Relation between skin structure and wool yellowing in Merino and Romney sheep

R.M.W. SUMNER and A.J. CRAVEN

AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand

ABSTRACT

Five adult Merino and five adult Romney ewes were skin sampled on the back, midside and belly regions of the body following a study measuring the relationship between rate of sweat secretion and wool yellowing under various controlled environmental conditions. Densities of primary follicles, secondary follicles, sweat (sudoriferous) glands and sebaceous glands, as well as dimensions of the sweat and the sebaceous glands were measured. The density of secondary follicles was negatively related to wool yellowing and there was a weaker positive relationship between the density of primary follicles and wool yellowing. Underlying these relationships were associations of measurements suggesting sebaceous gland output restricted wool yellowing while sweat gland output encouraged wool yellowing. In addition, large sweat glands were related to increased sweat secretion rate. The results of this study suggest there may be two potential avenues to minimise fleece discolouration while the wool is growing on the sheep; either increasing sebum production or reducing sweat production.

Keywords: Romney; Merino; wool yellowing; wool follicles; sweat glands; sweat; sebaceous glands.

INTRODUCTION

Sheep skin contains a multitude of pilosebaceous units comprising wool fibre producing follicles and their associated accessory glands (Carter & Clarke, 1957a; 1957b; Lyne, 1966). Each of these structures is derived from downgrowths of the epidermis (Millar, 2002; Fu *et al.,* 2005). Depending on the age of development, wool follicles develop into either primary or secondary follicles. Primary follicles are characterised as being associated with a sudoriferous or sweat gland secreting 'sweat', the basis of the water soluble fraction of 'greasy' wool termed suint, and a bilobed sebaceous gland secreting a water-insoluble sebum, or 'wool grease'. The natural mixture of these two chemically complex secretions is called 'yolk'. Although secondary follicles have no associated sweat glands, a proportion are associated with monolobed sebaceous glands. Sweat glands have been observed to be more developed in the warmer, summer months than during the winter in New Zealand pelts (Dempsey, 1956).

Sweat glands are tightly coiled tubular structures (Montagna & Parakkal, 1974) while sebaceous glands are branched alveolar structures (Scott, 1988) (Figure 1). At high ambient temperatures, the sweat glands in sheep have been observed simultaneously to discharge their secretion in pulses where the frequency of the pulses increases with the increasing temperature (Robertshaw, 1968), reflecting neural control.

Certain environmental conditions are known to induce yellow discolourations during growth, storage and processing of wool (Aliaga *et al.*, 1996) reducing the commercial value of the fibre. While the propensity of wool to discolour has a genetic basis (Benavides & Maher, 2003), wool from different sites of the body also varies in its propensity to develop yellow discolourations (Sumner *et al.,* 2003*)*, indicating that physiological or anatomical factors across the body may also be implicated in the reaction.

This report explores the relationship between characteristics of the population of wool follicles and associated secretory glands in the skin, with wool yellowing and sweating rate in a group of Merino and Romney sheep (Sumner *et al.,* 2004).

