SNP associations and genetic-parameter estimation for nose and hoof pigmentation in Corriedale sheep

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ABSTRACT

Context. Quantity and quality of both meat and wool are important for selection programs of the dual-purpose Corriedale sheep. In Corriedales, black pigmentation of nose skin and hooves is preferred as part of the breed standard. However, within the breed, pigmentation can vary from none to complete pigmentation. **Aims.** The aim was to discover single-nucleotide polymorphisms (SNPs) and genes associated with nose and hoof pigmentation and to estimate genetic parameters of nose and hoof pigmentation, wool traits and meat traits. **Methods.** The phenotype and genotype data on Corriedale lambs ($n = 764$) produced from 44 sires (12 studs) and 300 ewes in 2017–2019 were used in this study. Lambs were slaughtered at 6–7 months of age. Nose pigmentation and hoof pigmentation were scored on a five-point scale, where no pigmentation was scored 0 and complete pigmentation was scored 5. Wool- and meat-quality traits were measured, including greasy fleece weight, fibre diameter, weaning weight, hot standard carcass weight and intramuscular fat percentage. The lambs were genotyped with the GGPOvine50K SNP chip. A genome-wide association study (GWAS) was conducted. Genetic parameters and bivariate analyses were estimated using ASReml-R. **Results.** The lambs had a mean nose-pigmentation score of 3.69 and hoof-pigmentation score of 4.12. The nose pigmentation ($h^2 = 0.58$) and hoof pigmentation ($h^2 = 0.51$) were highly heritable and highly correlated ($r_G = 0.73$). The GWAS identified a genome-wide significant SNP (OAR19_33278780.1) associated with nose pigmentation (q-value $= 0.04$). This SNP is positioned in a known pigmentation gene, melanocyte-inducing transcription factor (*MITF*). The same SNP was the sixth-most associated SNP for hoof pigmentation but did not reach significance level. OAR19_33278780.1 also had a significant effect on back conformation and weaning weight. **Conclusions.** The results of these analyses have provided information and an understanding of the genetics for pigmentation of nose and hooves in Corriedale sheep. **Implications.** Nose and hoof pigmentation are highly genetically correlated with each other, but with no negative effects on production traits.

Keywords: Corriedale, hoof, meat quality, MITF, nose skin, *Ovis aries*, pigment, wool quality.

Introduction

Corriedales are a composite breed developed in the late-1800s in Australia and New Zealand, from crossing Lincoln or Leicester rams with Merino sheep (Marshall 1916; Meat and Livestock Australia 2006). Corriedales are a dual-purpose breed that are farmed for both meat and wool production. Therefore, improved meat-quality and wool-quality traits are desired. Corriedale sheep also have black-pigmented hooves and nose skin likely inherited from Lincoln or English Leicester rams (Marshall 1916). These traits were selected because pigmentation of the hooves may contribute to animal health and wellbeing (Scobie *et al.* 2017), and nose pigmentation tends to indicate pigmentation of hooves. It is assumed by producers that animals with pigmented hooves are less susceptible to hoof conditions than are animals with no hoof pigmentation or white hooves (Scobie *et al.* 2017). Black hooves may provide better protection as more force is required to puncture black hooves

than white hooves; however, other mechanical tests have found no difference between black and white hoof material (Scobie *et al.* 2017). Black hooves are also thought to be associated with better resistance to internal parasites. Internal-parasite burdens are more prevalent in high-rainfall, pasture systems (Larsen 2014) where Corriedale sheep are usually raised in Australia.

One earlier study investigated the genetic basis of nose and hoof pigmentation in a quantitative trait-loci (QTL) mapping experiment of Awassi-cross super-fine Merino sheep back-crossed with Merino (*n* = 177). QTLs on Chromosomes 2 and 19 were significantly associated with both traits (Raadsma *et al.* 2013). An additional loci on Chromosome 13 was also identified for nose pigmentation and a loci on Chromosome 6 for hoof pigmentation (Raadsma *et al.* 2013). These QTLs for nose and hoof pigmentation harbour the genes *TYRP1* (chr2), *KIT* (chr6), *ASIP* (chr13) and *MITF* (chr19) (Raadsma *et al.* 2013).

Pigmentation of hooves and nose skin in Corriedale sheep has not been studied. Within the breed, nose and hoof pigmentation can vary from no pigmentation to complete pigmentation. The aim of this study was to perform an extensive genome-wide association analysis to pinpoint significant single-nucleotide polymorphisms (SNPs) and genes associated with pigmentation traits. Genetic parameters for nose and hoof pigmentation, wool characteristics, and meat-production traits were also estimated.

Materials and methods

Animals

The data analysed were collected as part of the project initiated by the Performance Corriedale Group to improve this breed (Blackwood *et al.* 2021). Corriedale lambs (*n* = 764) were produced from three rounds of artificial insemination and one natural mating from April 2017 to 2019 (birth group). The matings resulted in 1–60 progeny per sire (*n* = 44). Sex, number of lambs born per ewe (type of birth), number of lambs reared per ewe (type of rearing), and slaughter date were recorded. Animals were kept on a commercial property in Evandale, Tasmania. Animal ethics approval was not required as measurements were normal management operations and, therefore, exempted.

Measures

Visual scores, wool-production, wool-quality, meat-production and meat-quality traits were measured as described in Table 1. Visual scores included nose pigmentation (NOSEP), hoof pigmentation (HVSP), face cover (FC), back conformation (BACK), and body wrinkle score (BDWR). All visual scores were based on a 1–5 scoring system (Australian Wool Innovation and Meat and Livestock Australia 2013) and were scored by two or three scorers. The mean of these scores was used in the

analysis. Pre-slaughter measures were weaning weight at ~5 months of age (WWT), post-weaning weight (PWWT) at ~6–7 months of age, scanned eye-muscle depth (PEMD), scanned fat depth (PFat) and height (Ht). PEMD and PFat were measured using an ultrasound scanner. Ht was measured from the ground to withers. Post-slaughter measures included eye-muscle depth (EMD), eye-muscle width (EMW), eye-muscle area (EMA), hot standard carcass weight (HSCW), fat depth (GRFat), shear force of *Longissimus lumborum* (SF5) and intramuscular-fat percentage (IMF). SF5 was measured, with six replications, by using a Lloyd texture analyser fitted with a Warner–Bratzler head. Intramuscular fat (IMF) was measured in *Longissimus lumborum* via chemical extraction. Wool-quality traits included greasy fleece weight (GFW), percentage of fibres under 30 microns (CF), wool-staple length (SL), staple-structure score (SSTRC), wool colour score (COL) and wool character score (CHAR).

