Follicle characteristics, seasonal changes in fibre cross-sectional area and ellipticity in Australasian specialty carpet wool sheep, Romneys and Merinos

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Abstract

Data was collected on secondary:primary follicle ratio, relative follicle density, and seasonal changes in fibre cross-sectional area and fibre ellipticity (ratio of major-to-minor fibre cross-sectional axis) in the Australasian specialty carpet wool breeds (Carpetmaster, Drysdale, Elliottdale, Tukidale), Romneys and Merinos. Merinos had a higher secondary:primary follicle ratio \((p<0.001)\), relative primary follicle density \((p<0.05)\), relative secondary and relative total follicle density \((p<0.01)\) than the other breeds. Both breed and follicle type (primary or secondary) had a significant effect on fibre cross-sectional area \((p<0.05)\). While both fibre types (primary and secondary) showed changes in cross-sectional area during the experiment, a more distinct seasonal pattern was seen in the secondary fibres with summer maxima and winter minima in all breeds. Relative change in fibre diameter was higher in secondary fibres than in primary fibres. Changes in fibre ellipticity were also noted throughout the duration of the experiment. It is apparent that there are differences between primary and secondary follicle populations in the way the fibres produced contribute to seasonal changes in average fibre diameter of fleeces. The changes suggest there are separate control mechanisms for each follicle type and that the number, size and arrangement of the cells in each type of fibre vary independently of one another throughout the year, bringing about dynamic changes in fibre growth and form. Further work is required to examine the influence of feed quality on these factors and to determine the mechanisms through which these changes in fibre structure are brought about. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Follicle; Carpet wool; Fibre diameter; Ellipticity

1. Introduction

There is considerable variation within, and between, breeds in the characteristics of wool follicles and fibre populations for both Merino and non-Merino genotypes (Carter and Clarke, 1957a,b). The ratio of secondary:primary follicles varies between sheep breeds (Chapman and Ward, 1979) and differences between primary and secondary fibres in medullation and other fibre characteristics have been noted in sheep (Ryder, 1969) and goats (Nixon et al., 1991). An interaction between nutrition and the fibre type grown by follicles has been noted by Short et al. (1958). In addition, the authors reported differences between breeds in the response of primary and secondary follicles to increased levels of feed intake, with...
primary and secondary fibres increasing in cross-sectional area by 16 and 23%, respectively, in the Lincoln, 37 and 54% in the Corriedale, 49 and 81% in the Polwarth, and 33 and 52% in the Merino. These breeds had secondary:primary diameter ratios of 1.23, 1.06, 1.05 and 0.96, respectively. An interaction between level of productivity and follicle type was also found by Williams and Winston (1987), with secondary follicles in a high productivity Merino strain showing a proportionally larger response to increased feed intake than those of a low productivity strain, even though the secondary:primary follicle ratio did not differ between the strains.

Seasonal wool growth patterns are evident in Romneys (Hawker and Crosbie, 1985; Woods and Orwin, 1988), and the specialty carpet wool breeds (Carpetmaster, Drysdale, Elliotdale and Tukidale) (Reid, 1981; Williams, 1981; Sumner, 1983) with summer wool growth rates two-to-three times greater than winter rates (Bigham et al., 1978; Reid, 1981). In Romneys, these seasonal changes are caused by changes of up to 40% in mean fibre diameter and length (Story and Ross, 1960), but no data is available for the specialty carpet wool breeds. While Merino and non-medullated crossbred wool types have near circular fibres (ellipticity of 1.18–1.25), some medullated wool shows considerable fibre ellipticity (Onions, 1962). This pattern also appears to be true within breeds exhibiting considerable fibre diameter variability, with ellipticity values of 1.30 and 1.20 in the fine fibres from Southdown and Wensleydale fleeces, respectively, but 1.50 and 1.54 from the coarse fibres of the same breeds. A similar pattern was reported within Romneys (Woods et al., 1990). This ellipticity may have some effect on fibre strength. Both Woods et al. (1990) and Gourdie et al. (1992) reported a significant negative correlation between single fibre tenacity and fibre ellipticity.

In order to gather base and seasonal data on the follicle and fibre characteristics of the specialty carpet wool breeds, a study was undertaken with the four Australasian specialty carpet wool breeds, and the Romney and Merino breeds, managed under the same conditions for 18 months. The primary objective of the study was to test the hypothesis that seasonal fibre diameter change is brought about by a relatively higher fibre diameter change in the secondary fibres than in primary fibres.

