-7659.

Gauly M, Schackert M, Hoffmann B, Erhardt G (2006) Influence of sex on the resistance of sheep lambs to an experimental Haemonchus contortus infection. DTW. **Deutsche tierärztliche Wochenschrift** 2006 113:178-181.

Goldberg V, Ciappesoni G, Aguilar I (2012) Genetic parameters for nematode resistance in periparturiant ewes and post-weaning lambs in Uruguayan Merino sheep. **Livestock Science** 147:181-187.

Gowane GR, Swarnkar CP, Misra SS, Kumar R, Kumar A, Prince LLL (2019) Genetic parameter estimates for fecal egg counts and their relationship with growth in Avikalin and Malpura sheep. **Animal** 13:1788-1796.

Gruner L, Bouix J, Brunel JC (2004) High genetic correlation between resistance to *Haemonchus contortus* and to *Trichostrongylus colubriformis* in INRA 401 sheep. **Veterinary Parasitology** 119:51-58.

Hunt PW, McEwan JC, Miller JE (2008) Future perspectives for the implementation of genetic markers for parasite resistance in sheep. **Tropical Biomedicine** 25:18–33.
Issakowicz J, Issakowicz ACKS, Bueno MS, Costa RLD, Katiki LM, Geraldo AT, Abdalla AL, McManus C, Louvandini H (2016) Parasitic infection, reproductive and productive performance from Santa Inês and Morada Nova ewes. **Small Ruminant Research** 136:96-103.

Jackson F, Bartley D, Bartley Y, Kenyon F (2009) Worm control in sheep in the future. **Small Ruminant Research** 86:40-45.

Janssen M, Weimann C, Gauly M, Erhardt G (2002) Associations between infections with *Haemonchus contortus* and genetic markers on ovine chromosome 20. 7TH WORLD CONGRESS ON GENETICS APPLIED TO LIVESTOCK PRODUCTION Montpellier, France.

Kahn LP (2003) Regulation of the resistance and resilience of periparturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. **Australian Journal of Experimental Agriculture** 43:1477-1485.

Karlsson LJ, Greeff JC (2006) Selection response in fecal worm egg counts in the Rylington Merino parasite resistant flock. **Australian Journal of Experimental Agriculture** 46:809-11.

Kemper KE, Palmer DG, Liu SM, Greeff JC, Bishop SC and Karlsson LJE (2010) Reduction of faecal worm egg count, worm numbers and worm fecundity in sheep selected for worm resistance following artificial infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. **Veterinary Parasitology** 171:238-246.

Kemper KE, Emery DL, Bishop SC, Oddy H, Hayes BJ, Dominik S, Henshall JM, Goddard ME (2011) The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. **Genetic Research** 93:203-219.

Kijas JW, Lenstra JA, Hayes B, Boitard S, Neto LR, San Cristobal M, Servin B, McCulloch R, Whan V, Gietzen K, Paiva S (2012) Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. **PLoS biology**10(2):e1001258.

Lôbo RN, Vieira LS, Oliveira AAD, Muniz EN, Silva JMD (2009) Genetic parameters for faecal egg count, packed-cell volume and body-weight in Santa Inês lambs. **Genetics and Molecular Biology** 32:228-294.

Lôbo RNB, Pereira IDC, Facó O, McManus CM (2011) Economic values for production traits of Morada Nova meat sheep in a pasture based production system in semi-arid Brazil. **Small Ruminant Research** 96:93-100.

Matika O, Nyoni S, van Wyk JB, Erasmus GJ, Baker RL (2003) Resistance of Sabi and Dorper ewes to gastro-intestinal nematode infections in an African semi-arid environment. **Small Ruminant Reserach** 47:95-102.

McManus C, Louvandini H, Paiva SR, Oliveira AA, Azevedo HC, Melo CB (2009) Genetic factors of sheep affecting gastrointestinal parasite infections in the Distrito Federal, Brazil. **Veterinary Parasitology** 166:308-313.

McManus C, Facó O, Shiotsuki L, Rolo JLJP, Peripolli V (2019) Pedigree analysis of Brazilian Morada Nova hair sheep. **Small Ruminant Research** 170:37-42.

Mederos AE, Ramos Z, Banchero GE (2014) First report of monepantel Haemonchus contortus resistance on sheep farms in Uruguay. **Parasites & Vectors** 7:598.

Miller JE, Bahirathan M, Lemarie SL, Hembry FG, Kearney MT, Barras SR (1998) Epidemiology of gastrointestinal nematode parsitism in Suffolk and Gulf Coast Native sheep with special emphasis on relative susceptibility to *Haemonchus contortus* infection. **Veterinary Parasitology** 74:55-74.

Morris CA, Vlassof A, Bisset SA, Baker RL, West CJ, Hurford AP (1997) Responses of Romney sheep to selection for resistance or susceptibility to nematode infection. **Animal Science** 62:319-329.

Morris CA, Wheeler M, Watson TG, Hosking BC, Leathwick DM (2005) Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. **New Zealand Journal of Agricultural Research** 48:1-10.

Morris CA, Bisset CA, Vlassof A, Wheeler M, West CJ, Devantier BP & Mackay AD (2010) Selecting for resilience in Romney sheep under nematode parasite challenge, 1994-2007. **New Zealand Journal of Agricultural Research** 53:245-261.

Oliveira EJ, Savegnago RP, Freitas LA, Freitas AP, Maia SR, Simili FF, Faro L, Costa RLD, Santana Júnior ML, Paz CCP (2018) Estimates of genetic parameters and cluster analysis for worm resistance and resilience in Santa Inês meat sheep. **Pesquisa Agropecuária Brasileira** 53:1338-1345.

Papadopoulos E, Gallidis E, Ptochos S (2012) Anthelmintic resistance in sheep in Europe: a selected review. **Veterinary Parasitology** 189:85-88.

Perry BD, Randolph TF, McDermott JJ, Sones KR, Thornton PK (2002) Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya, 148 pp.

Rosa GJ (2013) Foundations of animal breeding. In.: Christou P, Savin R, Costa-Pierce BA, Misztal I, Whitelaw CBA (Eds.) **Sustainable food production** Springer Science + Business Media New York pp. 58-78.

Sales N, Love S (2016) Resistance of Haemonchus sp. to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW, Australia. **Veterinary Parasitology** 228:193-6.

Sayers G, Good B, Hanrahan JP, Ryan M, Sweeney T (2005) Intron 1 of the interferon γ gene: Its role in nematode resistance in Suffolk and Texel sheep breeds. **Research in Veterinary Science** 79:191-6.

Schwaiger FW, Gostomski D, Stear MJ, Duncan JL, McKellar QA, Epplen JT, Buitkamp (1994) An Ovine Major Histocompatibility Complex DRB1 Allele is Associated with Low Faecal Egg Counts Following Natural, Predominantly *Ostertagia circumcincta* Infection. **International Journal for Parasitology** 7:815-822.

Scott I, Pomroy WE, Kenyon PR, Smith G, Adlington B, Moss A (2013) Lack of efficacy of monepantel against Teladorsagia circumcincta and Trichostrongylus colubriformis. **Veterinary Parasitology**. 198:166-71.

Snyman MA, Fisher AD (2019) Genetic parameters for traits associated with resistance to *Haemonchus contortus* in a South African Dohne Merino sheep flock. **Small Ruminant Research** 176:76-88.

Toledo NM (2014) **Estudo da estrutura genética de ovinos localmente adaptados do Brasil por meio de marcadores de base única (SNP – Single Nucleotide Polymorphism).** 88 pp. Thesis (Master of Animal Science) - Faculty of Agronomy and Veterinary Medicine, University of Brasília.

Toscano JHB (2019) **Caracterização de respostas imunes locais associadas ao fenótipo de resistência parasitária em cordeiros da raça Morada Nova.** 94 pp. Thesis (Master of Animal Science) – Faculty of Agronomy and Veterinary Medicine, São Paulo State University, Jaboticabal.

Toscano JHB, Santos IB, Haehling MB, Giraldelo LA, Lopes LG, Silva MH, Figueredo A, Esteves SN and Chagas ACS (2019) Morada Nova sheep breed: resistant or resilient to *Haemonchus contortus* infection? **Veterinary Parasitology X** 2:100019.

Vanimisetti HB, Andrew SL, Zajac AM, Notter DR (2004) Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus*. **Journal of Animal Science** 82:1602-1611.

Veríssimo CJ, Niciura SCM, Alberti ALL, Rodrigues CFC, Barbosa CMP, Chiebao DP, Cardoso D, Silva GS, Pereira JR, Margatho LFF, Costa RLD, Nardon RF, Ueno TEH, Curci VCLM, Molento MB (2012) Multidrug and multispecies resistance in sheep flocks from São Paulo state, Brazil.**Veterinary Parasitology** 187:209-216.

Williams AR, Greeff JC, Vercoe PE, Dobson RJ, Karlsson LJE (2010) Merino ewes bred for parasite resistance reduce larval contamination onto pasture during the peri-parturient period. **Animal** 4:122-127.

Wilson T, Wu XY, Juengel JL, Ross IK, Lumsden JM, Lord EA, Dodds KG, Walling GA, McEwan JC, O'Connell AR, McNatty KP (2001) Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. **Biology of Reproduction** 64:1225-1235.

Woolaston RR, Piper LR (1996) Selection of Merino sheep for resistance to *Haemonchus contortus*: genetic variation. **Animal Science** 62:451-460.

Zaros, LG, Neves, MRM, Benvenuti, CL, Navarro, AMC, Sider, LH, Coutinho, LL, Vieira, LS (2014) Response of resistant and susceptible Brazilian Somalis crossbreed sheep naturally infected by *Haemonchus contortus*. **Parasitology Research** 113: 1155-1161.

Zvinorova PI, Halimani TE, Muchadeyi FC, Matika O, Riggio V, Dzama K (2016) **Veterinary Parasitology** 225:19-28.

CHAPTER 2 – GENETIC PARAMETERS FOR FAECAL EGG COUNT (FEC) PACKED CELL VOLUME (PCV) AND DAILY WEIGHT GAIN IN MORADA NOVA SHEEP INFECTED WITH *Haemonchus contortus*

2.1. ABSTRACT

Gastrointestinal nematodes cause large economic losses in sheep production systems around the world. Selection of animals resistant to infection with GIN has proven to be a feasible and effective approach to address this problem. The aim of this study was to evaluate the possibility of selection for resistance and resilience traits in Morada Nova lambs and to estimate potential correlated responses. A total of 256 lambs and 123 ewes were submitted to two consecutive independent artificial infections with 4,000 infective larvae (L_3) of *Haemonchus contortus*. Records of faecal egg count (FEC), packed cell volume (PCV), and body weight (BW) were taken until day 42 after infection in both challenges. After model definition, estimates of genetic parameters were obtained for individual records as well as for overall traits with repeated measures, using mixed animal models. Phenotypic traits (FEC, PCV and BW/daily weight gain (DWG)) and estimated breeding values (EBVs) of lambs of all three traits were divided by their standard deviations to align variances and, hereafter, a phenotypic as well as a genetic index were established and compared. Heritability estimates for FEC in the first and second parasite challenge were, respectively, 0.25 ± 0.18 and 0.46 ± 0.19 for lambs, and 0.00 ± 0.09 and 0.20 ± 0.16 for ewes. For PCV, heritability estimates were 0.23 ± 0.14 and 0.32 ± 0.16 for lambs and 0.13 ± 0.11 and 0.37 ± 0.18 for ewes. The overall DWG heritability estimate was 0.70 ± 0.21. Genetic correlations of FEC and PCV between lambs and ewes were 0.36 ± 0.08 and 0.42 ± 0.08, respectively. No significant genetic correlation was found between DWG and the other traits, while there was a negative genetic correlation between FEC and PCV (-0.70 ± 0.03). The results of the present study show that selection for low FEC and high PCV is possible in Morada Nova lambs. Selection for low FEC should have a correlated response on PCV (leading to higher PCV), while no correlated response is expected on DWG. Selection for low FEC and high PCV in lambs should lead to low FEC and high PCV in ewes. An index consisting of overall DWG and two records of FEC and PCV (taken 4 to 6 weeks after infection during the second parasite challenge) can be used as selection criterion in the Morada Nova lambs, allowing simultaneous selection for lower FEC, higher PCV and higher DWG.

2.2. INTRODUCTION

Gastrointestinal nematodes (GIN) restrict the productivity of sheep farming systems worldwide. In Brazilian sheep flocks, haematophagous *Haemonchus contortus* has the highest clinical and economic importance, causing weight loss, retarded growth, anaemia and deaths (Amarante et al., 2004; Chagas et al., 2013). Control strategies are mainly based on the use of anthelmintics. However, the efficiency of this approach is threatened by the increasing prevalence of drug-resistant parasites (Jackson et al., 2009; Papadopoulos et al., 2012, Veríssimo et al., 2012), and the need for alternative strategies is evident.

Selection of animals resistant to GIN infection has proven to be a feasible and effective approach (Bisset et al., 2001; Eady et al., 2003; Kemper et al., 2010), and faecal egg count (FEC), widely used as a measure of resistance, has been included in several commercial breeding programmes (Goldberg et al., 2012; Brown and Fogarty, 2017). Bisset et al. (2001) related that after 21 years of selection for high and low resistance in Romney sheep, the differences in breeding values for log-transformed FEC was 35-fold. Kemper et al. (2010) found that FEC of resistant Rylington Merino sheep was approximately 82% lower compared to unselected animals, after 15 years of selection.

Resistant animals are less compromised by parasitized environments and require less anthelmintic treatments, thus lowering costs and increasing the sustainability of production. From the direct impact of selection on FEC, significant reductions in larval contaminations were shown in pastures grazed by lambs selected for high resistance, leading to higher weight gain (Bisset et al., 1997; Bishop and Stear, 2003). Williams et al. (2010) obtained similar results for resistant ewes during the periparturient period. Laurenson et al. (2012) estimated that susceptible lambs can benefit from contemporary grazing with resistant animals, reaching higher body weights (BW) than when grazed separately, while the resistant lamb's performance is not impaired. Despite these findings, the relationship between resistance and performance traits are not consistent throughout studies (Brown and Fogarty, 2017; Gowane et al., 2019; Snyman and Fisher, 2019). According to Bishop and Stear (2003), correlations between resistance and performance traits depend highly on larval contamination level of pastures, nutrition (especially dietary protein) and other environmental factors. Therefore, it is indispensable to evaluate correlations individually for each particular situation.

Packed cell volume (PCV) is an interesting trait due to the haematophagia of *H. contortus*. It depends on the level of infection, which is why it has been defined as a resistance trait (Albers et al., 1987), although there are mechanisms that allow some

animals to maintain high PCV despite high infection levels, leading PCV to be considered a resilience trait by most authors (Baker et al., 2003; Bishop, 2012; Aguerre et al., 2018). Selection for high PCV or other indicators of anaemia is recommended in order to capture these mechanisms, although the concomitant selection for low FEC is indicated in many situations, especially in breeds that are not highly resistant to parasites (Baker et al., 2003; Bishop, 2012; Snyman and Fisher, 2019).

Many studies have related that tropically adapted sheep breeds are more resistant and resilient to GIN infections compared to commercial breeds (Baker and Gray, 2004). Native breeds have generally been subject to little or no artificial selection, which is why they are frequently seen as “unproductive”. However, genotype by environment interactions can occur, with the adapted breeds showing better performance than commercial breeds under high parasite challenge, as evidenced by Baker et al. (2004), who compared the adapted Red Maasai breed (resistant) with Dorper sheep (susceptible). Adapted breeds are an important genetic resource for profitable and sustainable production in tropical environments, and their utilization (with concomitant selection for performance traits) is considered preferable to the introduction of commercial breeds in many situations (Baker and Gray, 2004; Bishop, 2012).

Morada Nova is a naturalized Brazilian hair sheep breed that is highly resistant and resilient to GIN infections (Issakowicz et al., 2016; Toscano et al., 2019). This breed is characterized by its small size, high adaptation to tropical climate, high prolificacy, non-existent reproductive seasonality, good maternal ability and excellent pelt quality, but also by low weight gain and carcass quality (Facó et al., 2008; Lôbo et al., 2011). In the present study, genetic parameters for FEC, PCV and weight gain were estimated in a Morada Nova sheep flock after artificial infection with *H. contortus* in order to investigate the possibility of selection for resistance, and to evaluate the relationship between resistance and performance in lambs.

2.3. MATERIAL AND METHODS

2.3.1. Experimental animals and phenotypes

The experiment was conducted in a Morada Nova flock at Embrapa Pecuária Sudeste (CPPSE), São Paulo state, Brazil. The location possesses tropical climate, with an annual dry season typically occuring between May and September. A total of 123 ewes were mated to 4 rams in two consecutive years, obtaining 256 lambs, born between April

and May of 2017 and March and May of 2018. In each year, two groups of animals were formed according to their birth date, with animals of the same group being weaned and submitted to further experimentation procedures together. At approximately 100 days of age, lambs were weaned, records of FEC, PCV and body weight (BW) were taken and animals were drenched using monepantel (Zolvix®, Novartis, 2.5 mg/kg BW) in order to eliminate natural infection. Subsequently, lambs were separated by sex and transferred to new paddocks. In September of 2018, all ewes were also drenched with monepantel and records of FEC and PCV were taken on the same day. Fifteen days after the first drench, lambs and ewes were infected with 4,000 infective larvae (L_3) of *Haemonchus contortus.* FEC, PCV and body weight were monitored regularly until day 42 after infection, when animals were drenched again in order to end the first artificial infection. Fifteen days after the end of the first trial, the second trial was iniciated, with infection and sampling following the same protocol that was used for the first trial. Management practices have been discussed in detail by Toscano et al. (2019).