Genomic relationship matrix

The 764 lambs were genotyped using the Illumina OvineSNP50 BeadChip. The SNPs with a minor allele frequency of <0.01, and on the X and Y chromosome, were removed. The SNP data were used to construct a genomic relationship matrix (GRM) by using VanRaden's first method (VanRaden 2008).

Heritabilities, genetic and phenotypic correlations

Univariate and bivariate analyses were conducted using ASReml-R (ASReml, version 4.1.0; Butler *et al.* 2018; https://asreml.kb.vsni.co.uk/) in R (version 4.2.1). One of three models was fitted to the traits (Table 2) and the levels of fixed effects are described in Table 3. The GRM was fitted as a random effect in each model. Sex was recorded directly from the lambs or, if it was not recorded, it was inferred using genotype information. For carcass traits, contemporary group was defined as a function of birth group and kill date. Specifically, one cohort (2019A) was slaughtered on two different days. Heterozygosity for each sample was estimated from the genotype data as the average heterozygosity across all SNPs.

Genome-wide association study

A genome-wide association study (GWAS) was conducted to identify regions associated with nose and hoof pigmentation. A mixed model was used to test association of pigmentation scores with the alleles of SNP genotype. All SNPs across the genome (Oar_v3.1) were tested with one SNP in the model at a time in the model. The model was fitted using GCTA software (Yang *et al.* 2011), by using the following model:

$$y = a + cz + bx + g + e$$

Table 1. List of traits included in this study, and their abbreviations and description.

Trait	Abbreviation	Unit	Description
Greasy fleece weight	GFW	kg	Weight of fleece after shearing
Fibre diameter	FD	Um	Fibre thickness
Comfort factor	CF	%	Fibres less than 30 micron
Staple length	SL	mm	Average fibre length
Weaning weight	WWT	kg	Lamb weight at weaning ~5 months of age
Post-weaning weight	PWWT	kg	Lamb weight post-weaning ~6 months of age
Height	Ht	cm	Height measured from withers to ground
Eye-muscle depth (scanned)	PEMD	mm	Measured using ultrasound
Fat depth (scanned)	PFat	mm	Measured using ultrasound
Hot standard carcass weight	HSCW	kg	Dressed carcass weight
Eye-muscle depth	EMD	mm	Measured with digital callipers to two decimal places
Eye-muscle width	EMW	mm	Measured with digital callipers to two decimal places
Eye-muscle area	EMA	cm^2	Multiplication of depth × width with no adjustment for shape
GR fat depth	GRFat	mm	Fat depth 110 mm from the backbone at 12th rib
Intramuscular fat	IMF	%	Intramuscular fat (%) of *Longissimus lumborum* measured by chemical extraction
Shear force	SF5	N	Force required to penetrate through *Longissimus lumborum*, measured using a lloyd texture analyser fitted with Warner–Bratzler head. Replicated six times, mean value reported
Nose skin pigmentation	NOSEP	Score (1–5)	1 = no pigmented area; 2 = 1–20% pigmented area; 3 = 21–40% pigmented; 4 = 41–70% pigmented; 71–100% pigmented
Hoof pigmentation	HVSP	Score (1–5)	1 = no pigmented area; 2 = 1–20% pigmented area; 3 = 21–40% pigmented; 4 = 41–70% pigmented; 71–100% pigmented
Wool colour	COL	Score (1–5)	1 = bright white wool; 2 = off white wool 3 = mild yellow wool; 4 = intense yellow wool; 5 = very intense yellow wool
Character	CHAR	Score (1–5)	1 = very well defined crimp; 2 = well defined crimp; 3 = crimp definition is lacking; 4 = crimp definition is severely lacking and the staples start to look 'flat'; 5 = no crimp definition and as a result looks 'flat'.
Staple structure	SSTRC	Score (1–5)	1 = staple comprises very fine bundles, i.e. staple width of less than 6 mm in diameter; 2 = staple comprises fine bundles i.e. staple width of 6–10 mm in diameter; 3 = staple comprises medium bundles i.e. staple width of 11–20 mm in diameter.; 4 = staple comprises large bundles i.e. staple width of 21–30 mm in diameter; 5 = staple comprises 'blocky', extremely large bundles, i.e. staple width of greater than 30 mm in diameter
Face cover	FACE	Score (1–5)	1 = open face with no wool in front of the ears and topknot, or on the jowls; 2 = wool cover over the top of head, some on the side of muzzle, but not joined between the ears and eyes; 3 = wool cover over the top of head and on the side of muzzle, wool joined between the ears and eyes; 4 = wool cover from the top of the head down the muzzle, clear channel remains between the eye and the mouth; 5 = heavy wool growth over the entire face with the exception of the eyes, nose and mouth areas, wool from the top and side of the muzzle joining
Body wrinkle	BDWR	Score (1–5)	1 = plain-bodied sheep with no body wrinkle; 2 = plain-bodied sheep with a few small wrinkles over the body; 3 = slight wrinkling over the body; 4 = heavy wrinkling over the body; 5 = very heavy wrinkling and heavy folds of skin over the body
Back conformation	BACK	Score (1–5)	1 = extremely 'dipped' backline; 3 = straight between shoulders and back; 5 = extremely 'arched' backline

In this model, y represents the quantitative score, a is the mean term, c represents effect of fixed effect z, b is the additive effect of a SNP tested for association, x denotes the genotype indicator variable coded as 0, 1 or 2, g is the polygenic effect (random effect) captured by the GRM, calculated using all autosomal SNPs, and e is the residual. The fixed effects were as described in the section above.

The *P*-values and effect size for all SNPs across the genome were estimated. The genome-wide false-discovery rate (FDR) was computed using the *q*-value package (Storey and Tibshirani 2003) of Bioconductor. A *q*-value is a measure used to estimate the FDR, representing the proportion of false positives among significant results in multiple hypothesis-testing scenarios. Chromosome-wide and genome-wide

Table 2. Models fitted for univariate and bivariate analyses of Corriedale traits.

Trait	Fixed effects	Random effects
Wool measures and live-animal traits	Type of birth × type of rearing, birth group, sex, heterozygosity	GRM
Carcass and eating-quality traits	Type of birth × type of rearing, contemporary group × slaughter date, sex, heterozygosity	GRM
Visual scores	Type of birth × type of rearing, birth group, sex, heterozygosity	GRM

GRM, genomic relationship matrix.

Table 3. Levels of fixed effects used in univariate and bivariate analyses.

