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Polymorphism of *KRT83* and its association with selected wool traits in Merino-cross lambs

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Highlights:

- PCR-SSCP was used to screen variation in three regions of ovine *KRT83*.
- No variation was found in the promoter region.
- Two and five variants were found for exon 2 and exon 3-4 regions.
- Seven SNPs were identified in these variants.
- Variation in *KRT83* affects GFW, wool Yield and fibre diameter-associated traits.

Abstract

Keratins (Ks) are structural proteins in the cortex of wool fibres. It is thought that variation in the Ks affects wool structure and characteristics. Polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) analysis was used to investigate three regions of ovine *KRT83*. These regions were a portion of the promoter, all of exon 2 (including part of intron 1) and a region encompassing exon 3-4 (including all of exon 3, intron 3, exon 4 and part of intron 4). Initially, in 300 New Zealand Romney, Merino and White Dorper sheep obtained from 26 farms, one, two and four PCR-SSCP banding patterns were observed for these regions respectively. The exon 2 region contained two single nucleotide polymorphisms (SNPs) and the exon 3-4 region contained five SNPs. Investigation of the effect of the variation in the exon 3-4 region on variation in some wool traits were subsequently undertaken in 489 Merino × Southdown-cross sheep from seven sire-lines. The four variants identified in the original 300 sheep (designated *A*-*D*) and a new variant (*E* containing a new SNP) were observed with a frequency of 64.6%, 15.4%, 6.6%, 10.1% and 3.3%, respectively. General linear mixed-effects models (GLMMs) were used to investigate associations between the presence or absence of the variants and the wool traits, with a second set of models testing associations between common genotypes and those traits. The presence of *A* was associated with a decrease in fibre diameter standard deviation (FDSD) and coefficient of variation of fibre diameter (CVFD). The presence of *C* was associated with an increase in mean fibre diameter (MFD), mean fibre curvature (MFC) and prickle factor (PF), and a decrease in wool Yield [clean fleece weight (CFW)/greasy fleece weight (GFW) ×100%]. A trend for association between the presence of *C* and increased FDSD was also detected. The presence of *D* was associated with an increase in MFD and PF, and a decrease in Yield. The presence of *E* was associated with a decrease in CVFD. Genotype *AD* had a higher GFW and a lower Yield than *AA*, and *AC* and *AD* tended to have increased MFD compared to *AA* and *AB*.

These results suggest that ovine *KRT83* might be a useful candidate gene for improving wool traits.

Keywords: *KRT83*, variation, wool traits, sheep.

1. Introduction

Hard α-keratins, the main structural proteins of wool fibre, assemble into keratin intermediate filaments (KIFs) (Powell and Rogers 1994). In each wool fibre, the KIFs are bundled together and combine with keratin associated proteins (KAPs) to form macrofibrils. These are further bundled together to form the cortex of the wool fibre (Rogers, 2004).

The α-keratins are low-sulphur proteins and they are grouped into type-I and type-II families. The α-keratin K83 (ENSOARG00000017113, Oar_v3.1:CM001584.1; Chromosome 3: 133,886,248-133,892,660) belongs to the type II family and is found in the cortex of wool fibres, along with K81 and K86 (Bragulla and Homberger, 2009). The K83 gene (*KRT83*, formerly known as *KRT2.10*) is tightly linked to *KRT87* (formerly *KRT2.13*) and has been mapped to ovine chromosome 3q14-q22 (Hediger et al., 1991; Mclaren et al., 1997). It is also tightly linked to other type II keratin genes in the sheep genome (Powell and Beltrame, 1994)

Several QTL experiments have revealed loci on chromosome 3 to be associated with variation in selected wool traits, including the brightness of wool (McKenzie, 2001), staple length (Ponz et al., 2001), mean fibre diameter (MFD) (Bidinost et al., 2008), coefficient of variation of fibre diameter (CVFD) (Allain et al., 1998; Allain et al., 2013) and greasy fleece weight (GFW) (Allain et al., 2006). However, to date only limited evidence has been reported describing genetic variation in the chromosome 3 *KRT* genes, with McLaren *et al.* (1997)

reporting two variants of *KRT83* found using a polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) typing method and McKenzie et al. (2012) describing nine variants of *KRT87* using a PCR-single stranded conformational polymorphism (PCR-SSCP) approach.