MATERIALS AND METHODS

Animals and measurement of wool yellowness and sweat secretion

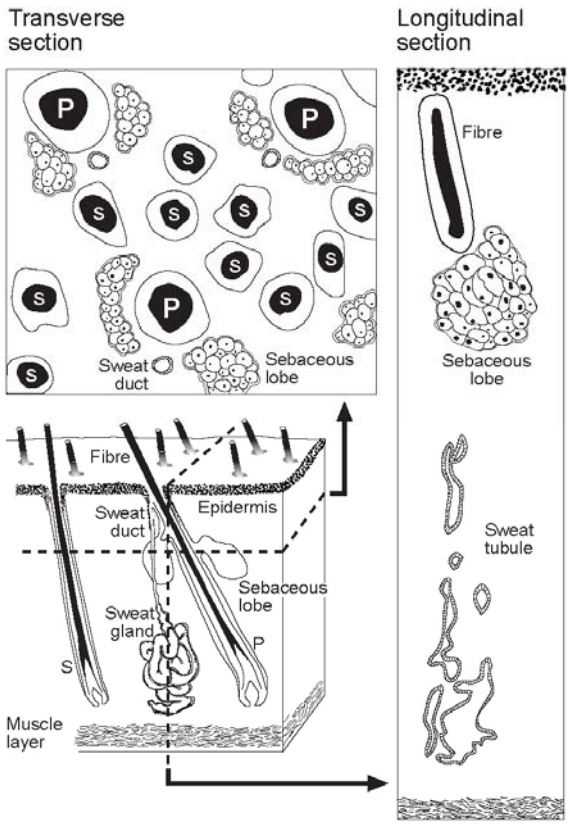
Five adult Merino and five adult Romney ewes born and raised on two farms in the central Waikato, New Zealand, were used. During June 2003 the sheep were subjected to nine different environmental challenges in a climate controlled room on consecutive days when the temperature humidity index (THI) was varied from 74.2 to 91.3 for up to 7 h at each exposure. Measurements on the relationship between wool yellowing and sweating rate were collected (Sumner *et al*., 2004). Briefly sweat secretion from three sites on the body of each sheep was collected with cellulose sponges held in contact with the skin for up to 5 h during each environmental challenge. The sites were beside the backbone over the *Longissimus dorsi* muscle directly above the attachment of the 13th rib (back region), over the ventral end of the 13th rib (midside region) and adjacent to the *linea alba* on the belly anterior to the umbilicus (belly region). Tristimulus (Y-Z) values (yellowness) of wool grown during this study adjacent to the site on the body of each sheep where sweat was collected, were measured according to the New Zealand Standard method (NZS8707:1984) using illuminant C and a 2° observer angle.

Skin sampling

In July 2003, 2 weeks after subjecting the sheep to the range of environmental challenges, two skin samples were taken from the centre of each of the three sites on the body of each sheep that had been previously

used for sweat collection measurements. One skin sample was taken by trephine (1 cm diameter) and the other skin sample taken by snip biopsy. Both sets of skin samples were fixed in phosphate-buffered 10% formalin solution and embedded in wax. All experimental procedures were carried out with the approval of the AgResearch Ruakura Animal Ethics Committee.

FIGURE 1: Accessory glands associated with wool follicles in intact skin, and in transverse and longitudinal section of a skin sample. P = primary follicle; S = secondary follicle.



Skin measurements

Skin samples taken with a trephine were cut serially in transverse section (8 μm) in the region of the sebaceous gland (Figure 1) and the sections stained by the sacpic method (Nixon, 1993). The density of primary (nP) follicles and the density of secondary (nS) follicles, were measured by projection microscope and adjusted for shrinkage (McCloghry *et al.,* 1997). A series of fields encompassing in excess of 15 primary follicles and their associated secondary follicles were counted. The ratio of secondary to primary follicles (S/P ratio) was calculated.

Skin samples taken as a snip biopsy were cut serially in longitudinal section (8 μm) (Figure 1) and the sections also stained by the sacpic method (Nixon, 1993). The length of the cut section, the number of sectioned sweat gland tubules and the perimeter of each sectioned sweat gland tubule, the number of sectioned sebaceous gland lobes and the area of each sectioned sebaceous gland lobe (Figure 1) were measured using a computer-aided projection microscope system (Olympus microscope with Spot RT camera and software (Diagnostic Instuments Inc, USA)). Sectioned sweat gland tubules were in distinct groups associated with individual glands. The total number of sectioned sweat gland tubules or sebaceous gland lobes associated with a minimum of 30 follicles was measured. Where there were more than 30 follicles with associated glands present in a single section, all the glands on that section were measured. In cases where more than one section had to be examined to locate the required number of follicles, sections 20 slices approximately 160 μm apart were selected for measurement to ensure an individual sweat gland was not measured twice. The mean value of each measurement was adjusted for the shrinkage of the sections taken by trephine from the same sheep. Both the sum of the perimeters of the sweat gland tubules and the total area of sebaceous gland lobes, per mm of longitudinally sectioned skin, were calculated.

Statistical analysis

Differences due to breed and body site were estimated by analysis of variance, with sheep as a blocking factor, using GenStat 7.1 (Lawes Agricultural Trust, 2003). A $\log_e$ transformation was applied to measurements involving an estimate of density to balance the variances across the data set. Least square mean values of the transformed data were back-transformed for presentation.