Fixed effect	Level	Definition
Type of birth × type of rearing	11	Born as single, raised as single
	21	Born as twin, raised as single
	22	Born as twin, raised as twin
	31	Born as triplet, raised as single
	32	Born as triplet, raised as twin
	33	Born as triplet, raised as triplet
Sex	F	Female
	M	Male
Birth group	2017	Born in 2017
	2018	Born in 2018
	2019A	Born in 2019
	2019B	Born in 2019 (natural mating)
Contemporary group	2017	Born in 2017; slaughtered in 2018
	2018	Born in 2018; slaughtered in 2019
	2019A1	Born in 2019; slaughtered in 2020, Group 1
	2019A2	Born in 2019; slaughtered in 2020, Group 2
	2019B	Born in 2019; slaughtered in 2020
Slaughter date	18 April 2018	
	19 April 2018	
	10 April 2019	
	1 April 2020	
	29 April 2020	

Manhattan plots of *P*-values were generated to identify key regions associated with the pigmentation traits.

Association of most significant SNPs with other traits

The univariate analyses were conducted by including the genotype of the top significant SNP to analyse the associations

with other traits. The ASReml-R function wald.asreml() was used to determine the significance of the genotype on the traits. Significance was defined as $P < 0.05$. The size of the SNP effect was also obtained from the analyses.

Results

Descriptive statistics

The mean and coefficient of variance for each trait are presented in Table 4. NOSEP scores of individual animals ranged from one to five (Fig. 1*a*), with an overall mean score of 3.69. HVSP scores of individual animals ranged

Table 4. Descriptive statistics of measured traits.

Trait (unit)	*n*	Mean	CV	Minimum	Maximum
GFW (kg)	551	1.81	0.21	0.6	3
FD (μm)	551	23.07	0.08	18.2	29.3
CF (%)	551	93.43	0.07	55.8	99.9
SL (mm)	316	49.16	0.18	25	70
PWWT (kg)	644	39.62	0.14	21	55
WWT (kg)	745	32.67	0.23	13.6	54.5
Ht (cm)	341	65.30	0.05	53	73
HSCW (kg)	736	20.64	0.17	11.3	32.9
PEMD (mm)	731	26.39	0.10	17.5	37
EMD (mm)	436	29.32	0.11	20	45
EMW (mm)	436	54.39	0.07	45	65.99
EMA (cm²)	436	12.78	0.14	7.68	18.408
PFat (mm)	731	3.52	0.26	2	7.5
GRFat (mm)	435	7.46	0.75	0.5	25
IMF (%)	732	4.65	0.32	1.43	11.61
SF5 (N)	714	39.42	0.34	16.96	99.26
NOSEP (1–5 score)	366	3.69	0.22	1	5
HVSP (1–5 score)	366	4.12	0.18	1.7	5
COL (1–5 score)	573	2.29	0.34	1	5
SSTRC (1–5 score)	574	2.62	0.25	1	4.3
CHAR (1–5 score)	573	2.69	0.23	1	4.3
FACE (1–5 score)	571	2.68	0.21	1	4.75
BDWR (1–5 score)	547	1.33	0.40	1	4
BACK (1–5 score)	366	1.54	0.41	1	3.7

NOSEP, nose pigmentation; HVSP, hoof pigmentation; GFW, greasy fleece weight; FD, fibre diameter; CF, comfort factor; SL, staple length; PWWT, post-weaning weight; WWT, weaning weight; Ht, height; HSCW, hot standard carcass weight; PEMD, eye-muscle depth ultrasound scan; EMD, eye-muscle depth; EMW, eye-muscle width; EMA, eye-muscle area; PFat, fat depth ultrasound scan; GRFat, fat depth at 12th rib; IMF, intramuscular fat; SF5, shear force; COL, wool-colour score; SSTRC, wool-staple structure; CHAR, wool character; FACE, wool face cover; BDWR, body wrinkle; BACK, back conformation.
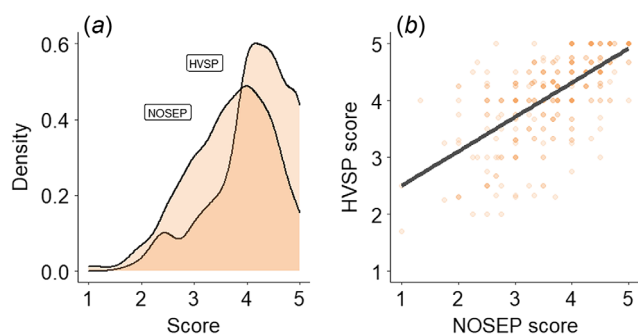
Fig. 1. Distribution of (a) nose-pigmentation score (NOSEP) and hoof-pigmentation score (HVSP), and (b) scatter plot of the NOSEP and HVSP scores.

from 1.7 to 5.0, with an overall mean score of 4.12. NOSEP and HVSP were positively correlated (Fig. 1b).

Genome-wide association study

The genome-wide association analysis tested a total of 39 484 SNPs for their association with NOSEP and HVSP. For NOSEP, after adjusting for FDR, there was only one significant SNP (OAR19_33278780.1; q-value = 0.042, P = 0.000001) on Chromosome 19 (chr19:31614145, Oar_v4.0; Fig. 2, Table 5).

OAR19_33278780.1 (rs419333175) SNP is located within the gene melanocyte-inducing transcription factor (*MITF*), which is known to be involved in pigmentation. No significant SNPs associated with HVSP were found (Fig. 3). However, OAR19_33278780.1 was the sixth-most significant SNP for HVSP, but remained below the significance threshold of the q-value of <0.05 (Table 5).

Of the lambs with NOSEP scores, there were 333 lambs with genotype GG, 32 with the genotype AG and only one lamb with the genotype AA of the OAR19_33278780 SNP. Lambs with the genotype GG at OAR19_33278780.1 SNP had a NOSEP mean score (3.75 ± 0.04 [s.e.]) 25% higher than for lambs with the genotype AG (3 ± 0.04), indicating that they had greater pigmentation (Fig. 4). Approximately 40% of lambs with the genotype AG had a NOSEP score of 3, while approximately half of the sheep with the genotype GG had a NOSEP score of 4 (Fig. 4b).