In this study, PCR-SSCP was used to investigate sequence variation in *KRT83* and the association, if any, between *KRT83* sequence variation and various wool traits.

2. Materials and methods

2.1. Sheep and wool samples

In the first part of this study to ascertain how much genetic variation occurred in *KRT83,* 300 New Zealand (NZ) Romney, Merino and White Dorper sheep, sourced from 26 farms, were genotyped for three regions of the gene. These regions were a 520 bp region of the promoter, a 371 bp region spanning exon 2 (including part of intron 2), and a 438 bp region encompassing all of exon 3, intron 3, exon 4, and part of intron 4.

Next, the association of *KRT83* exon 3-4 variation with selected wool traits was then investigated in a separate group of 489 Southdown × Merino-cross lambs from seven sire-lines, which had been shorn at 12 months of age. These lambs were all managed on the same farm. At shearing GFW was measured, and wool samples were collected from the mid-side region for wool trait measurement at the New Zealand Wool Testing Authority Ltd (NZWTA, Napier, NZ) using International Wool Testing Organisation (IWTO) standardised methods. This included measurement of wool yield (Yield), mean staple length (MSL), mean staple strength (MSS), MFD, fibre diameter standard deviation (FDSD), CVFD; mean fibre curvature (MFC) and prickle factor (PF; the percentage of fibres of diameter greater than 30 microns). Clean fleece weight was calculated from the GFW and Yield.

2.2. DNA extraction and polymerase chain reaction (PCR) amplification

Blood samples were collected onto FTA cards and genomic DNA was purified using a two-step procedure described by Zhou et al. (2006).The primers used to amplify the three regions of *KRT83* were designed based on sequences in the ovine genome assembly v4.0 and are described in Table 1. The primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

Amplification of each region was performed in 15-µL reactions that included the genomic DNA on one 1.2-mm punch of FTA paper, 10× reaction buffer with 0.5U of Taq DNA polymerase (Qiagen, Hilden, Germany), a 250 nM concentration of each primer, a 150 µM concentration of each dNTP (Eppendorf, Hamburg, Germany) and a final Mg^{2+} concentration of 2.5 mM.

The cycling parameters for PCR amplification consisted of denaturation at 94 °C for 2 min, followed by 35 cycles of 30 s each at 95 °C, the annealing temperature shown in Table 1 and extension at 72 °C; with a final extension at 72 °C for 5 min. Amplicons were visualized by gel electrophoresis in 1% agarose (Quantum Scientific, Queensland, Australia) gels, using 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA) that contained 200 ng/ml of ethidium bromide.

Following the initial screening of the 300 NZ Romney, Merino and White Dorper sheep, an inner forward PCR primer for the *KRT83* exon 3-4 region, 5'-AGGTAAGTGCGTGAGCCAAG-3' (Table 1), was designed to amplify a smaller fragment that spanned the nucleotide variations identified with the first set of primers. This primer was used along with the original reverse primer in the wool trait association analyses, to type the *KRT83* exon 3-4 region in the 489 Southdown × Merino-cross lambs.

2.3. SSCP analyses of the KRT83 gene regions

All SSCP analysis was carried out in 14% polyacrylamide gels (37.5:1; Bio-Rad Laboratories Inc.) at 200 V for 18 h in 0.5× TBE buffer at the temperatures given in Table 1. A 0.7-µL aliquot of the product from the PCR amplification was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol), denatured at 95 °C for 5 min, then cooled rapidly on wet ice and loaded onto gels. Once reference samples were determined for each variant, they were included on all subsequent gels to facilitate genotyping. All gels were silver-stained according to the method of Byun et al. (2009).

2.4. Sequencing of nucleotide variants and sequence analyses

For each of the three *KRT83* regions amplified, amplicons from sheep apparently homozygous for each of the SSCP banding patterns were sequenced directly at the Lincoln University DNA Sequencing Facility. Amplicons from three sheep representing each homozygous banding pattern, were sequenced with proof-reading enzymes, and the nucleotide sequences aligned to confirm the sequence and ascertain whether the sheep sequenced were homozygous at the sequence level.

