GENETIC DETERMINANTS OF THE RESPONSIVENESS OF WOOL FIBRES AND FOLLICLES TO NUTRITION

By

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ABSTRACT

The current research project was designed to investigate the extent to which there is a genetic component to the response of wool growth and fibre diameter (FD) to the environment. In the first experiment the follicle morphology of 210 Merino sheep was assessed and classified after the "break of the season". The incidence of follicle shutdown (FS) was low (1.4%) and there were no significant effects of sire, sex on FS and low correlation was found between the incidence of FS with FD or clean fleece weight ($p>0.05$). However, sheep born and raised as singles had a higher incidence of FS than those born as twins and raised as single or twins ($p<0.01$).

In experiment 2, Coefficient of variation of FD (ACVFD), representing FD variation throughout the year was investigated from 593 South Australian Merino strain ewes. ACVFD was measured by cutting the staple into 10 snippets at equidistant intervals along the staple. The results showed that FD varied significantly throughout the year with minimum fibre diameter in wool staple was mostly found around autumn. The susceptibility of FD changes throughout the year was not significantly different between stud ($p=0.065$) or between sire ($p=0.868$). Heritability of ACVFD was low ($h^2 = 0.17$), suggesting this variation has only a small genetic component.

In experiment 3, twelve sheep from each Finewool Merinos and Mediumwool Merinos were selected on the basis of their FD and wool sulphur content. L-cysteine was continuously infused into the jugular vein of the sheep for 21 days. Cysteine infusion increased greasy wool growth (GWG), paracortex ratio, wool sulphur content and expression of selected keratin genes, but surprisingly not FD. The increase in GWG but not FD was an exciting finding as it is a breakthrough to the generally held view that selection to increase GWG is associated with FD increase, unwanted response in the wool industry. Mediumwool sheep were more responsive to the cysteine infusion in GWG and expression of KAP2.12, a high-sulphur encoding gene. Low-sulphur sheep had statistically significant increases in GWG, paracortex ratio, wool sulphur content and expression of KAP2.12. A mechanism operating to produce these results is proposed.

In a general conclusion, resistance of wool traits to environmental changes in relation to the interaction between genes and the nutritional environment, might apply not only to sheep and wool production, but also to other animal production systems. The decrease in the capability of animals to adapt to their environment such as disease resistance, low-quality feed and heat tolerance, might be related to some improvement on animal production system. This becomes one of general concern in the sustainable animal production issue to achieve 'a balance' between animal production system for human requirements as well as animal welfare.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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7 March 2006

PUBLICATIONS

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ABBREVIATIONS

AUS$	= Australian Dollars
ACVFD	= Coefficient of variation of fibre diameter along staple
ACTH	= Adrenocorticotrophic hormones
ADJDIFF	=Adjacent difference
ATLAS	= Automatic testing of length and strength
AWTA	= Australian wool testing authority
BMP	= Bone morphogenic proteins
CFW	= Clean fleece weight
CNS	= Central nervous system
CP	= Crude protein
CT	= Connective tissue
CTSE	= Central Test Sire Evaluation
CV	= Coefficient of variation
CVFD	= Coefficient of variation of fibre diameter
DM	= Dry matter
DNA	= Dioxyribose nucleic acid
DSE/ha	= Dry sheep equivalent/hectare
EGF	= Epidermal growth factor
FD	= Fibre diameter
FDMAX	= Maximum fibre diameter
FDMEAN	= Mean fibre diameter
FDMIN	= Minimum fibre diameter
FDV	= Fibre diameter variance

FFDA	= Fibre finess distribution analyser
FGF	= Fibroblast growth factor
FSH	= Follicle stimulating hormone
GLM	= General linear model
GWG	= Greasy wool growth
h^2	= Heritability
HGT	= High glysine tyrosine
HS	= High sulphur
IF	= Intermediate filament
IFAP	= Intermediate filament associated protein
IFS	= Intrinsic fibre strength
IRS	= Inner root sheath
Kg	= Kilogram
LEF-1	= Lymphoid enhancer binding factor
LH	= Luteinizing hormone
LS	= low sulphur
µm	= micrometer
MAX-MIN	= difference between maximum and minimum finbre diameter
ME	= Metabolisable Energy
mg	= miligram
MJ	= Mega Joule
Mpa	= Mega Pascals
mRNA	= Messanger ribo-necleic acid
N/ktex	= Newton/Kilotex
OFDA	= Optic fibre diemeter analyser

ORS	= Outer root sheath
POB	= Point of break
r_g	= genetic correlation
SA	= South Australia
SDS	= Sodium dodecyl sulphate
SL	= Staple length
S/P	= Secondary/Primary follicle
SS	= Staple strength
SSC	= Sodium citrate/Sodium chloride buffer
TGF	= Transforming growth factor
TEAM	= Trials Evaluating Additional Measurement
TRN	= Trichohyalin
TSH	= Thyroid stimulating hormone
UV	= Ultra violet
UHS	= Ultra high sulphur
VALT	= Valine, arginine, lysine and threonine
VM	= Vegetable matter base (%)
WA	= Western Australia

CHAPTER 1

LITERATURE REVIEW

1. Introduction

While it has previously been considered that Merino wool follicles are in a constant state of anagen (active fibre production), recent evidence suggests that there are times when Merino follicles become transiently inactive. This results not only in reduced staple strength (SS), but also in a reduction in total fibre output (Schlink and Dollin, 1995; Hynd *et al.,* 1997). Together these effects are estimated to cost the wool industry AUS$367 million per annum (Schlink, pers. comm).

In this review the importance of staple strength (SS) in determining the processing performance of wool fibres is outlined along with a consideration of the economic impact of weak wool fibres. The impact of genetics and environment and the relative roles of minimum fibre diameter, fibre diameter variation, intrinsic strength and follicle shutdown in determining staple strength are discussed. A detailed description of the histology and cell biology of follicle shutdown is presented along with a consideration of the similarities and differences between 'shutdown', as it may relate to staple strength, and normal hair follicle regression in the hair cycle.