Eight records of FEC, 6 records of PCV and 4 records of BW were thus obtained under artificial challenge, with samples of FEC being taken on day 21, 28, 35 and 42 after each infection (FECd21-1, FECd28-1, FECd35-2 and FECd42-1 during first, and FECd21-2. FECd28-2, FECd35-2 and FECd42-2 during second parasite challenge). For PCV, samples were taken on day 14, 28 and 42 after infection (PCVd14-1, PCVd28-1 and PCVd42-1 during first, and PCVd14-2, PCVd28-2 and PCVd42-2 during second infection). For BW, the sampling days were day 28 and 42 after challenge (BWd28-1, BWd42-1, BWd28-2 and BWd42-2). Records of FEC, PCV and BW were taken at weaning, under natural infection (FECweaning, PCVweaning, Bwweaning). For FEC, PCV and BW, analyses were conducted separately for each sampling date. For FEC and PCV, additional analyses were conducted for the overall trait, including all records taken during both parasite challenges (AllRepFEC, AllRepPCV), as well as for each parasite challenge, using data of all records taken during first or second challenge (RepFEC1, RepFEC2, RepPCV1, RepPCV2)

After the recording of phenotypes, lambs were ranked based on their FEC values and categorized into phenotypic groups of susceptible (20%), intermediate (60%) and resistant (20%) animals. Further analyses of these phenotypic groups have been conducted and described by Toscano et al. (2019).

2.3.2. Statistical analysis

Phenotypic data of FEC and PCV from 256 lambs and 123 ewes were used for analyses, while body weight (BW) and weight gain were only accessed in the lambs. The ewes' BWs remained relatively constant as they were adult animals, not in a growing phase. The distributions of FEC data were found to be positively skewed, which is why a log-transformation (log_{10}(FEC+25)) was performed in order to achieve more symmetrical distributions. Daily weight gain (DWG) was calculated in lambs for the period between weaning and the last day (day 42) of the second challenge (totalDWG), and for each parasite challenge (DWG1, DWG2), between days 0 and 42.

In order to define the models of the genetic analyses, fixed effects were tested using the "R" software (R Core Team, 2018), applying a significance level of 0.05. For lambs, fixed effects of sex, group (2 age groups per year, totalling 4 groups), birth type (single or twin), age of the dam (1 to 6, including dams older than 6 years in the group 6), and first-order interactions were tested, while age at sampling and weight of the dam at birth were tested as covariates. For ewes, the effect of age was tested as a covariate. In the case of interactions between two fixed effects, contemporary groups containing both effects were formed for genetic analysis. A linear normal model was used for model definition in the case of individual sampling dates of FEC, PCV, BW and DWG, using the lm() function. In order to define analyses with repeated measurements, a linear mixed model (lme() function of the nlme package (Pinheiro et al., 2018)) with maximum likelihood estimation was established and effects were tested using the likelihood ratio test, by applying the anova() function on the respective models. In some cases, fixed effects and/or covariates could not be utilized in the same model due to confounding effects (for example age and weight of the dam at birth). In those cases, effects were chosen based on higher adjusted coefficient of determination (linear normal model) or lower Akaike Information Criteria (AIC) (linear mixed model).

The MTDFREML software was used to estimate variance components and genetic parameters (Boldman et al., 1995). For FEC, PCV and BW of each individual sampling date and for totalDWG, DWG1 and DWG2 of lambs, univariate mixed animal models were implemented based on the formula:

$$y = Xb + Z_1a + e$$

where y was a vector of the respective observed traits of animals, b, a and e were vectors of fixed effects, additive genetic effects and residuals, while X and Z_1 where the respective incidence matrices for fixed and additive genetic effects.

Animal models of repeated measures were applied using FEC and PCV of all sampling dates (AllRepFEC, AllRepPCV), and separately for sampling dates in the first (RepFEC1, RepPCV1) and second (RepFEC2, RepPCV2) parasite challenge using the formula:

$y = Xb + Z_1a + Z_2pe + e$

where y was a vector of the respective observed traits of animals, b, a, pe and e were vectors of fixed effects, additive genetic effects, permanent environment effects and residuals, while X, Z_1 and Z_2 where the respective incidence matrices for fixed, additive genetic effects and permanent environment effects. For ewes, only the repeated measures animal models for FEC and PCV were run.

Phenotypic and genetic correlations were calculated within traits, as well as between traits using Pearson´s correlation. Phenotypic correlations were based on phenotypic data of lambs, while genetic correlations were based on Estimated Breeding Values (EBVs) obtained from analyses using records of lambs, according to van Vleck et al. (1987). Genetic correlations of ewe and lamb traits were based on overall EBVs of 146 (FEC) and 147 (PCV) animals. Although 37 of them were animals born in 2017 that had records as lambs and ewes, EBVs for FEC and PCV in lambs were estimated based on information of progeny for most animals.

Lambs were ranked based on their phenotypic values of totalDWG and the overall means of (untransformed) FEC and PCV (TotalMeanFEC, TotalMeanPCV). All values were divided by the standard deviation of the respective variable to align variances. Hereafter, an index was formed based on a score calculated as: (DWG · 0.4) – (FEC · 0.4) + (PCV · 0.2). The same was done for EBVs of totalDWG, AllRepFEC and AllRepPCV. Spearman´s rank correlation was calculated between the phenotypic and the genetic index. With the weighting of each trait, there was a lower emphasis (0.2) on the resilience trait (PCV) than the performance trait (DWG) and the resistance trait (FEC). An emphasis on selection for resilience traits can lead to high pasture contamination, which might eventually cause a breakdown in resilience, with high morbidity (Albers et al., 1987; Bisset et al., 2001). Also, a negative correlation between FEC and PCV was expected, which would lead to a correlated response of selection for FEC on PCV (Albers et al. 1987; Baker et al., 2003).

Given that weekly samplings are cumbersome and expensive, phenotypic and genetic indices using information of only some of the sampling dates were formed and compared to the overall genetic index in order to verify the possibility of reduced sampling. Sampling dates were chosen based on their phenotypic and genetic correlations with the overall trait and indices based on different combinations of sampling dates were formed and evaluated.

2.4. RESULTS

FEC data showed a very wide distribution, with a maximum of 81950 eggs at day 35 of the second parasite challenge in lambs. The coefficient of variance of FEC was high throughout the recordings, ranging from 96.93 to 180.91% in lambs, and from 142.74 to 516.30% in ewes. Descriptive statistics for FEC, PCV, BW and DWG in lambs are given in Table 2.1, while those for FEC and PCV in ewes are presented in Table 2.2.

Heritability estimates of FEC in lambs were low to moderate, with one high estimate of 0.46 ± 0.19 for the overall heritability of the second parasite challenge. The same was found for PCV, with the exception of 0.78 ± 0.23 estimated for PCV of day 28 of the second parasite challenge. The heritability estimate of FEC at weaning was not significantly different from zero in lambs and they also showed very low heritability estimates for PCV at weaning and FEC on most of the individual sampling dates of the first challenge. Heritability estimates of BW and DWG were mostly high. Estimates of genetic parameters for FEC, PCV, BW and DWG in lambs are listed in Table 2.3. For ewes, estimates for FEC and PCV under natural challenge (Day -14), as well as FEC in the first challenge did not differ from zero, while those of FEC and PCV of the second challenge were moderate (Table 2.4). Standard errors for heritability estimates were generally high. An overview of the genetic merit in progeny of two out of the four sires of lambs is given for EBVs of FEC, PCV and DWG (Figure 2.1). EBVs of progeny of the ram “2014” are mainly below average for FEC, PCV and DWG during challenges, while the opposite is true for the ram “287”. Repeatability estimates were moderate to high for FEC and PCV in lambs and ewes.

Table 2.1. Descriptive statistics for faecal egg count (FEC), packed cell volume (PCV), body weight (BW) and daily weight gain (DWG) in Morada Nova lambs.

trait	recording	minimum	maximum	mean	sd	CV (%)
FEC	weaning	0	71350	6777	9319	137.51
	d21-1	0	19200	1876	3046	162.40
	d28-1	0	28100	5974	6063	101.50
	d35-1	0	46150	9010	8733	96.93
	d42-1	0	56100	8561	9351	109.22
	d21-2	0	22500	1375	2487	180.91
	d28-2	0	40000	3274	5082	155.20
	d35-2	0	81950	4473	7497	167.61
	d42-2	0	44050	4666	6420	137.61
PCV	weaning	17	45	33.06	4.87	14.74
	d14-1	29	49	36.60	2.92	7.99
	d28-1	22	42	31.38	4.29	13.67
	d42-1	20	41	31.20	4.11	13.17
	d14-2	26	44	35.49	3.02	8.52
	d28-2	19	45	33.09	4.35	13.16
	d42-2	18	44	32.66	4.65	14.24
BW	weaning	8.0	24.5	15.83	2.99	18.88
	d28-1	10.9	32.8	20.32	3.83	18.85
	d42-1	10.9	35.3	21.15	4.14	19.57
	d28-2	12.0	40.0	24.17	4.58	18.94
	d42-2	10.0	40.3	24.51	4.74	19.33
DWG	DWG1	- 0.04	0.19	0.093	0.037	39.27
	DWG2	- 0.05	0.17	0.061	0.032	52.38
	totalDWG	- 0.03	0.15	0.078	0.028	35.26

dxx-y = recording at day xx of the yth challenge; DWG1 = daily weight gain during first parasite challenge; DWG2 = daily weight gain during second parasite challenge; totalDWG = weight gain from weaning to the end of the second parasite challenge

Table 2.2. Descriptive statistics for faecal egg count (FEC) and packed cell volume (PCV) in Morada Nova ewes.

trait	recording	minimum	maximum	mean	sd	CV (%)
FEC	d -14	0	10350	1189	1696	142.74
	d21-1	0	1400	76	183	240.28
	d28-1	0	2600	93	323	349.42
	d35-1	0	10400	220	1060	482.27
	d42-1	0	21300	408	2108	516.30
	d21-2	0	2000	259	440	169.94
	d28-2	0	8400	357	924	258.95
	d35-2	0	9200	632	1398	221.33
	d42-2	0	11800	430	1284	298.47
PCV	d -14	24	44	34.62	3.70	10.70
	d14-1	30	44	36.33	2.72	7.49
	d28-1	28	43	34.57	3.52	10.18
	d42-1	23	43	34.19	3.46	10.11
	d14-2	28	44	34.44	3.16	9.18
	d28-2	28	43	34.55	3.16	9.16
	d42-2	24	44	34.71	3.41	9.82

d-14 = recording under natural infection, 14 days before first artificial infection; dxx-y = recording at day xx of the yth challenge

Table 2.3. Estimates (± standard error) of additive genetic variance (σ^2_a), residual variance (σ^2_e), permanent environmental variance (σ^2_{pe}), heritability (h^2) and repeatability (t) for faecal egg count (FEC), packed cell volume (PCV), body weight (BW) and daily weight gain (DWG) of Morada Nova lambs.

trait	recordings	σ^2_a	σ^2_e	σ^2_{pe}	h^2	t
FEC	weaning	0.00	0.46		0.00 ± 0.07	
	overall trait	0.10	0.46	0.11	0.15 ± 0.10	0.31
	challenge 1	0.19	0.26	0.29	0.25 ± 0.18	0.65
	challenge 2	0.30	0.20	0.16	0.46 ± 0.19	0.70
	d21-1	0.19	0.37		0.34 ± 0.28	
	d28-1	0.02	0.48		0.03 ± 0.08	
	d35-1	0.02	0.41		0.05 ± 0.09	
	d42-1	0.03	0.42		0.07 ± 0.12	
	d21-2	0.15	0.33		0.31 ± 0.19	
	d28-2	0.07	0.40		0.15 ± 0.14	
	d35-2	0.11	0.31		0.26 ± 0.21	
	d42-2	0.11	0.28		0.29 ± 0.19	
	d35+42-2	0.14	0.11	0.17	0.33 ± 019	0.74
PCV	weaning	0.87	14.19		0.06 ± 0.10	
	overall trait	2.34	14.29	1.13	0.13 ± 0.08	0.20
	challenge 1	3.97	10.21	3.32	0.23 ± 0.14	0.42
	challenge 2	5.10	9.32	1.32	0.32 ± 0.16	0.41
	d14-1	3.07	5.73		0.35 ± 0.22	
	d28-1	0.28	12.85		0.02 ± 0.07	
	d42-1	1.99	10.98		0.15 ± 0.14	
	d14-2	1.98	5.40		0.27 ± 0.24	
	d28-2	12.14	3.42		0.78 ± 0.23	
	d42-2	2.80	8.83		0.24 ± 0.19	
	d28+42-2	6.78	8.27	1.01	0.42 ± 0.19	0.49
BW	weaning	7.70	0.45		0.94 ± 0.21	
	d28-1	12.20	1.57		0.89 ± 0.21	
	d42-1	10.34	4.87		0.68 ± 0.22	
	d28-2	17.15	2.42		0.88 ± 0.20	
	d42-2	16.50	4.14		0.80 ± 0.21	
DWG	totalDWG	0.0004	0.0002		0.70 ± 0.21	
	DWG 1	0.0005	0.0008		0.39 ± 0.18	
	DWG 2	0.0004	0.0005		0.44 ± 0.22	

dxx-y = analysis of records of day xx of the yth parasite challenge; dxx+xx-y = analysis including recordings of dxx-y and dxx-y; totalDWG = analysis of daily weight gain from weaning to day 42-2; DWG 1/DWG 2 = analysis of daily weight gain during first/second parasite challenge

Table 2.4. Estimates (± standard error) of additive genetic variance (σ^2_a), residual variance (σ^2_e), permanent environmental variance (σ^2_{pe}), heritability (h^2) and repeatability for faecal egg count (FEC) and packed cell volume (PCV) in Morada Nova ewes.

recordings	σ^2_a	σ^2_e	σ^2_{pe}	h^2	t
FECd -14	0.00	0.48		0.00 ± 0.17	
AllRepFEC	0.07	0.24	0.09	0.18 ± 0.14	0.40
RepFEC1	0.00	0.10	0.17	0.00 ± 0.09	0.63
RepFEC2	0.10	0.26	0.14	0.20 ± 0.16	0.48
PCVd -14	0.00	13.93		0.00 ± 0.12	
AllRepPCV	2.65	7.14	1.68	0.24 ± 0.13	0.38
RepPCV1	1.42	7.14	2.66	0.13 ± 0.11	0.36
RepPCV2	3.98	5.16	1.55	0.37 ± 0.18	0.52

AllRepFEC/PVC = of records of both parasite challenges, RepFEC1//PVC1 = analyses of records of the first parasite challenge, RepFEC2//PVC2 = analysis of records of the second parasite challenge, FEC//PVC d-14 = analysis of FEC//PVC records 14 days before artificial infections.

Phenotypic correlations between FEC and DWG or BW were zero to negative, ranging from 0.001 ± 0.06 at weaning to -0.20 ± 0.06 on day 42 of the second challenge (Table 2.5). There was a significant negative correlation between mean FEC and DWG in the first, but not in the second challenge. The opposite was found for FEC of individual sampling dates and BW. None of the genetic correlations between FEC and DWG or BW differed significantly from zero. Genetic correlations between PCV and DWG or BW were also not significant. Phenotypic correlations between these traits were mainly positive, ranging from 0.08 to 0.37, and only correlation between PCV and DWG of the second challenge did not differ significantly from zero (0.08 ± 0.06). For FEC and PCV, phenotypic and genetic correlations were negative and significant, with the former ranging from -0.39 to -0.67, and the latter ranging from -0.30 to -0.85. The genetic correlation between overall FEC in lambs and ewes was 0.36 ± 0.08, and for the overall PCV it was 0.42 ± 0.08. A list of phenotypic and genetic correlations between individual sampling dates and overall means or EBVs within traits are given in the Supplementary tables A.1. and A.2., respectively.