## 2. Materials and methods

### 2.1. Animals and Site

Five sheep (1.5 to 2.5 years) of each of the Carpetmaster, Drysdale, Elliotdale, Tukidale, Romney and fine-wool Merino breeds were selected for skin sampling by stratified randomization based on live weight from experimental flocks of about 20 sheep from each breed. One selected Drysdale died during the experiment and was not replaced. The sheep selected for skin sampling were managed as part of the larger flock (129 sheep) and were set stocked from April 1993 (autumn) in a 6-ha paddock of improved pasture (perennial ryegrass/sub-clover) on a commercial property near Oberon, NSW. Skin was sampled between July 1993 (winter) and September 1994 (spring).

### 2.2. Skin sampling processing, embedding and sectioning

In order to facilitate skin sampling, the sheep were restrained using a ‘Hamilton Sheep Handler’ that had been modified to allow the animal to be held in a horizontal, rather than vertical position. A site just forward of the midside on the right of the animal was clipped and sprayed liberally with a 1% Lignocaine anaesthetic spray. A 1-cm diameter trephine was used to make an incision to the connective tissue beneath the skin. The skin section was raised with forceps and a hand-held scalpel blade used to cut parallel to the skin surface through the connective tissue, removing the skin section. Skin samples were placed in small plastic tissue embedding cassettes. A Shandon tissue processor (Shandon Scientific, Cheshire, UK) was used to process the samples which were embedded in Paraplast tissue embedding medium (Sherwood, St. Louis, MO) using a Shandon Histo-centre II tissue embedding system. Sections were cut using a Microm Model Hm325 rotary microtome (Microm Laborgerate GmbH, Walldorf, Germany) fitted with Feather disposable microtome blades (Feather Safety Razor, Tokyo, Japan).
2.3. Staining

Sections were stained after mounting using a haematoxylin, eosin and picric acid staining technique based on the method of Maddocks and Jackson (1988). The alterations involved a staining in Celestine Blue solution for 5 min, followed by a rinse in water prior to staining in Mayer’s Haemotoxylin, and a subsequent rinse in 0.5% rather than 1% acid alcohol. Additionally, a 1-min wash in 70% alcohol was inserted prior to the staining in acidified alcoholic eosin phloxine. After staining, cover slips were placed over the sections using DPX mounting medium.

2.4. Section analysis

Analysis of the slides was performed on a Macintosh Power PC 6100AV computer using the public domain NIH Image program (written by Wayne Rasband at the U.S. National Institute of Health, available by anonymous FTP from ftp://zippy.nimh.nih.gov). A Galai monochrome CCD camera mounted to an Olympus BH2 light microscope with a motorised stage was used to collect the images. Multiple images were collected at the same time. The microscope stage, controlled by a joystick, was moved through a zigzag pattern similar to that used with projection microscopes. This ensured no follicle or fibre was measured twice. Only follicles or fibres fully within the boundary of the image were analysed, the stage being adjusted slightly, if necessary, to ensure all of a follicle or fibre was within the boundary.

2.5. Relative follicle densities and secondary:primary follicle ratios

For each sheep at least 300 follicles per slide were identified to derive estimates of relative follicle density and secondary:primary ratio. Skin sections collected on 30 August 1993 (winter), were used for these analyses due to the high quality of the sections. Once 300 follicles had been typed, all the follicles on the final image were typed. Primary and secondary follicles were identified through the associated gland structures.

All densities in this study were expressed relative to one another and to a Merino ‘baseline’, assuming a constant shrinkage across all skin samples. As cross-sectional areas were not determined at the time of sampling and as not all samples were circular when processed, the determination of shrinkage by calculation of a conversion factor as suggested by Maddocks and Jackson (1988) was not possible for the sections in this study.

2.6. Fibre diameter changes in primary and secondary fibres over time

Sections taken every 10 weeks were analysed for changes over time in fibre diameter for each of the two follicle types. Twenty images of each skin section were taken in which primary and secondary follicle types could be identified according to the accessory gland structure. These images were then calibrated using a standard slide marked with 10 µm minor increments and 100 µm major increments. Images for the specialty carpet wool breeds and the Romney were collected using the 10× objective, while for ease of measurement, a 20× objective was used with the Merinos. Calibration slides were created for both the 10× and 20× objective. A free-hand measurement tool was used to trace around the circumference of the fibres, upon the completion of which the software calculated the cross-sectional area of the fibre and the lengths major and minor ellipse axes. Fifty primary fibres and 100 secondary fibres were measured.

2.7. Statistical Analysis

A SAS package (SAS, 1989) was used to carry out one-way (breed as the main effect) and two-way analyses of variance. In the two-way analysis, breed and follicle were analysed as main effects and their interaction was examined for statistical significance.