2. Importance of Staple Strength

2.1 Relative Value of Raw Wool Attributes to the Industry

Staple strength is a measure of "the force in Newtons required to break a wool staple relative to its linear density in kilotex, where a kilotex is the density of a standard yarn weighing 1 g/m" (Bigham *et al.,* 1983).

This is an objective measurement of staple strength obtained by clamping the staple at each end and extending/pulling until it breaks. The maximum force to break is recorded and adjusted for the amount of material in staple per unit length (Ralph, 1986)

Staple strength (SS) is the second most important determinant of wool price after fibre diameter, and can contribute up to 21% of the wool price paid at auction in comparison with the other fleece traits such as fibre diameter (48%), vegetable matter (10%), length (7%), style (4%), colour (4%), yield (3%) and other (4%) (Couchman *et al.* 1992). However the precise relative contribution of SS to wool price depends on the micron range with in which one is operating. In general the discounts for poor SS are greater for finer wools than broader wools (Table 1.1).

Table 1.1 Effect of staple strength on price discount from different sheep genotypes (Woolmark, 2000)

Staple Strength (N/ktex)	Price Discount (%) Fine Wool	Price Discount (%) Medium Wool	Price Discount (%) Broad Wool
15	18.9	13.6	10.5
20	13.5	9.1	5.3
25	10.8	9.1	5.3
30	5.4	0	0
35	0	0	0

Sound wool/yarn is demanded by modern high speed processing machinery at all stages of wool processing, so staple strength can affect carding, combing and spinning performance. Rottenbury *et al.* (1986) compared wool of five different staple strengths (tender, weak, average 1, average 2, and strong). They found that carding losses for the lowest SS wool were 9.3% compared to 6.6% for wool with the highest SS. Similarly,

spinning potential drops as staple strength decreases. The study also found that there was a tendency for wool with lower staple strength to have increased levels of noil (short, tangled and broken fibres that are removed during combing) (Table 1.2). Ross (1982) reported that staple strength is mainly related to fibre length in the top, with tender wool having shorter fibres after carding than sound wool (50 vs 70 mm, respectively).

Table 1.2. The impact of staple strength on carding loss, spinning performance and noilage (Rottenbury *et al*, 1986)

Staple Strength (N/ktex)	Carding Loss (%)	Spinning at 18.5 tex & 34 mm carding setting (r/min)	Noil (%) at 34 mm carding setting
Tender (20)	9.3	10166	6.7
Weak (34)	7.6	12604	6.3
Average1 (43)	5.9	12520	5.1
Average2 (47)	7.6	11916	5.4
Strong (63)	6.6	13125	5.9

Greater penalties therefore apply to weaker wools and the magnitude of the discount is greater for finer wools than for coarser wools.

The penalty applying to lower staple strength wool has become a serious problem, as approximately 34% of all wool produced in Australia was categorised as tender (<32 N/ktex), with Western Australia producing the highest figure (42%) (Oldham, 2000). Thompson (1998) estimated that the SS problem could cause $40 million in losses annually in Western Australia alone and may cost the national wool industry more than $100 million per year.

2.2 Staple Strength and Wool Processing Performance

Staple strength indirectly influences the processing performance as a result of its strong correlation with Hauteur (fibre length in the top) (Couchman *et al.* 1992). In the carding process, the staple strength of raw wool is the second most important fibre characteristic after staple length in determining Hauteur (Douglas, 1988). Hauteur, in turn, affects many variables in late-stage processing, including yarn strength, yarn performance, spindle speed and the incidence of yarn breakage during spinning (Plate *et al.* 1987). The strong correlation between staple strength and Hauteur occurs at staple strengths between 20 and 50 N/ktex (Rottenbury *et al.* 1986). This relationship is also influenced by the position of break (POB), in that a stronger correlation is found when the break is near the middle of the wool staple (Plate *et al.* 1987). Woods *et al.* (1990) also found that 72% of the fibres broke at the point of minimum fibre diameter. The relationship between Hauteur, staple strength and other fleece traits (staple length, fibre diameter, point of break and vegetable matter) is defined by the TEAM (Trials Evaluating Additional Measurement) equation which was introduced by the Australian Wool Corporation in 1988 (Equation 1) (Couchman *et al.* 1992).

$$\textbf{Hauteur (mm)} = (0.52\,SL)+(0.47\,SS)+(0.95\,FD)-(0.19\,M^*) - (0.45\,VM) - 3.5 \qquad (1)$$

Where SL = staple length (mm); SS = staple strength (N/ktex); FD = mean fibre diameter (μm); M* = adjusted percentage of middle breaks (%) (This value is adjusted up to 45 % if it is less than 45 %, as the value of less than 45 % had little effect on the processing performance); and VM = vegetable matter base (%).

Staple strength can also be used to calculate the uniformity of fibre length in the top, which is predicted from the equation for CV (coefficient of variation) Hauteur (%)

(Equation 2) (Couchman *et al.*, 1992). Staple strength and the percentage of mid breaks are the most important variables influencing the uniformity of Hauteur (Douglas, 1988).

CV Hauteur (%) = (0.12 SL) - (0.41 SS) - (0.35 FD) + (0.20 M*) + 49.3 (2)

Staple strength is also used to predict the percentage of romaine, which is the percentage of short fibres, noil or waste as a result of combing wool from staple measurement (Equation 3) (Couchman *et al.* 1992). Although vegetable matter content is the most important variable in determining the romaine, the other fleece traits (staple length, staple strength and mean fibre diameter) are also important (Douglas, 1988). Although neither the CV Hauteur nor the Romaine formulas are as robust as that for Hauteur, they are reasonable predictors of processing performance (Couchman *et al.* 1992).