Table 2.5. Phenotypic (rp) and genetic (rg) correlations (± standard error) at weaning, for overall traits, per challenge and on individual sampling dates, according to phenotypic values and estimated breeding values (EBVs), between the traits faecal egg count (FEC) and body weight (BW) or daily weight gain (DWG), packed cell volume (PCV) and BW or DWG, and FEC and PCV in Morada Nova lambs.

recordings	FEC and BW or DWG		PCV and BW or DWG		FEC and PCV	
	r_p	r_g	r_p	r_g	r_p	r_g
Weaning	- 0.00 ± 0.06 [1]	- 0.02 ± 0.05 [1]	0.33 ± 0.06	0.00 ± 0.05 [1]	- 0.39 ± 0.06	- 0.30 ± 0.05
Overall trait	- 0.16 ± 0.06	0.01 ± 0.05 [1]	0.27 ± 0.06	-0.05 ± 0.05 [1]	- 0.64 ± 0.05	- 0.70 ± 0.03
Challenge 1	- 0.17 ± 0.06	- 0.02 ± 0.05 [1]	0.17 ± 0.06	- 0.01 ± 0.05 [1]	- 0.64 ± 0.05	- 0.79 ± 0.03
Challenge 2	- 0.05 ± 0.06 [1]	- 0.02 ± 0.05 [1]	0.08 ± 0.06 [1]	- 0.06 ± 0.05 [1]	- 0.67 ± 0.04	- 0.73 ± 0.03
Day 28-1	- 0.07 ± 0.06 [1]	0.01 ± 0.05 [1]	0.17 ± 0.06	0.00 ± 0.05 [1]	- 0.67 ± 0.04	- 0.85 ± 0.03
Day 42-1	- 0.07 ± 0.06 [1]	0.04 ± 0.05 [1]	0.29 ± 0.06	0.01 ± 0.05 [1]	- 0.51 ± 0.05	- 0.48 ± 0.04
Day 28-2	- 0.14 ± 0.06	0.02 ± 0.05 [1]	0.37 ± 0.05	- 0.05 ± 0.05 [1]	- 0.59 ± 0.05	- 0.51 ± 0.04
Day 42-2	- 0.20 ± 0.06	0.03 ± 0.05 [1]	0.25 ± 0.06	0.01 ± 0.05 [1]	- 0.61 ± 0.05	- 0.68 ± 0.04

[1] Correlations not significantly different from zero ($P > 0.05$)

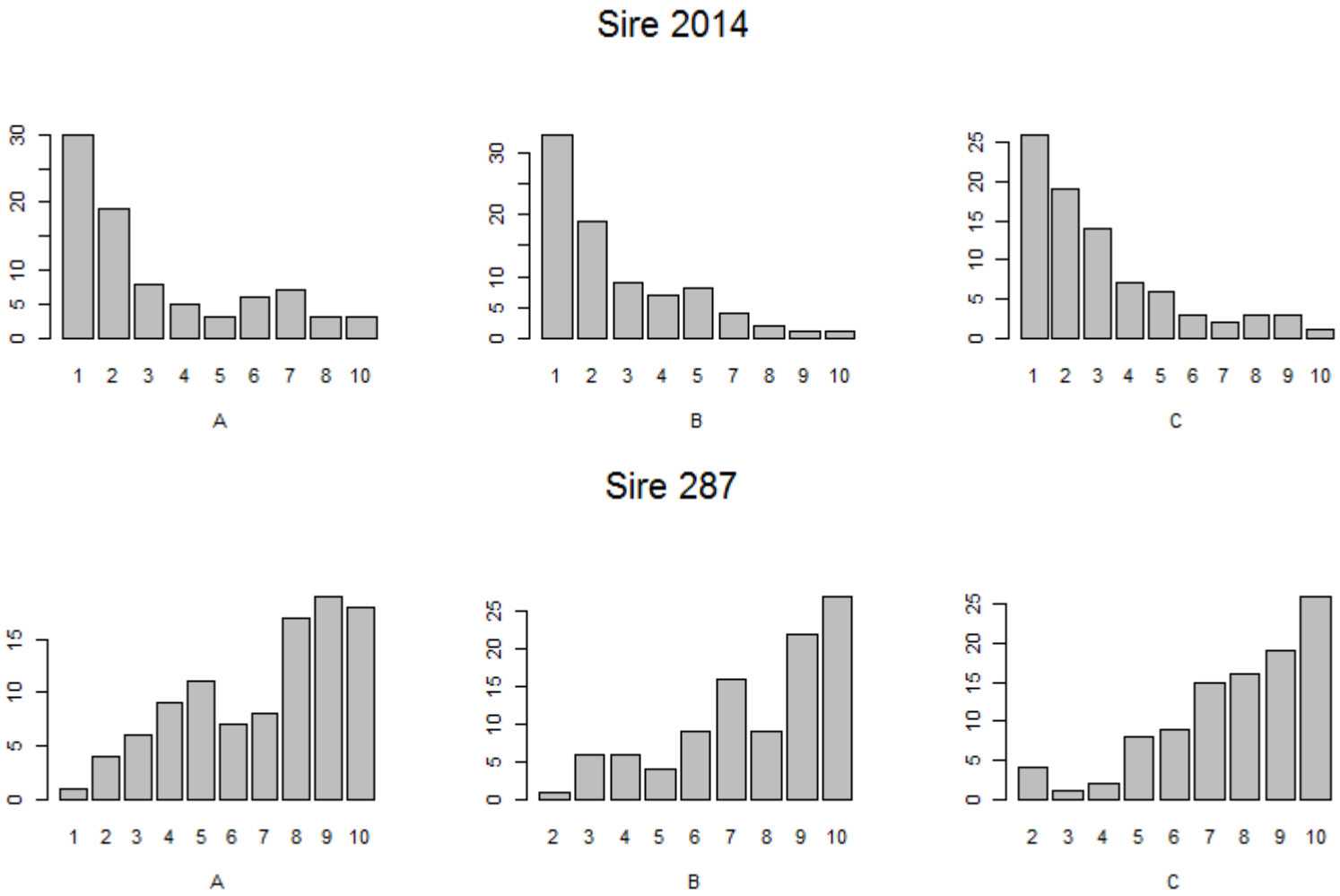

Figure 2.1. Frequencies of lambs sired by the rams 2014 and 287 in groups formed according to estimated breeding values (EBVs) of overall FEC (A), overall PCV (B) and total daily weight gain (C), with group 10 representing the most favourable, and group 1 the most unfavourable EBVs.

The phenotypic index based on TotalDWG and overall means of FEC and PCV (TotalMeanFEC and TotalMeanPCV) had a correlation of 0.86 ± 0.03 with the genetic index based on EBVs for the overall models of the traits (TotalDWG, AllRepFEC, AllRepPCV). In order to reduce samplings, various models including records of different sampling dates were compared. A repeated measurement model for FEC, based on only two records, taken on day 35 and 42 of the second challenge, showed the highest correlation (0.87 ± 0.03) with RepFEC2, compared to other models tested. For PCV, a model including day 28 and 42 of the second challenge was found most appropriate, showing a correlation of 0.85 ± 0.03 with AllRepPCV. Estimates of genetic parameters for these models are presented in Table 2.3. Correlation between a phenotypic index based on TotalDWG and on mean values for the cited records of FEC and PCV with a genetic index based on EBVs of totalDWG, RepFEC2 and AllRepPCV was 0.84 ± 0.03.

2.5. DISCUSSION

The overall heritability estimate for FEC found in the Morada Nova flock (0.15 ± 0.10 for the lambs and 0.18 ± 0.14 for the ewes) lies within the scale of findings in literature, that range from 0.00 to 0.37 ± 0.00 (Bishop et al., 1996; Brown and Fogarty, 2017; Snyman and Fisher, 2019). Phenotypic characterization of the Morada Nova sheep flock showed that ewes were highly resistant to *H. contortus*, while lambs were considerably more susceptible (Toscano et al., 2019). At weaning, lambs could not be differentiated according to resistance status, while in the first and second parasite challenge, a clear distinction could be made between resistant, intermediate and susceptible lambs based on FEC. Furthermore, there was a significant decrease in FEC of intermediate and susceptible lambs in the second parasite challenge. Estimated genetic parameters confirmed this increase in lamb's resistance over time: despite a considerable level of infection (mean FEC of 6777), heritability of FEC at weaning was zero (0.00 ± 0.07). Although heritability estimates are commonly different from zero at three months of age (heritability estimates of 0.29 ± 0.01 and 0.19 ± 0.08 were related for Merino and 0.12 ± 0.10 for Scottish Blackface lambs by Brown and Fogarty (2017), Pollott et al. (2004) and Bishop et al. (1996), respectively), they tend to increase with the age of lambs. Monthly recordings of FEC showed absence of heritability at one and two months of age (0.00 and 0.01), with subsequent monthly increase up to a level of 0.22 ± 0.13 at the age of six months (Bishop et al., 1996). Similar results were obtained by Pollott et al. (2004), who found that heritability estimates increased monthly from 0.19 ± 0.08 at 3 – 4 months to

0.60 ± 0.17 at 7 – 8 months of age. In the present study, heritability estimates of FEC on individual records of the first challenge were mostly very low for lambs and the overall heritability of the first challenge (RepFEC1), when lambs were 4 to 5 months old, was low (0.15 ± 0.10), while that of the second challenge (RepFEC2), at an age of 6 to 7 months, was considerably higher (0.45 ± 0.19). Apart from a lack of sufficiently long exposure to parasites, immunological hyporesponsiveness in lambs has been suggested as a reason for the increase in resistance with age (Colditz et al., 1996). It is possible that the lack of heritability at weaning reflects this decreased immunity to the parasite (as a result of insufficient exposure to parasites or related to young age of the lambs), but there could also be a response that is equal in all individuals, and thus does not possess any genetic variation (Bishop et al., 1996).

Heritability of PCV in lambs was close to zero at weaning and increased from the first to the second challenge, thus following a pattern similar to that of FEC. This effect is partly due to the same mechanisms that determine FEC, which is evidenced by the moderate to high favourable genetic correlations between the two traits. The estimate of overall heritability of the first parasite challenge was lower than that observed by Vanimisetti et al. (2004) after a single artificial infection with *H. contortus* in 4 – 6 months old lambs (0.39), but higher than that found by Baker et al. (2003) in several subsequent natural challenges at a similar age (0.14 ± 0.05). Other studies found heritability estimates of 0.35 ± 0.12 to 0.45 ± 0.11 (Albers et al., 1987) and 0.00 to 0.31 (Lôbo et al., 2009). In the present study, the very low heritability of PCV at weaning, when variation in genetic resistance was not yet evidenced, indicates that genetic variation in PCV is mostly due to host´s response to parasite.

It is remarkable that FEC heritability estimates in ewes showed an increase over time, similar to that of lambs. Under natural infection as well as in the first challenge, heritability did not differ from zero, while it was moderate for FEC in the second challenge. Aguerre et al. (2018) also found that rams artificially infected with *H. contortus* had a higher heritability in a second (0.35 ± 0.08) than in a first (0.14 ± 0.04) parasite challenge. On the other hand, these animals were housed indoors and they most probably were not exposed to considerable levels of parasites before the trial, as opposed to the Morada Nova ewes used in the present study. Similar to FEC, heritability of PCV in ewes under natural infection was estimated to be zero. Although the heritability estimates were different from zero in the first challenge, they increased in the second challenge, for which a moderate heritability was estimated. The high level of resistance and resilience in Morada Nova ewes is evidenced by their low FEC and high PCV under natural and

artificial infection with *H. contortus* (Toscano et al., 2019). It is probable that a response mechanism to *H. contortus* that is under genetic control exists in Morada Nova ewes, but that it is common to all individuals and thus does not possess genetic variability. The moderate heritability of FEC and PCV in the second challenge could then be classified as an additional response to the two artificial challenges that was differently expressed between individual animals.

Precision was low for most estimates in this study. Given that accuracy of heritability is a function of number of records and repeatability (Baker et al., 2003), this can be partly explained by the relatively low number of animals used compared to some studies (Brown and Fogarty, 2017; Gowane et al., 2019; Snyman and Fisher, 2019). Other authors estimated genetic parameters using animal numbers comparable to those of the present study (Bishop et al., 1996; Vanimisetti et al., 2004; Lôbo et al., 2009), one of which related relatively high standard errors as well (Bishop et al., 1996). Using repeated measures models had a favourable effect on precision, with slightly lower standard errors.

Given the haematophagia of *H. contortus*, the negative phenotypic correlations between FEC and PCV were expected. Several other studies obtained similar results, although correlations in Morada Nova lambs in the present study were stronger than those related by other authors: – 0.24 ± 0.03 to – 0.47 ± 0.02 (Baker et al., 2003) and 0.28 (Lôbo et al., 2009). The positive correlation found by Lôbo et al. (2009) is due to the use of transformed values for FEC values, which inversed the scale of data. As for genetic correlations, however, studies are not consistent. While Lôbo et al. (2009) and Aguerre et al., (2018) found no significant genetic correlation between FEC and PCV, correlations ranged from – 0.63 ± 0.58 to – 0.98 ± 0.24 in other studies (Albers et al. 1987; Baker et al., 2003). In the present study, correlations showed that mechanisms controlling FEC and PCV under *H. contortus* challenge are largely the same.

It is of crucial importance to estimate correlated responses prior to selection for resistance traits, in order to evaluate the possibility of correlated responses. In the present study, high FEC under natural infection did not result in a significant reduction in BW (0.001 ± 0.06), while after the first artificial infection, DWG was lower in animals with high FEC (0.17 ± 0.06). The negative phenotypic correlations between FEC and BW during the second challenge were probably due to the fact that susceptible animals had lower DWG in the first challenge, resulting in lower BW in subsequent measurements. Interestingly, DWG was not significantly influenced by FEC during the second parasite challenge. Lambs had lower DWG during second (mean of 0.06) than during first (0.09) challenge, probably due to deceleration of growth in older lambs. The lower variation in DWG of the

second challenge might have masked a significant phenotypic correlation with FEC. Another explanation could be an increased level of resilience in the second compared to the first challenge, with lambs being able to maintain their growth rate despite infection levels. No significant genetic correlations were detected between FEC and BW or DWG, which means that performance would not be compromised by selection for reduced FEC in Morada Nova lambs.

The genetic relationship between FEC and weight or weight gain has been studied in a variety of environments and breeds. There has been little consistency across studies: some authors related weak, moderate or strong negative genetic correlations (Bishop et al., 1996; Snyman and Fisher, 2019), while others found insignificant (Lôbo et al., 2009; Brown and Fogarty, 2017), or even positive-correlations (Morris et al., 2005; Gowane et al., 2019). According to Bishop and Stear (2003) and Bishop (2012), the relationship between resistance and performance traits is highly dependent on the environment: in heavily parasitized environments, the effort of building and maintaining an effective immune response is beneficial as it leads to lower FEC and subsequent better performance of resistant animals. Under low challenge conditions, performance may not be compromised by infection, which makes the mounting of an immune response an unnecessary waste of energy and resources, leading to poorer performance of resistant animals.

Based on results of the present study, FEC of lambs and ewes cannot be considered the same trait, although selection for one should have a favourable effect on the other. That is, selection of animals based on FEC in lambs would result in lower FEC in lambs and (to a lower degree) in ewes (genetic correlation of 0.36 ± 0.08). The same is true for PCV of lambs and ewes, that showed a positive genetic correlation of 0.42 ± 0.08. Vanimisetti et al., (2004) did not detect a significant genetic correlation between FEC in lambs and FEC in ewes after artificial infection with *H. contortus.* Goldberg et al. (2012), on the contrary, found a correlation of 0.81 ± 0.11 between FEC of lambs and ewes under natural infection. The diverging results of these studies might be explained by the fact that Goldberg et al. (2012) used periparturient ewes while the ewes investigated by Vanimisetti et al. (2004) were infected at about 70 days after lambing. The periparturient period, which is characterized by a rise in FEC (Williams et al., 2010; Goldberg et al., 2012), might be more sensitive for the detection of common mechanisms in lambs and ewes. This is supported by the fact that Williams et al. (2010) found animals from a low FEC selection line (based on FEC at 12 months of age) to have significantly lower FEC during the periparturient period than animals from a control line. For the Morada Nova flock, a higher correlation than that presented in this study might be found when FEC of lambs and periparturient ewes are considered, instead of using ewes in normal physiological status.

Given the very low heritability estimates found for most records of the first challenge, the recording of just one parasite challenge is not considered sufficient for the estimation of breeding values for FEC. The results of this study indicate that EBVs of FEC obtained in the second challenge (RepFEC2) are more informative than FEC of the overall model (AllRepFEC). The results of the present study suggest that selection can be based on only two records of BW (at weaning and at the end of the trial), and two records of FEC (of day 35 and 42) and PCV (day 28 and 42), taken during the second parasite challenge in Morada Nova lambs.

2.6. CONCLUSION

Selection for FEC, PCV and DWG under parasite challenge is possible in lambs and ewes of the Morada Nova population investigated in this study. Selection for low FEC in lambs would increase PCV in lambs and reduce FEC in ewes. Based on our results, there is no evidence of a favourable or unfavourable correlated response of selection for FEC on BW or DWG. However, lower infection levels should be favourable to performance due to negative phenotypic correlations between these traits. For lambs, a ranking formed by FEC (2 records), PCV (2 records), and overall DWG during the trial, with records of FEC and PCV taken 4 to 6 weeks after infection in the second parasite challenge, can be used as selection criterion, allowing simultaneous selection for lower FEC, higher PCV and higher DWG.

2.7. REFERENCES

Aguerre S, Jacquiet P, Brodier H, Bournazel JP, Grisez C, Prévot F, Michot L, Fidelle F, Astruc JM, Moreno CR (2018) Resistance to gastrointestinal nematodes in dairy sheep: Genetic variability and relevance of artificial infection of nucleus rams to select for resistant ewes on farms. **Veterinary Parasitology** 256:16-23.

Albers GAA, Gray GD, Piper LR, Barker JSF, Le Jambre LF, Barger IA (1987) The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. **International Journal for Parasitology** 17:1355-1363.

Amarante AFT, Bricarello PA, Rocha RA, Gennari SM (2004) Resistanceof Santa Inês, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. **Veterinary Parasitology** 120:91-106.

Baker RL, Nagda S, Rodriguez-Zas SL, Southey BR, Audho JO, Aduda EO, Thorpe W (2003) Resistance and resilience to gastro-intestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai × Dorper crossbred lambs in the sub-humid tropics. **Animal Science** 76:119-136.

Baker RL, Gray GD, 2004. Appropriate breeds and breeding schemes for sheep and goats in the tropics. In,: Sani RA, Gray GD, Baker RL(Eds.) **Worm Control for Small Ruminants in Tropical Asia.** Canberra, Australia: ACIAR Monograph 113, pp. 63-95.

Baker RL, Mugambi JM, Audho JO, Carles AB, Thorpe W (2004) Genotype by environment interactions for productivity and resistance to gastro-intestinal nematode parasites in Red Maasai and Dorper sheep. **Animal Science** 79:343-353.

Bishop SC, Bairden K, McKellar QA, Park M, Stear M J (1996) Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with live-weight in young lambs. **Animal Science** 63: 423-428

Bishop SC, Stear MJ (2003) Modeling of host genetics and resistance to infectious diseases: understanding and controlling **nematode** infections **Veterinary Parasitology** 115:147-166.

Bishop SC (2012) Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. **Animal** 6:741-747.

Bisset SA, Vlassof A, West CJ, Morrison L (1997) Epidemiology of nematodosis in Romney lambs selectively bred for resistance or susceptibility to nematode infection. **Veterinary Parasitology** 70:255-269.

Bisset SA, Morris CA, McEwan JC, Vlassof A (2001) Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. **New Zealand Veterinary Journal** 49:236-246.