For variants that were only found in apparently heterozygous sheep, the DNA was sequenced using an approach described in Gong et al., (2011). Briefly, the SSCP band corresponding to the variant not found in a homozygous form was excised from the SSCP gel, macerated, and then used as a template for re-amplification. The product of the second amplification was then directly sequenced in triplicate.

Sequence alignments, translations (to determine presumed amino acid sequences) and comparisons were carried out using DNAMAN (version 5.2.10, LynnonBioSoft, Vaudreuil, Canada). The BLAST algorithm was used to search the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) databases for sequence homology, in particular homology found with the ovine genome assembly v4.0.

2.5. Statistical analyses

For each wool trait, statistical analyses were performed using Minitab version 16. Unless otherwise indicated, all *P* values were considered statistically significant when $P < 0.05$ and trends were noted when $0.05 < P < 0.10$.

General Linear Mixed-effects Models (GLMMs) were used to evaluate the effect, if any, of the presence/absence of the *KRT83* exon 3-4 variants, or *KRT83* genotypes, on various wool traits. In all the models, gender and birth rank were fitted as fixed factors, and sire was fitted as a random factor.

The *KRT83* exon 3-4 variants were coded as either present (1) or absent (0) for each animal's genotype. For each wool trait, single-variant presence/absence models were then run. Any gene variant that had an association in the single-variant models with a *P* value of less than 0.2 ($P < 0.2$) and which could thus potentially impact on the trait was then factored into multi-variant models, such that a correction was made for the second variant in the genotype. This statistical approach has been used previously by Forrest et al. (2009).

In a third set of models, any *KRT83* exon 3-4 genotype present at a frequency of 5% or more (thereby ensuring adequate sample size), were tested to ascertain associations with the wool traits. Multiple pairwise comparisons between genotypes were performed using a Tukey test with Bonferroni corrections.

3. Results

3.1. Variants of the KRT83 promoter, exon 2 and exon 3-4 regions

For the *KRT83* promoter, exon 2 and exon 3-4 regions investigated, one, two and four SSCP banding patterns were observed, respectively. Sequencing of the amplicons that produced unique SSCP banding patterns for each of the three regions of *KRT83*, revealed sequences

that were unique, but at least 99% homologous to the ovine genome (ovine genome assembly v 4.0, 133717044-133717563, 133721248-133721618, 133722529-133722966; for the *KRT83* promoter, exon 2 and exon 3-4 regions respectively).

No sequence variation was found in the portion of the promoter amplified, but sequence analysis of the exon 2 region from the 300 NZ Romney, Merino and White Dorper sheep, revealed a single nucleotide polymorphism (SNP) c.385-23G/C (Figure 1) in the segment of intron 1 that was amplified. This gave rise to two variants (designated *a* and *b*; Figure 1). Four nucleotide sequences were confirmed for the *KRT83* exon 3-4 region in the 300 NZ Romney, Merino and White Dorper sheep and these were designated *A*-*D* (Figure 1). Variant *A* of exon 3-4 had 100% homology with the ovine genome assembly v4.0 sequence over coordinates 133721248-133721618. There were five SNPs identified in the four variants (c.655-64delT, c.655-22C/T, c.666C/T, c.672C/T and c.750+45C/T; Figure 1), with two SNPs located in intron 3, two SNPs in exon 4 and one SNP in intron 4.

Genotyping of the 489 Southdown × Merino-cross lambs with the new forward primer revealed an additional variant designated *E* that was observed in 16 lambs. Sequencing of *E* revealed an additional SNP c.655-61G/A in intron 3.

The frequencies of the *KRT83* exon 3-4 variants *A* to *E* in the Southdown × Merino-cross sheep were 64.6%, 15.4%, 6.6%, 10.1% and 3.3% respectively. Thirteen different genotypes were observed with the following genotypes having frequencies over 5%: *AA* (40.7%); *AB* (20.1%); *AC* (8.6%) and *AD* (14.5%). The remaining eight genotypes observed were *AE*, *BB*, *BC*, *BD*, *BE*, *CC*, *CD*, *DD* and *DE*; with *CE* and *EE* not being present in the 489 sheep studied.