Effects of OAR19_33278780.1 genotypes on other measured traits

The genotype of OAR19_33278780.1 was added as a fixed factor in the univariate models and Wald tests were performed to determine effects of the genotype of this SNP on the wool,
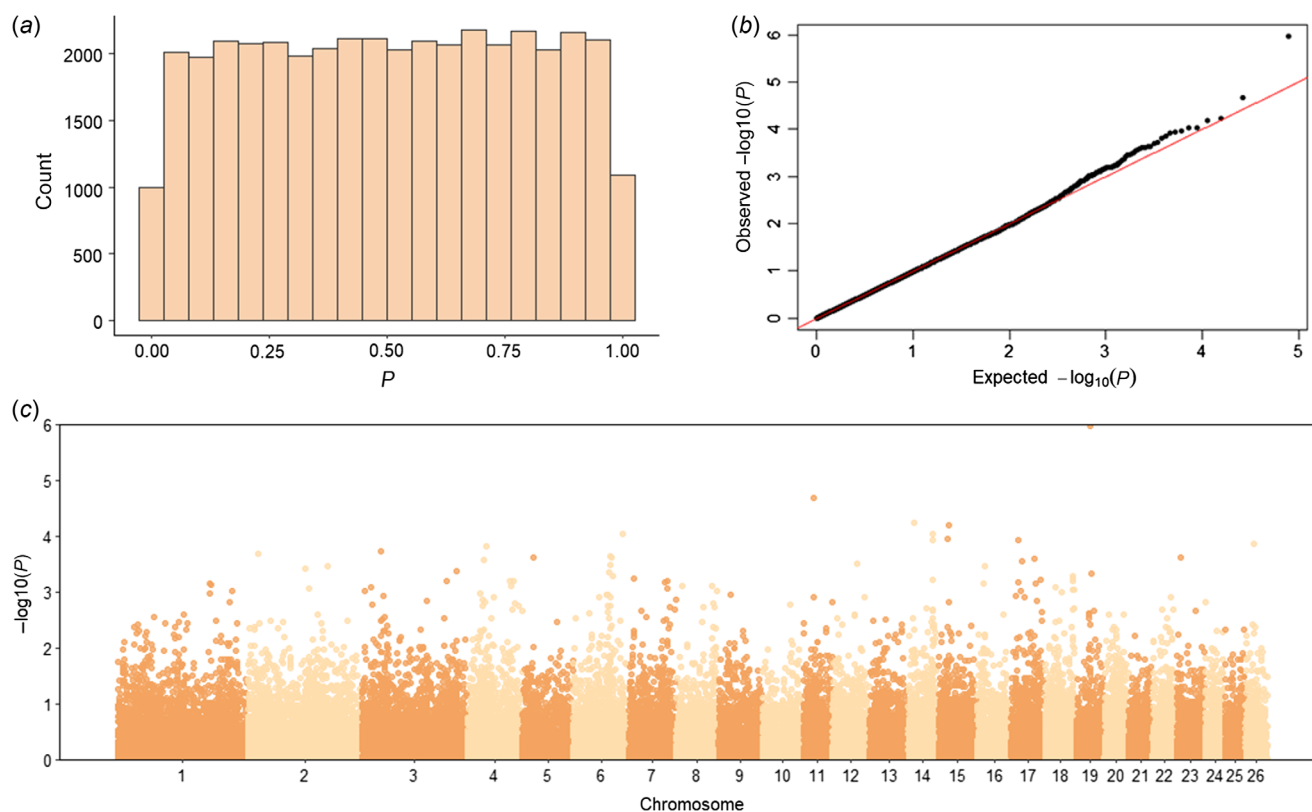

Fig. 2. Genome-wide association study results for nose pigmentation in Corriedale sheep. (a) Histogram of P-values, (b) QQ-plot of P-values and (c) Manhattan plot of $-\log_{10} P$-values. The P-values in the Manhattan plot are plotted by chromosome and the horizontal line indicates genome-wide significance ($P = 5 \times 10^{-6}$). Only one SNP was significant for NOSEP on Chromosome 19 (q-value = 0.042).
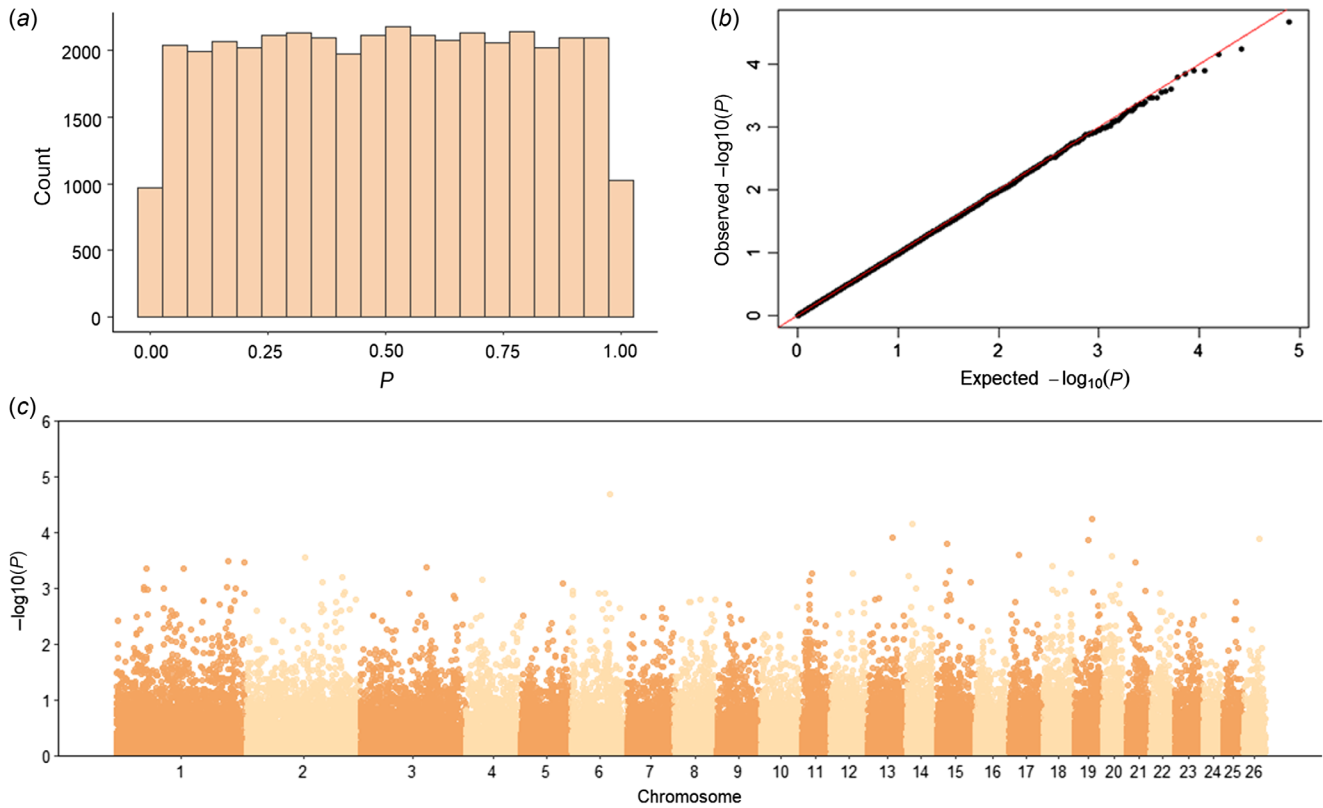
Fig. 3. Genome-wide association study results for hoof pigmentation in Corriedale sheep. (*a*) Histogram of *P*-values, (*b*) qq-plot of *P*-values and (*c*) Manhattan plot of $-\log_{10}$ *P*-values. The *P*-values in the Manhattan plot are plotted by chromosome and the horizontal line indicates genome-wide significance ($P = 5 \times 10^{-6}$).
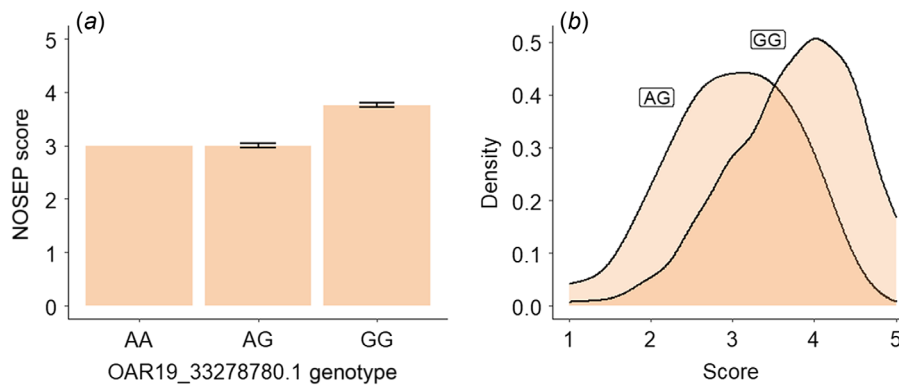