Boldman KG, Kriese LA, van Vleck LD, Kachman SD (1995) A manual for use of MTDFREML: a set of programs to obtain estimates of variances and covariances (DRAFT). Lincoln Department of Agriculture, Agricultural Research Service, 125 pp.

Brown DJ, Fogarty NM (2017) Genetic relationships between internal parasite resistance and production traits in Merino sheep. **Animal Production Science** 57:209-215.

Chagas ACS, Katiki, LM, Silva, IC, Giglioti R, Esteves SN, Oliveira MCS Barioni-Júnior W, (2013) Haemonchus contortus: A multiple-resistant Brazilian isolate and the costs for its characterization and maintenance for research use. **Parasitology International** 62:1–6.

Colditz IG, Watson DL, Gray GD, Eady SJ (1996) Some relationships between age, immune responsiveness and resistance to parasites in ruminants. **International Journal for Parasitology** 26:869-877.

Eady SJ, Woolaston RR, Barger IA (2003) Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. **Livestock Production Science** 81:11-23.

Facó O, Paiva SR, Alves LRN, Lôbo RNB, Villela LCV (2008) Raça Morada Nova: Origem, Características e Perspectivas. **Embrapa** Documentos 75 ISSN 1676-7659.

Goldberg V, Ciappesoni G, Aguilar I (2012) Genetic parameters for nematode resistance in periparturiant ewes and post-weaning lambs in Uruguayan Merino sheep. **Livestock Science** 147:181-187.

Gowane GR, Swarnkar CP, Misra SS, Kumar R, Kumar A, Prince LLL (2019) Genetic parameter estimates for fecal egg counts and their relationship with growth in Avikalin and Malpura sheep. **Animal** 13:1788-1796.

Issakowicz J, Issakowicz ACKS, Bueno MS, Costa RLD, Katiki LM, Geraldo AT, Abdalla AL, McManus C, Louvandini H (2016) Parasitic infection, reproductive and productive performance from Santa Inês and Morada Nova ewes. **Small Ruminant Research** 136:96-103.

Jackson F, Bartley D, Bartley Y, Kenyon F (2009) Worm control in sheep in the future. **Small Ruminant Research** 86:40-45.

Kemper KE, Palmer DG, Liu SM, Greeff JC, Bishop SC and Karlsson LJE (2010) Reduction of faecal worm egg count, worm numbers and worm fecundity in sheep selected for worm resistance following artificial infection with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. **Veterinary Parasitology** 171:238-246.

Laurenson YCSM, Kyriazakis I, Forbes AB, Bishop SC, (2012) Exploration of the epidemiological consequences of resistance to gastro-intestinal parasitism and grazing management of sheep through a mathematical model. **Veterinary Parasitology** 189: 238-249.

Lôbo RN, Vieira LS, Oliveira AAD, Muniz EN, Silva JMD (2009) Genetic parameters for faecal egg count, packed-cell volume and body-weight in Santa Inês lambs. **Genetics and Molecular Biology** 32:228-294.

Lôbo RNB, Pereira IDC, Facó O, McManus CM (2011) Economic values for production traits of Morada Nova meat sheep in a pasture based production system in semi-arid Brazil. **Small Ruminant Research** 96:93-100.

Morris CA, Wheeler M, Watson TG, Hosking BC, Leathwick DM (2005) Direct and correlated responses to selection for high or low faecal nematode egg count in Perendale sheep. **New Zealand Journal of Agricultural Research** 48:1-10.

Papadopoulos E, Gallidis E, Ptochos S (2012) Anthelmintic resistance in sheep in Europe: a selected review. **Veterinary Parasitology** 189:85-88.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2018) _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-137, <URL:https://CRAN.Rproject.org/package=nlme>.

Pollott GE, Karlsson LJE, Eady S, Greef JC (2004) Genetic parameters for indicators of host resistance to parasites from weaning to hogget age in Merino sheep. **Journal of Animal Science** 82: 2852–2864

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Snyman MA, Fisher AD (2019) Genetic parameters for traits associated with resistance to *Haemonchus contortus* in a South African Dohne Merino sheep flock. **Small Ruminant Research** 176:76-88.

Toscano JHB, Santos IB, Haehling MB, Giraldelo LA, Lopes LG, Silva MH, Figueredo A, Esteves SN and Chagas ACS (2019) Morada Nova sheep breed: resistant or resilient to *Haemonchus contortus* infection? **Veterinary Parasitology X** 2:100019.

Vanimisetti HB, Andrew SL, Zajac AM, Notter DR (2004) Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with *Haemonchus contortus*. **Journal of Animal Science** 82:1602-1611.

Van Vleck LD, Pollak EJ, Oltenacu EA (1987) Genetics for the animal sciences. WH freeman and Company, New York, 391 pp.

Veríssimo CJ, Niciura SCM, Alberti ALL, Rodrigues CFC, Barbosa CMP, Chiebao DP, Cardoso D, Silva GS, Pereira JR, Margatho LFF, Costa RLD, Nardon RF, Ueno TEH, Curci VCLM, Molento MB (2012) Multidrug and multispecies resistance in sheep flocks from São Paulo state, Brazil.**Veterinary Parasitology** 187:209-216.

Williams AR, Greeff JC, Vercoe PE, Dobson RJ, Karlsson LJE (2010) Merino ewes bred for parasite resistance reduce larval contamination onto pasture during the peri-parturient period. **Animal** 4:122-127.

CHAPTER 3 – FOUR SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) ARE ASSOCIATED WITH RESISTANCE AND RESILIENCE TO *Haemonchus contortus* IN BRAZILIAN MORADA NOVA SHEEP[1]

3.1. ABSTRACT

Gastrointestinal nematodes are a major constraint in sheep production. Breeding for resistance has proven to be an effective and feasible approach to address this problem. The use and investigation of genetic markers for resistance traits could accelerate genetic progress and lead to a better understanding of underlying molecular mechanisms. Thus, the aim of this study was to evaluate if five single nucleotide polymorphisms (SNPs OAR2_14765360, OAR6_81718546, OAR11_62887032, OAR12_69606944 and OAR15_59871543) are associated with resistance and resilience traits in a flock of the Morada Nova sheep breed. Lambs were submitted to two consecutive parasite challenges by oral infection with 4,000 infective larvae L_3) of *Haemonchus contortus*. Faecal egg counts (FEC), packed cell volume (PVC) and BW were measured every one or two weeks for 42 days in each trial. DNA samples from 287 lambs, 131 ewes and 4 rams were amplified by ARMS-PCR or PCR-RFLP and genotypes were determined. Analysis of variance (ANOVA) was used for association analyses between genotypes and phenotypes. In case of significant association, the allele substitution effect was calculated based on a linear model. OAR2_14765360 and OAR12_69606944 were associated with FEC, and OAR12_69606944 also had significant effects on PCV and weight gain, showing favourable associations of the CC genotype with all evaluated traits. Both OAR6_81718546 and OAR11_62887032 were associated with weight gain, and OAR6_81718546 had an additional effect on PCV. OAR15_59871543 was not polymorphic in the population. OAR6_81718546 and OAR12_69606944 presented significant allele substitution effects of -1.06 ± 0.52 kg for the T allele on final BW and 0.74 ± 0.32 for the C allele in PCV of the same sampling date, respectively. This is the first report of SNPs associated with gastrointestinal nematode resistance in this sheep breed. Our findings support the existence of quantitatice trait loci (QTL) for resistance and resilience in linkage disequilibrium with the polymorphic SNPs and suggest their future use for explorations of these traits in Morada Nova sheep.

[1] This chapter corresponds to the research article published by Veterinary Parasitology 279:109053 (2020)

3.2. INTRODUCTION

Helminthosis is the most important disease in sheep production systems on a global scale (Perry et al., 2002), with gastrointestinal nematodes (GIN) being the major problem. Hematophagous *Haemonchus contortus* is the parasite with the highest clinical and economic importance in Brazilian flocks (Amarante et al., 2004; Chagas et al., 2013), being responsible for production deficit due to weight loss or retarded growth, anaemia and deaths. Control measures are still heavily reliant on the use of anthelmintics. However, this approach is limited due to the increasing prevalence of drug resistant parasites (Jackson et al., 2009; Papadopoulos et al., 2012; Zvinorova et al., 2016). In São Paulo state, for example, there are reports of flock holders abandoning sheep production due to this problem (Amarante et al., 2004). Therefore, the need for alternative approaches to reduce anthelmintic treatments and thus attenuate the development of anthelmintic resistance in parasites is evident.

Resistance, the host's ability to control the parasite life cycle, is commonly quantified by faecal egg count (FEC) (Bishop and Stear, 2003). Resilience is defined as the capability of a host to maintain performance despite infection and is often measured as a performance trait (like body weight or weight gain) under parasite challenge (Bishop and Stear, 2003). Packed cell volume (PCV), a measure of the impact of infection on animal health, is also considered a resilience trait (Bishop, 2012b). Selection of animals resistant to GIN has shown to be a feasible and effective control measure (Woolaston and Piper, 1996; Woolaston and Windon, 2001; Eady et al., 2003; Snyman and Fisher, 2019), and it has been included in several commercial breeding programmes (Goldberg et al., 2012; Brown and Fogarty, 2016). Given the high prevalence of GIN infections, resilience traits can bear an additional benefit when implemented concomitantly with resistance traits (Bishop, 2012a). To obtain estimated breeding values for resistance or resilience traits, it is necessary to submit animals to either natural or artificial nematode infection, which is to some extent cumbersome and can raise issues of comparability between environments and generations (Hunt et al., 2008).

Apart from selection within breeds, the choice of breed can also be of major importance for GIN control. In many cases, tropical sheep breeds evolved in high challenge environments have shown to be more resistant and resilient to GIN than commercial breeds (Matika et al., 2003; Baker et al., 2004; Bishop and Morris, 2007), offering an important resource for stockholders and allowing a more effective and sustainable production under tropical conditions. Morada Nova is a naturalized hair sheep breed from the Northeastern region of Brazil. It is characterized by its small size, high

adaptation to tropical climate, high prolificacy, non-existent reproductive seasonality, good maternal ability and excellent pelt quality, but also by low weight gain and carcass quality (Facó et al., 2008; Lôbo et al., 2011). Besides, this breed has shown a high ability to withstand GIN infections, evidenced by lower FEC, higher PCV and largely unaffected performance compared to other breeds kept under the same management conditions, including Santa Inês, the most commonly used breed of Brazil, also known for its resistance to GIN infection (Issakowicz et al., 2016). Issakowicz et al. (2016) highlighted that the health of Morada Nova sheep was unaffected by infection, even during the periparturient period, demonstrating Morada Nova's potential as a maternal breed.

Many studies have focused on detecting genetic markers associated with GIN resistance in sheep (Coltman et al., 2001; Janssen et al., 2002 ;Benavides et al., 2015; Berton et al., 2017; Ali et al., 2019). These investigations could support the screening for relevant genes involved in the biological mechanisms underlying resistance. The regions most consistently associated with GIN resistance in sheep are those in and near the major histocompatibility complex II (MHC II) and Interferon (IFN)-γ genes (Coltman et al., 2001; Janssen et al., 2002; Ali et al., 2019), both known to be components of the immune response. Other QTLs for GIN resistance point to other mechanisms, including innate and required immune responses, gastric mucosal protection and hemostasis pathways (Benavides et al., 2016). In addition to the detection of relevant mechanisms of resistance, genetic markers might also be used in breeding programmes. Marker-assisted selection (MAS) could accelerate genetic progress and reduce the number of animals that need to be submitted to parasite challenge, with the proviso that sufficient genetic variation is accounted for by the markers (Hunt et al., 2008; Benavides et al., 2016). To avoid unfavourable effects of MAS on other traits than the one originally assessed by the markers, correlated response to selection needs to be investigated (Hunt at al., 2008). According to the sheep quantitative trait loci (QTL) database (QTLdb; https://www.animalgenome.org/cgi-bin/QTLdb/OA/summary), there are 51 QTLs associated with *H. contortus* FEC, distributed in the 26 ovine autosomes, except for chromosomes 14, 17 and 19, and in the X chromosome. However, no major QTL has yet been described and study results suggest a polygenic nature of this trait (Kemper et al., 2011; Benavides et al., 2015).

In a genome-wide association study (GWAS), performed on a crossed population of Red Maasai and Dorper, the top five most relevant markers for FEC were detected on chromosomes 2, 6, 11, 12 and 15 (OAR2_14765360, OAR6_81718546, OAR11_62887032, OAR12_69606944 and OAR15_59871543, respectively; Benavides et al., 2015). Together, these five Single Nucleotide Polimoprhism (SNP) markers accounted

for 2.33 % of phenotypic variation in the Red Maasai x Dorper population. Thus, the aim of the present study was to verify if these five SNPs are associated with resistance and resilience traits (FEC, PCV, and weight gain) after artificial infection with *H. contortus* in a Morada Nova hair sheep population.

3.3. MATERIAL AND METHODS

3.3.1. Experimental animals and phenotypes

The experiment was conducted on the Morada Nova flock at Embrapa Pecuária Sudeste (CPPSE), São Paulo state, Brazil, with all procedures approved by the CPPSE Ethics and Animal Experimentation Committee (process no. 04/2017). Management practices and determination of phenotypes have been discussed by Toscano et al. (2019). Briefly, 287 lambs, progeny of 131 ewes and 7 rams born in two consecutive years, were submitted to two successive artificial parasite challenges with *H. contortus.* At the age of approximately 100 days, lambs were weaned, faecal samples taken to perform FEC, and animals were drenched to eliminate natural infections. Fifteen days after weaning, animals were infected with 4,000 infective larvae L_3) of *H. contortus.* FEC, PCV and body weight were monitored regularly until day 42 after infection, when animals were drenched. Fifteen days after the end of the first trial, the second trial was initiated, with infection and sampling following the same protocol that was used for the first trial.

3.3.2. Genotypes

Genotyping was performed according to Niciura et al. (2018). Briefly, white blood cells recovered from blood samples collected by jugular vein puncture into EDTA-containing tubes were submitted to DNA extraction by salt precipitation. Animals (lambs, ewes and rams) were genotyped by tetra-Primer Amplification Refractory Mutation System – PCR (ARMS-PCR) for four SNPs: OAR2_14765360, OAR6_81718546, OAR11_62887032 and OAR12_69606944. Restriction fragment length polymorphism – PCR (PCR-RFLP) was used to genotype one SNP: OAR15_59871543, which was not polymorphic in lambs born in 2017 and therefore was not further investigated.

3.3.3. Statistical analysis

Genotyping of ewes and rams was used to detect discordant parent-progeny pairs. After comparisons, 18 lambs (6.2%) presented discordant genotypes in at least one SNP. It was not possible to identify whether this was due to misidentification of animals at mating or at birth, or if the error occurred during sampling and the data from these animals were removed from analysis. Another 13 lambs were removed because they are descendants of three rams with few progenies, concentrated in only one of the four age groups. Thus, information of 256 lambs was used for further analysis of FEC, PCV, body weight and weight gain. In cases of salvage anthelmintic treatment, values obtained after treatment were excluded from analysis, while values from earlier sampling dates were maintained.

Genotypic and allelic frequencies were calculated for ewes and lambs. Also, a chi-squared test ($p < 0.05$) was conducted to detect deviation from Hardy-Weinberg-equilibrium.

For analysis of SNP effects on phenotypes in lambs, FEC were log-transformed ($\log_{10}(FEC+25)$) to normalize data. An overall mean of FEC and PVC (totalmeanFEC, totalmeanPCV), as well as a mean for each parasite challenge (meanFEC1, meanFEC2, meanPCV1 and meanPCV2) was calculated, including days 21, 28, 35 and 42 of each challenge for FEC, and days 14, 28 and 42 for PCV. Daily weight gain (DWG) was calculated for the period between weaning and the end of the second challenge (totalDWG), and for each parasite challenge (DWG1, DWG2), between days 0 and 42. Analyses were conducted using the "R" package, applying a significance level of 0.05. Fixed effects of sex, age at weaning (categorized from 1 to 5: 1 = 70–79 days; 2 = 80–89 days; 3 = 90–99 days; 4 = 100–109 days; 5 = 110–119 days), group (1 to 4, 1 and 2 in the first year, 3 and 4 in the second year of the experiment), father, birth type (single or twin), age of the dam (1 to 6, including dams older than 6 years in the group 6), and first-order interactions were tested by ANOVA, using the aov() function. That way, models were defined for each FEC and PCV mean and for total DWG, DWG1 and DWG2, as well as for individual measurements of FEC, PCV and body weight at the respective days of sampling and challenge. There were significant interactions between sex and group in the case of total DWG, body weight at weaning and mean PCV2, which is why a composed fixed effect containing sex and group (“contemporary group”) was used for the analyses of SNP effects in these cases. Also, a significant interaction was detected between OAR2_14765360 and age group in mean FEC2, as well as OAR11_62887032 and age of the dam in total DWG. In both cases, analyses were not conducted separately for age

groups considering the relatively small number of animals compared to the number of age groups (6). Each SNP was tested individually by ANOVA (aov() function), and in case of significance, the Tukey-test, adjusted for unbalanced group sizes, was performed to detect differences between each pair of group means. In the case of OAR6_81718546, the group size was found to be extremely unbalanced (with the GG genotype being represented by only 4 animals), so Scheffé-test was more appropriate. Allele substitution effects were tested for SNPs significant in ANOVA. Therefore, the animals' phenotypes were considered twice: once for each of the two alleles. The alleles were then tested for significance in a linear model using the lm() function. The partial regression coefficient for the allele effect in the lm model was considered to be the allele substitution effect, according to Stear et al. (1989).

