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

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Copenhagen, Denmark
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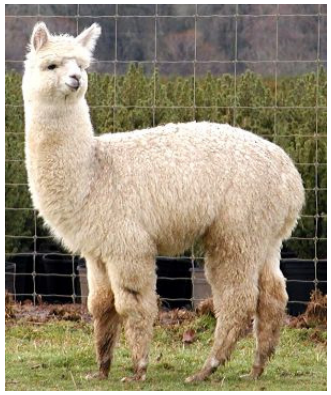
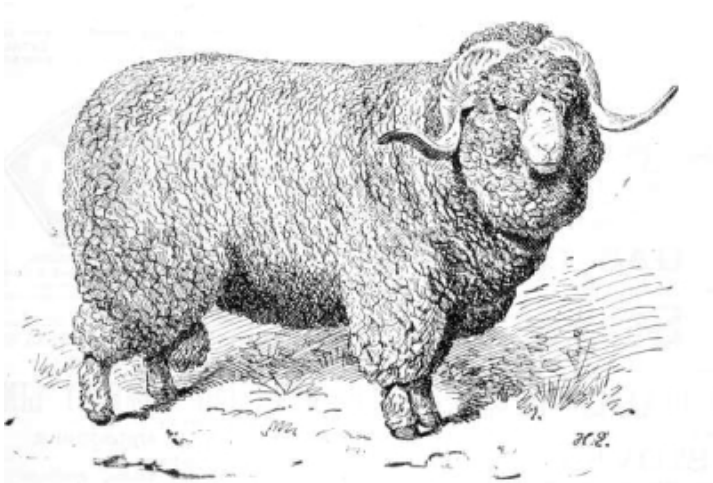
Introduction

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**

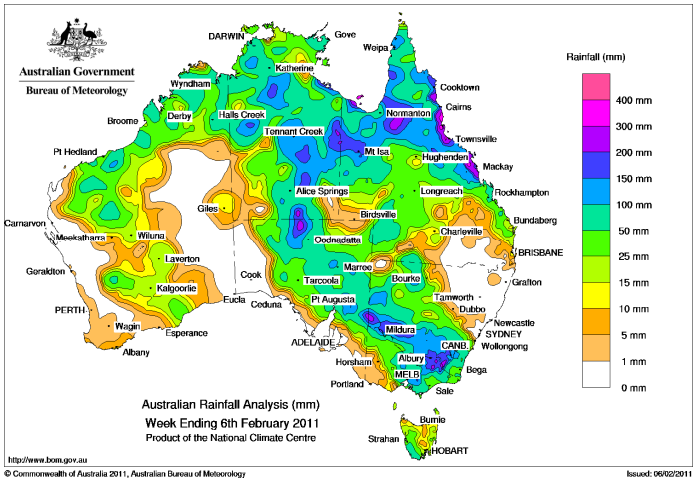


CHAMPION MERINO RAM, THE PROPERTY OF MESSRS. WM. CUMMING AND SON, Mount Fynns, Darlington.