Romaine = -(0.11 SL) - (0.14 SS) - (0.35 FD) + (0.94 VM) + 27.7 (3)

Given the unambiguous importance of staple strength in wool processing, it is important to understand how genetic and environmental factors contribute to the strength of fibres and staples.

3. Determinants of Staple Strength (SS)

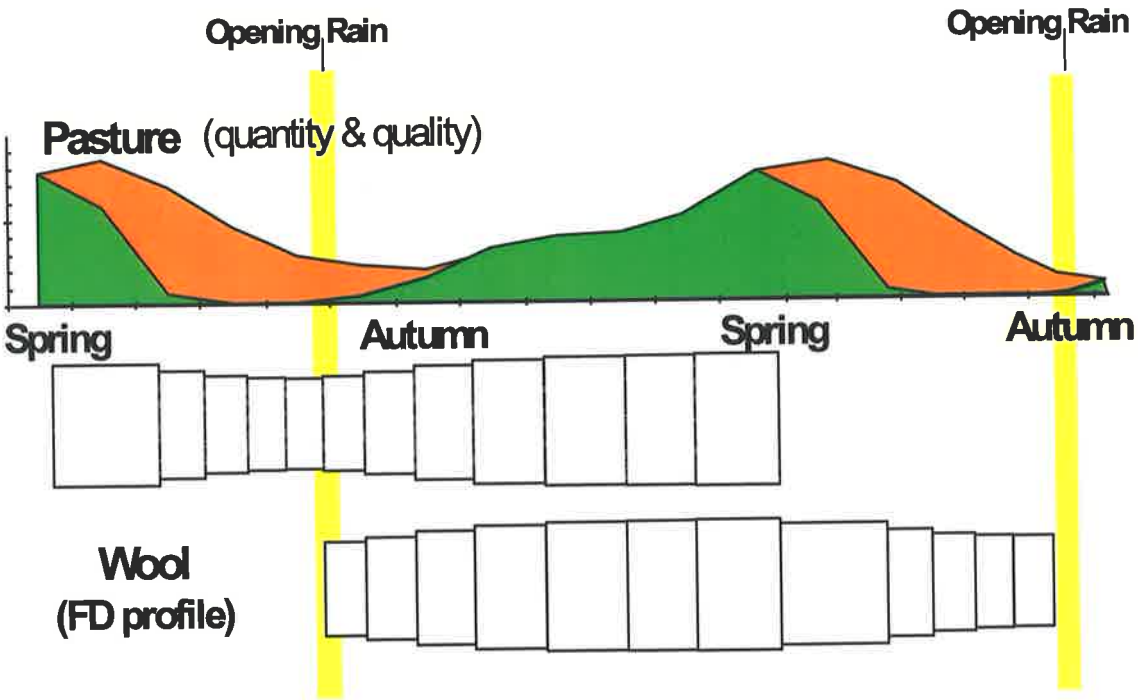
3.1 The Role of Minimum Fibre Diameter in Determining SS

Previously it has been shown that in a Mediterranean environment, adverse nutrition during summer and rain at the "break of season" (autumn) causes a sudden shock to follicle growth as well as a reduction in fibre diameter to the lowest level (minimum

fibre diameter) (Schlink *et al.,* 1996[a]). A reduction in FD can also be caused by disease and or parasitism, pregnancy and lactation, season or any factor inducing stress in sheep (Hynd and Schlink, 1992). Some of these effects are a direct consequence of a reduction in feed intake, while others are hormonally-mediated. For instance, stimulating stress by using cortisol injections decreased FD causing shutdown follicles (Ansari-Renani and Hynd, 1996).

Minimum FD is one of the important determinants of staple strength (Hansford and Kennedy, 1988) as wool fibres are likely to break at the point of the lowest diameter (Bigham *et al.,* 1983). Woods *et al.* (1990) found that 72% of the fibres broke at the point of minimum FD. In this study, minimum FD at the point of break (POB) contributed to 50% of the variation in SS. Other studies have indicated that only 41% of variation in SS is accounted by minimum FD and 54% by rate of change in diameter (Hunter *et al.,* 1983; Hansford and Kennedy, 1988). This finding is in agreement with Orwin *et al.* (1985) who found that there was a highly significant correlation between minimum FD and the force required to break individual fibres ($r = 0.82$).

The position of minimum FD and POB is related to shearing time. In Mediterranean climate, Oldham (1999) showed that under spring shearing management, the minimum FD will occur in the middle of staple and this can reduce the Hauteur. He recommended application of autumn shearing in order to obtain the minimum FD around the tip or the base of staple with a resultant increase in Hauteur and SS (Figure 1.1).



Adapted by Chris Oldham from data supplied by Kimbal Curtis 1993

Figure 1.1 Diagram of seasonal feed availability in a Mediterranean climate and its relation to FD profile under different shearing management (Oldham, 1999).

3.2 The Role of Fibre Diameter Variation between and along the Staple in Determining Staple Strength (SS)

Fibre diameter variation measured as coefficient of variation in FD (CVFD) has become one of the objective measurements applied in the wool industry as this parameter is an important SS determinant. This parameter is a relative measure of variation between actual FD variation (standard deviation) and mean FD. CVFD can now be measured simultaneously with FD using OFDA or laserscan systems during routine FD measurements from midside sample. Midside wool provides a good sampling site for FD variation representing the variation between fibres as well as within fibres (Dawes, 1975).

CVFD has a strong genetic association with staple strength (McKinley *et al.* 1976), but the precise relationship seems to vary with geographical location. Ritchie and

Lewer (1994) reported a high negative genetic correlation under Western Australian high rainfall conditions (r_g = -0.64; Table 1.5), but in the Northern Tablelands of New South Wales (high rainfall), the correlation was only -0.29 in accord with a similar value found under South Australia conditions (Mediterranean) (r = -0.36) (Hill and Ponzoni, 1999).