3. Results

3.1. Secondary:primary follicle ratios and relative follicle densities

Merinos had a higher \( p<0.001 \) secondary:primary follicle ratio than all other breeds, between which there were no significant differences (Table 1). Mean breed values for relative primary, secondary and total follicle density are shown in Table 2. Merinos had a
higher relative primary, relative secondary and total follicle density ($p<0.01$) than the other breeds ($p<0.05$).

### 3.2. Changes in the cross-sectional area of primary and secondary fibres over time

There were significant effects of both breed and follicle type on fibre cross-sectional area at each of the sampling dates in the study ($p<0.05$). There were also significant interactions for fibre cross-sectional area between breed and follicle type at each date ($p<0.05$), except for 26 September 1994 (spring). This indicates that the difference in fibre cross-sectional area between follicle types was not the same for all breeds. The primary fibres always had a higher mean cross-sectional area than the secondary fibres.

The change in primary fibre cross-sectional area is shown in Fig. 1. While variation throughout the year was negligible in the Merinos, and minimal in the Romneys, it was considerable in the other breeds. The Carpetmasters showed a distinct increase in cross-sectional area in the summer months as did the Drysdales, although the peak for the Carpetmasters was considerably longer than for the Drysdales. The

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**Table 1**

Mean breed values for secondary:primary follicle ratio (S:P ratio)

<table>
<thead>
<tr>
<th>Breed</th>
<th>S:P Ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carpetmaster</td>
<td>6.82±0.41</td>
</tr>
<tr>
<td>Drysdale</td>
<td>5.82±0.62</td>
</tr>
<tr>
<td>Elliottdale</td>
<td>6.56±0.78</td>
</tr>
<tr>
<td>Merino</td>
<td>29.51±3.79</td>
</tr>
<tr>
<td>Romney</td>
<td>8.59±0.63</td>
</tr>
<tr>
<td>Tukidale</td>
<td>6.72±0.62</td>
</tr>
</tbody>
</table>

$^a$ Means differ at $p<0.01$.

**Table 2**

Relative primary (P Density), secondary (S Density) and total follicle (Follicle Density) densities relative to the Merino (100)

<table>
<thead>
<tr>
<th>Breed</th>
<th>P Density$^a$</th>
<th>S Density$^b$</th>
<th>Follicle Density$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carpetmaster</td>
<td>16.21±1.09</td>
<td>60.02±5.11</td>
<td>14.62±0.99</td>
</tr>
<tr>
<td>Drysdale</td>
<td>16.32±0.73</td>
<td>70.09±5.28</td>
<td>14.38±0.72</td>
</tr>
<tr>
<td>Elliottdale</td>
<td>17.09±0.91</td>
<td>67.07±7.10</td>
<td>15.28±0.9</td>
</tr>
<tr>
<td>Merino</td>
<td>100.00±7.64</td>
<td>100.00±14.94</td>
<td>100.00±7.92</td>
</tr>
<tr>
<td>Romney</td>
<td>20.32±1.54</td>
<td>60.98±5.3</td>
<td>18.85±1.53</td>
</tr>
<tr>
<td>Tukidale</td>
<td>17.92±1.21</td>
<td>67.59±5.06</td>
<td>16.13±1.22</td>
</tr>
</tbody>
</table>

$^a$ Means differ at $p<0.05$.

$^b$ Means differ at $p<0.01$.

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Fig. 1. Change in primary fibre cross-sectional area over time.
pattern was different in the Elliottdales and Tukidales, with a peak occurring in October in the Elliottdales and May in the Tukidales.

Specific between breed differences for primary fibre diameter at each time period are shown in Table 3. There were differences between the Merinos and Romneys during summer when primary fibre cross-sectional area in the Romneys increased slightly, but not during winter. Generally, primary fibre cross-sectional area was lower in the Merinos and Romneys than in the other breeds, except for the Carpetmasters. Other breed differences varied with time of year.

Variation in secondary fibre cross-sectional area had a clear seasonal pattern (Fig. 2), as all breeds had a cycle with a December (summer) maximum and a July (winter) minimum.

Specific differences among breeds in secondary fibre diameter at each time period are shown in Table 4. While differences among breeds appear to be apparent in Fig. 2, they were usually not statistically significant due to high within-breed variation. In July (winter) and October (spring) 1993, and May (autumn) and September (spring) 1994, there were no differences among breeds. It was only when the

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Table 3

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<td>bc</td>
<td>b</td>
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*Breeds with differing letters within columns are significantly different at p<0.05.*

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Fig. 2. Change in secondary fibre cross-sectional area over time.
difference between the Merinos and the other breeds increased during the summer production peak that differences became apparent, and even then were only for the Merinos compared to the other breeds. Differences in secondary fibre diameters between Romneys and the carpet wool breeds were noticeably less than for primary fibre cross-sectional areas.