3.2. Associations between in KRT83 exon 3-4 variation and wool traits

In the single-variant GLMMs, the presence of *A* was associated with a decrease in MFD, FDSD, CVFD and PF (Table 2). This association persisted for FDSD and CVFD, but was lost for MFD and PF in the multi-variant GLMMs (Table 2).

No associations were detected with *B* in the GLMMs, but the present of *C* was found to be associated with an increase in MFD, FDSD, MFC and PF, and a decrease in Yield. These effects persisted in the multi-variant GLMMs, except for FDSD for which a trend ($P = 0.102$) was observed (Table 2).

The presence of *D* was associated with decreased Yield and an increase in MFD and PF in both the single and multi-variant GLMMs (Table 2). A trend for an increase in GFW and FDSD associated with the presence of *D* was found with the single-variant GLMMs, but the association between FDSD and D was lost in the multi-variant GLMM (Table 2).

The presence of *E* tended to be associated with a decrease in CVFD in the single-variant GLMM, and the association became significant in the multi-variant GLMM (Table 2).

3.3. *Effect of common genotypes on wool traits*

With the four common genotypes (frequencies greater than 5%), genotype effects were detected for GFW and Yield. Sheep with genotype *AD* had higher mean GFWs (*AD*: 2.49 ± 0.04 kg; *AA*: 2.35 ± 0.03 kg; $P = 0.040$) and lower mean Yields (*AD*: 71.6 ± 0.67 %; *AA*: 73.7 ± 0.49 %; $P = 0.031$) than those of genotype *AA*. A trend for association between genotype and MFD was also detected ($P = 0.089$), with *AC* and *AD* sheep tending to have a higher mean MFD compared to *AA* and *AB* sheep.

4. Discussion

This study identified sequence variation in two regions of ovine *KRT83*. Two exon 2 region sequences, defined by one SNP (c.385-23G/A) were identified, but the SNP was located in a non-coding region (the region of intron 1 that was amplified). The SNPs identified in the *KRT83* exon 3-4 region were located in exon 4 (the coding region SNPs were synonymous) and non-coding DNA (intron 3 and intron 4). Variation in introns can influence gene expression in many ways; including changing the activity of enhancer elements found within the intron, and by affecting primary transcript production by changing both transcription initiation and RNA polymerase II processivity (Fong and Zhou, 2001; Furger et al., 2002; Kwek et al., 2002), by influencing primary transcript splicing (Furger et al., 2002) including causing alternate splicing, by changing RNA secondary structures and thus stability and also by disturbing mRNA export (Nott et al., 2003).

Despite there being no evidence of variation in the amino acid sequence of K83, variation in exon 3-4 was found to be associated with a number of wool traits. The possibility exists that the associations observed may be due to linkage between *KRT83* and other genes on chromosome 3, specifically other *KRT*s. The chromosome *KRTs* are clustered and potentially all of them are variable and expressed in the wool fibre (Yu et al., 2011). This means that it would not be an easy task to isolate and illustrate the independent effects of the individual *KRT* genes. Greater insight into the possibility of linkage driving the effects could however be obtained by replicating this research with other genes found proximal to *KRT83* on chromosome 3.

The associations revealed between variation in *KRT83* and some wool traits are consistent with previously reported correlations between these wool traits (Gong et al. 2015). For example, Gong et al. (2015) reported a moderate negative correlation between wool Yield, and MFD and MFC; a strong positive correlation between MFD, and FDSD and PF, and a

strong positive correlation between FDSD and CVFD. The association of variant *A* with both decreased FDSD and CVFD, may therefore simply reflect the observation of Gong et al (2015) that MFD and FDSD are strongly positively correlated traits, and not that the variant is associated with both MFD and FDSD independently. Similarly, the association of variant with increased MFD, PF and MFC, and decreased Yield; and the association of *D* with increased MFD and PF, and decreased Yield; may simple reflect the observation that Yield is moderately negatively correlated with MFD and PF, and that MFD is strongly positively correlated with PF (Gong et al. 2015).

