

SKIN AND FOLLICLE ATTRIBUTES CONTRIBUTE TO DIFFERENCES IN CLEAN FLEECE WEIGHT IN SUPERFINE MERINO SHEEP

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SUMMARY

Variation between animals in clean fleece weight at the same fibre diameter can be attributed to a number of skin and follicle factors. The aim of this project was to determine what contributions various skin and follicle attributes make to variation in clean fleece weight. Two groups of ten sheep were selected from the CSIRO Finewool flock at the extremes of estimated breeding values for clean fleece weight and mean fibre diameter (high clean fleece weight and low fibre diameter, low clean fleece weight and low fibre diameter) and the differences between the groups in a wide range of skin and follicle attributes were determined. The associations between the skin and follicle attributes with clean fleece weight and fibre diameter were also calculated. There were significant associations of follicle density, S/P ratio, dp/ds ratio, follicle branching and follicle shutdown on clean fleece weight. The correlations between staple length and clean fleece weight (0.50) were of similar magnitude to the correlations between clean fleece weight and follicle density (0.37 to 0.48), follicle branching (0.50), S/P ratio (0.28) and dp/ds ratio (0.40). The data presented here suggest that several skin and follicle attributes make contributions to the wide distribution of fleece weights that we commonly observe in flocks at any given MFD.

Keywords: follicle activity, wool, skin, Finewool

INTRODUCTION

Sheep breeding programs are increasingly being focussed on selection for clean fleece weight (CFW) and mean fibre diameter (MFD). At present there is increased interest in the Merino breeding industry in use of alternative strategies for identification of superior animals to the more conventional selection methods based on CFW and MFD. Many of these strategies include assessments of skin and follicle attributes. It is known that a number of follicular components contribute to the wool growth potential of sheep. These include the density of follicles in the skin, follicle size and germinative volume, rate of bulb cell division, proportion of bulb cells that are used for fibre synthesis, and cortical cell size (Black 1987). To date most emphasis on skin measurements on Finewool sheep has been concentrated on follicle density. This is understandable as in most flocks follicle density has a close association with MFD, although associations with CFW are lower (Purvis and Swan 1997). The relationship between CFW and other skin characters is not clear. The aim of this project was to determine what contributions various skin and follicle attributes make to variation in CFW at a given MFD.

MATERIALS AND METHODS

Fine wool flock

The CSIRO Fine Wool Flock has been described in detail by Swan *et al.* (1993). The flock consists of sheep from nine fine and superfine bloodlines, and two medium wool Merino bloodlines, each comprising 200 ewes and their progeny. These animals are representative of the major fine and superfine wool producing regions of eastern Australia and provide linkages with genetic studies based on medium wool sheep. In this study 20 wethers born in 1993 were examined. Two groups of ten animals were selected from 130 wethers based on their estimated breeding values (EBV) for CFW and MFD, ie high CFW and low MFD, and low CFW and low MFD, to represent the extremes of the CFW distribution with MFD in the superfine range.

Skin and wool measurements

Skin biopsies and wool samples were collected at 21 months of age (early Spring) from the midside of each sheep. MFD was measured on the wool samples using a Laserscan machine. Staple lengths and yield were measured by the Australian Wool Testing Authority. Skin samples were fixed in buffered formalin, embedded in paraffin and sectioned (8µm) in a transverse plane to the follicles at the level of the sebaceous glands.

Measurements of follicle density, the ratio of the number of secondary follicles to primary follicles (S/P) and the ratio of the diameter of primary follicles to secondary follicles (dp/ds) were performed on eosin and picric acid-stained slides using image analysis (Maddocks and Jackson 1988). Follicle density was also measured using a new technique which involved taking an impression from a small shaved area of the skin using an epoxy resin. Digitized images of the skin impressions were captured using scanning electron microscopy. Follicle density and the number of original and derived follicles were determined by image analysis (Nagorcka *et al.* personal communication).