In tender wool, FD variation between fibres accounts for the greatest proportion of variation (64%), whereas in sound wool it accounted for only 43%. Lower variation of FD between fibres creates higher uniformity of fibre length, which produces sound wool (Schlink *et al.*, 1996[a]).

Similarly, the variation in FD along fibres is higher (43%) in tender wool than in sound wool (16%) (Quinnell *et al.* 1973). Significant correlation between variation in FD along the staple (measured as the rate of FD change) and SS was also shown by Hansford and Kennedy (1990): accounting for 54% of the variation in SS. This indicates that both FD variations between and along fibres are important in determining SS.

As previously discussed, changes in FD along the staple are generated by the pasture changes in Mediterranean environments and this is also related to minimum FD and POB and shearing management. Naylor and Stanton (2000) showed clearly the difference in FD changes throughout the year between different shearing time and found that autumn shearing increased SS (Figure 1.2).

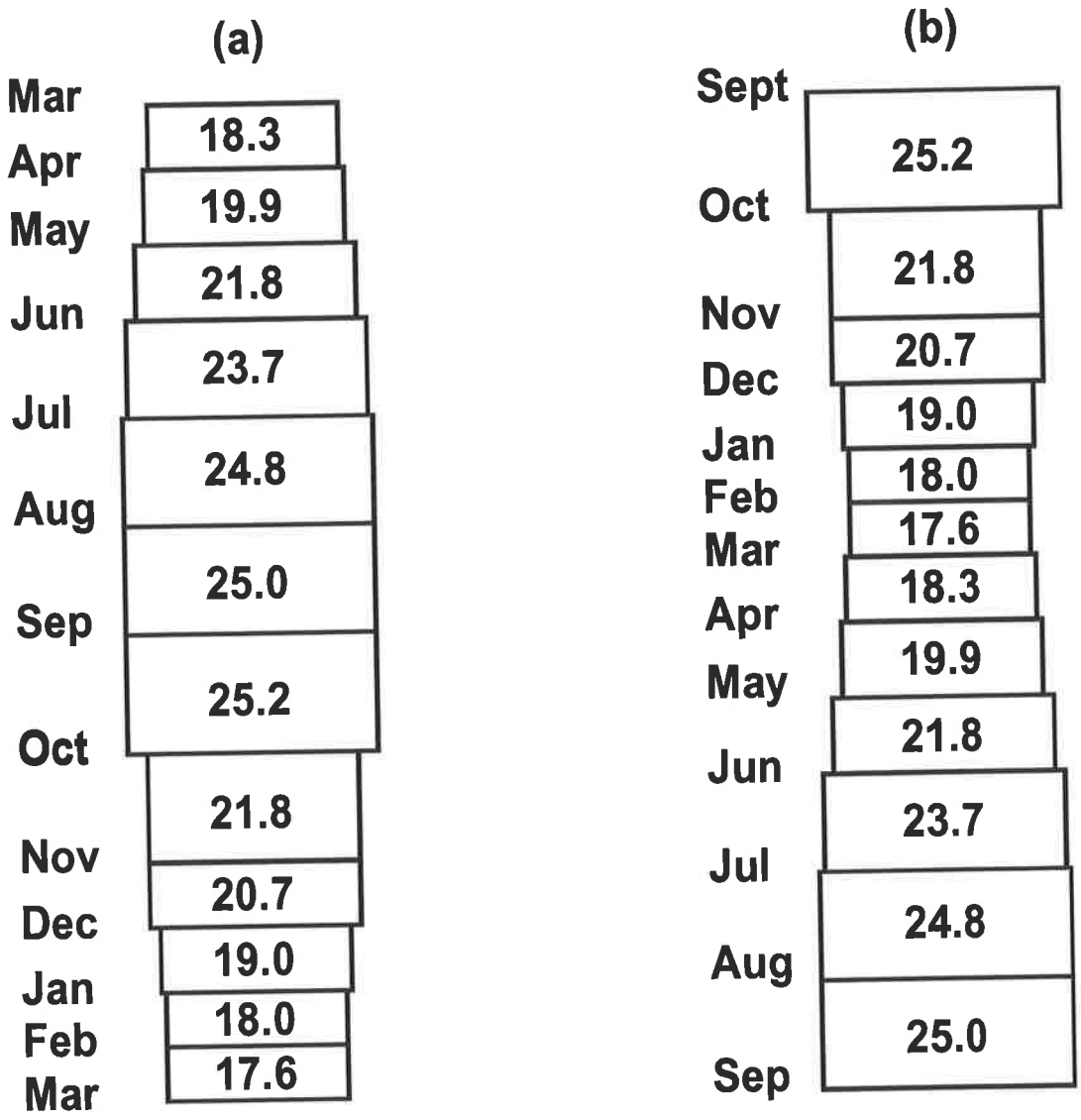


Figure 1.2 Fibre diameter profile from spring shearing (a) and autumn shearing (b) (Adapted from Naylor and Stanton, 2000)

Hynd and Schlink (1992) proposed that since the FD variation in response to the environment is a significant determinant of SS, it is very important to know the susceptibility of sheep in changing their wool FD to the environmental changes. This may be a key component of genetic differences in SS as shown. Hynd (1994[a]) found that FD variation as a result of nutritional change was less in longer than shorter fibres as fibres with a high rate of elongation have slower diameter changes. This concept is discussed

further in Chapter 3 (Genetic and environment interaction in FD variation along the staple).

In summary FD variation between and along fibres contribute SS through their relation to the evenness of fibre length and diameter between fibres as well as to minimum fibre diameter along the staple.

3.3 The Role of Intrinsic Fibre Strength (IFS) in Determining SS

Minimum FD and rate of change in FD as a result of variation in the pasture changes in Mediterranean environment does not account for all of the variation in SS. Fibres of the same FD at the POB, can differ markedly in breaking force suggesting that their intrinsic fibre strength (IFS) differs. IFS is defined as 'the tensile force required to break a sample of wool, normalized by dividing the force by some measure of the amount of material being broken' (Huson *et al.*, 2000[a]).