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

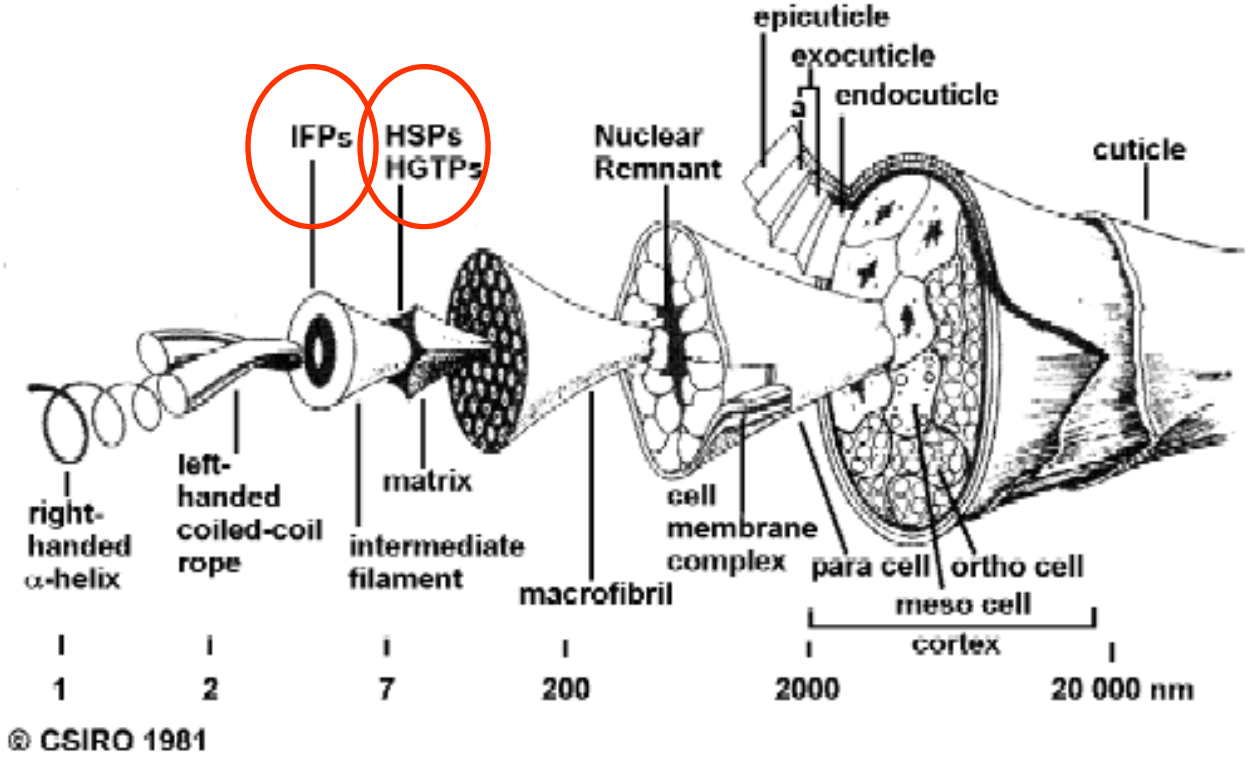
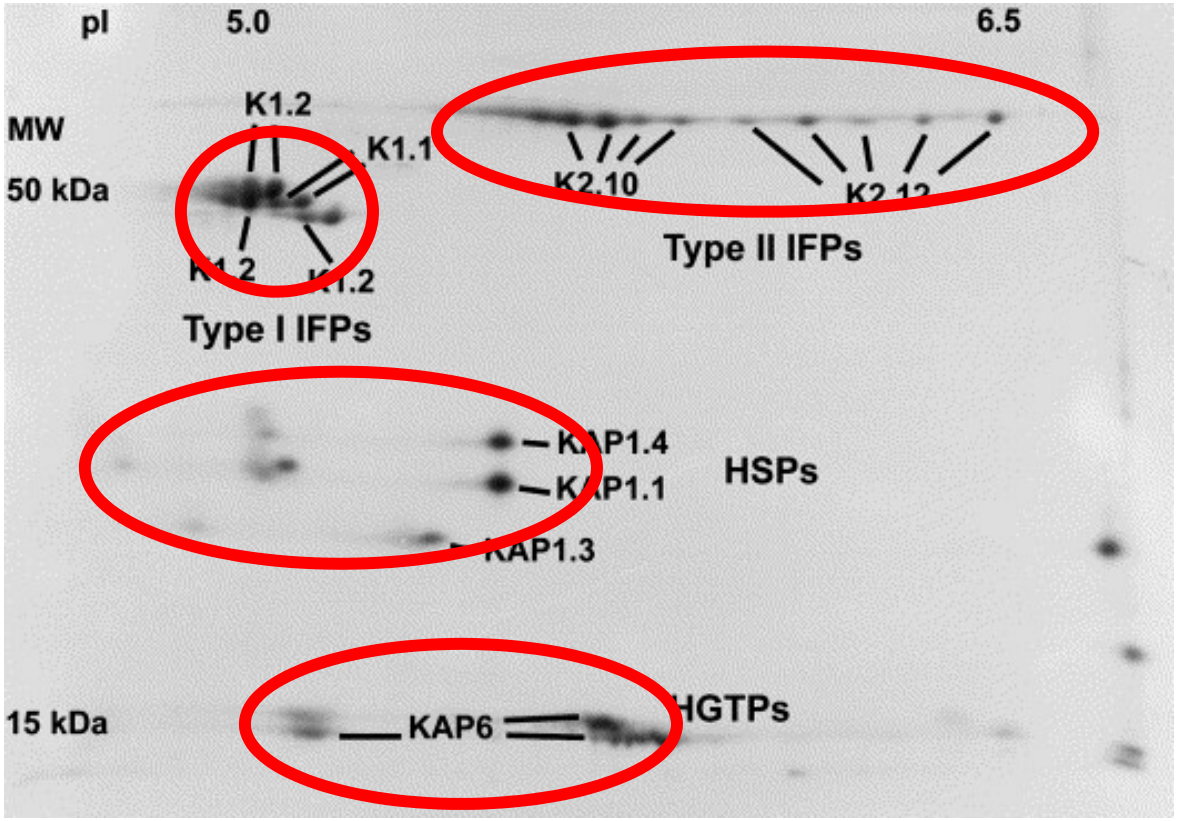


Fig. 1. Schematic diagram of a wool fibre showing the major structural features found in the cortical cells. (Reproduced with the permission of CSIRO Textile and Fibre Technology).