Follicle activity measurements

Additional skin biopsies were similarly collected and processed for follicle activity measurements. Transverse skin sections were prepared and every fifth section collected. Sections were Saccpic stained and follicles scored as active or inactive (Nixon 1993). A follicle was scored as inactive if it met any of the following criteria:

- (i) the follicle contained a brush-end characterised by irregular fibre shape,
- (ii) no fibre was present in the follicle and the inner root sheath morphology was abnormal,
- (iii) no fibre was present in the follicle and the inner root sheath had collapsed,
- (iv) there was irregular staining of the inner root sheath, or
- (v) if the follicle appeared as a small condensate of dark staining nuclear cells. Approximately 1000 follicles were scored per sample.

Production ratios were calculated on the Saccpic-stained sections (Butler and Wilkinson 1979). The cross-sectional area of fibre and fibre plus inner root sheath were measured using image analysis. The production ratio was then determined by dividing the fibre area by the fibre plus inner root sheath area. At least 100 follicles per sample were measured at the level in the skin where fibres were beginning to keratinise.

Statistical analysis

Statistical analyses were performed using S-Plus (1995). A linear model including the effects of bloodline and groups was fitted for each trait. Where bloodline was not significant it was omitted from the model. The results presented are least squares means for group.

RESULTS

The selected sheep did not differ significantly in MFD, however the group with high CFW EBV had phenotypic values that were 32% higher than the mean of the low CFW group (Table 1). The high CFW sheep also had staples that were on average 10% longer than the low CFW group (Table 1). In the animals examined, there were no significant effects of bloodline on MFD, CFW or staple length.

Follicle density, S/P ratio, dp/ds ratio and follicle branching were significantly higher in the high CFW sheep than the low CFW group (Table 2). The greater follicle density in the high CFW group is reflected in the significantly increased proportion of branched epidermal follicles, number of follicles per bundle, follicles per epidermal follicle and, although not significant, the smaller area of skin devoid of follicles (Table 2). The number of epidermal follicles did not alter, so these results can be interpreted to mean that in the high CFW group for every original primary and secondary follicle (epidermal follicle) there are more secondary derived follicles compared to the low CFW group due to follicle branching. This has led to an elevation in follicle density, a decrease in the skin area not occupied by follicles, an increase of the S/P ratio and, as the derived follicles yield fibre of finer diameter, an increase in the dp/ds ratio. Surprisingly, the density results obtained using skin biopsies and skin impressions were not the same. The correlation between follicle density measured on skin biopsies and the total density of fibres measured using skin impressions was only 0.37.

Table 1. Mean fibre diameter (μm), clean fleece weight (kg) and staple length (mm) for the high and low CFW EBV groups at 21 months of age (least squares means \pm s.e.)

Parameter	High CFW	Low CFW	P value
Fibre diameter	16.7 \pm 0.21	16.9 \pm 0.21	0.535
Clean fleece weight	2.9 \pm 0.08	2.2 \pm 0.08	0.00001
Staple length	91.6 \pm 2.63	82.9 \pm 2.63	0.031

The level of follicle shutdown differed significantly between the high and low CFW sheep. On average the high CFW sheep had 5% more active follicles than the low CFW sheep (Table 3). There were significant effects of bloodline on follicle shutdown ($P=0.032$). Some bloodlines were more susceptible to follicle shutdown than others managed in the same environment. The differences between bloodlines in the total number of shutdown follicles can be largely attributed to differences in the number of shutdown secondary follicles (Table 3). The production ratio of fibre area to fibre plus inner root sheath area did not differ significantly between the groups (Table 3).

Moderate positive correlations existed for CFW and staple length, follicle density, S/P ratio, dp/ds ratio, and follicle branching (Table 4). The correlations between these traits and MFD were either negative or negligible (Table 4). A weak negative correlation was observed between the proportion of inactive follicles and the fraction of skin bare of follicles with CFW indicate that CFW is affected by increases in both parameters, albeit at low levels. Poor relationships existed between the production ratio and CFW and MFD (Table 4).

Table 2. Skin and follicle parameters determined from skin biopsies and skin impressions (least squares means \pm s.e.)