IFS differs to SS in terms of the amount of material which is linear density of the staple or bundle, resulting its unit of N/Ktex. Whereas in IFS, using single fibres, measures FD to calculate cross sectional area to determine the amount of material at the point of break. It results in units either of cN/ktex or MPa (Mega Pascals), which cannot be interconverted, because of different methodologies used to measure IFS in which MPa is a measure of stress or load required to break the fibre compared to SS, it does not account for possible thin regions along the length of the fibres, poorly aligned fibres or in the case of number of shed fibres (Huson *et al.*, 2000[a]).

The relationship between IFS (single fibre tenacity) and SS (staple tenacity) is quite high, with the correlation (r) values of 0.84-0.97 (Huson *et al.* (2000[b]). Similar high correlations were also found in some studies (r = 0.96) in which the IFS of Australian Merino type wool were lower (6.7 cN/tex) in tender wool than in sound wool (10.3

cN/tex), while SS results were 1.3 and 4.2 cN/tex, respectively for both types (Hunter *et al.*, 1983). Huson *et al.* (1997) also showed that tender wool had lower IFS (136 Mpa) than sound wool (166 Mpa). A significant correlation between IFS and SS, but in lower correlation was also shown by Ansari-Renani (1996) with 50% of the variation in SS between sheep being accounted for by mean IFS. Such correlation was also found (r = 0.52) in a study reported by Gourdie *et al.* (1992).

In contrast, Thompson (1998) found that IFS was similar in sound and tender selection flocks (211 and 203 Mpa, respectively), but IFS was lower from sheep on a lower plane of nutrition. Scobie *et al.* (1996) also showed a non-significant relationship between IFS and SS.

4. Impact of Environment and Genetics on SS

4.1 Impact of Environment on SS

4.1.1 Effect of temperature and photoperiod on SS

Environmental factors such as temperature and photoperiod may have important effects on SS as discussed below.

Bottomley (1979) reviewed the effect of cold and heat on wool growth and found that by cooling an area of sheep skin, the length growth rate of wool was depressed immediately in the cooled area of skin, without affecting FD. Given the positive correlation between SS and FD, it could be hypothesized that cold exposure might not significantly affect SS. This review also showed that heat stress caused wool growth depressions which may have related to a reduction in feed intake particularly in pregnant sheep. Heat stress may thus indirectly depress SS.

In the case of photoperiod, it has been long known that wool growth rate of most sheep breeds is affected by daylength (Ferguson, 1949; Ferguson *et al,* 1965; Hart, 1955;

Wildman, 1957; Hart *et al.,* 1963 and Doney, 1966). These studies showed that during the short daylength season (winter) wool growth declined and increased in the long daylength season (summer), although there are significant differences between breeds in their responses to photoperiod. The effect of photoperiod was also found for wool growth of more primitive sheep breeds including Wiltshire sheep (Ryder, 1969), Shetland sheep (Ryder, 1971[a]), Soay sheep (Ryder, 1971[b]) and wild Mouflon sheep (Ryder, 1973).

Hormonal status is closely related to photoperiod which is a significant factor controlling pelage changes throughout the year. Melatonin was secreted from the pineal gland, a key element in the photoperiodic control of annual cycles of hair growth. Higher concentration of secreation of this hormone occurs during periods of shortdays length (winter) and resulting in increased formation of kemp follicles in Limousin sheep in anagen during winter (Allain *et al.,* 1994). Whereas prolactin secreted from the pituitary gland was elevated during longer daylength (autumn), resulting probably in increased spring follicle activity (Pearson *et al.,* 1996). This study also concluded that the mechanism of prolactin effects on wool growth appears to be by an indirect causative action on wool growth. In Merinos, however, the effect of photoperiodism as indicated by the amplitude of photoperiodic rhythm in wool growth (<17%) is less than that exhibited by other breeds of sheep such as the Corriedale (85%), Suffolk (52%) or Cheviot (68%) (Nagorcka, 1979). The effect of nutrition on wool growth is more dominant than photoperiod as shown by Williams and Schinkel (1962) or Thwaites (1972) and this is discussed further in the next section. Thus, it is concluded that it is unlikely that seasonal rhythms in wool growth induced by photoperiod or temperature are significant in terms of their effects on SS. It is more likely that in Merinos genetics and/or nutrition are the major determinants of SS.

4.1.2 Effect of Nutrition on SS

4.1.2.1 Variation of Feed Availability and SS

Mediterranean environments are characterised by large seasonal variations in pasture quantity and quality (Figure 1.3). There is low green feed availability in autumn/winter, dry feed over summer/autumn and surplus feed in spring (Belloti *et al.* 1992). This variation in feed supply is associated with variation in wool growth rate and fibre diameter which could affect staple strength.

Studies on seasonally-induced nutritional changes in wool growth of Merinos have been conducted over several decades. Williams and Schinkel (1962) showed that in a winter rainfall environment (Roseworthy, South Australia), the wool growth rate was high in spring and low during March-April. A similar wool growth pattern was also reported in Western Australian Merino wethers (Stewart *et al.,* 1961). This is also in agreement with Butler and Head (1993) who showed that wool growth rate measured as staple length rate dropped around 19% in autumn in comparison with spring.

Similarly, Williams and Schinkel (1962) found that fibre diameter declined in May/June associated with poor nutrition in South Australia. Schlink *et al.* (1996[a]) showed that fibre diameter was around 63% lower in autumn (poor nutrition) than in spring (good feed availability).