For those SNPs that were found to be significantly associated to one of the characteristics, the genotypic frequencies were analysed in the 40% animals of extreme phenotypes of that trait. Animals were ranked according to overall phenotypic values of each characteristic (total mean FEC, total mean PCV and total DWG) in order to define the 40% extreme animals (20% of animals with most favourable (low FEC, high PCV and high DWG), and the 20% with most unfavourable (high FEC, low PCV and low DWG) phentotypic values). Given the differences in FEC, PCV and DWG between males and females, animals were ranked separately for sex. The 26 males and females with most favourable and 26 males and females with most unfavourable phenotypes were chosen to build the two groups of 52 animals of favourable and 52 animals of unfavourable phenotypes for each trait. Genotypic frequencies between the extreme phenotype groups were compared using the Fisher's exact test, applying a significance level of 0.05.

3.3.4. Annotation and functional analysis of SNPs

A search for all genes located in a 1,000 kb window around each SNP was performed using the Ensembl database (www.ensembl.org; Zerbino et al., 2018). Gene functions were retrieved from literature.

3.4. RESULTS

Table 3.1 presents genotypic frequencies, varying from 0 to 72.2%, and allelic frequencies, varying from 0.15 to 0.85, for the four polymorphic SNPs (OAR2_14765360,

OAR6_81718546, OAR11_62887032 and OAR12_69606944) in lambs and ewes. All four SNPs were found to be in Hardy-Weinberg-equilibrium, except for OAR6_81718546 in the ewes ($p < 0.05$). The OAR15_59871543 SNP was monomorphic to the GG genotype.

Table 3.1. Genotypic and allelic frequencies of OAR2_14765360 (OAR2), OAR6_81718546 (OAR6), OAR11_62887032 (OAR11) and OAR12_69606944 (OAR12) SNPs in Morada Nova ewes and lambs.

SNP	Ewes					Lambs				
	Genotypic frequency (%)			Allelic frequency		Genotypic frequency (%)			Allelic frequency	
OAR2	TT	CT	CC	f(T)	f(C)	TT	CT	CC	f(T)	f(C)
	52.6	37.8	9.6	0.71	0.29	50.4	43.0	6.6	0.72	0.28
OAR6	TT	CT	CC	f(T)	f(C)	TT	CT	CC	f(T)	f(C)
	69.9	30.1	0.0	0.85	0.15	72.2	26.2	1.6	0.85	0.15
OAR11	AA	AG	GG	f(A)	f(G)	AA	AG	GG	f(A)	f(G)
	43.4	44.1	12.5	0.65	0.35	46.1	42.2	11.7	0.67	0.33
OAR12	AA	AC	CC	f(A)	f(C)	AA	AC	CC	f(A)	f(C)
	33.8	53.0	13.2	0.60	0.40	40.2	46.9	12.9	0.64	0.36

ANOVA revealed significant effects of OAR2_14765360 and OAR12_69606944 on mean FEC of the second parasite challenge (Fig. 3.1), but Tukey test was only significant for OAR12_69606944, showing the AA genotype to be associated to higher FEC than the CC genotype. When analysing individual sampling days (Fig. 3.2), significant effects were detected by ANOVA on days 21/2 and 28/2 for OAR2_14765360, and on days 28/2 and 35/2 for OAR12_69606944. Tukey test was significant only in the case of OAR12_69606944 on day 28/2, with the AA genotype being unfavourable (higher mean FEC) compared to the CC genotype (Fig. 3.2).

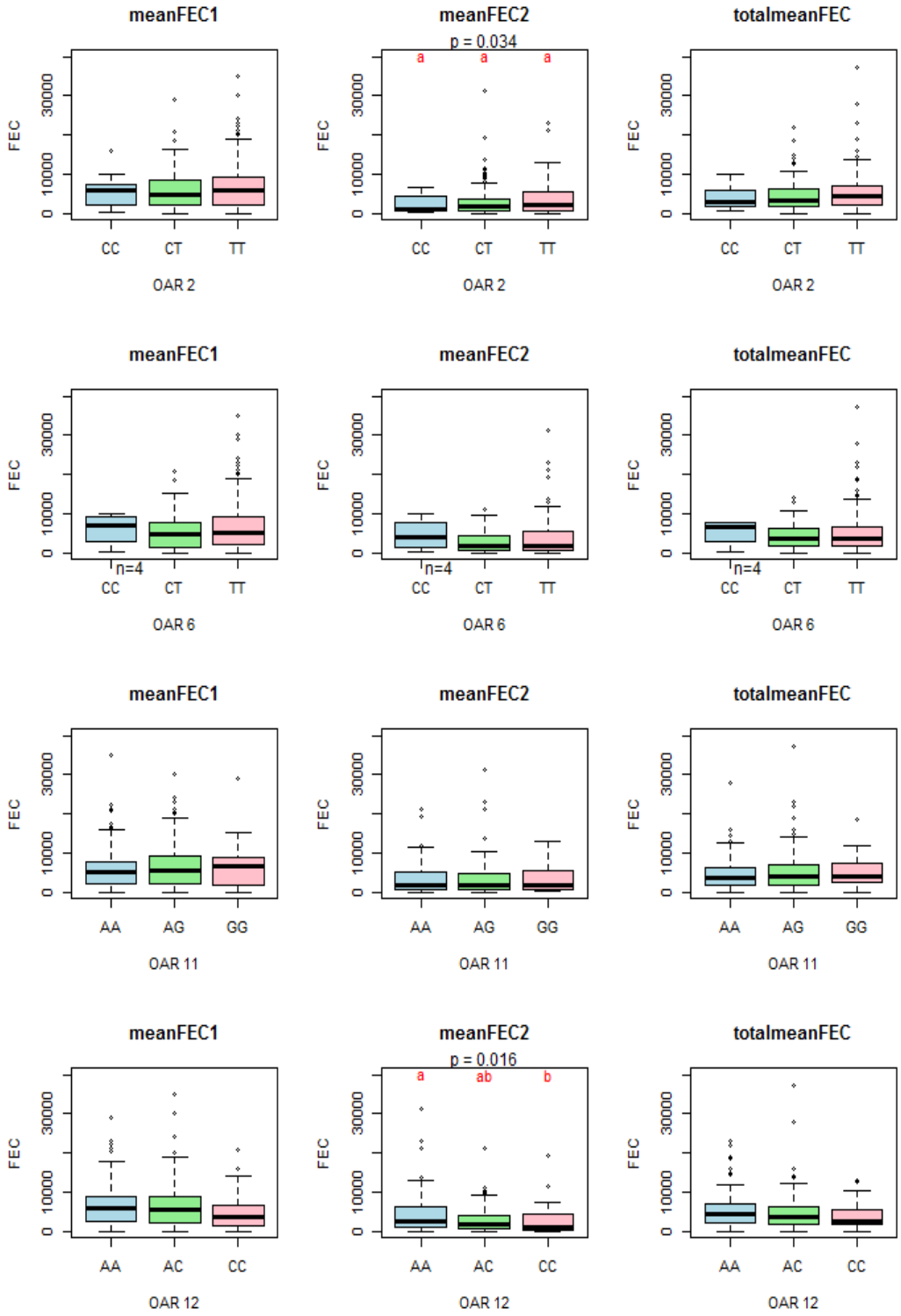

Figure 3.1. Box-and-whisker plot of genotypic effects of polymorphic SNPs (OAR2_14765360 (OAR2), OAR6_81718546 (OAR6), OAR11_62887032 (OAR11) and OAR12_69606944 (OAR12)) on mean faecal egg counts (FEC) in the first (meanFEC1) and second (meanFEC2) challenges, and total (totalmeanFEC), detected in 256 Morada Nova lambs, with p-values of the ANOVA. Different lower case letters indicate differences in group means according to post-hoc tests.

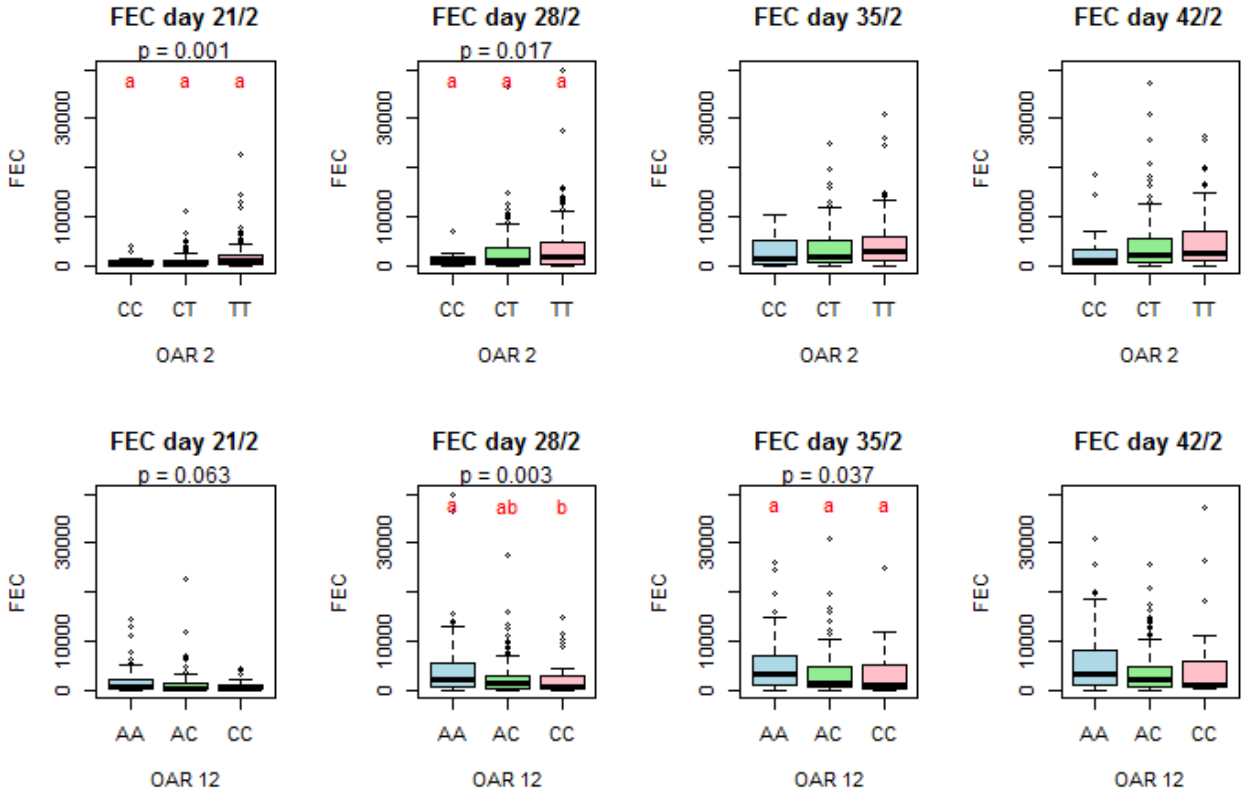

Figure 3.2. Box-and-whisker plot of genotypic effects of polymorphic SNPS (OAR2_14765360 (OAR2) and OAR12_69606944 (OAR12)) on faecal egg counts (FEC) of individual sampling dates (days 21, 28, 35 and 42 of the challenge 2), detected in 256 Morada Nova lambs, with p-values of the ANOVA. Different lower case letters indicate differences in group means according to post-hoc tests.

Regarding PCV, ANOVA (Fig. 3.3) detected significant effects of OAR6_81718546 and OAR12_69606944 for meanPCV2, and of OAR6_81718546 for totalmeanPCV, but post hoc tests were only significant for OAR12_69606944. For individual sampling days (Fig. 4), association with PCV, detected by ANOVA, was found for OAR6_81718546 on day 28/2 and for OAR12 on day 42/2, with the CT genotype of OAR6_81718546 having significantly higher PCV than the TT and CC genotypes according to the Scheffé test. For OAR12_69606944, the AA genotype was unfavourable both in mean PCV2 and PCV of day 42/2, resulting in low PCV values (Fig. 3.3 and 3.4).

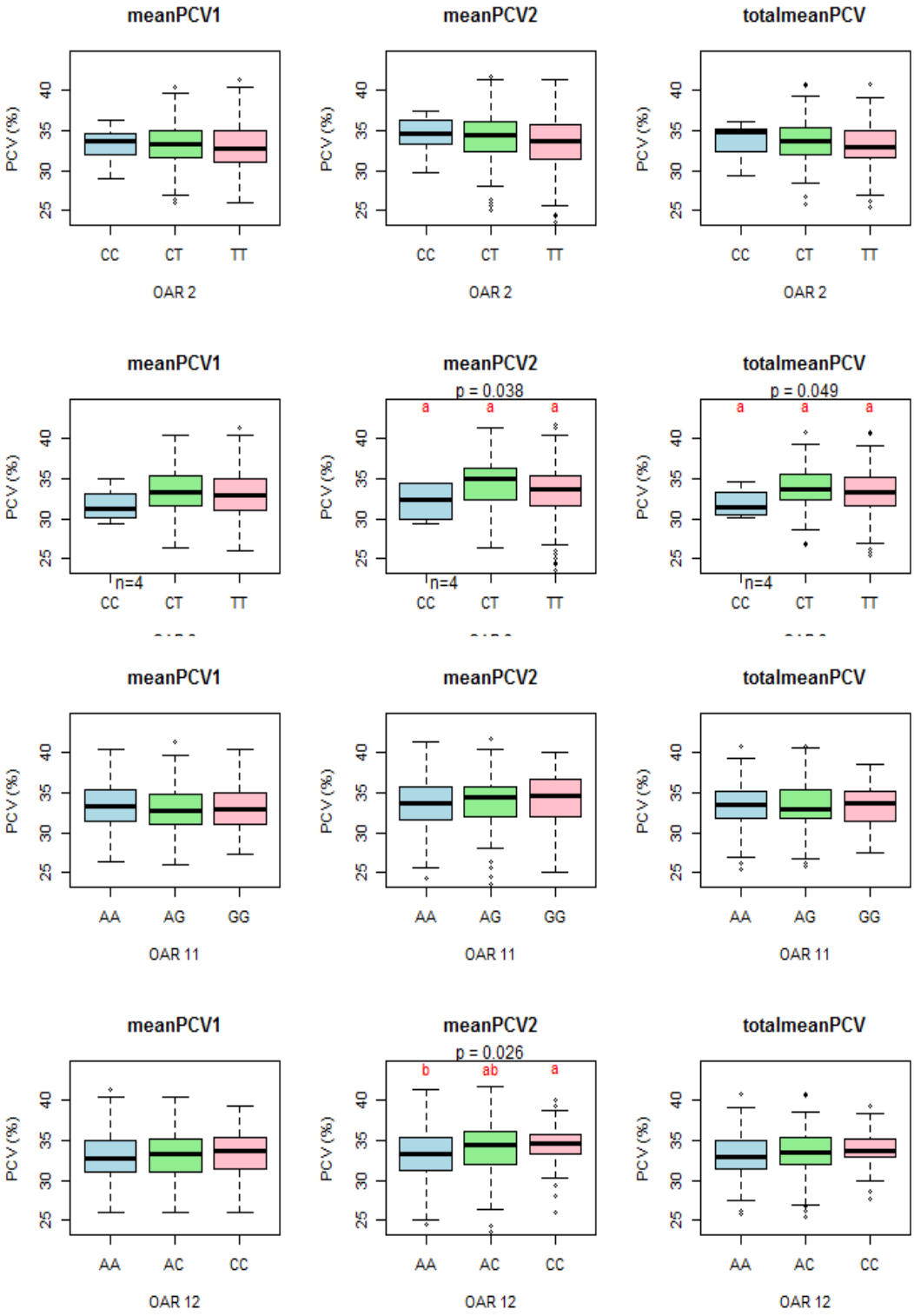

Figure 3.3. Box-and-whisker plot of genotypic effects of polymorphic SNPs (OAR2_14765360 (OAR2), OAR6_81718546 (OAR6), OAR11_62887032 (OAR11) and OAR12_69606944 (OAR12)) on mean packed cell volume (PCV) in the first (meanPCV1) and second (meanPCV2) challenges, and total (totalmeanPCV), detected in 256 Morada Nova lambs, with p-values of the ANOVA. Different lower case letters indicate differences in group means according to post-hoc tests.

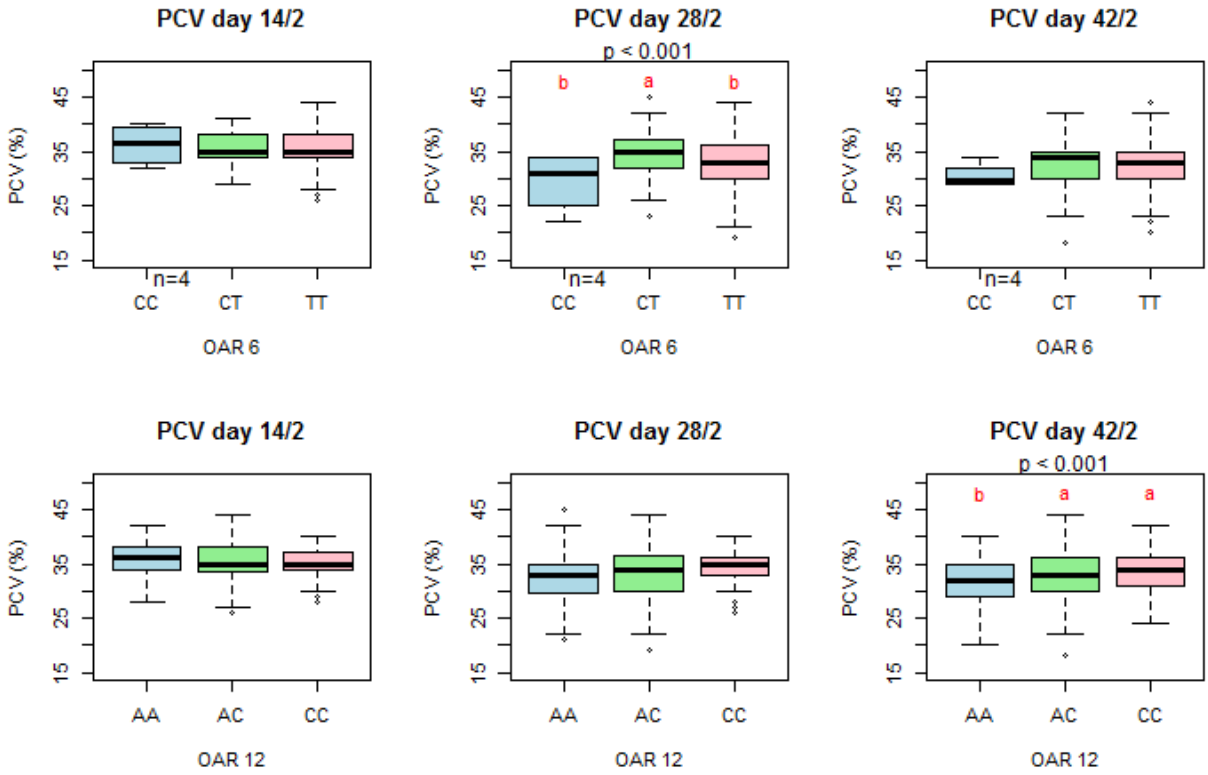

Figure 3.4. Box-and-whisker plot of genotypic effects of polymorphic SNPs (OAR6_81718546 (OAR6) and OAR12_69606944 (OAR12)) on packed cell volume (PCV) of individual sampling dates (days 14, 28 and 42 of the challenge 2), detected in 256 Morada Nova lambs, with p-values of the ANOVA. Different lower case letters indicate differences in group means according to post-hoc tests.