The best predictors of the tristimulus (Y-Z) values for wool growing at the time of skin sampling, before and after an incubation challenge, and the mean rate of sweat secretion for these same sheep as previously reported by Sumner *et al.* (2004), were determined using the GenStat implementation of the best subset regression developed by Furnival and Wilson (1974). The smaller the expected mean square error of prediction, as assessed by Mallow's C_P value, the greater the accuracy of prediction.

RESULTS

Mean live weight of the sheep at the time of skin sampling was 42.8 ± 6.2 kg and 65.2 ± 6.6 kg for the Merino and Romney ewes respectively. The least square mean values for measures of wool yellowing and sweat secretion rate published previously (Sumner *et al*., 2004), and the wool follicle population characteristics and sweat and sebaceous gland dimensions measured in this study, are given in Table 1. There was no significant breed by body site interactions of biological importance for any of the measured characteristics.

TABLE 1: Least square mean values for measures of wool yellowing, sweat secretion rate, wool follicle population characteristics and sweat and sebaceous gland dimensions in Merino and Romney sheep sampled at three sites over the body.

Treatment	Tristimulus (Y-Z)		Sweat secretion rate (mg /cm^2/h)	Primary follicle density[1] (No/mm^2)	Secondary follicle density[1] (No/mm^2)	Number of secondaries per primary
	Pre-incubation	Post-incubation				
Breed						
Merino	1.9	6.6	0.64	4.6	61.9	13.9
Romney	7.1	13.5	0.87	3.0	14.3	4.8
LSD[2]	1.7	1.5	0.23	-	-	1.5
LSR[3]	-	-	-	1.2	1.1	-
Body site						
Back	0.6	7.7	0.74	3.7	34.8	10.7
Midside	0.3	7.6	0.73	3.7	30.4	9.9
Belly	12.6	14.8	0.80	3.6	24.9	7.5
LSD[2]	1.4	1.5	0.06	-	-	1.3
LSR[3]	-	-	-	1.2	1.1	-
Breed effect	***	***	†	***	***	***
Body site effect	***	***	†	NS	***	***

Treatment	Density of sweat gland tubules in skin[1] (No/mm)	Perimeter of sweat gland tubules (mm)	Total perimeter of sweat gland tubules in skin (mm/mm)	Density of sebaceous gland lobes in skin[1] (No/mm)	Area of sebaceous gland tissue per lobe (mm^2)	Total area sebaceous gland tissue in skin (mm^2/mm)
Breed						
Merino	3.4	0.48	1.6	1.6	0.030	0.053
Romney	2.9	0.71	2.2	1.1	0.049	0.055
LSD[2]	-	0.08	0.6	-	0.012	0.027
LSR[3]	1.5	-	-	1.5	-	-
Body site						
Back	2.7	0.56	1.6	1.5	0.039	0.061
Midside	2.6	0.68	1.7	1.5	0.044	0.062
Belly	4.4	0.54	2.5	1.1	0.036	0.038
LSD[2]	-	0.09	0.7	-	0.010	0.018
LSR[3]	1.3	-	-	1.4	-	-
Breed effect	NS	***	*	*	**	NS
Body site effect	**	**	*	†	NS	*

[1] Back-transformed values.
[2] Least significant difference. Effect significant at 5% level of significance where the difference between the means exceeds the quoted value.
[3] Least significant ratio. Effect significant at 5% level of significance where the ratio of the means exceeds the quoted value.

There were significant breed and body site effects for tristumulus (Y-Z) values both before and after incubation. Romney wool had a higher tristimulus (Y-Z) value than Merino wool with the tristimulus (Y-Z) value increasing when the wool samples were incubated at 40°C for 7 days. Wool clipped from the belly region had a significantly higher tristimulus (Y-Z) value than the wool clipped from both the back and midside regions for which the tristimulus (Y-Z) values were similar. The greater rate of sweat secretion in both the Romney and on the belly region approached significance.

Both nP and nS within the skin were greater for the Merino sheep than for the Romney sheep with the Merino sheep also having a higher S/P ratio than the Romney. Although nP did not differ over the body, nS was less on the belly region than on the midside and back regions hence S/P was lower on the belly than at the other sites.