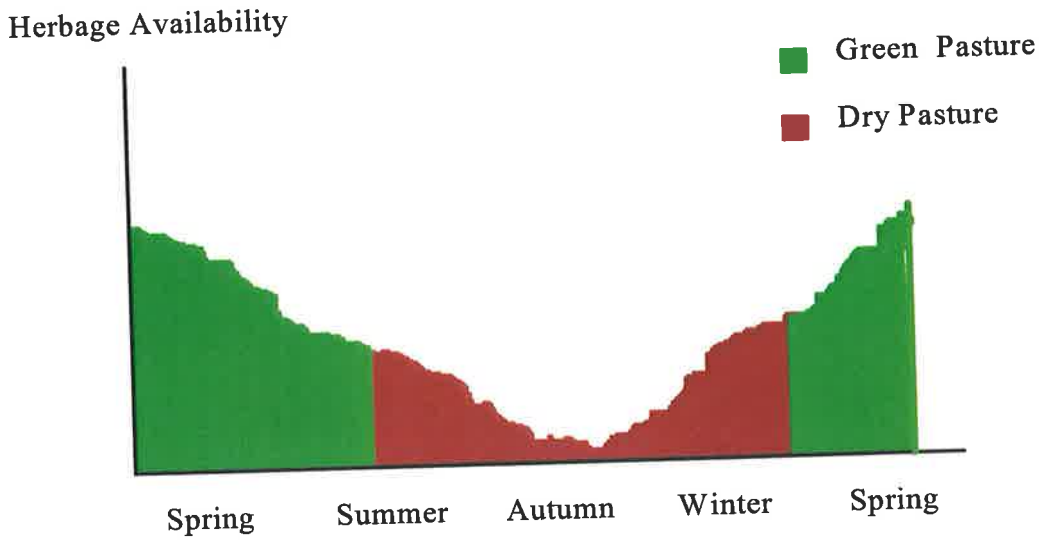


Figure 1.3. Seasonal variation in feed availability in a Mediterranean environment (Belloti *et al.* 1992).

Given the strong correlation between FD, FD variation along the staple and SS, it is then clear that variation in nutrient supply could affect SS (Doyle *et al.* 1994). Butler (1994) also concluded that SS was determined by minimum FD and FD variation induced by nutritional changes throughout the year. This may be related to lower FD occurring in the middle of the staple as shown in Figure 1.4; the lower FD at POB, the lower the SS (Schlink *et al.* 1996[b]). These authors also compared fibre strength at different minimum fibre diameters but at the same average FD (23 μm), and found that fibre strengths were 16.6 and 22 mg/μm^2, respectively at minimum FD of 20 and 23 μm. This is in accord to what Thompson (1998) reported that minimum FD accounted for 66% of the total variance in SS generated by selection and nutrition. It appears then that minimum FD is likely to be a major contributor to fibre strength and therefore SS.

Butler (1994) proposed a management strategy to minimise the effect of minimum FD on SS by changing the shearing date and by manipulation of feeding regimes. Shearing can be changed from a conventional spring shearing to an autumn/winter

shearing, so that, minimum FDs will occur in autumn will be positioned at the tip or base of wool staple rather than in the middle of staple. This can significantly increase SS and reduce the proportion of mid-breaks (Oldham, 1999; Doyle *et al.*, 1994).

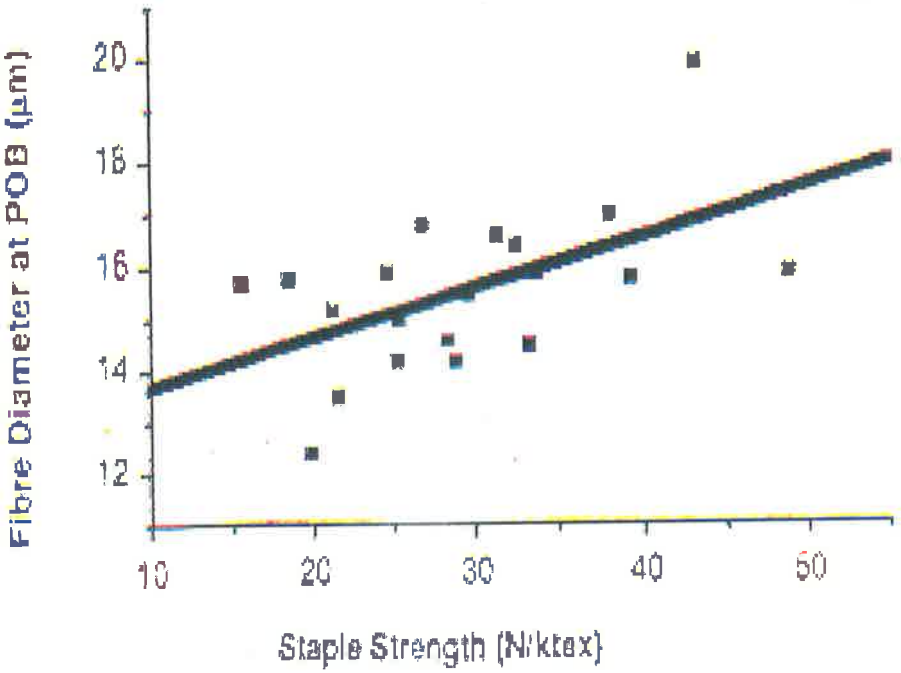


Figure 1.4 Relationship between FD at POB (Point of Break) and SS (adapted from Schlink *et al.* 1996[b])

Alternatively animals can be given supplements to increase minimum FD or stocking rate can be altered. Butler and Head (1993) showed that the SS of Merino, Polwarth and their crosses at maintenance feeding was lower (29.6 N/ktex) than those fed at maintenance + 10% level (35.0 N/ktex). Hutchinson (1962) demonstrated that a uniform diet applied to Strongwool Merino ewes elimiated the low wool growth that usually occurs during December-February at pasture.