Weight gain (DWG1, DWG2 and totalDWG) was affected by OAR6_81718546, OAR11_62887032 and OAR12_69606944 (Fig. 3.5); with unfavourable TT genotype for OAR6_81718546 and favourable CC genotype for OAR12. The AG genotype of OAR11_62887032 was unfavourable for DWG2 and totalDWG. For individual sampling days (Fig. 3.6), OAR6_81718546 affected weight at weaning and on days 42/1 and 42/2, and OAR11_62887032 and OAR12_69606944 affected weight on day 42/2. Post hoc tests were significant for OAR6_81718546, with the TT genotype as unfavourable, and for OAR12_69606944, with the AA genotype as unfavourable.

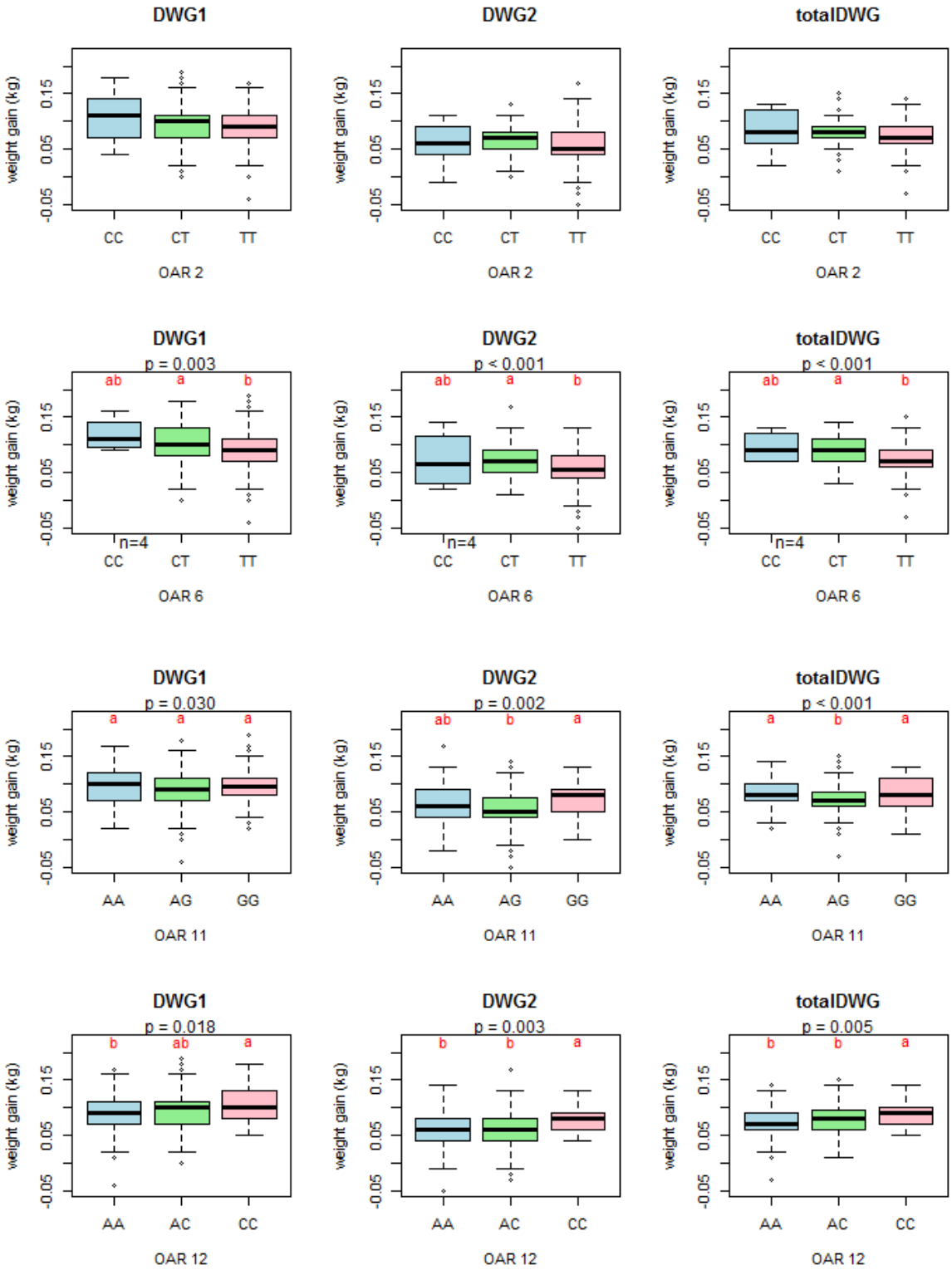

Figure 3.5. Box-and-whisker plot of genotypic effects of polymorphic SNPs (OAR2_14765360 (OAR2), OAR6_81718546 (OAR6), OAR11_62887032 (OAR11) and OAR12_69606944 (OAR12)) on daily weight gain (DWG) in the first (DWG1) and second (DWG2) challenges, and total (totalDWG), detected in 256 Morada Nova lambs, with p-values of the ANOVA. Different lower case letters indicate differences in group means according to post-hoc tests.

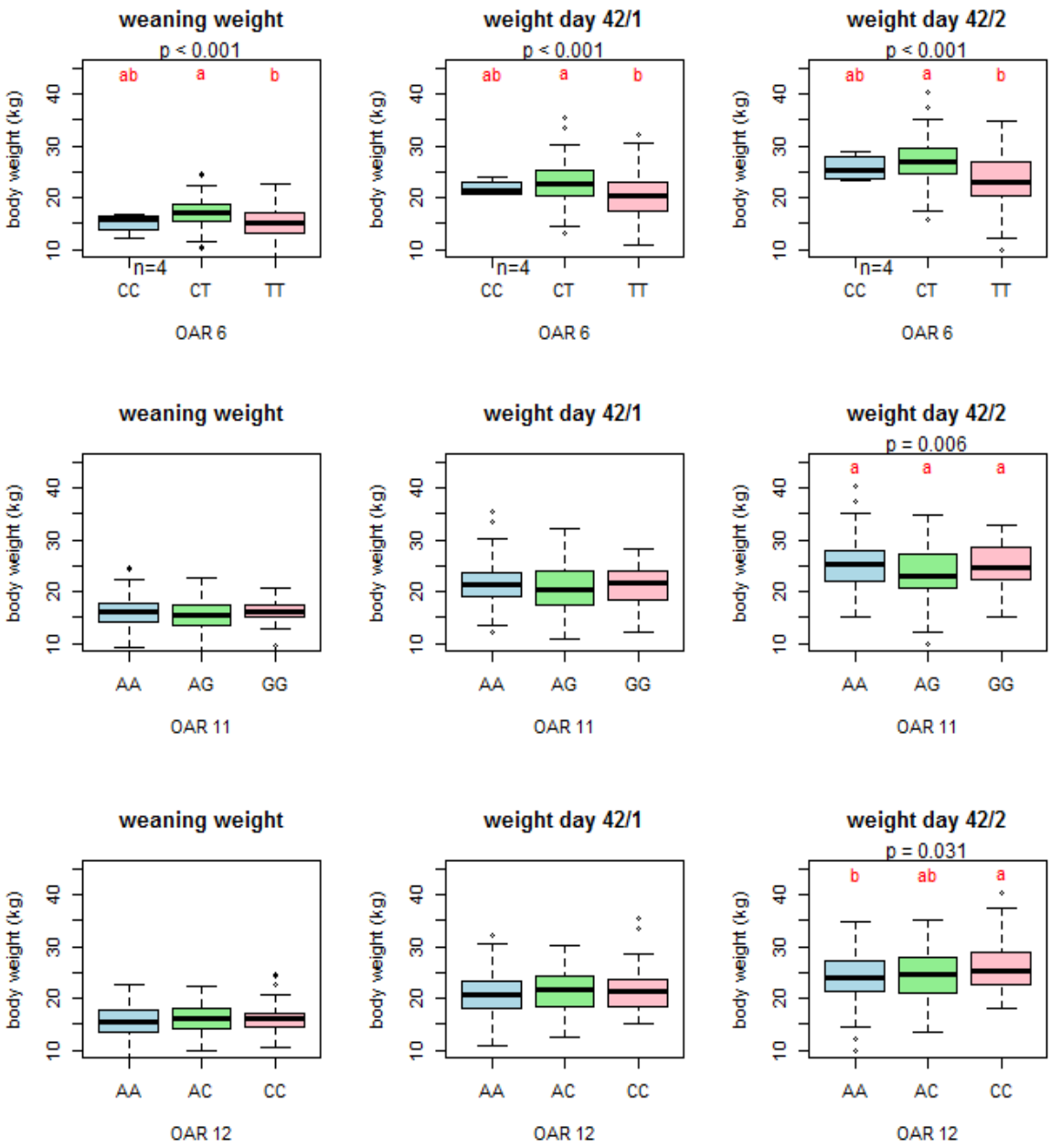

Figure 3.6. Box-and-whisker plot of genotypic effects of polymorphic SNPs (OAR6_81718546 (OAR6), OAR11_62887032 (OAR11) and OAR12_69606944 (OAR12)) on body weight at weaning and at individual sampling dates (day 42 in challenges 1 and 2), detected in 256 Morada Nova lambs, with p-values of the ANOVA. Different lower case letters indicate differences in group means according to post-hoc tests.

Allele substitution effect was significant (p = 0.02) for OAR12_69606944 in PCV at day 42 of the second parasite challenge, with the substitution of an A allele by a C allele accounting for an increase of 0.74 ± 0.32 in PCV, representing 3.41 % of total phenotypic variance. The T allele of OAR6_81718546 was shown to be unfavourable compared to the

C allele on day 42 of the second challenge, with a significant (p = 0.04) allele substitution effect of -1.06 ± 0.52 kg (4.72 % of total phenotypic variance) in body weight.

Table 3.2. Number of animals with OAR2_14765360 and OAR12_69606944 genotypes in the 20% lambs with most favourable and 20% of lambs with most unfavourable phenotypes for FEC (low and high FEC, respectively).

	OAR2_14765360			OAR12_69606944		
	NS			p = 0.029		
	CC	CT	TT	AA	AC	CC
Favourable	4	24	24	15	27	10
Unfavourable	2	22	28	28	19	5

NS SNP genotype frequencies were not significantly different (p > 0.05) between animals with favourable and unfavourable phenotypes.

The differences in distributions of SNP genotypes between animals of extreme phenotypes for FEC, PCV, and weight gain were assessed (Table 3.2, 3.3 and 3.4). For OAR12_69606944, the CC genotype was shown to be significantly more prevalent in animals of favourable phenotypes in FEC and DWG. The TT genotype of OAR6_81718546 was more frequent in animals of unfavourable phenotype in PCV and weight gain, while the AA genotype of OAR11_62887032 was more prevalent in animals of high daily weight gain.

Table 3.3. Number of animals with OAR6_81718546 and OAR12_69606944 genotypes in the 20% lambs with most favourable and 20% of lambs with most unfavourable phenotypes for PCV (high and low PCV, respectively).

	OAR6_81718546			OAR12_69606944		
	p = 0.014			NS		
	CC	CT	TT	AA	AC	CC
Favourable	1	16	34	19	25	8
Unfavourable	2	5	45	24	22	6

NS SNP genotype frequencies were not significantly different (p > 0.05) between animals with favourable and unfavourable phenotypes.

Given that only the OAR2_14765360 and OAR12_69606944 SNPs are located inside genes, ENSOARG00000025741 coding for a lincRNA and *COLGALT2* coding for a galactosyltransferase 2, respectively, a search for all genes located in a 1,000 kb window around each SNP was performed (Supplementary material: Table B.2.). Genes coding for proteins or regulatory RNAs were detected (Supplementary material: Table S1): 14 genes close to OAR2_14765360 (5 protein-coding genes: *KLF4*, *RAD23B*, *ZNF464*, *ELP1* and

ACTL7B), 1 gene close to OAR6_81718546 (coding for a SnRNA), 6 genes close to OAR11_62887032 (2 protein-coding genes: *SOX9* and ENSOARG00000013770) and 19 genes close to OAR12_69606944 (14 protein-coding genes: *COLGALT2*, *RGL1*, *TSEN15*, *APOBEC4*, *ARPC5*, *NCF2*, *SMGF*, ENSOARG00000002522, *NMNAT2*, *C1orf21*, *LAMC2*, *EDEM3*, *LAMC1* and *FAM129A*).

Table 3.4. Number of animals with OAR6_81718546, OAR11_62887032 and OAR12_69606944 genotypes in the 20% lambs with most favourable and 20% of lambs with most unfavourable phenotypes for daily weight gain (high and low DWG, respectively).

	OAR6_81718546			OAR11_62887032			OAR12_69606944		
	p < 0.001			p = 0.024			p = 0.029		
	CC	CT	TT	AA	AG	GG	AA	AC	CC
Favourable	2	22	26	31	17	4	17	22	13
Unfavourable	0	5	47	17	29	6	21	28	3

3.5. DISCUSSION

This study has investigated whether five SNPs previously associated with FEC in a Red Maasai x Dorper backcross population (Benavides et al., 2015) were applicable to a Morada Nova sheep flock. Despite the moderate sample size (n = 256), it was possible to observe that two of the SNPs (OAR2_14765360 and OAR12_69606944) had significant effects on *H. contortus* FEC. In addition, significant effects were also detected for PCV (OAR6_81718546 and OAR12_69606944) and weight gain (OAR6_81718546, OAR11_62887032 and OAR12_69606944), traits that were not significantly associated with these SNPs in the previous study (Benavides et al., 2015). OAR15_59871543 was monomorphic in the Morada Nova population, and it was fixed to the GG genotype, which was associated with high FEC in the Red Maasai x Dorper population. This does not necessarily mean that the GG genotype is equally associated with high FEC in Morada Nova, as linkage phases are family specific (Bishop, 2012a). In addition, sheep breeds are genetically fragmented which is why genetic marker information cannot be extrapolated from one breed to the others, but needs to be specifically validated for each breed (Zvinorova et al., 2016). Different linkage phases could be an explanation for the fact that the OAR12_69606944 genotypes were reversely associated with FEC compared to the findings of Benavides et al. (2015).

In some cases, ANOVA showed significant differences in the response variable between genotypes, while Tukey or Scheffé tests could not detect differences between any of the group means. These differing results arise due to diverging mathematical approaches of ANOVA and post-hoc tests (Ruxton and Beauchamp, 2008), which reflects that small SNP effects are difficult to capture, given the moderate number of animals used compared to other studies (Kemper et al., 2011; Benavides et al., 2015).

SNP effects on FEC and PCV and allele substitution effects were only detected in the second parasite challenge. The establishment of an effective immune response after infection demands a certain amount of time, and some studies have shown that a first "priming" challenge is necessary to induce a response, which is more reliably quantified after a second challenge (Morris et al., 1997; Woolaston and Windon, 2001). On the other hand, this "priming" effect was found to be of less importance in *H. contortus* compared to *Trichostrongylus colubriformis* (Gruner et al., 2004). Apart from exposure to nematodes, host's response in the second challenge could also be influenced by age, as older animals tend to have a higher ability to mount an effective immune response (Colditz et al., 1996; Goldberg et al., 2012). In the present study, mean FEC was lower and mean PCV higher in the second challenge, indicating that the animals were able to establish a certain level of immunity over time (Toscano et al., 2019). It is possible that SNP effects in the first challenge were not detected in this study due to moderate sample size. If existent, these effects are, however, likely to be of lower magnitude than those detected in the second challenge. In the GWAS from where the SNPs were selected (Benavides et al., 2015), data were obtained from animals submitted to one continuous natural infection challenge (76–98% *Haemonchus* sp.) and sampled 40-70 days after infection at a mean age of 6 months (Mugambi et al., 2005). The moment of sampling in the previous study is more comparable to the second challenge in our study, especially when considering the age of the animals.