Fig. 4. (*a*) Bar chart of mean and standard error for nose pigmentation (NOSEP) score grouped by genotypes (AA, *n* = 1; AG, *n* = 32; and GG, *n* = 333) at OAR19_33278780.1 SNP. (*b*) Density plot for NOSEP for AG and GG genotypes.

meat traits and visual scores. The genotypes for OAR19_33278780.1 were AA (*n* = 1), AG (*n* = 60) or GG (*n* = 736). The genotype at OAR19_33278780.1 was not significant for any of the visual scores. The genotype was significant for WWT (*P* = 0.01) and PWWT (*P* = 0.01), where GG was associated with higher weights. The genotype at OAR19_33278780.1 did not have a significant effect on the remaining traits.

An additional analysis was conducted by excluding the single lamb with the genotype AA. The genotype at OAR19_33278780.1 was significant for visual score, BACK (*P* = 0.02), and marginally significant for COL (*P* = 0.053, Table 6). Lambs with the genotype GG had 24.9% higher nose skin-pigmentation scores (darker pigmentation), 17.3% higher hoof-pigmentation scores (darker pigmentation), 7.0%

lower scores for backline (more dipped) and 8.1% higher wool-colour scores (more yellow) than those with the genotype AG (Fig. 5, Table 6). The genotype at OAR19_33278780.1 was significant for WWT ($P = 0.03$) and close to significance level for PWWT ($P = 0.06$) and HSCW ($P = 0.07$) (Fig. 6, Table 6). Lambs with the genotype GG had 4.9% higher weaning weight, 3.9% higher post-weaning weight and 3.7% higher carcass weight (Fig. 3c, d, Table 6). The estimated means for these traits are given in Table 6.

Heritability estimates

The heritability estimates for different traits included in this study are presented in Table 7. NOSEP ($h^2 = 0.58$) and HVSP ($h^2 = 0.51$) were highly heritable, but the other visual scores were low to moderately heritable ($h^2 = 0.17–0.44$). Wool measures, GFW ($h^2 = 0.80$), FD ($h^2 = 0.82$) and CF ($h^2 = 0.60$) had high heritability estimates (Table 7), while staple length had a low heritability ($h^2 = 0.20$). The meat-production and eating-quality traits, PWWT, WWT, Ht, HSCW, EMW, PFat and GRFat, had high heritability estimates, whereas PEMD, EMD, EMA and IMF had moderate heritability estimates and, SF5 had a low heritability estimate.

Genetic and phenotypic correlations

The genetic and phenotypic correlations of NOSEP and HVSP with wool and meat traits, and visual scores, are presented in Table 7. NOSEP had genetic correlations with three traits (HVSP, PFat and FACE) and HVSP had genetic correlations with 13 traits (NOSEP, PWWT, WWT, Ht, HSCW, PEMD, EMD, EMA, PFAT, GRFat, IMF, CHAR and FACE).

NOSEP was highly positively correlated (genetically and phenotypically) with HVSP. NOSEP was highly positively correlated (genetically) with FACE score, but the phenotypic correlation was weak. HVSP was highly positively correlated (genetically) with FACE and highly negatively correlated (genetically) with CHAR. The phenotypic correlations between HVSP and visual scores, except for NOSEP, were close to zero.

NOSEP was moderately negatively correlated (genetically) with PFat. HVSP was highly negatively correlated (genetically) with Ht and EMD, and moderately negatively correlated (genetically) with PWWT, WWT, HSCW, PEMD, EMA, PFat, GRFat and IMF. The phenotypic correlations between NOSEP or HVSP and meat-production and meat-quality traits were either weak or close to zero.

Discussion

This study aimed to test for evidence of major genes affecting nose and hoof pigmentation and associated effects on wool- and meat-production traits in Corriedale sheep. A GWAS was conducted to identify genomic regions associated with pigmentation, specifically in the nose and hooves. In the following sections, we first discuss the GWAS results,
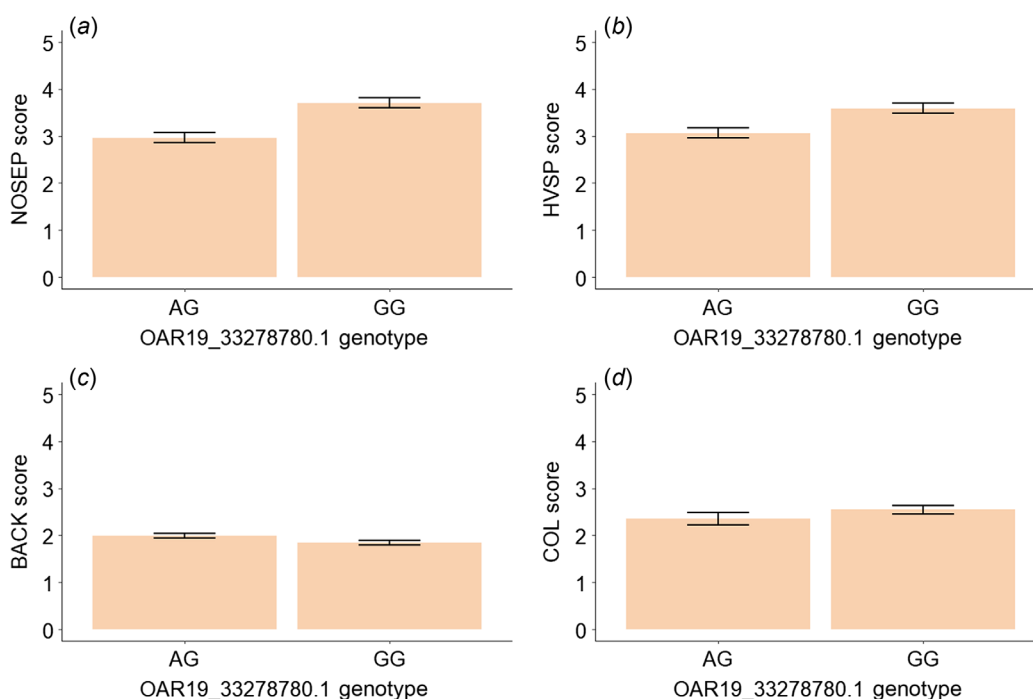