(Plowman, 2003)

Introduction

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



Type II keratin

Type I keratin

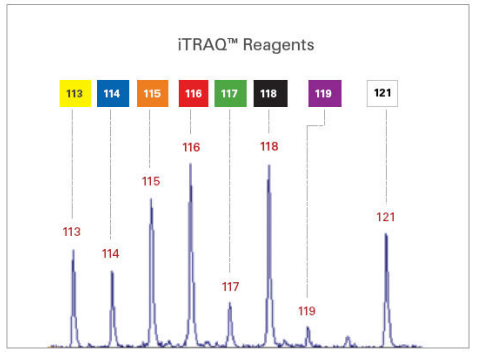
HSP – High Sulphur Proteins

HGTP – High Glycine Tyrosine Proteins

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

Objectives

Quantitation



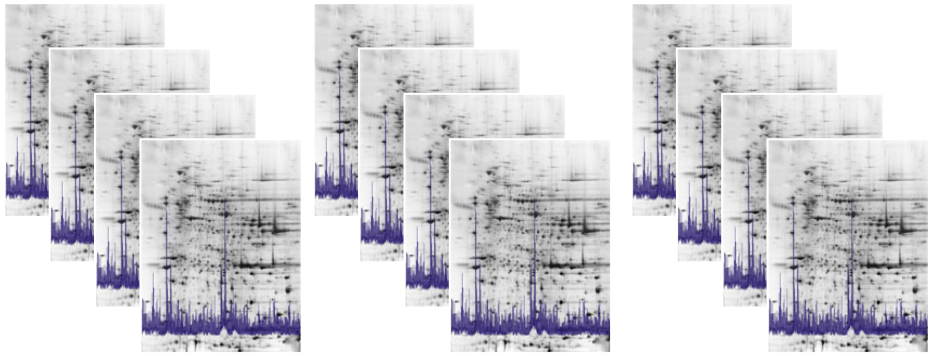
- Study the effects of experimentally induced weight loss on wool protein profiles in Australian Merino ram lambs;
- Approach combining isobaric tags for relative and absolute quantitation (iTRAQ);
- Combined to a study on changes in wool fiber diameter and curvature

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



UP Regulated / Down Regulated proteins????

MS Protein Identification

Low Nutritional Level

Material and Methods

Small Ruminant Research 109 (2013) 101–106

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Live weight parameters and feed intake in Dorper, Damara and Australian Merino lambs exposed to restricted feeding

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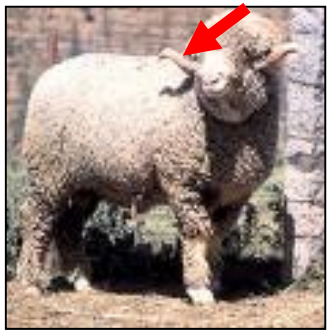
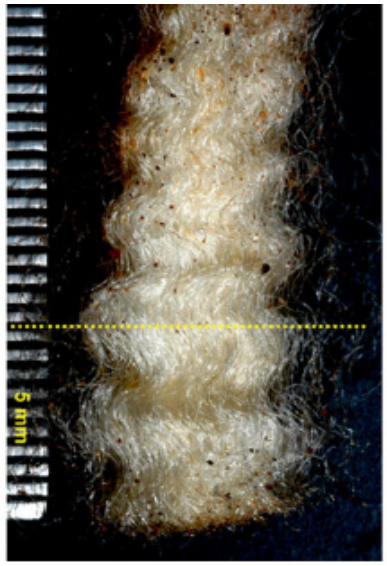
- Two experimental groups – Restricted and Control;
- Animals fed on pellets;
- Restricted Fed animals lost 15% initial body weight;
- Control Animals had an increase of 8-10 % IBW;
- n = 12;
- At day 42, 100 cm^2 were shaven from the left scapula and separated into staples;
- Focus on lower 5mm, expected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