Although the density of the sweat gland tubules in each section of skin was similar in both breeds, the perimeter of the tubules was greater for the Romney than for the Merino sheep. Hence the sum of the perimeters of the sectioned sweat gland tubules was also greater for the Romney than for the Merino sheep. Skin in the belly region had both a greater density of sweat gland tubules and a larger total perimeter of sweat gland tubules than in either the midside or back regions. The perimeter of sweat gland tubules was, however, greater in the midside region than in either the back or belly regions.

The Merino sheep had more smaller sized sebaceous gland lobes than the Romney sheep with a similar total area of sebaceous gland tissue in both breeds. Both the density of sebaceous gland lobes and the total area of sebaceous gland tissue were less in the belly region than in either the midside or back regions. There was no difference in the area of individual sebaceous gland lobes between regions of the body.

Tristimulus (Y-Z) values before and after incubation, and sweat secretion rate, were each negatively related to nS which explained 64%, 80% and 24% of the variation in the three production characteristics respectively. It is recognised that the intensity of yellow discolouration in the belly region may be enhanced by both body warmth when the sheep is lying down and the transfer of water soluble components from the upper regions of the fleece during rain-wetting (Sumner, 2002). Consequently data relating to the belly region of the body were excluded from the analysis related to the prediction of tristimulus (Y-Z) values.

The two skin structure characteristics which together best predicted wool yellowing over the upper body and sweat secretion rate over the whole body, are listed in Table 2. Both wool yellowing measurements were negatively related to nS and positively related to nP. Introduction of the nP term increased the explained variation in tristimulus (Y-Z) before incubation from 64% to 73% and the explained variation in tristimulus (Y-Z) after incubation from 80% to 85%. The introduction of a second prediction parameter into the relationship predicting sweat secretion rate did not significantly increase the proportion of explained variation above 24%.

An indication of the impact of skin secretory gland characteristics on wool yellowing and sweat secretion independent of nP and nS, was obtained by deleting the nP and nS terms from the model. Wool yellowing before and after incubation was then best predicted by a negative relationship with the density of sebaceous gland lobes and a positive relationship with the mean area of the sebaceous gland lobes (Table 3). These two characteristics together explained 35% and 45% of the variation in wool yellowing before and after incubation respectively. Sweat secretion, on the other hand, was best predicted by positive relationships with the perimeter of the sectioned sweat gland tubules and the sum of the perimeters of the sectioned sweat gland tubules (Table 3). These two characteristics together explained 21% of the variation in sweat secretion rate.

DISCUSSION

Primary and secondary follicle population parameters measured in this study were within the ranges previously reported for the Merino and Romney (Carter & Clarke, 1957a; 1957b). Similarly the trend in this study for wool follicle density to be less in lower regions of the body was similar to that reported by Sumner and Craven (2000).

No reports have been located in the literature of comparative measurements of aspects of the population of sweat and sebaceous glands in the skin of Merino and Romney sheep or over the body of the sheep, with which to compare the data obtained in this study.

Within each breed, wool yellowing and, to a lesser extent, sweat secretion rate, were strongly related to the density of secondary follicles within the skin. Primary follicles are associated with bilobed sebaceous glands and a proportion of secondary follicles are associated with a monolobed sebaceous gland (Carter & Clarke, 1957a; 1957b). Thus sheep with a high overall follicle density, which will also have a high nS and a high S/P ratio, are likely to have a greater density of sebaceous lobes within their skin than sheep with a low overall follicle density. This trend was evident in these data. As the basic characteristic used to identify a primary follicle is the association with a sweat gland, the density of sweat glands in the skin is more closely related to nP than the density of sebaceous gland lobes is related to nS.

TABLE 2: Adjusted R^2 and C_P[1] values for predicting wool yellowing on the back and midside regions of the body, and sweat secretion rate on the back, midside and belly regions of the body from two measured skin structure characteristics.

