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Supplementary Material S1- Detailed methods

Manuscript Title: Characterization of white and black merino wools: a proteomics study

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Wool diameter and curvature determinations, scouring, protein extraction and quantification

The mean fibre diameter and curvature of the white and black merinos were determined by an optical fibre diameter analyser OFDA 2000 (BSC electronics, Ardross, WA, Australia) at the New Zealand Wool Testing Authority (Napier, New Zealand) following the manufacturer's instructions. Extraneous dirt and sebum was removed from the wool fibres from each animal by washing first with 0.15% Teric GN9 twice for 2 min each time at 60°C, followed by washing twice with water for 2 min each time at 60°C. The fibres were air dried overnight and then washed twice with dichloromethane, twice with ethanol and twice with water for 30 sec each time before being snipped to a powder with scissors. One sample was prepared by mixing an equal weight of powdered fibre from each of 15 white merinos, while a second sample was prepared by mixing an equal weight of fibre powder from 15 of the black merinos. From these two composite samples, four 10 mg replicates of each sample were shaken for 18 hrs with 1 mL of 8 M urea, 50 mM triethylamonium bicarbonate buffer (TEAB) and 50 mM dithiothreitol at pH 9.3. The wool protein extracts were quantitated using a 2DQuant kit (GE Healthcare Lifesciences, Auckland, New Zealand) following the manufacturer's instructions.

Isobaric tag for relative and absolute quantitation study

The methodology used for the iTRAQ analysis has been used by our group (Almeida et al., 2014). An aliquot equivalent to 250 µg of protein was taken from each sample and precipitated using the methanol/chloroform procedure, before resuspension in 0.5 M TEAB. The extracts were reduced with 0.1 M tris(2-carboxyethyl)phosphine, alkylated with 20 µL of 0.15 M iodoacetamide and digested for 18 hrs with 10 µg of TPCK-trypsin (Promega, Madison, WI, USA). Next they were dried down, resuspended in 0.5 M TEAB and labeled with an iTRAQ® 8 Plex Multiplex kit (ABSciex, Foster City, CA, USA). The iTRAQ-labelled peptide digests were combined into one pooled sample, as previously

described (Almeida et al., 2014) and using standard methodology used in iTRAQ-based wool proteomics (Almeida et al., 2014; Li et al., 2018). Samples were then resuspended in 0.1% formic acid (FA), bound to a C18 Empore™ disk, eluted with 50% acetonitrile (AcN) in 0.1% FA and dried down. The labelled peptide extract was then reconstituted in 0.1% FA and separated on a 5 µM BioX-SCX column (Dionex, Auckland, NZ) into 10 fractions with 0, 1, 5, 10, 20, 40, 60, 80 and 100% 2 M ammonium formate in 2% AcN. The iTRAQ labeled peptides from each fraction were bound to a C18 Empore™ disk, eluted with 50% acetonitrile (AcN) in 0.1% FA, dried down and reconstituted in 0.1% FA. LC–MS/MS was performed on a nanoAdvance UPLC coupled to a maXis impact mass spectrometer equipped with a CaptiveSpray source (Bruker Daltonik, Bremen, Germany). A C18AQ nanotrap (Bruker, 75 µm × 2 cm, C18AQ, 3 µm particles, 200 Å pore size) was loaded with 2 µL of sample. The trap column was then switched in line with the analytical column (Bruker Magic C18AQ, 100 µm × 15 cm C18AQ, 3 µm particles, 200 Å pore size). The column oven temperature was 50°C. Elution was with a gradient from 0% to 40% B (98% LCMS-grade ACN, 0.1% FA) in 90 min at a flow rate of 800 nL/min. Solvent A was LCMS-grade water with 0.1% FA and 1% ACN. Samples were measured in automatic MS/MS mode, with a mass range of m/z 50–2200. For each MS collected, 10 MS/MS of the most intense ions in that mass spectrum were also collected. Acquisition speed was 2 Hz in MS mode and 10 or 5 Hz in MS/MS mode depending on precursor intensity. Precursors were selected in the m/z 400–1400 range, with charge states 2–5 (singly charge ions were excluded). Active exclusion was activated after one spectrum for 0.3 min. Peak lists from the 10 BioX-SCX column fractions were concatenated using ProteinScape 3.1.0 rev3 (Bruker Daltonik, Bremen, Germany) and then queried against O. aries entries in the NCBI nr database using the Mascot search engine (v2.4, Matrix Science) maintained on an in-house server. The Mascot search parameters included semitrypsin as the proteolytic enzyme with two missed cleavages; with a taxonomy of O. aries, standard modifications being carbamidomethylation (C), deamidation (NQ) and oxidation (M); monoisotopic peptides with charges of +1, +2 or +3, and peptide mass tolerance was set to 0.025 Da and 0.15 Da for fragment mass tolerance.

Statistical Analysis

Overall, the study included two groups: Black Merino wool and White Merino wool. Results for wool diameter and curvature were compared using ANOVA-single factor proceedings of SAS 9.4 (SAS, 1991). For the iTRAQ experiment results statistical analysis we have used an approach of standard use (Almeida et al., 2014). The quantitative results generated by the Mascot search engine (v2.4, Matrix Science) were reported in terms of the mean black:white ratio of each identified protein from the four technical repeats, the number of labelled peptides used in obtaining this mean and the geometric standard deviation. For ease of interpretation, given that the geometric standard deviation is a multiplication factor and hence dimensionless, the 95% confidence intervals for each mean were also determined. Because of the high sequence homology

among the keratins and KAPs the results were assessed in terms of the incorporation of all peptides for each protein in the analysis and unique peptides only for only those proteins that appeared in all four replicates.