Material and Methods

Normal Nutritional Level



Low Nutritional Level



Microscopy study on wool curvature and diameter

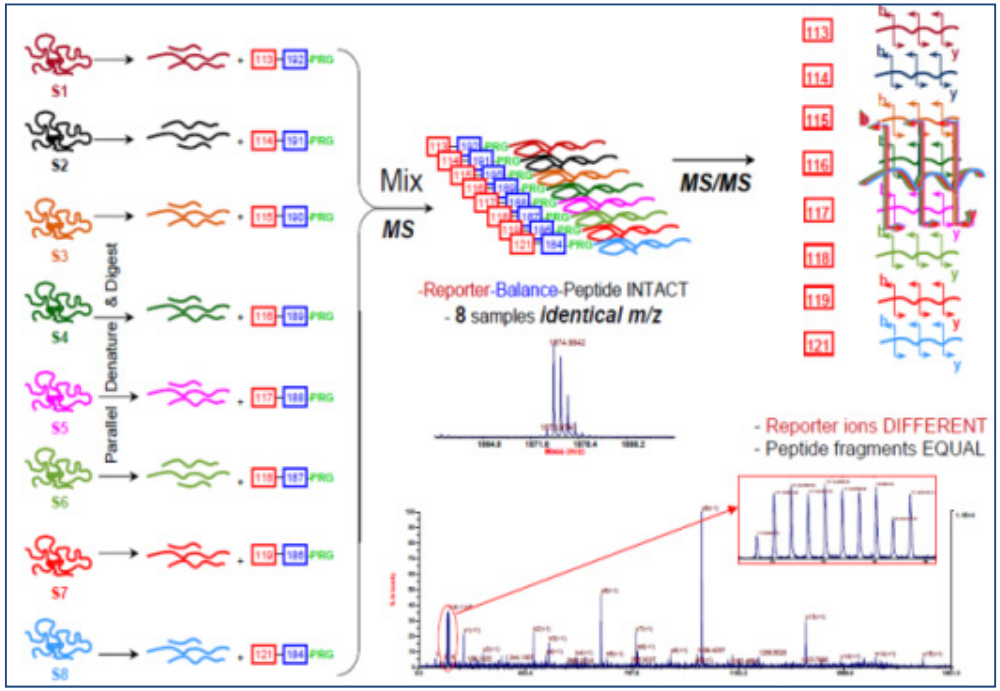
Staples cut into snipets

Wool Scouring

Protein extraction and quantification

iTRAQ study

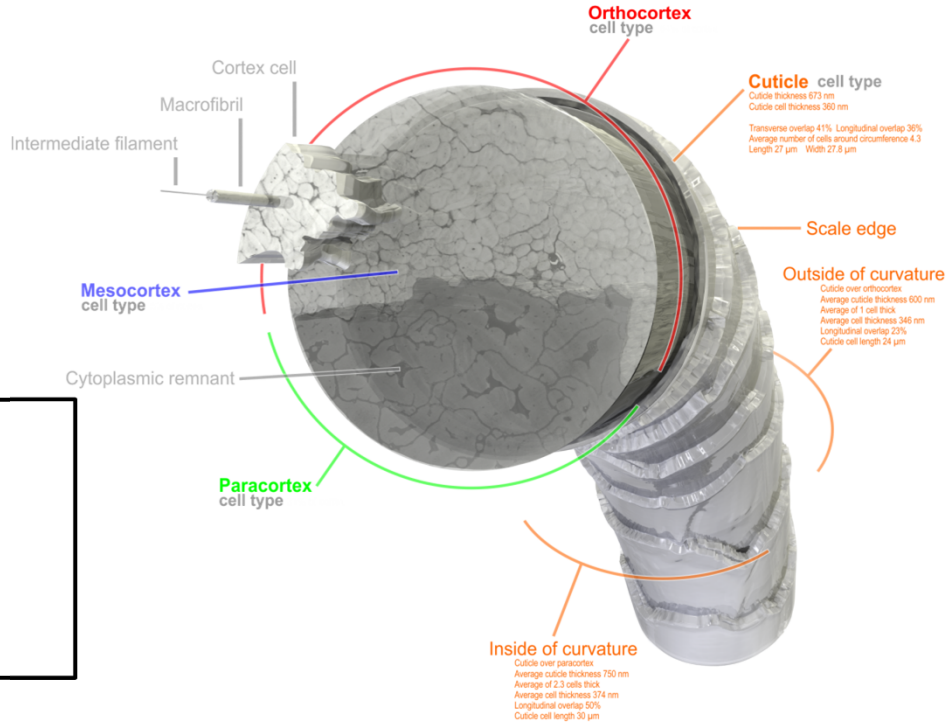
Material and Methods: iTRAQ study



- 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

Keratin

KAP – Keratin Associated Proteins

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

Description	Accession no.	Score	No. of matches	All peptides used for quantitation				Unique peptides only used for quantitation			
				Control: Restricted	No. of pept	SD (geo)	95% confid interval	Control: Restricted	No. of pept	SD (geo)	95% confid interval
K31	gi|125090	3213	221	1.071	115	1.12	[0.94–1.200]	1.066	11	1.423	[0.975–1.157]
K32	gi|426238005	337	61	**1.106**	26	1.085	[1.012–1.200]	–	–	–	–
K33a	gi|125091	2023	184	1.076	87	1.07	[1.001–1.151]	1.008	7	1.172	[0.844–1.181]
K33b	gi|312283590	2702	218	1.074	110	1.099	[0.968–1.180]	1.01	9	1.155	[0.853–1.167]
K34	gi|309323371	2043	169	1.071	85	1.134	[0.927–1.214]	1.023	20	1.247	[0.771–1.275]
K35	gi|168693435	1073	85	**1.06**	41	1.079	[0.976–1.144]	0.939	10	1.115	[0.831–1.046]
K81	gi|1308	2914	288	1.02	135	1.081	[0.937–1.102]	0.846	7	1.226	[0.655–1.037]
K83	gi|312283588	2278	278	1.015	120	1.107	[0.933–1.097]	**0.928**	30	1.073	[0.860–0.996]
K85	gi|246276	1217	180	1.032	76	1.082	[0.929–1.117]	1.16	2	1.154	[0.981–1.339]
K86	gi|426224446	604	124	**1.021**	42	1.049	[0.971–1.071]	1.037	2	1.152	[0.879–1.194]
K86-like #	gi|312283584	2849	288	1.016	135	1.086	[0.935–1.103]	0.846	7	1.226	[0.69–1.037]