While variant *D* was associated with decreased Yield, this variant was showing a trend towards being associated with increased GFW. This might suggest that *D* has effects on both Yield and GFW. The effects seen on Yield and GFW do not appear to be due to these traits being correlated, as the correlation between these traits is reported (Gong et al., 2015) to be weak and positive. In Gong at al. (2015) the trait most highly correlated with GFW is CFW, yet *D* was only found to be associated with GFW, but not CFW. Given that Yield is the proportion of GFW that is CFW, as a percentage, then an increase in GFW due to the present of *D* should therefore lead to a decrease in Yield. This is what was observed in this study.

Variants *C* and *D* were found to be associated with increased MFD, but no with FDSD and CVFD. Gong et al. (2015) revealed MFD to be strongly correlated with both CVFD and FDSD, which is unsurprising given that CVFD is the proportion of MFD that is FDSD, described as a percentage. Similarly, *A* and *E* were associated with decreased FDSD and CVFD, but were not associated with MFD. This suggests that *KRT83* affects wool fibre diameter-associated traits including MFD, FDSD and CVFD, but the effect with MFD may be independent of the effects with FDSD and CVFD. This may mean that *C* and *D* affect the mean of the fibre diameter for any given wool sample, but not the distribution of fibre

diameters around that mean, whereas *A* and *E* do not affect the mean of fibre diameters, but instead affect the distribution of fibre diameters around a mean. This might suggest that mean fibre diameter and the two fibre diameter distribution traits (CVFD and FDSD) can be independently selected for, despite these traits being correlated.

CFW and MFD are two of the most economically important traits in wool production systems. There have been some studies reporting a positive correlation between these two traits (Wuliji et al., 2001; Safari et al., 2007; Huisman and Brown, 2009), implying that selecting for either CFW or MFD may lead to a change in the other trait. However, this study suggests that despite variation in *KRT83* having an association with variation in MFD, CVFD and FDSD, there is not an association observed with CFW. This suggests that selecting for improvements in MFD, CVFD and FDSD may be possible, without having a negative impact on fleece production. While this would require further testing in more sheep of differing breeds, genders and ages, it is consistent with the study of Gong et al. (2015), and it is supported by the Trangie QPLU$ project (CSIRO, Australia) which reveals that both CFW and MFD can be improved concurrently using genetic selection (Mortimer et al., 2006).

The associations detected in this study are in agreement with previous findings that revealed QTL for MFD (Bidinost et al., 2008), CVFD (Allain et al., 1998; Allain et al., 2013) and GFW (Allain et al., 2006) on chromosome 3. The effect of the variation in *KRT83* on wool traits is also somewhat similar to the effects reported for *KRTAP1-2* (Gong et al., 2015), *KRTAP6-1* (Zhou et al., 2015) and *KRTAP22-1* (Li et al., 2017) despite these *KRTAPs* being located on other chromosomes. This would sugguest that key wool traits are controlled by multiple genes and not indivual *KRTs* and *KAPs*. Further study is therefore required to assess how groups of genes may work together to affect wool traits.

Conflict of Interest Statement

The authors have declared that no conflict of interest exists.

Dr Huitong Zhou
(for the authors)