Fig. 5. Estimated means and standard errors for (*a*) nose-pigmentation (NOSEP) score, (*b*) hoof-pigmentation (HVSP) score, (*c*) back-conformation (BACK) score and (*d*) wool-colour (COL) score (yellowness) for sheep with genotypes AG and GG at OAR19_33278780.1.

Table 5. Top-10 significant SNPs for nose pigmentation and hoof pigmentation in Corriedale sheep.

Chr	SNP name	Minor allele frequency	SNP effect size	s.e.	*P*-value	*q*-value
NOSEP						
6	OAR6_115827874.1	0.36	0.24	0.063	0.000091	0.53
11	oar3_OAR11_25301463	0.02	−0.95	0.223	0.000021	0.41
14	Chr14:14283245	0.14	−0.34	0.085	0.000058	0.53
14	oar3_OAR14_56359275	0.21	−0.28	0.072	0.000091	0.53
14	OAR14_57012757.1	0.4	−0.24	0.062	0.00012	0.53
15	Chr15:23406267	0.28	−0.27	0.066	0.000064	0.53
15	OAR15_22081912.1	0.46	−0.24	0.062	0.00011	0.53
17	Chr17:16013001	0.03	−0.75	0.19	0.00012	0.53
19	OAR19_33278780.1	0.05	−0.69	0.14	1.1E-06	0.04*
26	OAR26_21018684.1	0.27	−0.26	0.068	0.00014	0.55
HVSP						
2	oar3_OAR2_134146443	0.24	−0.24	0.066	0.00028	0.97
6	oar3_OAR6_89367464	0.04	−0.66	0.155	0.00002	0.84
13	oar3_OAR13_55804408	0.4	−0.23	0.06	0.00012	0.91
14	Chr14:14283245	0.14	−0.31	0.079	0.00007	0.91
15	Chr15:23406267	0.28	−0.23	0.062	0.00016	0.91
17	oar3_OAR17_22491315	0.07	−0.42	0.113	0.00025	0.97
19	OAR19_41399545.1	0.27	−0.26	0.063	0.00006	0.91
19	OAR19_33278780.1	0.05	−0.5	0.13	0.00014	0.91
20	oar3_OAR20_20498243	0.47	−0.22	0.061	0.00027	0.97
26	oar3_OAR26_35076973	0.38	−0.22	0.058	0.00013	0.91

Chr, chromosome; s.e., standard error of effect size; NOSEP, nose-pigmentation score; HVSP, hoof-pigmentation score.
*$q < 0.05$.

genetic parameters, , and, finally, mean and distribution of the traits.

SNP OAR19_33278780.1 (rs419333175) is associated with nose pigmentation

The GWAS pinpointed a single SNP (OAR19_33278780.1; chr19:31614145, Oar_v4.0) on Chromosome 19 associated with nose-pigmentation score (Fig. 2). Raadsma *et al.* (2013) reported a QTL at 20 cM (confidence interval = 0–53 cM) on Chromosome 19 linked to nose and hoof pigmentation, among other pigmentation traits, of Merino × Awassi F1 sheep. The authors proposed that *MITF* is located within this QTL on Chromosome 19. As OAR19_33278780.1 is located within an intron of the pigmentation gene, *MITF*, it is likely that the SNP also resides within the QTL. The current study provides further support for the involvement of the region on Chromosome 19 in pigmentation.

The GG genotype at OAR19_33278780.1 was associated with a 25% increase in nose-pigmentation scores compared with AG genotype. As the genotype at OAR19_33278780.1 does not follow Mendelian inheritance, the trait is likely to be affected by additional genetic variants. Other sources of variation could result from incomplete linkage disequilibrium between the SNP and the causative variant, as well as environmental factors and scoring inaccuracies.

Interestingly, only one individual possessed the genotype AA, highlighting the selective pressure favouring the G allele. This SNP was identified to be under positive selection in a study involving 74 diverse breeds of sheep (Kijas *et al.* 2012).

OAR19_33278780.1 may also be important for hoof pigmentation. OAR19_33278780.1 ranked among the top-10 most significant SNPs for HVSP, although it did not exhibit a significant *q*-value. Nevertheless, the genetic correlation between nose and hoof pigmentation was strong (Table 7). Previous studies on sheep have also identified OAR19_33278780.1 as a significant marker for pigmentation traits. This SNP was significantly associated with coat colour (white, black, 'grey' caused by white and black fibres, and brown) in a GWAS study of Finnsheep (Li *et al.* 2014). Additionally, QTLs for eye skin, ear skin, face skin, leg fibre, horn fibre and ear fibre pigmentation also mapped to Chromosome 19 in Merino × Awassi sheep (Raadsma *et al.* 2013). Taken together, *MITF* may be involved in hoof pigmentation, but the current study may have lacked sufficient observations or accurate scoring to detect significance for this trait.

Table 6. Estimated means for traits in Corriedale sheep grouped by genotype at SNP OAR19_33278780.1.

Trait	Genotype	Estimated mean	s.e.	Effect size	Mean difference (%)	Significance
WWT	AG	30.59	0.65	0	4.90	0.027*
	GG	32.09	0.17	1.58		
PWWT	AG	36.89	0.17	0	3.88	0.059
	GG	38.32	0.17	1.47		
HSCW	AG	18.93	0.36	0	3.75	0.069
	GG	19.64	0.09	0.709		
COL	AG	2.35	0.13	0	8.09	0.053
	GG	2.54	0.09	0.19		
BACK	AG	1.99	0.05	0	−7.04	0.024*
	GG	1.85	0.05	−0.14		

WWT, weaning weight; PWWT, post-weaning weight; HSCW, hot standard carcass weight; COL, wool-colour score; BACK, back conformation.
*$P < 0.05$.

Table 7. Heritability estimates of traits, and genetic and phenotypic correlations of NOSEP and HVSP with wool and meat traits, and visual scores.