KAP3.2	gi|165917	57	2	1.016	3	1.153	[0.960–1.072]	1.016	2	1.153	[0.960–1.072]
KAP3.3	gi|125661	106	1	0.896	2	1.308	[0.620–1.172]	0.896	1	1.308	[0.620–1.172]
KAP6	gi|224556010	245	11	**0.545**	5	1.235	[0.417–0.673]	–	–	–	–
KAP6.3 var1	gi|404313479	198	10	**0.545**	5	1.235	[0.417–0.673]	–	–	–	–
KAP6.2	gi|404313479	198	8	**0.544**	5	1.235	[0.417–0.673]	–	–	–	–
HGT keratin (AA1–62)*	gi|1304	119	38	**0.802**	17	1.079	[0.739–0.865]	**0.802**	17	1.079	[0.739–0.865]
KAP8.2	gi|999000029	186	19	0.996	7	1.045	[0.951–1.041]	0.996	7	1.045	[0.951–1.041]
KAP13.1	gi|999000030	478	26	**0.782**	5	1.042	[0.749–0.815]	**0.782**	5	1.042	[0.749–0.815]
KAP16.2	gi|999000035	77	10	0.736	3	2.261	[−0.192–1.664]	0.736	3	2.261	[−0.192–1.664]
KAP19.3	gi|999000016	234	19	0.9	4	1.379	[0.559–1.241]	0.808	2	1.118	[0.713–0.903]
KAP19.6	gi|999000034	185	9	0.782	4	1.464	[0.363–1.145]	0.63	2	1.17	[0.523–0.737]
K14	gi|426238017	57	14	1.051	2	1.069	[0.978–1.124]	–	–	–	–
K75	gi|999000081	66	40	1.021	5	1.118	[0.901–1.141]	1.17	4	1.147	[0.998–1.342]

Note: * HGT keratin (AA1–62) is a member of the KAP6 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:feed-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 **KAP13.1** expression;

• Seasonal Weight loss causes an increase in **HGTP - High Glycine–Tyrosine Protein KAP6 family**

• **Fiber Reduced Prickle;**

• **Reduction in wearability and appearance retention**

Commercial Implications

For further information …

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Influence of feed restriction on the wool proteome: A combined iTRAQ and fiber structural study

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