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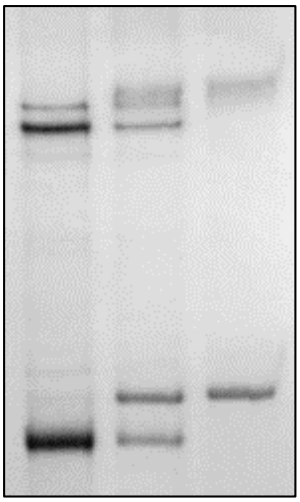
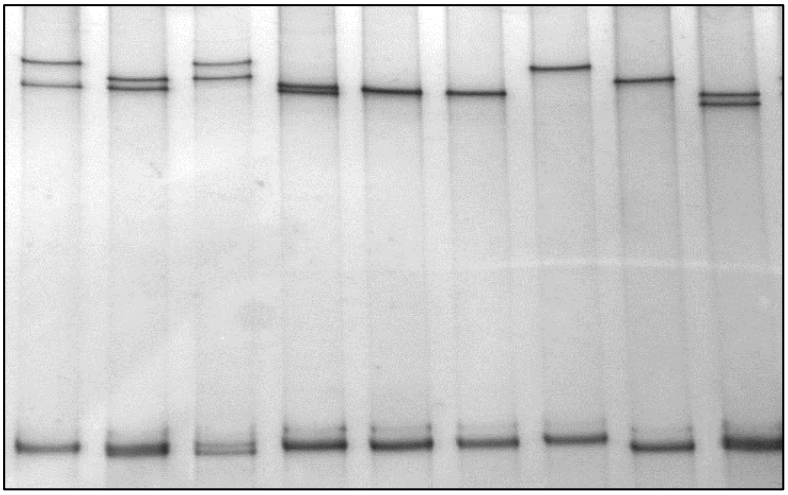
Exon 2



a/*a* *a*/*b* *b*/*b*

Exon 3-4



A/B *A/C* *B/C* *A/D* *A/A* *A/A* *B/B* *C/C* *A/E*

	c.385-23
Variant *a*	G
Variant *b*	C

	c.655-64	c.655-61	c.655-22	c.666	c.672	c.750+45
Variant *A*	T	G	C	C	C	C
Variant *B*	-	G	C	T	C	C
Variant *C*	T	G	T	C	T	C
Variant *D*	T	G	C	C	C	T
Variant *E*	T	A	C	C	C	C

Figure 1. Variation identified in ovine *KRT83*. Two PCR-SSCP patterns corresponding to two (*a* and *b*) variant sequences were identified in the exon 2 region, while five PCR-SSCP patterns responding to five (*A* to *E*) variant sequences were identified in an exon 3-4 region. Only the nucleotide sequences that differ between variants are shown, and nucleotide positions refer to GenBank accession no. NC_019460 following the nomenclature described in www.hgvs.org/mutnomen/.

Table 1. The primer sequence and PCR-SSCP conditions for analysis of three regions of ovine *KRT83*.

Gene region		Primer binding coordinates[1]	Primer sequence (5'-3')	Predicted amplicon size (bp)	PCR annealing temperature (℃)	SSCP gel electrophoresis conditions[2]
Promoter	up	133717044-133717063	AGAGGCAATTAGGAGTGTGG	520	63	280 V, 14%, 20 ℃
	dn	133717563-133717544	GTGAGTGGTCAGTTATGTCC			
Exon 2	up	133721248-133721267	ATCCTGGGTCTTGGTGGTG	371	63	320 V, 14%, 4 ℃
	dn	133721618-133721601	CACAGGTCTGGACGCTCC			
Exon 3-4	up	133722529-133722549	CATGGTCAAGTTTAGAGTTGC	438	58	220 V, 14%, 20 ℃
	dn	133722966-133722947	CTCTGCCTAACCCTTCAGTC			
	up	133722643-133722662	AGGTAAGTGCGTGAGCCAAG	324	58	220 V, 14%, 20 ℃
	dn	133722966-133722947	CTCTGCCTAACCCTTCAGTC			

[1] Primer binding coordinates refer to the Oar v4.0 sequence NC_019460.

[2] Voltage, V; Acrylamide gel percentage, %; Electrophoresis temperature, ^{o}C.

Table 2. Association of *KRT83* exon3-4 variants with various wool traits[1]

Trait[2]	Variant			Single-variant model			Multi-variant model			
		Absent n	Present n	Absent (Mean ± SE)	Present (Mean ± SE)	*P*	Other variant fitted	Absent (Mean ± SE)	Present (Mean ± SE)	*P*
GFW	*A*	60	429	2.29 ± 0.11	2.28 ± 0.09	0.883	*D*	2.25 ± 0.11	2.27 ± 0.09	0.744
(kg)	*B*	386	103	2.67 ± 0.09	2.32 ± 0.10	0.307	*D*	2.26 ± 0.09	2.31 ± 0.10	0.200