Parameter being predicted	First predictor parameter	Effect	Significance	Second predictor parameter	Effect	Significance	Overall R^2 value	C_P
Tristimulus (Y-Z) Pre incubation	Density of secondary follicles	-ve	***	Density of primary follicles	+ve	*	72.6	5.1
Tristimulus (Y-Z) Post incubation	Density of secondary follicles	-ve	***	Density of primary follicles	+ve	*	84.6	5.4
Sweat secretion rate	Density of secondary follicles	-ve	†	Perimeter of sweat gland tubules	+ve	NS	24.1	0.5

[1] Expected mean square error of prediction

TABLE 3: Adjusted R^2 and C_P[1] values for predicting wool yellowing on the back and midside regions of the body, and sweat secretion rate on the back, midside and belly regions of the body from two measured skin structure characteristics after removing the density of primary and secondary follicles from the model.

Parameter being predicted	First predictor parameter	Effect	Significance	Second predictor parameter	Effect	Significance	Overall R^2 value	C_P
Tristimulus (Y-Z) Pre incubation	Density of sebaceous gland lobes	-ve	*	Area of sebaceous gland lobes	+ve	†	35.4	4.9
Tristimulus (Y-Z) Post incubation	Area of sebaceous gland lobes	+ve	**	Density of sebaceous gland lobes	-ve	NS	44.8	5.5
Sweat secretion rate	Total perimeter of sweat gland tubules in skin	+ve	†	Perimeter of sweat gland tubules	+ve	NS	20.9	6.0

[1] Expected mean square error of prediction

The basic premise in conducting this trial was that dimensional aspects of the two types of skin glands associated with wool follicles were likely to be a reflection of the gland's secretory output. In adopting this premise it was recognised that the measurements used did not take account of between-sheep differences in the rate of cell division occurring within each gland. Gland size was measured as either the perimeter of the sectioned sweat gland tubules or the area of sectioned sebaceous gland lobes. Both of these measurements were positively related to the secretion rate of the respective glands. For example, the difference in the perimeter of the sectioned sweat gland tubules indicated that sweat tubules in Romney sheep had a larger lumen with the capacity to secrete a greater volume of sweat than Merino sheep. In turn the mean perimeter of each tubule was positively related to the measured sweat secretion rate (Sumner *et al.*, 2004). In the case of wool yellowing however, the density of sebaceous glands appeared to be more important than gland size *per se*, with a greater density of smaller glands being more effective in secreting sebum as reflected in the higher wax content of Merino compared with Romney wool (Henderson, 1965).

Ignoring the statistically significant effect of nP and nS on wool yellowing and sweat secretion, which in themselves have no direct effect on skin gland secretions, there was no strong relationship in this study between any single skin gland dimensional characteristic and wool yellowing. Nevertheless the increase in discolouration that occurs while wool is growing, or after the wool has been subjected to a post-harvest simulated 'environmental challenge', was negatively related to sebaceous gland parameters likely to reflect sebum output and positively related to sweat gland parameters likely to reflect sweat output. Similarly Aitken *et al*. (1994) reported the propensity of

wool to develop yellow discolourations was negatively related to the wax/suint ratio associated with the fibres when in a greasy state. These trends support the contention that secretions from the sweat glands play a key role in inducing wool yellowing under appropriate environmental conditions with secretions from the sebaceous glands protecting the growing wool fibres from the effects of the sweat gland secretions.

These results suggest there may be two potential avenues to minimise fleece discolouration while the wool is growing on the sheep; either increasing sebum production or reducing sweat production. The output of both of these secretions is regulated by genetics and the environment. Genetic parameters associated with wool yellowing tend to be low (Benavides & Maher, 2003) due in part to variations in the environmental conditions to which sheep are exposed to stimulate wool yellowing. This study has shown there is also limited variation in the dimensions of accessory glands associated with wool follicles within a genotype.

Changes in management procedures may therefore be the most effective way of reducing wool yellowing in warm moist environments. One way of doing this is by reducing the time interval that the growing wool is exposed to freshly secreted sweat. This can be achieved on-farm by shearing sheep in the spring when sweat production is potentially low (Dempsey, 1956). In the case of coarse long-woolled breeds it is appropriate to shear them again in the autumn thereby removing the greater volume of sweat that is secreted over the summer (Dempsey, 1956) before it induces fleece yellowing in the lengthening fleece. Short wool produced under a twice-yearly shearing regime is commercially viable as it is suited for producing woollen type yarns. Further studies are now required to identify the specific factors within sweat responsible for inducing wool yellowing.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution of Shellee Olliver for preparing the skin sections and undertaking the associated measurements, and Martin Upsdell for undertaking the data analysis. The work was funded by Meat and Wool NZ Ltd.