Parameter	High CFW	Low CFW	Pvalue
<i>Skin biopsy measurements</i>			
Follicle density ($\#/mm^2$)	91.9 ± 4.07	74.6 ± 4.07	0.008
S/P ratio	35.0 ± 2.19	27.6 ± 2.19	0.003
dp/ds ratio	1.13 ± 0.047	0.95 ± 0.047	0.014
<i>Skin impression measurements</i>			
Total density of fibres ($\#/mm^2$)	111.4 ± 7.14	87.1 ± 7.14	0.027
Epidermal follicles ($\#/mm^2$)	38.0 ± 2.02	38.6 ± 2.02	0.823
Branched epidermal follicles (%)	56.4 ± 2.30	45.9 ± 2.30	0.005
Follicles per bundle	4.29 ± 0.174	3.84 ± 0.174	0.088
Follicles per epidermal follicle	2.89 ± 0.168	2.33 ± 0.168	0.030
Fraction of bare skin	0.52 ± 0.014	0.56 ± 0.014	0.060

Table 3. Effects of follicle shutdown (%) and production ratio on clean fleece weight (least squares means \pm s.e.)

Parameter	High CFW	Low CFW	Pvalue
Total inactive follicles	6.17 ± 0.693	6.53 ± 0.564	0.023
Inactive primary follicles	23.9 ± 2.77	17.6 ± 2.77	0.125
Inactive secondary follicles	5.59 ± 0.717	6.12 ± 0.583	0.016
Production ratio	0.31 ± 0.011	0.32 ± 0.011	0.374

Table 4. Correlations between skin and follicle characters with clean fleece weight and mean fibre diameter

Parameter	Clean fleece weight	Mean fibre diameter
Staple length	0.50	0.09
Follicle density	0.37	-0.24
S/P ratio	0.28	-0.39
dp/ds ratio	0.40	-0.19
Total density of fibres	0.48	0.01
Branched epidermal follicles	0.50	-0.17
Follicles per bundle	0.33	-0.12
Follicles per epidermal follicle	0.43	-0.15
Fraction of bare skin	-0.22	0.05
Total inactive follicles	-0.18	0.03
Production ratio	-0.06	0.17

DISCUSSION

Variation in CFW can be attributed to a number of biological factors including the morphology of the skin, composition of the follicle population within the skin, and the efficiency of fibre production by those follicles, in addition to the physiological state of the animal and numerous environmental factors. To eliminate environmental effects and ascertain which biological processes contribute to genetic differences in fleece weight, animals were examined with different EBV for CFW and EBV for MFD in the superfine range. The data presented here suggest that several skin and follicle attributes make contributions to the wide distribution of fleece weights that we commonly observe in flocks at any given MFD.

In this study, the sheep selected for genetically higher CFW had significantly higher phenotypic CFW largely due to increases in staple length and follicle density. The differences observed in follicle density between the groups are not in agreement with other data from the Finewool Project where the phenotypic correlation between CFW and follicle density has been reported to be much smaller than that reported here (0.37 compared to 0.04 in Purvis and Swan 1997). As the small group of animals used here were selected from one extreme of the MFD distribution they are not representative of the entire Finewool flock, which may contribute to the differences in the correlations between skin and fleece characters with MFD and CFW. Nevertheless, a large proportion of CFW variation in these superfine sheep can be attributed to follicle density which implies that we may be able to elevate CFW by increasing follicle density.

It is also interesting to note that there were differences in the density results from skin sections and the impression technique. The discrepancy between the values can be accounted for in part by the scoring, or not, of inactive follicles. Firstly, inactive follicles, particularly those of type (v), were often not scored in the skin density measurements which leads to an underestimate of the total density. Secondly, inactive follicles retaining a fibre will be counted as active follicles in the impression density as the scoring system counts the number of fibres protruding from the skin surface, thereby inflating the density estimates above those determined using the corresponding skin section.

The levels of follicle shutdown varied considerably between and within bloodlines. That some bloodlines were more susceptible to follicle shutdown than others run in the same management and environmental conditions warrants further examination. Follicle shutdown can be caused by a number of environmental and physiological factors including stress, pregnancy, lactation and poor nutrition. These results suggest that there is room to improve productivity by elevating the proportion of follicles actively producing a fibre and improving the efficiency of those follicles eg by improving nutrition and management practices during periods of environmental and physiological stress.

These results indicate that placing selection emphasis on skin and follicle traits such as follicle density, follicle shutdown, follicle branching, S/P ratio and dp/ds ratio will not compromise MFD and may improve CFW. Whether the inclusion of skin and follicle attributes will be useful or cost effective in sheep breeding programs will require further examination.

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