	C	417	72	2.28 ± 0.09	2.29 ± 0.10	0.860	*D*	2.26 ± 0.09	2.30 ± 0.10	0.496
	D	369	120	*2.24 ± 0.10*	*2.31 ± 0.10*	*0.099*				
	E	457	32	2.28 ± 0.09	2.26 ± 0.12	0.830	*D*	2.27 ± 0.09	2.26 ± 0.11	0.948
CFW	*A*	60	429	1.69 ± 0.09	1.73 ± 0.08	0.448				
(kg)	*B*	386	103	1.71 ± 0.08	1.74 ± 0.08	0.508				
	C	417	72	1.73 ± 0.08	1.69 ± 0.09	0.477				
	D	369	120	1.72 ± 0.08	1.73 ± 0.08	0.770				
	E	457	32	1.72 ± 0.08	1.74 ± 0.09	0.798				
Yield	*A*	60	429	*74.5 ± 1.55*	*76.0 ± 1.37*	*0.077*	*C,D*	74.1 ± 1.33	74.6 ± 1.15	0.563
(%)	*B*	386	103	75.8 ± 1.37	75.6 ± 1.46	0.709	*A,C,D*	74.6 ± 1.19	73.9 ± 1.27	0.358
	C	417	72	**76.0 ± 1.36**	**74.4 ± 1.52**	**0.041**	*A,D*	**75.3 ± 1.18**	**73.4 ± 1.28**	**0.020**
	D	369	120	**76.6 ± 1.39**	**74.9 ± 1.39**	**0.005**	*A,C*	**75.3 ± 1.20**	**73.4 ± 1.21**	**0.004**
	E	457	32	75.7 ± 1.37	77.0 ± 1.69	0.209	*A,C,D*	74.3 ± 1.17	75.3 ± 1.52	0.345
MFD	*A*	60	429	**19.5 ± 0.46**	**18.9 ± 0.40**	**0.008**	*C,D*	*19.8 ± 0.40*	*19.5 ± 0.34*	*0.174*
(µm)	*B*	386	103	19.0 ± 0.41	18.9 ± 0.43	0.708	*A,C,D*	19.7 ± 0.36	19.7 ± 0.38	0.996
	C	417	72	**18.8 ± 0.40**	**19.8 ± 0.45**	**<0.001**	*A,D*	**19.2 ± 0.35**	**20.1 ± 0.38**	**<0.001**
	D	369	120	**18.8 ± 0.41**	**19.2 ± 0.41**	**0.024**	*A,C*	**19.4 ± 0.36**	**19.9 ± 0.36**	**0.019**
	E	457	32	19.0 ± 0.41	18.7 ± 0.50	0.396	*A,C,D*	19.7 ± 0.35	19.4 ± 0.45	0.340
FDSD	*A*	60	429	**4.33 ± 0.17**	**4.03 ± 0.15**	**0.001**	*C,D,E*	**4.31 ± 0.15**	**4.02 ± 0.14**	**0.004**
(µm)	*B*	386	103	4.06 ± 0.15	4.11 ± 0.16	0.441	*A,C,D,E*	4.17 ± 0.14	4.16 ± 0.15	0.984
	C	417	72	**4.04 ± 0.15**	**4.26 ± 0.17**	**0.011**	*A,D,E*	*4.09 ± 0.14*	*4.24 ± 0.15*	*0.102*
	D	369	120	*4.01 ± 0.15*	*4.13 ± 0.15*	*0.092*	*A,C,E*	4.13 ± 0.14	4.21 ± 0.14	0.243
	E	457	32	*4.08 ± 0.15*	*3.90 ± 0.18*	*0.112*	*A,C,D*	**4.29 ± 0.13**	**4.04 ± 0.17**	**0.034**
CVFD	*A*	60	429	**22.2 ± 0.65**	**21.3 ± 0.58**	**0.014**	*B,E*	**21.9 ± 0.36**	**20.9 ± 0.27**	**0.010**
	B	386	103	21.3 ± 0.58	21.7 ± 0.62	0.182	*A,E*	21.4 ± 0.26	21.5 ± 0.33	0.789
	C	417	72	21.4 ± 0.58	21.5 ± 0.65	0.796	*A,B,E*	21.5 ± 0.26	21.3 ± 0.40	0.557
	D	369	120	21.4 ± 0.59	21.4 ± 0.59	0.823	*A,B,E*	21.5 ± 0.27	21.4 ± 0.32	0.732
	E	457	32	*21.5 ± 0.13*	*20.8 ± 0.43*	*0.087*	*A,B*	**21.9 ± 0.19**	**20.9 ± 0.44**	**0.035**
MSL	*A*	60	429	80.9 ± 3.07	82.8 ± 2.71	0.265				
(mm)	*B*	386	103	82.3 ± 2.72	83.3 ± 2.89	0.480				