The effect of nutrition on SS was also clearly shown from the SS increases achieved when sheep were supplemented with canola meal based pellets (Masters and

Mata, 1998). Similarly, an increase in SS was also found on supplementation with Lupins plus barley plus fresh silage Barley+fresh silage (Gardner *et al.,* 1993) or Lupins plus oats at a high rate (Doyle *et al.,* 1995). Ralph (1986) showed that an oats/lupin supplement increased the SS from 14 to 35 N/ktex.

Similarly, a decrease in nutrition due to stocking rate also decreased SS as demonstrated by Morcombe *et al.* (1996). SS was lower (24.9 N/ktex) at higher stocking density than that at lower stocking densities (27.1 N/ktex). Earl *et al.* (1994) also generally found lower SS at higher stocking rates in Merino wethers. This conclusion is also in agreement with that reported by Hynd *et al.* (1997). High stocking rates were associated with low SS (less than 20 N/ktex) in both Fine and Strongwool Merinos strains.

4.1.2.2 The Effect of Feed Intake and Protein/Energy Ratio of the Diet on Wool Growth and SS

As previously reviewed, low green feed availability in autumn/winter can cause a reduction in wool growth, FD and SS, presumably as a result of limited feed intake. Feed intake is one of the major determinants of wool growth in addition to the digestibility of the diet and metabolic efficiency of the animal (Allden, 1979). Other factors such as the physiological status of the animal, previous nutrition and size at maturity can also affect feed intake with a relatively complex mechanism, making it difficult to predict feed intake (Allden, 1979). However, generally there is a positive correlation between feed intake and wool growth, although the precise nature of the relationship varies from study to study (Allden, 1968; Ferguson, 1972).

The increase in wool growth as a result of increased feed intake is usually accompanied by changes in fibre diameter and fibre length (Stewart *et al.* 1961; Sumner

and Wickham, 1969). As previously reviewed, both wool growth and FD are positively correlated with SS, suggesting that the effects of feed intake may also increase SS. This is in agreement with Thwaites (1972) who showed that poor nutrition was a major cause of wool tenderness and fibre breakage.

4.1.2.3 Amino Acids and Other Essential Nutrients for Wool Growth and SS

Wool proteins, especially high sulphur and ultra high sulphur proteins, are rich in cystine and contain very little methionine or lysine (Table 1.3) (Reis, 1989). Detail of fibre properties and structure is discussed later in Section 5.

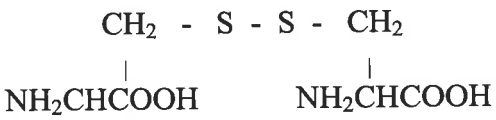
Table 1.3. Composition of amino acids in different types of protein in wool and wool proteins extracted from Merino wool (Adapted from Reis, 1989)

Protein	Cyst(e)ine	Methionine	Lysine
Whole wool			
- Low-sulphur wool	9.5 [a]	0.5	3.0
- Enriched-sulphur wool	13.8 [a]	0.4	2.8
High-sulphur protein			
- Low-sulphur wool	20.1 [b]	0.0	0.6
- Enriched-sulphur wool	24.5 [b]	0.0	0.6
Ultra-high sulphur protein	29.9 [b]	0.0	0.9
High-tyrosine protein			
- Type I	6.0 [b]	0.0	0.4
- Type II	9.8 [b]	0.0	0.4
Low-sulphur protein	6.0 [b]	0.6	3.5

[a] Cysteine was measured as half-cystine in wool
[b] or as S-carboxymethyl cysteine in soluble proteins.
Sulphur-enriched wool was obtained by manipulating the amino acid nutrition of the sheep

Sulphur-amino acids are the most important amino acids regulating wool growth and cyst(e)ine is the rate-limiting amino acid required for the synthesis of wool proteins. Cysteine may occur in protein in two forms, either as itself or as cystine in which two

cysteine molecules are joined together by a disulphide bond (McDonald *et al.*, 1995). The structure of cystine is as follow:



Cyst(e)ine is required for the synthesis of important compounds like glutathione and coenzyme A and may also have a role in mitotic activity of follicle bulb cells by the provision of sulphydryl groups (Reis, 1979; Hynd, 1989). Cyst(e)ine can be obtained via gastrointestinal absorption or from methionine via homocysteine and cystathionine (the transulphuration pathway, Figure 1.5).

Reis (1979) showed that by supplementing sheep on roughage-based diets or pasture with cysteine or methionine (up to 3 g/day), increased wool growth including fibre length and fibre diameter significantly, with more response in cysteine supplementation (*could you please check whether it is right. I lost the paper, Phil. You have the book: Reis P.J. 1979. Effect of amino acids ...to wool growth. In "Physiological and environmnet limitation to wool growth, eds J.L. Black and P.J. Reis. pp. 223-242.).* Reis and Colebrook (1972) found a reduction in wool growth when sheep were fed a gelatin based diet lacking in most essential amino acids, or zein which is deficient in lysine and tryptophan. Fibre strength is reduced by feeding an amino acid mixture lacking lysine and methionine (Reis 1979). Masters *et al.* (1996) showed that SS and wool growth increased when sheep were given a supplement of egg white or fish meal. The egg white increased cysteine in plasma and sulphur in wool, whereas fish meal increased arginine, histidine, lysine and threonine in plasma.