REFERENCES

Aitken, F.J.; Cottle, D.J.; Reid, T.C.; Wilkinson, B.R. 1994: Mineral and amino acid composition of wool from New Zealand Merino sheep differing in susceptibility to yellowing. *Australian journal of agricultural research 45:* 391-401

Aliaga, J.L.; Sanderson, R.H.; Maher, A.P.; Reid, T.C. 1996: Optimising of the challenge test for the susceptibility of wool to yellow discolouration. *Proceedings of the New Zealand Society of Animal Production: 56:* 319-323

Benavides, M.V.; Maher, P.; 2003: Genetic parameters of wool colour and skin traits in Corriedale sheep. *Genetics and molecular biology 26:* 267-274

Carter, H.B.; Clarke, W.H. 1957a: The hair follicle group and skin follicle population of Australian Merino sheep. *Australian journal of agricultural research 8:* 91-108

Carter, H.B.; Clarke, W.H. 1957b: The hair follicle group and skin follicle population of some non-Merino breeds of sheep. *Australian journal of agricultural research 8:* 109-119

Dempsey, M. 1956: Some seasonal variations in lambskins. *Journal of the Royal Microscopical Society (Great Britain) 75:* 103-110

Fu, X.; Li, J.; Sun, X.; Sun, T.; Sheng, Z. 2005: Epidermal stem cells are the source of sweat cells in human fetal skin: Evidence of synergetic development of stem cells, sweat glands, growth factors and matrix metalloproteinases. *Wound repair and regeneration 13:* 102-108

Furnival, G.M.; Wilson, R.W. 1974: Regression by leaps and bounds. *Technometrics 16:* 499-511

Henderson, A.E. 1965: Wool and woolclassing. A.H. and A.W. Reed, Wellington, New Zealand. pp 67

Lawes Agricultural Trust, 2003: GenStat, Release 7.1. Reference Manual. VSN International, Hemel Hempstead, Herts., UK

Lyne, A.G. 1966: The development of hair follicles. *Australian journal of science 28:* 370-377

McCloghry, C.E.; Brown, G.H.; Uphill, G.C. 1997: Computer-assisted image analysis for the measurement of wool follicle density and fibre diameter in skin sections. *New Zealand journal of agricultural research 40:* 239-244

Millar, S.E. 2002: Molecular mechanisms regulating hair follicle development. *Journal of investigative dermatology 118:* 216-225

Montagna, W.; Parakkul, P. 1974: The structure and function of skin. Academic Press, New York, USA

Nixon, A.J. 1993: A method for determining the activity state of hair follicles. *Biotechnic and histochemistry 68:* 316-325

NZS8707:1984: Method for the measurement of colour in wool.

Robertshaw, D. 1968: The pattern and control of sweating in the sheep and the goat. *Journal of physiology 198:* 531-539

Scott, D. 1988: Large animal dermatology. W.B. Saunders Company, Philadelphia, USA

Sumner, R.M.W. 2002: Factors associated with yellowing within Romney fleeces. *Proceedings of the New Zealand Society of Animal Production 62:* 61-64

Sumner, R.M.W.; Ashby, M.G.; Craven, A.J. 2004: Relation between sweating and wool yellowing in Merino and Romney sheep. *Proceedings of the New Zealand Society of Animal Production 64:* 286-292

Sumner, R.M.W.; Craven, A.J. 2000: Variation of fibre and follicle characteristics related to wool bulk over the body of Perendale ewes: Implications for measurement of wool bulk. *Proceedings of the New Zealand Society of Animal Production 60:* 166-170

Sumner, R.M.W.; Young, S.R.; Upsdell, M.P. 2003: Wool yellowing and pH within Merino and Romney fleeces. *Proceedings of the New Zealand Society of Animal Production 63:* 155-159