Trait	$h^2 \pm$ s.e	V_P	NOSEP		HVSP	
			$r_P \pm$ s.e.	$r_G \pm$ s.e.	$r_P \pm$ s.e.	$r_G \pm$ s.e.
NOSEP	0.58 ± 0.15	0.59	–	–	**0.62 ± 0.04**	**0.73 ± 0.13**
HVSP	0.51 ± 0.16	0.50	**0.62 ± 0.04**	**0.73 ± 0.13**	–	–
GFW	0.80 ± 0.10	0.09	0.08 ± 0.06	−0.21 ± 0.18	0.03 ± 0.06	−0.24 ± 0.19
FD	0.82 ± 0.10	3.13	0.08 ± 0.06	0.01 ± 0.18	0.00 ± 0.06	0.00 ± 0.19
CF	0.60 ± 0.11	37.20	−0.08 ± 0.06	−0.01 ± 0.2	−0.02 ± 0.06	−0.02 ± 0.21
SL	0.20 ± 0.14	26.48	**−0.28 ± 0.10**	−0.33 ± 0.35	−0.15 ± 0.11	−0.37 ± 0.42
PWWT	0.68 ± 0.10	26.48	0.00 ± 0.07	−0.35 ± 0.19	−0.07 ± 0.07	**−0.46 ± 0.18**
WWT	0.60 ± 0.09	22.29	0.02 ± 0.06	−0.25 ± 0.18	−0.06 ± 0.06	**−0.44 ± 0.17**
Ht	0.56 ± 0.16	6.33	−0.07 ± 0.10	−0.27 ± 0.28	0.00 ± 0.10	**−0.62 ± 0.25**
HSCW	0.63 ± 0.09	7.37	−0.03 ± 0.06	−0.17 ± 0.18	−0.12 ± 0.06	**−0.37 ± 0.17**
PEMD	0.47 ± 0.09	5.95	0.03 ± 0.06	−0.22 ± 0.19	−0.07 ± 0.06	**−0.38 ± 0.18**
EMD	0.21 ± 0.11	9.01	−0.08 ± 0.07	−0.35 ± 0.28	**−0.15 ± 0.07**	**−0.72 ± 0.20**
EMW	0.60 ± 0.13	12.10	−0.02 ± 0.07	−0.16 ± 0.21	−0.04 ± 0.07	−0.15 ± 0.24
EMA	0.47 ± 0.14	3.02	−0.07 ± 0.07	−0.33 ± 0.22	**−0.15 ± 0.07**	**−0.58 ± 0.18**
PFat	0.50 ± 0.10	0.79	−0.05 ± 0.06	**−0.36 ± 0.17**	**−0.14 ± 0.06**	**−0.50 ± 0.17**
GRFat	0.51 ± 0.14	6.73	−0.07 ± 0.06	−0.24 ± 0.23	−0.12 ± 0.06	**−0.41 ± 0.19**
IMF	0.46 ± 0.10	0.85	−0.01 ± 0.06	−0.24 ± 0.19	**−0.19 ± 0.06**	**−0.59 ± 0.16**
SF5	0.16 ± 0.08	165.06	−0.02 ± 0.06	0.31 ± 0.28	−0.03 ± 0.06	0.23 ± 0.31
COL	0.44 ± 0.10	0.39	0.18 ± 0.06	0.30 ± 0.20	0.04 ± 0.06	−0.22 ± 0.22
SSTRC	0.26 ± 0.09	0.33	0.05 ± 0.06	−0.28 ± 0.23	0.00 ± 0.06	−0.21 ± 0.26
CHAR	0.44 ± 0.11	0.35	−0.02 ± 0.06	−0.14 ± 0.23	−0.07 ± 0.06	**−0.53 ± 0.22**
FACE	0.37 ± 0.11	0.30	0.11 ± 0.06	**0.55 ± 0.18**	**0.15 ± 0.06**	**0.56 ± 0.19**
BDWR	0.17 ± 0.09	0.21	−0.13 ± 0.06	−0.09 ± 0.35	−0.04 ± 0.07	0.20 ± 0.38
BACK	0.22 ± 0.10	0.11	−0.14 ± 0.06	−0.49 ± 0.25	−0.08 ± 0.06	−0.02 ± 0.30

The correlations with a standard error less than half of their estimates are highlighted in boldface.
h^2, estimated heritability; V_P, phenotypic variance; r_P, phenotypic correlation; r_G, additive genetic correlation; s.e., standard error; NOSEP, nose pigmentation; HVSP, hoof pigmentation; GFW, greasy fleece weight; FD, fibre diameter; CF, comfort factor; SL, staple length; PWWT, post-weaning weight; WWT, weaning weight; Ht, height; HSCW, hot standard carcass weight; PEMD, eye-muscle depth ultrasound scan; EMD, eye-muscle depth; EMW, eye-muscle width; EMA, eye-muscle area; PFat, fat depth ultrasound scan; GRFat, fat depth at 12th rib; IMF, intramuscular fat; SF5, shear force; COL, wool-colour score; SSTRC, wool-staple structure; CHAR, wool character; FACE, wool face cover; BDWR, body wrinkle; BACK, back conformation.
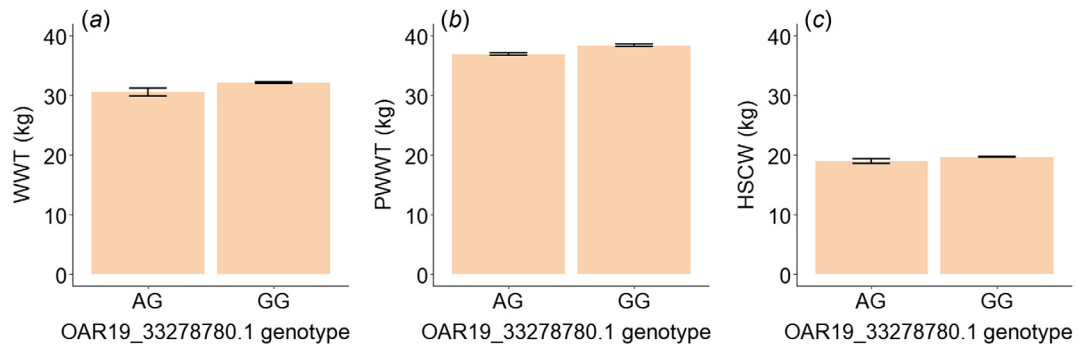
Fig. 6. Estimated means and standard errors for (*a*) weaning weight (WWT, kg), (*b*) post-weaning weight (PWWT, kg) and (*c*) hot standard carcass weight (HSCW, kg) for sheep with genotypes AG and GG at SNP OAR19_33278780.1.