The relative change in fibre cross-sectional area with respect to the change from the value at the start of the experiment can be seen in Fig. 3(a and b). It is clear that the relative change in cross-sectional area of the secondary fibres (Fig. 3a) was greater than the relative change in the primary fibres (Fig. 3b) in all breeds, although this difference was marginal in the Merinos. It is also interesting to note that, at some stages, the Tukidale and Elliottdale samples showed contrary motion between fibre types with respect to change in cross-sectional area.

### 3.3. Changes in fibre ellipticity with time

It was clear during the collection of data, that many of the fibres from the specialty carpet wool breeds were not circular, and, therefore, fibre diameter was essentially meaningless. It was for this reason that cross-sectional areas, rather than derived diameter values (which assume circularity), were used in the analysis. A simple measure of ellipticity was derived using the image analysis software to give the major and minor axis lengths of the fibre cross sections. Thus, the calculation was:

$$\text{Ellipticity} = \frac{\text{major axis}}{\text{minor axis}} \times 100$$

There was a significant difference among breeds for fibre ellipticity at each sampling date. There was a difference ($p<0.01$) in ellipticity between fibre types at every time period, except December 1993 (summer), with primary fibres being more elliptical than secondary fibres. The interaction between breed and follicle type was not significant.

Fig. 4 shows the change in ellipticity with time for primary fibres. Merino primary fibres had the lowest ellipticity values. In contrast, a seasonal cycle in ellipticity appears to have been present in the Romneys and specialty carpet wool breeds. The timing of the peak ellipticity varied from summer to very early autumn: December in the Carpetmasters, and March in the Drysdales, Elliottdales, Tukidales and Romneys. The increase, and high within-breed variation, apparent at the last sampling date, coincided with an increase in nutrition due to supplementary feeding with hay.

Fig. 5 shows the change in ellipticity with time for secondary fibres. In general, the secondary fibres had lower ellipticity values than the primary fibres, and Merino secondary fibres had lower ellipticity values than the other breeds. Skin samples from a Merino (Fig. 6) and Drysdale (Fig. 7), both sampled on 30 August 1993 (winter), illustrate the within-sample and between-breed differences for fibre ellipticity. Peaks in the cycle for ellipticity occurred in December (summer) for the Carpetmasters, March (autumn) for the Tukidales, Elliottdales and Romneys, and May (autumn) for the Drysdales. The December (summer) peak for Carpetmasters was much more pronounced than the peak for the other breeds.

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### Table 4

Differences among breeds for secondary fibre cross-sectional area ($\mu m^2$)

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<tr>
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</tbody>
</table>

*a Breeds with differing letters within columns are significantly different at $p<0.05$.}
Fig. 3. (a) Relative change in the primary fibre cross-sectional area. (b) Relative change in the secondary fibre cross-sectional area.
4. Discussion

4.1. Secondary:primary follicle ratios

The results of this study show that Merinos have a higher secondary:primary ratio than the Australasian specialty carpet wool breeds, confirming previous studies (Carter and Clarke, 1957a,b; Andrews et al., 1998) that have found Merinos to have a higher secondary:primary ratio than other breeds and their crosses with Merinos. While the data should not be interpreted as an objective breed performance com-

![Fig. 4. Changes in ellipticity of primary fibres with time.](chart1)

![Fig. 5. Changes in ellipticity in secondary fibres with time.](chart2)
parison, comparative follicle data has not previously been reported for the specialty carpet wool breeds. The similar secondary:primary ratios in the follicle populations for the four specialty carpet wool breeds largely explains the visual similarity of staple structure and density in their fleeces.

Follicle attributes vary between Merino strains, S:P ratio being highest in fine wool strains and lowest in

Fig. 6. Merino skin section, sampled on 30 August 1993, showing near circular fibres in both primary and secondary follicles (at the same magnification as Fig. 7).

Fig. 7. Drysdale skin section, sampled on 30 August 1993, showing differences within and between primary and secondary follicle populations. Note the ribbon-like, and highly elliptical, primary fibre at lower centre-left.
coarse wool strains, and the Merino secondary:primary ratio is within the range of values reported by Carter and Clarke (1957a) for fine wool Merino genotypes. The Romney secondary:primary ratio reported here is at the high end of the values reported for Romneys and may reflect genetic change due to selective breeding for wool growth characteristics over the last 40 years, as increases in secondary:primary ratio due to selection for fleece-weight, fibre diameter and live weight have been reported in Merinos (Hynd et al., 1989). The values reported in this study for the specialty carpet wool breeds are similar to values reported for Downs breeds such as the Suffolk and Ryeland (Carter and Clarke, 1957b), but are higher than for breeds which might be expected to be similar, such as the Scottish Blackface, Welsh Mountain and Swaledale. These latter three British breeds have coarse fleeces similar to the specialty carpet wool breeds in terms of fibre diameter, overall level of medullation and fleece structure.