	C	417	72	82.8 ± 2.71	80.9 ± 3.02	0.240				
	D	369	120	82.7 ± 2.77	82.3 ± 2.78	0.764				
	E	457	32	82.5 ± 2.71	83.2 ± 3.36	0.583				
MSS	*A*	60	429	25.8 ± 2.20	24.4 ± 1.95	0.257	*B,D,E*	*27.3 ± 2.01*	*25.1 ± 1.86*	*0.121*
(N/ktex)	*B*	386	103	*24.3 ± 1.95*	*25.5 ± 2.08*	*0.199*	*D,E*	25.3 ± 1.77	26.5 ± 1.97	0.249
	C	417	72	24.7 ± 1.95	24.1 ± 2.17	0.645	*B,D,E*	25.9 ± 1.81	25.7 ± 2.08	0.876
	D	369	120	*25.2 ± 1.98*	*23.9 ± 1.99*	*0.142*	*B,E*	*26.5 ± 1.83*	*25.2 ± 1.89*	*0.145*
	E	457	32	*24.5 ± 1.94*	*26.4 ± 2.40*	*0.192*	*B,D*	*24.8 ± 1.66*	*26.9 ± 2.20*	*0.157*
PF (%)	*A*	60	429	**3.08 ± 0.81**	**1.86 ± 0.71**	**0.007**	*C,D*	*3.45 ± 0.41*	*2.70 ± 0.27*	*0.110*
	B	386	103	2.00 ± 0.72	2.17 ± 0.77	0.621	*A,C,D*	3.01 ± 0.28	3.23 ± 0.38	0.556
	C	417	72	**1.89 ± 0.71**	**2.97 ± 0.80**	**0.010**	*A,D*	**2.52 ± 0.24**	**3.63 ± 0.41**	**0.010**
	D	369	120	**1.67 ± 0.73**	**2.41 ± 0.73**	**0.024**	*A,C*	**2.70 ± 0.27**	**3.44 ± 0.35**	**0.030**
	E	457	32	2.06 ± 0.72	1.63 ± 0.89	0.443	*A,C,D*	3.11 ± 0.26	2.65 ± 0.58	0.412
MFC	*A*	60	429	91.8 ± 3.75	90.1 ± 3.32	0.418	*C*	97.3 ± 3.32	96.4 ± 2.85	0.679
(°/mm)	*B*	386	103	90.8 ± 3.33	88.8 ± 3.54	0.227	*C*	96.9 ± 2.84	95.4 ± 3.12	0.386
	C	417	72	**89.7 ± 3.30**	**94.6 ± 3.68**	**0.011**				
	D	369	120	89.5 ± 3.39	91.2 ± 3.39	0.251	*C*	96.0 ± 2.86	97.9 ± 3.00	0.211
	E	457	32	90.4 ± 3.31	89.0 ± 4.11	0.572	*C*	96.6 ± 2.82	96.4 ± 3.78	0.946

1 Estimated marginal means and standard errors (SE) derived from the GLMMs. $P < 0.05$ are in bold, whereas $0.05 \leq P < 0.20$ are italicised.

2GFW - greasy fleece weight; CFW - clean fleece weight; Yield - wool yield; MFD - mean fibre diameter; FDSD - fibre diameter standard deviation; CVFD - coefficient of variation of fibre diameter; MSL- mean staple length; MSS - mean staple strength; PF - prickle factor (the percentage of fibres of diameter greater than 30 microns); MFC- mean fibre curvature.