Variants of *MITF* have been linked to white spotting in horses (Hauswirth *et al.* 2012; Negro *et al.* 2017; Henkel *et al.* 2019; Magdesian *et al.* 2020; Patterson Rosa *et al.* 2022) and water buffalo (Yusnizar *et al.* 2015). In horses, an intronic SNP (g.20147039C > T) was associated with white facial markings in the Spanish Purebreds, and white forelimbs in Menorca Purebreds (Negro *et al.* 2017). A large deletion including *MITF* was associated with white coat colour in a Holstein calf (Wiedemar and Drögemüller 2014). A *de novo* missense variant in Exon 8 of *MITF* was associated with white coat colour in a white American Standardbred foal (Dürig *et al.* 2017).

MITF, a transcription factor, plays a role in the migration and differentiation of neural crest cells, which serves as the pluripotent precursor of melanocytes (Widlund and Fisher 2003; Steingrímsson *et al.* 2004; Levy *et al.* 2006). Genetic variants of *MITF* influence pigmentation early in development by guiding the migration and differentiation of melanocyte precursors in specific regions. Consequently, skin and fibres lacking melanocytes appear white. In Corriedale sheep, the pigmented nose skin may be caused by a variant of *MITF* that specifies localised melanocyte development at the nose.

OAR19_33278780.1 significantly associated with back conformation, and weaning weight

The genotype at OAR19_33278780.1 was added to the univariate model to determine the effect on production traits. The genotype GG was also associated with 5.0% increased weaning weights and 7.3% reduced back-conformation scores, indicating that back lines are slightly more dipped. Nevertheless, these effect sizes are small and may not have significant impacts on breeding programs.

The GG genotype was associated with 3.9% increase in post-weaning weights, 3.7% rise in carcass weights, and an 8.1% increase in wool colour, although these associations were not statistically significant. As the effect on weaning weight was significant, lambs with the GG genotype may exhibit higher early growth rates. However, the significance

of the SNP diminished over time in subsequent weight measurements (PWWT and HSCW). Additionally, there might be insufficient observations to establish significance of the SNP effect for weight traits as the lamb ages and is exposed to more environmental influences. While lambs with the GG genotype tended to have more yellow wool, the effect size remained small.

MC1R may also be involved in pigmentation traits

A SNP on Chromosome 14 (Chr14:14283245) near melanocortin 1 receptor (*MC1R*) (Chr14:14,231,721–14,232,541; Oar_v3.1) was among the top 10 most significant SNPs for NOSEP and HVSP, but was not significant when the *P*-value was adjusted for FDR. MC1R is a 7-transmembrane G protein-coupled receptor expressed by melanocytes (Cieslak *et al.* 2011; Koseniuk *et al.* 2018) that acts as a pigmentation switch and stimulates eumelanin (black pigment) production when it binds to α melanocyte-stimulating hormone (Cieslak *et al.* 2011; Koseniuk *et al.* 2018). Although, the SNP near *MC1R* is not significant, it may still be involved with these pigmentation traits. This hypothesis could be investigated with more observations and objective measurement methods such as by using image analysis.

Pigmentation traits have some genetic correlations with meat traits

Darker hooves had moderate and high genetic correlations with meat and fat-quantity traits, which indicated reduced growth and fat coverage (Table 5). Some of these genetically correlated traits also had phenotypic correlations with hoof pigmentation; however, these were weak. Darker hooves were also significantly correlated with lower intramuscular fat percentage, indicating lower meat quality; however, the phenotypic correlation was weak. Nose pigmentation was not genetically correlated with wool- and meat-production traits, except for scanned fat depth. Darker noses had moderate

genetic correlation with scanned fat depth. As the phenotypic correlations for NOSEP and HVSP with meat-production and meat-quality traits were weak or close to zero, any negative genetic associations that NOSEP and HVSP have on traits may be compensated by other genetic and environmental factors.

Corriedale sheep have mean trait measures similar to those of other breeds

These Corriedale sheep had good growth rates, with weaning weights (~5 months old) of 32.6 kg and post-weaning weights (~6–7 months) of 39.6 kg. This is greater than the WWT of Merino × lambs (27.8 kg; Mortimer *et al.* 2010) and Merinos (6.51–27.05 kg) and mean PWWT of Merinos (21.98–38.28 kg; Vaez *et al.* 1996; Huisman *et al.* 2008; Mortimer *et al.* 2017*a*, 2018). These Corriedale sheep had lower PWWT than those of Polled Dorset (45.2 kg; Gilmour *et al.* 1994).

The lambs were slaughtered at lighter weights (20.64 kg) than in similar trials with other meat breeds (21.6–23.1 kg; Mortimer *et al.* 2010, 2014) and Merino lambs (21.1 kg; Mortimer *et al.* 2017*b*, 2018). The EMA of Corriedale lambs was within the range of means reported in Merinos (11.6–13.1 mm; Mortimer *et al.* 2010, 2017*b*, 2018). The GRFat depth in Corriedales (7.46 mm) was lower than GRFat of Merinos (10.7 mm; Mortimer *et al.* 2018) and Merino × lambs (21.6 mm; Mortimer *et al.* 2010). IMF in Corriedales was within the range reported for Merinos and Merino × lambs (4.23–5.7%; Mortimer *et al.* 2010, 2014, 2018). The lower HSCW in these Corriedale may be attributed to the lower fat coverage.

The FD of Corriedale lambs (23 μm) was lower than an earlier reported mean of 25 μm in this breed (Brash *et al.* 1994), but greater than FD in yearling Merino sheep (16.9–18.1 μm; Huisman *et al.* 2008; Mortimer *et al.* 2017*a*). Mean GFW was 1.81 kg at approximately 5–6 months.

The Corriedale lambs had COL, SSTRC, CHAR and FACE scores similar to those of Merinos (COL: 2.37–2.85; SSTRC: 2.5–2.65; CHAR: 2.45–2.69; and FACE: 1.85–2.73; Hatcher and Preston 2018). The mean BDWR score of Corriedale lambs was lower than yearling, adult, and hogget Merino scores (2.09–2.64), indicating that Corriedale lambs are less wrinkled.

Conclusions

This investigation of nose and hoof pigmentation in Corriedale sheep supports the potential involvement of *MITF* in the development of nose pigmentation. While *MITF* might also contribute to hoof pigmentation, the SNP within this gene (OAR19_33278780.1) did not exhibit a significant association. Further research with more observations and improved scoring or measurement of hoof pigmentation could help clarify this relationship.

While NOSEP and HVSP had low to moderate genetic correlations with wool traits, live-animal traits and carcass traits, no phenotypic correlations were observed. This suggests that the pigmentation traits have little impact on the overall phenotype. The genotype at OAR19_33278780.1 has a significant effect on nose-pigmentation, weaning-weight, and back-conformation scores. The animals with the genotype GG had a greater mean NOSEP, HVSP, and WWT.

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