Within-breed variation for follicle ratio was similar in the Romneys, Tukidales and Drysdales, but compared to them, variation was lower and greater in the Carpetmasters and Elliottdales, respectively. This may be associated with the origin of the breeds. Carpetmasters and Elliottdales have different ancestries to Drysdales and Tukidales. Carpetmasters were developed in New Zealand from the Perendale, itself a stabilised cross of the Cheviot and Romney. Elliottdales were developed from Tasmanian (Australian) coarse-wooled Romneys, and their wool characteristics occur as the result of a different mutation to that determining fleece characteristics in the other three specialty carpet wool breeds. Tukidales and Drysdales were developed from separate mutations in New Zealand Romneys which gave rise to the fleece characteristics; however, alleles of the same gene are believed to be responsible in both breeds (Sides, 1988).

4.2. Relative follicle densities

The relative follicle density values recorded in this study highlight the similarities in skin and follicle populations of the specialty carpet wool breeds, and to some degree their similarity with the Romney. The higher total follicle density in the Romneys, compared to the specialty carpet wool breeds, was due to a higher primary density. The coarseness and fleece structure of the specialty carpet wool breeds relative to the Romney is, therefore, not due to primary follicle density, but may be due to higher fibre diameter, increased levels of medullation or variability in fibre length within the staple.

4.3. Changes in fibre cross-sectional area

The erratic changes in primary fibre cross-sectional area, and the sometimes contrary motion of the primary and secondary fibre cross-sectional areas within breeds suggests that there are different control mechanisms for fibre production from the two follicle types. There were breed differences for the changes in fibre cross-sectional area and, as illustrated in Fig. 7, the irregularity of some medullated fibres would have confounded any measurement of fibre diameter. In fact, a probable effect of irregular fibres on measures of fibre diameter is to limit the validity of some published fibre diameter values for medullated fibres. Also, the contrary movement in the two fibre populations reduced the mean fibre diameter change compared to what it might have been if the fibre cross-sectional areas had changed in the same direction for both follicle types. The data show that relative fibre diameter change is higher in secondary fibres. In this study, feed intake was known to be lower in winter than in summer (Champion et al., 1995) and, hence, the changes in cross-sectional area are likely to be due to increased feed intake. This would support the findings of Short et al. (1958) whose work examined changes in the follicle populations due to increased feed intake. However, the effect of changes in feed quality on these characteristics is unknown and worthy of further investigation.

4.4. Ellipticity

Ellipticity could have been present in the fibre cross sections through failure to cut the fibre on a plane perpendicular to the fibre cuticle. However, any such effect would have been random across the six breeds and, thus, the observed differences would appear to be real.

The Merinos had the most circular fibres, while the specialty carpet wool breeds and the Romneys showed considerable ellipticity which varied throughout the
year. Black and Reis (1979) and Black and Nagorcka (1993) emphasised that bulb cell numbers vary between genotypes and this may play a role in the seasonal changes seen in this study. However, other factors such as size and shape of cells and cell arrangement during fibre formation may also play a role in the seasonal changes noted in the study and these factors may vary with season. Nutrition also appears to have affected ellipticity as the increase in ellipticity late in the experiment (between July (winter) and September (spring) 1994) coincided with an increased level of feed availability following the introduction of hay supplementation into the grazing environment. A similar increase in fibre ellipticity with increased feed availability, at least in the secondary fibres, occurred as a peak in ellipticity during the period of high feed availability and increased intake in summer 1993/1994.

5. Conclusion

It is apparent that there are differences between primary and secondary follicle populations in the way the fibres they produce contribute to seasonal changes in average fibre diameter of fleeces. In general, the proportional change in fibre diameter with season is greatest in fibres produced by secondary follicles. Furthermore, there are differences between the primary and secondary fibres for ellipticity and cross-sectional area. The seasonal changes in these attributes suggests that there are separate control mechanisms for each follicle type, and that the number, size and arrangement of the cells in each type of fibre vary independently of one another throughout the year, bringing about dynamic changes in fibre growth and form. Further work is required to examine the influence of feed quality on these factors and to determine the mechanisms through which these changes in fibre structure are brought about, particularly in the specialty carpet wool breeds.

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