For OAR2_14765360, significant effects were detected on mean FEC, as well as on FEC of days 21 and 28 of the second parasite challenge. However, the significant results of the ANOVA were not confirmed by the Tukey test. Thus, the suggested association of OAR2_14765360 with FEC should be confirmed with larger sample sizes. OAR2_14765360 is located in the intron of the ENSOARG00000025741 lincRNA gene. In general, long-noncoding RNAs (lincRNA), defined as non-coding RNAs larger than 200 nucleotides, can regulate gene expression (Deniz and Erman, 2017). However, in this particular case, no specific function has yet been attributed to this gene. For the other genes close to this SNP, Krüppel-like fact 4 gene (*KLF4*) is of specific interest. Apart from this gene's importance in cell growth, proliferation and differentiation and its role in the

induction of pluripotent stem cells, it also influences immune response (Ghaleb and Yang, 2017). *KLF4* down-regulates NFκB, responsible for an inflammatory response, and also influences TGFβ, a regulatory factor that inhibits the NFκB pathway. TGFβ is superiorly expressed by animals that mount an effective immune response to nematodes, while susceptible animals have a prolonged pro-inflammatory response mediated by NFκB (Maizels, 2005; Ghaleb and Yang, 2017). A study on copy number variations between selection lineages of cattle resistant or susceptible to GIN infections revealed an impact of the KLF4 pathway (Liu et al., 2011).

OAR6_81718546 was associated with PCV, body weight and weight gain, while no significant effects on FEC were found. There were only 4 animals with the CC genotype, and although this group did not follow a clear pattern, heterozygous (CT) animals had higher body weight and weight gain, as well as PCV on day 28/2, than TT homozygous animals. The effect on PCV might be due to a superior health condition in heavier lambs, which enabled them to better withstand infection. This hypothesis is supported by the fact that OAR6_81718546 had a significant effect on weight at weaning, before the first artificial infection. On the other hand, considerable levels of natural infection (FEC mean = 6643) were present before weaning (Toscano et al., 2019), and the superior body weight of the CT genotype group at weaning could also be the result of a response mechanism that attenuates the effect of infection on body weight (resilience). The analyses of genotype frequencies in animals of extreme phenotypes showed that the majority of the animals with low DWG were of the TT genotype, confirming the unfavourable effect of the T allele on weight gain and body weight, that was further evidenced by the unfavourable allele substitution effect of -1.06 ± 0.52 on body weight at the end of the second trial (day 42). Together, these findings highlight the relevance of this SNP's effect on weight gain and body weight in Morada Nova sheep, with a phenotypic variance of 4.72 % in final body weight being determined by OAR6_81718546 (although the allele substitution effect is likely to be slightly overestimated due to the assumption of independency of alleles made in the analysis (Stear et al., 1989). The deviation from Hardy-Weinberg equilibrium detected in ewes for this SNP could be the result of selection, given that ewes with particularly low body sizes are discarded from the flock each year. The only gene currently known to be within the 1,000 kb window around the OAR6_81718546 SNP encodes U6 spliceosomal RNA, which is highly conserved, with copies existing all over the genome (Brow and Guthrie, 1988).

For OAR11_62887032, no significant effects were detected on FEC and PCV. However, there was an association with weight gain, which was significant for totalDWG, DWG1 and DWG2, as well as for body weight on day 42/2, with significant differences

when comparing GG and AA genotypes to AG on DWG2 and totalDWG. The homozygous animals (AA and GG) tended to have a higher weight gain than the homozygous (AG) animals. This could be explained either by an imperfect linkage disequilibrium or by an underdominance effect at the causative mutation locus. Among the genes in proximity to OAR11_62887032, *SOX9*, at about 350 kp in distance, deserves special attention. This gene has an important role in bone and cartilage development, and haploinsufficiency of this gene causes campomelic dysplasia, a skeletal dysplasia in humans, resulting in disproportionally short stature and bowing of the limbs, among other symptoms (Akiyama, 2008).

The OAR12_69606944 SNP has shown to be significantly associated to FEC, PCV and weight gain in the present study. Animals of the CC genotype consistently showed the lowest FEC and the highest PCV and weight gain. Looking at the individual sampling dates, the significant effects occurred on days 28 and 35 for FEC and on day 42 for PCV. The favourable effect of the C allele was confirmed by an allele substitution effect of 0.74 ± 0.32 in PCV at day 42 of the second challenge. For this SNP, there is no effect on body weight at the beginning of the challenge, but on weight gain during the experimental period and on body weight on the last day of challenge. These results indicate that the effects on PCV and weight gain may be a consequence of lower FEC. OAR12_69606944 is an intron variant of the *COLGALT2* gene, which encodes the collagen-beta (1-O) galactosyltransferase 2. Forty four physical interactions with other molecules have been found for this enzyme in humans, mainly with diverse collagen subtypes, and also with the von-Willebrand-factor (https://thebiogrid.org/116746/summary/homo-sapiens/colgalt2.html; Huttlin et al., 2017), component of haemostasis. One gene close to the OAR12_69606944 SNP is the *NCF2* that codes for a subunit of NADPH oxidase involved in the production of microbiocidal agents in phagocytes. Although other subunits of this enzyme were associated with the immune response of GIN resistant sheep (Ingham et al., 2008), in a study exploring *NCF2*, an association with GIN resistance was not detected (Kadarmideen et al., 2011).

For a marker with a relevant effect on a trait, the favourable genotype is expected to be more prevalent in animals of favourable phenotypes. In the present study, this was the case for OAR6_81718546 in PCV and DWG, OAR11_62887032 in DWG and OAR12_69606944 in FEC and DWG, confirming the relevance of these SNP effects. The genotypes found to be more prevalent in animals of favourable or unfavourable genotypes were consistent with those genotypes classified as favourable or unfavourable in the association analyses, although distributions of genotypes were not sufficiently unbalanced to select animals based on their genotypes. OAR6_81718546 and OAR12_69606944

accounted for 4.72 and 3.41% of total phenotypic variation in final body weight and final PCV, based on allele substitution effects. These effects are not sufficient to suggest marker-assisted selection. Given the polygenic nature of this trait (Kemper et al., 2011; Benavides et al., 2015), a high number of genetic markers would be necessary to verify the feasibility of marker-assisted selection for GIN resistance in Morada Nova sheep, with genomic prediction being the most promising approach. However, in the present study, four out of five SNPs were polymorphic and associated to at least one of the three traits investigated, indicating possible QTLs for resistance and resilience that were partly consistent with those found in a Red Maasai X Dorper population (Benavides et al., 2015).

3.6. CONCLUSION

The present study confirmed the association of two resistance markers (OAR2_14765360 and OAR12_69606944), previously reported by Benavides et al. (2015) in a Red Maasai X Dorper population, with FEC in a Morada Nova flock. Furthermore, the “resistant genotype” (CC) of OAR12_69606944 was also associated with higher PCV and weight gain. Another two SNPs (OAR6_81718546 and OAR11_62887032) were not associated with FEC, but with resilience traits, namely PCV (OAR6_81718546), body weight and weight gain (OAR6_81718546 and OAR11_62887032) under parasite challenge. These findings support the possibility of QTL for resistance and resilience in linkage disequlibrium with the polymorphic SNPs and suggest their usefulness in future explorations of these traits in Morada Nova sheep. For this purpose, the identification of additional SNPs associated to resistance and resilience traits using larger sample sizes would be of great interest.

REFERENCES

Akiyama H (2008) Control of chondrogenesis by the transcription factor SOX9. **Modern Rheumatology** 18:213-219.

Ali AOA, Murphy L, Stear A, Fairlie-Clarke K, Brujeni GN, Donskow-Łysoniewska K, Groth D, Buitkamp J, Stear MJ (2019) Association of MHC class II haplotypes with reduced faecal nematode egg count and IgA activity in British Texel sheep. **Parasite Immunology** 41: e12626 https://doi.org/10.1111/pim.12626

Amarante AFT, Bricarello PA, Rocha RA, Gennari SM (2004) Resistanceof Santa Inês, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. **Veterinary Parasitology** 120:91-106.

Baker RL, Mugambi JM, Audho JO, Carles AB, Thorpe W (2004) Genotype by environment interactions for productivity and resistance to gastro-intestinal nematode parasites in Red Maasai and Dorper sheep. **Animal Science** 79:343-353.

Benavides MV, Sonstegard TS, Kemp S, Mugambi JM, Gibson JP, Baker RL, Hanotte O, Marshall K, Tassel C (2015) Identification of Novel Loci Associated with Gastrointestinal Parasite esistance in a Red Maasai x Dorper Backcross Population. **PLOS one** 10:1-20.

Benavides MV, Sonstegard TS, Van Tassell C (2016) Genomic regions associated with sheep resistance to gastrointestinal nematodes. **Trends in Parasitology** 32:470–480.

Berton MP, de Oliveira Silva RM, Peripolli E, Stafuzza NB, Martin JF, Álvarez MS, Gavinã BV, Toro MA, Banchero G, Oliveira PS, Eler, JP (2017) Genomic regions and pathways associated with gastrointestinal parasites resistance in Santa Inês breed adapted to tropical climate. **Journal of Animal Science and Biotechnology** 8:73.

Bishop SC (2012a) Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. **Animal** 6:741-747.

Bishop SC (2012b) A consideration of resistance and tolerance for ruminant nematode infections. **Frontiers in Genetics** 3:168/1-7.

Bishop SC, Stear MJ (2003) Modeling of host genetics and resistance to infectious diseases: understanding and controlling **nematode** infections **Veterinary Parasitology** 115:147-166.

Bishop SC, Morris CA (2007) Genetics of disease resistance in sheep and goats. **Small Ruminant Research** 70:48-59.

Brow DA, Guthrie C, (1988) Spliceosomal RNA is remarkably conserved from yeast to mammal. **Nature** 334:213-218.

Brown DJ, Fogarty NM (2017) Genetic relationships between internal parasite resistance and production traits in Merino sheep. **Animal Production Science** 57:209-215.

Chagas ACS, Katiki, LM, Silva, IC, Giglioti R, Esteves SN, Oliveira MCS Barioni-Júnior W, (2013) Haemonchus contortus: A multiple-resistant Brazilian isolate and the costs for its characterization and maintenance for research use. **Parasitology International** 62:1–6.

Colditz IG, Watson DL, Gray GD, Eady SJ (1996) Some relationships between age, immune responsiveness and resistance to parasites in ruminants. **International Journal for Parasitology** 26:869-877.

Coltman DW, Wilson K, Pilkington JG, Stear MJ, Pemberton JM (2001) A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. **Veterinary Parasitology** 122:571-582.

Deniz E, Erman B (2017) Long noncoding RNA (lincRNA), a new paradigm in gene expression control. **Functional and Integrative Genomics** 17:135-143.

Eady SJ, Woolaston RR, Barger IA (2003) Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. **Livestock Production Science** 81:11-23.

Facó O, Paiva SR, Alves LRN, Lôbo RNB, Villela LCV (2008) Raça Morada Nova: Origem, Características e Perspectivas. **Embrapa** Documentos 75 ISSN 1676-7659.
Ghaleb AM, Yang VW (2017) Krüppel-like factor 4 (KLF4): what we currently know. **Gene** 611:27-37.

Goldberg V, Ciappesoni G, Aguilar I (2012) Genetic parameters for nematode resistance in periparturiant ewes and post-weaning lambs in Uruguayan Merino sheep. **Livestock Science** 147:181-187.

Gruner L, Bouix J, Brunel JC (2004) High genetic correlation between resistance to *Haemonchus contortus* and to *Trichostrongylus colubriformis* in INRA 401 sheep. **Veterinary Parasitology** 119:51-58.

Hunt PW, McEwan JC, Miller JE (2008) Future perspectives for the implementation of genetic markers for parasite resistance in sheep. **Tropical Biomedicine** 25:18–33.

Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, Colby G, Gebreab F, Gygi MP, Parzen H, Szpyt J, Tam S, Zarraga G, Pontano-Vaites L, Swarup S, White AE, Schweppe DK, Rad R, Erickson BK, Obar RA, Guruharsha KG, Li K, Artavanis-Tsakonas S, Gygi SP, Harper JW (2017) Architecture of the human interactome defines protein communities and disease networks. **Nature** 545:505-509.

Ingham A, Reverter A, Windon R, Hunt P, Menzies M (2008) Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep. **International Journal for Parasitology** 38:431-442.

Issakowicz J, Issakowicz ACKS, Bueno MS, Costa RLD, Katiki LM, Geraldo AT, Abdalla AL, McManus C, Louvandini H (2016) Parasitic infection, reproductive and productive performance from Santa Inês and Morada Nova ewes. **Small Ruminant Research** 136:96-103.

Jackson F, Bartley D, Bartley Y, Kenyon F (2009) Worm control in sheep in the future. **Small Ruminant Research** 86:40-45.

Janssen M, Weimann C, Gauly M, Erhardt G (2002) Associations between infections with *Haemonchus contortus* and genetic markers on ovine chromosome 20. 7TH WORLD CONGRESS ON GENETICS APPLIED TO LIVESTOCK PRODUCTION Montpellier, France.

Kadarmideen HN, Watson-Haigh NS, Andronicos NM (2011) Systems biology of ovine intestinal parasite resistance: disease gene modules and biomarkers. **Molecular Biosystems** 7:235-246.

Kemper KE, Emery DL, Bishop SC, Oddy H, Hayes BJ, Dominik S, Henshall JM, Goddard ME (2011) The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. **Genetic Research** 93:203-219.

Liu GE, Brown T, Hebert DA, Cardone MF, Hou Y, Choudhary RK, Shaffer J, Amazu C, Connor EE, Ventura M, Gasbarre LC (2011) Initial analysis of copy number variations in cattle selected for resistance or susceptibility to intestinal nematodes. **Mammalian Genome** 22:111-121.

Lôbo RNB, Pereira IDC, Facó O, McManus CM (2011) Economic values for production traits of Morada Nova meat sheep in a pasture based production system in semi-arid Brazil. **Small Ruminant Research** 96:93-100.

Maizels RM (2005) Infections and allergy – helminths, hygiene and host immune regulation. **Current Opinion in Immunology** 17:656-661.

Matika O, Nyoni S, van Wyk JB, Erasmus GJ, Baker RL (2003) Resistance of Sabi and Dorper ewes to gastro-intestinal nematode infections in an African semi-arid environment. **Small Ruminant Reserach** 47:95-102.

Morris CA, Vlassof A, Bisset SA, Baker RL, West CJ, Hurford AP (1997) Responses of Romney sheep to selection for resistance or susceptibility to nematode infection. **Animal Science** 62:319-329.

Mugambi JM, Audho JO, Baker RL (2005) Evaluation of the phenotypic performance of a Red Maasai and Dorper double backcross resource population: natural pasture challenge with gastro-intestinal nematode parasites. **Small Ruminant Research** 56:239-251.

Niciura SCM, Cruvinel GG, Moraes CV, Bressani FA, Malagó Junior W, Benavides MV, Chagas ACS (2018) PCR-based genotyping of SNP markers in sheep. **Molecular Biology Reports** 45:651-656.

Papadopoulos E, Gallidis E, Ptochos S (2012) Anthelmintic resistance in sheep in Europe: a selected review. **Veterinary Parasitology** 189:85-88.

Perry BD, Randolph TF, McDermott JJ, Sones KR, Thornton PK (2002) Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya, 148 pp.

Ruxton GD, Beauchamp G (2008) Time for some a priori thinking about post hoc testing. **Behavioral Ecology** 19:690-693.

Snyman MA, Fisher AD (2019) Genetic parameters for traits associated with resistance to *Haemonchus contortus* in a South African Dohne Merino sheep flock. **Small Ruminant Research** 176:76-88.

Stear MJ, Pokorny TS, Muggli NE, Stone RT (1989) The relationships of birth weight, preweaning gain and postweaning gain with the bovine major histocompatibility system. **Journal of Animal Science** 67:641-649.

Toscano JHB, Santos IB, Haehling MB, Giraldelo LA, Lopes LG, Silva MH, Figueredo A, Esteves SN and Chagas ACS (2019) Morada Nova sheep breed: resistant or resilient to *Haemonchus contortus* infection? **Veterinary Parasitology X** 2:100019.

Woolaston RR, Piper LR (1996) Selection of Merino sheep for resistance to *Haemonchus contortus*: genetic variation. **Animal Science** 62:451-460.

Woolaston RR, Windon RG (2001) Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. **Animal Science** 73:41-48.

Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J, Billis K, Cummins K, Gall A, Girón CG, Gil L, Gordon L, Haggerty L, Haskell E, Hourlier T, Izuogu OG, Janacek SH, Juettemann T, To JK, Laird MR, Lavidas I, Liu Z, Loveland JE, Maurel T, McLaren W, Moore B, Mudge J, Murphy DN, Newman V, Nuhn M, Ogeh D, Ong CK, Parker A, Patricio M, Riat HS, Schuilenburg H, Sheppard D, Sparrow H, Taylor K, Thormann A, Vullo A, Walts B, Zadissa A, Frankish A, Hunt SE, Kostadima M, Langridge N, Martin FJ, Muffato M, Perry E, Ruffier M, Staines DM, Trevanion SJ, Aken BL, Cunningham F,Yates A, Flicek P (2018) Ensembl 2018. **Nucleic Acids Research** 46:D754-D761.

Zvinorova PI, Halimani TE, Muchadeyi FC, Matika O, Riggio V, Dzama K (2016) **Veterinary Parasitology** 225:19-28.

CHAPTER 4 – FINAL CONSIDERATIONS

Morada Nova sheep show high levels of resistance and resilience. Selection for resistance would further reduce losses and the need for treatments, thus lowering costs. Reductions in contamination levels of pastures could be beneficial for animal performance. Higher levels of resilience (with simultaneous selection for resistance) could contribute to the maintenance of performance, especially in critical situations, like the periparturient period, when rises in infection levels are inevitable. Given that reproductive performance has a high economic impact, estimates of the correlation between reproductive and resistance traits would be of great interest.

