Influence of weight loss on the wool proteomics profile: a combined iTRAQ and fiber structural study

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Introduction

There are numerous animal natural fibers with economic interest: *mohair*, *cashmere*, *qiviut*, etc.

• Wool is the most important natural occurring fiber;
• WOOL however occurs only in sheep (*Ovis aries*) with highest commercial value in the merino breed;
• Economic relevance in Australia, NZ, ZA, Argentina and Southern Europe
Introduction

• Wool is one of the major agricultural products of Australia and New Zealand, who are two of the most important exporters in the world;

• Since 1834 Australia’s wool production is based on extensive farming systems / ranching, based on “stations” many of which are located on semi-desert rangelands;

• Briefly the animals are left on station land and are gathered once a year for shearing or other purposes;

• Shearing is done during the early phases of the dry season but in more remote areas it is often conducted at later stages when the animal is subjected to SWL – Seasonal Weight Loss

• SWL is the major drawback in Animal Production in Arid regions
Introduction

Wool structure: organized in fibrils made of keratin family proteins, HGTP – High Glycine-Tyrosine Proteins and HSP – High Sulphur Proteins

Fig. 1. Schematic diagram of a wool fibre showing the major structural features found in the cortical cells. (Reproduced with the permission of CSIRO Textile and Fibre Technology).

(Plowman, 2003)
Plowman et al. (2000) described the wool proteome in 2D gels for the first time

The wool proteome is relatively simple as it is made essentially of keratin and keratin associated proteins – 4 major classes

Type II keratin
Type I keratin
HSP – High Sulphur Proteins
HGTP – High Glycine Tyrosine Proteins
Objectives

• Study the effects of experimentally induced weight loss on wool protein profiles in Australian Merino ram lambs;

• Approach combining isobaric tags for relative and absolute quantitation (iTRAQ);

• Combined to a study on changes in wool fiber diameter and curvature

Determine if nutritional stress affects wool protein expression

Assess SWL influence on wool properties from both a physiological and a commercial point of view.
Basic Design Overview – Proteomics of sheep tolerance to seasonal weight loss

Normal Nutritional Level

Low Nutritional Level

UP Regulated / Down Regulated proteins????

MS Protein Identification
Material and Methods

- Two experimental groups – Restricted and Control;
- Animals fed on pellets;
- Restricted Fed animals lost 15% initial body weight;
- Control Animals had an increase of 8-10 % IBW;
- \( n = 12 \);
- At day 42, 100 cm\(^2\) were shaven from the left scapula and separated into staples;
- Focus on lower 5mm, expected to have grown during the trial.
Material and Methods

Normal Nutritional Level

Microscopy study on wool curvature and diameter

Staples cut into snippets

Wool Scouring

Protein extraction and quantification

iTRAQ study
Material and Methods: iTRAQ study

• Protein extracts were reduced, alkylated and Digested with Trypsin
• Peptide extracts labelled with iTRAQ® 8 Plex Multiplex kit
• Extracts separated on a 5 μM BioX-SCX column
• LC–MS/MS was performed on a nanoAdvance UPLC coupled to a maXis impact mass spectrometer equipped with a CaptiveSpray source
• Peak lists concatenated using ProteinScape 3.1.0 rev3
• Search against *O. aries* entries in the NCBI database using the Mascot search engine
Results and Discussion
Wool curvature & Diameter

- Restricted group: median wool curvature of 123°/mm
- Control group: median wool curvature of 121°/mm

- Restricted group: wool fiber diameter of 18.1 μm
- Control group: wool fiber diameter of 19.0 μm
Results and Discussion - iTraq Experiment

Table 1 – The relative quantities of protein between the control and restricted diet Merino sheep.

<table>
<thead>
<tr>
<th>Description</th>
<th>Accession no.</th>
<th>Score</th>
<th>No. of matches</th>
<th>Control: Restricted</th>
<th>No. of pept</th>
<th>SD (geo)</th>
<th>95% confid interval</th>
<th>Unique peptides only for quantitation</th>
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<tr>
<td>K1</td>
<td>g[125090</td>
<td>3213</td>
<td>221</td>
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<td>115</td>
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<td>[0.94–1.200]</td>
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<td>61</td>
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<td>26</td>
<td>1.085</td>
<td>[1.012–1.200]</td>
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<td>K3</td>
<td>g[125091</td>
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<td>184</td>
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<td>87</td>
<td>1.07</td>
<td>[1.001–1.151]</td>
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<td>K3a</td>
<td>g[81238059</td>
<td>2702</td>
<td>218</td>
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<td>110</td>
<td>1.099</td>
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<td>85</td>
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<td>K9</td>
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<td>K6</td>
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<td>604</td>
<td>124</td>
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<td>K6-like #</td>
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<td>KAP3.2</td>
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<td>HCT keratin</td>
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<td>38</td>
<td>0.802</td>
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<td>KAP3.2*</td>
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<td>7</td>
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<td>0.786</td>
<td>3</td>
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<td>1.051</td>
<td>2</td>
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<td>[0.975–1.118]</td>
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</tbody>
</table>

Note: * HCT keratin (AA1–62) is a member of the KAPs family of proteins. K — Keratin; KAPs — keratin associated proteins. # 94.3% identity with K86. Identifications shown indicate the case when all the peptides or only the unique peptides in each protein are taken into consideration. Significant results are indicated in bold type. Accession numbers beginning with 999 all refer to an internal AgResearch database of Ovis aries wool proteins. From left to right in the table the results are reported in terms of the Mascot score for the protein identification, the number of matches to identify the protein; and in the two sections of All peptides and Unique peptides: the quantitation ratio of the control: fed restricted animals; the number of peptides used in the quantitation, the geometric standard deviation (the exponential value of the standard deviation of the log transformed values) and the reliability of the quantitation ratio based on its 95% confidence limits.
Major Findings

• Seasonal weight loss caused by poor pasture availability has strong effects on wool productivity parameters and quality traits;

• Seasonal weight loss causes a decrease in fiber diameter;

• Seasonal Weight loss causes an increase in HSP - High Sulfur Protein \textbf{KAP13.1} expression;

• Seasonal Weight loss causes an increase in \textbf{HGTP - High Glycine–Tyrosine Protein KAP6 family}

• Fiber Reduced Prickle;

• Reduction in wearability and appearance retention

\textbf{Commercial Implications}
Influence of feed restriction on the wool proteome: A combined iTRAQ and fiber structural study

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