In the Morada Nova population investigated in this study, selection could be based on records of the second parasite challenge only. It was, however, not possible to elaborate whether this was a result of an older age of the animals during the second parasite challenge, or if two challenges are necessary in order to evaluate the genetic merit of the animals. The fact that estimates of heritability were higher during the second parasite challenge in ewes, as well as lambs, favours the second option. In this case, selection could be based on two parasite challenges, but samplings of FEC and PCV would only be necessary during the second parasite challenge. For most farms, artificial infection and recording of FEC and PCV is not viable. Selection based on the suggested index is therefore currently restricted to research flocks, which could contribute to genetic progress in other farms, for example by progeny testing of sires. In the long run, it might be possible to establish on-farm selection for resistance using records of FEC under natural infection. For this aim, the investigation of genetic parameters for FEC under natural infection in commercial farms would be a first step.

The associations found between 4 SNPs and the investigated traits suggest the existence of quantitative trait loci in linkage disequilibrium. However, given that FEC is a quantitative trait, a genome wide association study (GWAS) could contribute to the detection of underlying mechanisms of resistance, as well as verify the possibility of marker-assisted selection.

APPENDICES

APPENDIX A

Table A.1. Phenotypic correlations (± standard error) within traits for faecal egg count (FEC), packed cell volume (PCV), body weight and daily weight gain (DWG) in Morada nova lambs.

	Phenotypic correlations									
	Total MeanFEC	Mean FEC1	Mean FEC2		Total MeanPCV	Mean PCV1	Mean PCV2		TotalDWG	DWG1	DWG2
Total MeanFEC	1	0.82 ± 0.03	0.75 ± 0.04	Total MeanPCV	1	0.85 ± 0.03	0.87 ± 0.03	TotalDWG	1	0.78 ± 0.04	0.72 ± 0.04
Mean FEC1	0.82 ± 0.03	1	0.38 ± 0.05	Mean PCV1	0.85 ± 0.03	1	0.49 ± 0.05	DWG1	0.78 ± 0.04	1	0 43 ± 0.05
Mean FEC2	0.75 ± 0.04	0.38 ± 0.05	1	Mean PCV2	0.87 ± 0.03	0.49 ± 0.05	1	DWG2	0.72 ± 0.04	0.43 ± 0.05	1
Weaning	0.14 ± 0.06	0.10 ± 0.06	0.21 ± 0.06	Weaning	0.26 ± 0.06	0.13 ± 0.06	0.31 ± 0.06	Weaning	0.26 ± 0.06	0.32 ± 0.06	0.07 ± 0.06
Day 14-1				Day 14-1	0.47 ± 0.05	0.63 ± 0.05	0.20 ± 0.06	Day 14-1			
Day 21-1	0.57 ± 0.05	0.68 ± 0.04	0.23 ± 0.06	Day 21-1				Day 21-1			
Day 28-1	0.76 ± 0.04	0.92 ± 0.02	0.34 ± 0.06	Day 28-1	0.71 ± 0.04	0.88 ± 0.03	0.36 ± 0.06	Day 28-1	0.54 ± 0.05	0.57 ± 0.05	0.22 ± 0.06
Day 35-1	0.74 ± 0.04	0.92 ± 0.02	0.30 ± 0.06	Day 35-1				Day 35-1			
Day 42-1	0.72 ± 0.04	0.88 ± 0.03	0.34 ± 0.06	Day 42-1	0.82 ± 0.03	0.85 ± 0.03	0.58 ± 0.05	Day 42-1	0.62 ± 0.05	0.67 ± 0.04	0.26 ± 0.06
Day 14-2				Day 14-2	0.60 ± 0.05	0.34 ± 0.06	0.69 ± 0.04	Day 14-2			
Day 21-2	0.62 ± 0.05	0.48 ± 0.05	0.70 ± 0.04	Day 21-2				Day 21-2			
Day 28-2	0.67 ± 0.04	0.35 ± 0.06	0.90 ± 0.03	Day 28-2	0.79 ± 0.04	0.48 ± 0.05	0.87 ± 0.03	Day 28-2	0.73 ± 0.04	0.68 ± 0.04	0.40 ± 0.05
Day 35-2	0.69 ± 0.04	0.33 ± 0.06	0.93 ± 0.02	Day 35-2				Day 35-2			
Day 42-2	0.66 ± 0.04	0.30 ± 0.06	0.92 ± 0.02	Day 42-2	0.71 ± 0.04	0.36 ± 0.06	0.85 ± 0.03	Day 42-2	0.78 ± 0.04	0.69 ± 0.04	0.49 ± 0.05

TotalMeanFEC = overall mean of all FEC records taken during parasite challenges; MeanFEC1 = mean of FEC records of the first parasite challenge; MeanFEC2 = mean of FEC records of the second parasite challenge; TotalMeanPCV = overall mean of all PCV records taken during parasite challenges; MeanPCV1 = mean of PCV records of the first parasite challenge; MeanPCV2 = mean of PCV records of the second parasite.

Table A.2. Genetic correlations (± standard error) within traits for faecal egg count (FEC), packed cell volume (PCV), body weight and daily weight gain (DWG) based on estimated breeding values (EBVs) of Morada nova lambs.

	Genetic correlations									
	AllrepFEC	RepFEC1	RepFEC2		AllrepPCV	RepPCV1	RepPCV2		TotalDWG	DWG1	DWG2
AllrepFEC	1	0.82 ± 0.03	0.75 ± 0.04	AllrepPCV	1	0.85 ± 0.03	0.87 ± 0.03	TotalDWG	1	0.78 ± 0.04	0.72 ± 0.04
RepFEC1	0.82 ± 0.03	1	0.38 ± 0.05	RepPCV1	0.85 ± 0.03	1	0.49 ± 0.05	DWG1	0.78 ± 0.04	1	0 43 ± 0.05
RepFEC2	0.75 ± 0.04	0.38 ± 0.05	1	RepPCV2	0.87 ± 0.03	0.49 ± 0.05	1	DWG2	0.72 ± 0.04	0.43 ± 0.05	1
Weaning	0.14 ± 0.06	0.10 ± 0.06	0.21 ± 0.06	Weaning	0.26 ± 0.06	0.13 ± 0.06	0.31 ± 0.06	Weaning	0.26 ± 0.06	0.32 ± 0.06	0.07 ± 0.06
Day 14-1				Day 14-1	0.47 ± 0.05	0.63 ± 0.05	0.20 ± 0.06	Day 14-1			
Day 21-1	0.57 ± 0.05	0.68 ± 0.04	0.23 ± 0.06	Day 21-1				Day 21-1			
Day 28-1	0.76 ± 0.04	0.92 ± 0.02	0.34 ± 0.06	Day 28-1	0.71 ± 0.04	0.88 ± 0.03	0.36 ± 0.06	Day 28-1	0.54 ± 0.05	0.57 ± 0.05	0.22 ± 0.06
Day 35-1	0.74 ± 0.04	0.92 ± 0.02	0.30 ± 0.06	Day 35-1				Day 35-1			
Day 42-1	0.72 ± 0.04	0.88 ± 0.03	0.34 ± 0.06	Day 42-1	0.82 ± 0.03	0.85 ± 0.03	0.58 ± 0.05	Day 42-1	0.62 ± 0.05	0.67 ± 0.04	0.26 ± 0.06
Day 14-2				Day 14-2	0.60 ± 0.05	0.34 ± 0.06	0.69 ± 0.04	Day 14-2			
Day 21-2	0.62 ± 0.05	0.48 ± 0.05	0.70 ± 0.04	Day 21-2				Day 21-2			
Day 28-2	0.67 ± 0.04	0.35 ± 0.06	0.90 ± 0.03	Day 28-2	0.79 ± 0.04	0.48 ± 0.05	0.87 ± 0.03	Day 28-2	0.73 ± 0.04	0.68 ± 0.04	0.40 ± 0.05
Day 35-2	0.69 ± 0.04	0.33 ± 0.06	0.93 ± 0.02	Day 35-2				Day 35-2			
Day 42-2	0.66 ± 0.04	0.30 ± 0.06	0.92 ± 0.02	Day 42-2	0.71 ± 0.04	0.36 ± 0.06	0.85 ± 0.03	Day 42-2	0.78 ± 0.04	0.69 ± 0.04	0.49 ± 0.05

AllrepFEC = EBVs for all FEC records taken during parasite challenges; RepFEC1 = EBVs for FEC records of the first parasite challenge; RepFEC2 = EBVs for FEC records of the second parasite challenge; AllrepPCV = EBVs for PCV records taken during parasite challenges; RepPCV1 = EBVs for PCV records of the first parasite challenge; RepPCV2 = EBVs for PCV records of the second parasite challenge; TotalDWG = EBVs for total daily weight gain; DWG1 = EBVs for weight gain during first parasite challenge; DWG2 = EBVs for weight gain during second parasite challenge; Weaning = EBVs for records taken at weaning; Day xx-y = EBVs for records of day xx of parasite challenge y.

APPENDIX B

Table B.1. SNP positions and genes (name, type, distance to the SNP and function) located within a 1,000 kb window around each SNP.

SNP	Position	Gene name	Gene type*	Annotation/ Distance	Name/Function
OAR2_14765360	2:15322091	ENSOARG00000025741	lincRNA	Intron	-
		ENSOARG00000022554	snoRNA	40kb	-
		ENSOARG00000022529	RRNA	80kb	-
		ENSOARG00000025742	lincRNA	80kb	-
		ENSOARG00000025018	SnRNA	190kb	“U6 spliceosomal RNA”.
		ENSOARG00000025743	lincRNA	230kb	-
		KLF4	protein coding	300kb	Krüppel-like factor 4. Zinc-finger transcription factor. Cell growth, proliferation, differentiation, apoptosis, regulation of key inflammatory signalling: NfkB, TGFβ1 (induction of pluripotent stem cells) (Ghaleb and Yang, 2017).
		RAD23B	protein coding	410kb	Part of the nucleotide excision repair protein complex XPC. DNA damage repair (Mu et al., 2018).
		ENSOARG00000022229	SnRNA	530kb	“U6 spliceosomal RNA”.
		ZNF462	protein coding	700kb	Zinc-finger protein (www.ensembl.org).
		ENSOARG00000022818	SnRNA	910kb	“U6 atac minor spliceosomal RNA”.
		ENSOARG00000007153	pseudogene	910kb	-
		ENSOARG00000007143	pseudogene	970kb	-
		ELP1	protein coding	970kb	Elongator Protein 1. Part of the Elongator Complex. Supposed functions: scaffold for other elongator subunits and docking site for factors that regulate elongator activity (Glatt and Müller, 2013).
		ACTL7B	protein	980kb	Part of the actin-related protein family.

			coding		
OAR6_81718546	6:74867312		-	Intergenic region	-
		ENSOARG00000024398	SnRNA	500kb	“U6 spliceosomal RNA”.
OAR11_62887032	11:58151039	-	-	Intergenic region	-
		SOX9	protein coding	350kb	Transcription factor. Regulation of the anti-Müllerian hormone (AMH), regulation of chondrocyte differentiation (Wagner, 1994; Akiyama, 2008).
		ENSOARG00000026393	lincRNA	450kb	-
		ENSOARG00000026392	lincRNA	500kb	-
		RF00019	MiscRNA	700kb	-
		ENSOARG00000026394	lincRNA	800kb	-
		ENSOARG00000013770	protein coding	800kb	Member 11 of the solute carrier family (SLC) 39 (www.bioinfo.hpc.cam.ac.uk).
OAR12_69606944	12:63088234	*COLGALT2*	protein coding	intron	Galactosyltransferase 2. Predicted interaction with collagen subtypes and von-Willebrand-factor (https://thebiogrid.org/116746/summary/homo-sapiens/colgalt2.html; Huttlin et al., 2017).
		RGL1	protein coding	10kb	Ral guanine nucleotide dissociation stimulator like 1. One of seven RALGEFs.
		TSEN15	protein coding	100kb	tRNA-splicing endonuclease subunit Sen 15
		ENSOARG00000021674	SnRNA	100kb	”U6 spliceosomal RNA”.
		APOBEC4	protein coding	300kb	Member of the AID/APOBEC family.
		ARPC5	protein coding	300kb	Actin related protein 2/3 complex subunit 5. Regulation of translational suppression during male germ cell differentiation (in interaction with microRNAs) (Chang et al., 2012).
		NCF2	protein	300kb	Neutrophil cytosolic factor 2. Subunit of NADPH oxidase. Phagocyte

	coding		respiratory burst (Thomas, 2017).
SMGF	protein coding	400kb	Nonsense mediated mRNA decay factor. Exonucleolytic degradation of mRNAs (in complex with SMG 5) (Nicholson et al., 2018).
ENSOARG00000002522	protein coding	400kb	-
ENSOARG00000025463	lincRNA	450kb	-
ENSOARG00000022053	SnRNA	450kb	”U6 spliceosomal RNA”.
NMNAT2	protein coding	450kb	Nicotinamide nucleotide adenylyltransferase 2. Endogenous axon maintenance factor. Preservation of axon health (Gilley et al., 2019).
RF00001	RRNA	500kb	-
C1orf21	protein coding	550kb	-
ENSOARG00000024729	ncRNA	660kb	“MicroRNA”.
LAMC2	protein coding	700kb	Laminin subunit γ 2. Extracellular matrix protein, anchoring filaments of basement membranes as a part of laminin 332 heterotrimer (Medeiros and Riet-Correa, 2015).
EDEM3	protein coding	750kb	Endoplasmatic reticulum (ER) degradation enhancing alpha-mannosidase-like protein 3. Degradation of folding-defective glycoproteins (Olivari and Molinari, 2007).
LAMC1	protein coding	800kb	Laminin subunit γ 1.Extracellular matrix protein, part of 10 out of 11 known isoforms of laminin, vital part of the basement membrane (Smyth et al., 1999).
FAM129A	protein coding	900kb	Protein Niban.

*lincRNAs are associated with gene expression regulation through epigenetic transcriptional and post transcriptional regulation (Deniz and Erman, 2017). snoRNAs are associated with modification of RNAs, precursor of miRNAs, splicing, telomerase activity (Cao et al., 2018). RRNAs are involved in protein biosynthesis (Lafontaine and Tollervey, 2001). SnRNAs are involved in RNA-splicing (Matera et al., 2007). Pseudogenes present potential regulatory function (Pei et al., 2012). microRNAs have a role in the regulation of mRNA expression (Ambros, 2004).

REFERENCES

Akiyama H (2008) Control of chondrogenesis by the transcription factor SOX9. **Modern Rheumatology** 18:213-219.
Ambros V (2004) The functions of animal microRNAs. **Nature** 431:350-355.

Chang YF, Lee-Chang JS, Imam JS, Buddavarapu KC, Subaran SS, Sinha-Hikim AP, Gorospe M, Rao MK (2012) Interaction between microRNAs and actin-associated protein Arpc5 regulates translational suppression of male germ cell differentiation. **Proceedings of the National Academy of Sciences USA** 109:5750-5755.

Cao T, Rajasingh S, Samanta S, Dawn B, Bittel DC, Rajasingh J (2018) Biology and clinical relevance of noncoding sno/scaRNAs. **Trends in Cardiovascular Medicine** 28:81-90.

Deniz E, Erman B (2017) Long noncoding RNA (lincRNA), a new paradigm in gene expression control. **Functional and Integrative Genomics** 17:135-143.

Ghaleb AM, Yang VW (2017) Krüppel-like factor 4 (KLF4): what we currently know. **Gene** 611:27-37.

Gilley J, Mayer PR, Yu G, Coleman MP (2019) Low levels of NMNAT2 compromise axon development and survival. **Human Molecular Genetics** 28:448-458.

Glatt S, Müller CW (2013) Structural insights into Elongator function. **Current Opinion in Structural Biology** 23:235-242.

Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, Colby G, Gebreab F, Gygi MP, Parzen H, Szpyt J, Tam S, Zarraga G, Pontano-Vaites L, Swarup S, White AE, Schweppe DK, Rad R, Erickson BK, Obar RA, Guruharsha KG, Li K, Artavanis-Tsakonas S, Gygi SP, Harper JW (2017) Architecture of the human interactome defines protein communities and disease networks. **Nature** 545:505-509.

Lafontaine DLJ, Tollervey D (2001) The function and synthesis of ribosomes. **Nature Reviews Molecular Cell Biology** 2:514-520.

Matera AG, Terns RM, Terns MP (2007) Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. **Nature Reviews Molecular Cell Biology** 8:209-220.

Medeiros GX, Riet-Correa F (2015) Epidermolysis bullosa in animals: A review. **Veterinary Dermatology** 26:e2.

Mu H, Geacintov NE, Broyde S, Yeo JE, Schärer OD (2018) Molecular basis for damage recognition and verification by XPC-RAD23B and TFIIH in nucleotide excision repair. **DNA Repair** 71:33-42.

Nicholson P, Gkratsou A, Josi C, Colombo M, Mühlemann O (2018) Dissecting the functions of SMG5, SMG7, and PNRC2 in nonsense-mediated mRNA decay of human cells. **RNA** 24:557-573.

Olivari S, Molinari M (2007) Glycoprotein folding and the role of EDEM1, EDEM2 and EDEM3 in degradation of folding-defective glycoproteins. **FEBS Letters** 581:3658-3664.

Pei B, Sisu C, Frankish A, Howald C, Habegger L, Mu XJ, Harte R, Balasubramanian S, Tanzer A, Diekhans M, Reymond A, Hubbard TJ, Harrow J, Gerstein MB (2012) The GENCODE pseudogene resource. **Genome Biology** 13:R51.

Smyth N, Vatansever HS, Murray P, Meyer M, Frie C, Paulsson M, Edgar D (1999) Absence of basement membranes after targeting the *LAMC1* gene results in embryonic lethality due to failure of endoderm differentiation. **The Journal of Cell Biology** 144:151-160.

Thomas DC (2017) The phagocyte respiratory burst: historical perspectives and recent advances. **Immunology Letters** 192:88-96.

Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, Pasantes J, Bricarelli FD, Keutel J, Hustert E, Wolf U, Tommerup N, Schempp W, Scherer G (1994) Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene *SOX9.* **Cell** 79:1111-1120