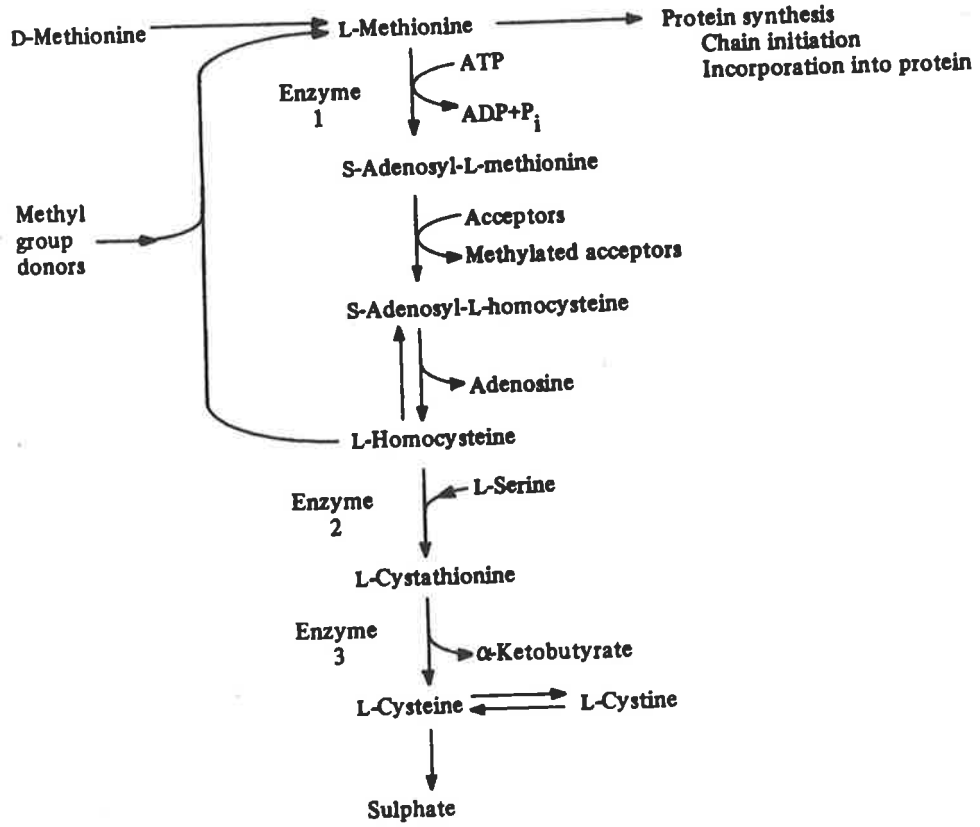


Figure 1.5 Diagram of mechanism of amino acids metabolism for wool growth (Reis, 1979)

Reis and Tunks (1978) studied supplementation with individual amino acids via the abomasum and found that the greatest reduction in wool growth as well as staple strength of wool fibres was found when methionine or lysine were omitted from the feed, despite the fact that wool is low in both methionine and lysine (Table 1.3). *There were three line text in this paragraph that have been deleted, as they are unrelated.*

The small amount of methionine present in wool fibres (Table 1.3) may indicate that methionine has a function mainly as a cysteine source (Reis, 1979). However,

methionine was found to stimulate wool growth 20% more than an equimolar amount of cysteine (Williams *et al.*, 1972). This study also found that omission of methionine reduced wool growth as well as FD and SS, and cysteine did not increase the wool growth to the some amount when fed as an alternative to methionine as the sulphur amino acid source. This result indicates that methionine has another important role other than as a cysteine source (Reis, 1989). Hynd and Nancarrow (1996) showed that in methionine-deficient culture medium, provision of the polyamine spermidine produced an increase in wool growth, indicating the importance of methionine in wool growth in relation to its capacity to produce spermidine. When the polyamine was absent as result of a treatment of using as specific inhibitor, fibre growth and composition were also perturbed (Reis 1989; Reis and Hynd, 1989). It is suggested that polyamines have an important role in normal follicle function including cell division and protein synthesis (Hynd, 2000).

It is clear from this review that the efficiency of wool growth depends on the balance of essential amino acids available exiting the rumen as well as those absorbed for wool synthesis. Sulphur amino acids stimulate wool growth and are first limiting amino acids for wool growth. Changes in wool growth with nutrition largely reflect a change in cysteine and methionine supply to the follicle. This topic is discussed further in Chapter 4.

Besides a requirement for the amino acids as discussed above, some essential minerals are also needed for maintaining optimal staple strength as well as wool growth. White *et al.* (1992) reported that by giving grazing weaned Merino wethers a mineral mix (multielement supplement) consisting of phosphorus, sulphur, selenium and vitamin B12, staple strength and CFW increased significantly. In more comprehensive reviews, Purser (1979) and Hynd (2000) highlighted the role of minerals in wool growth and concluded that zinc and copper mainly had effects on wool growth independent of feed intake. Purser (1979) concluded that fluorine, phosphorus and selenium may affect wool growth

as a result of changes in feed intake independent of changes in rumen function. Sulphur, sodium, potassium and cobalt were the minerals that are concerned mostly with rumen function and not directly related to wool growth (Purser, 1979).

In terms of vitamin requirement for wool growth, Hynd (2000) concluded that biotin, riboflavin, pyridoxine, folate and pantothenic acid may have roles in wool growth regulation, but further studies are required to elucidate their functions.

4.1.3 Physiological Status of Animals

A reduction in wool growth was found in pregnant and lactating sheep with the decrease ranging from 3.4 to 13.7 % annually in Merinos during pregnancy and 9.0 to 18 % during lactation (Corbett, 1979). Masters *et al.* (1993) similarly showed that during the last 3 weeks of pregnancy, Merinos had lower wool growth, wool sulphur, FD and SS than non reproducing ewes. The increase in the rate of protein synthesis and energy usage during pregnancy/lactation may result in a decrease in total wool production in the ewes (Corbett, 1979). While rearing lambs, FD decreased by up to 1.9 μm, with SS and staple length being reduced by 22 N/ktex and 6 mm, respectively in grazing ewes. This reduction in SS was also found when the pregnant sheep were raised in pens (controlled environment to maintain ewe liveweight) (Masters *et al.,* 1993), suggesting the reduction was caused by pregnancy rather than by poor nutrition.

Hansford (1989) reported that wool growth and staple strength were decreased during pregnancy and lactation in sheep and that the reduction was more severe when feed quantity and quality were poor, because the higher requirement of nutrients for both ewes and their foetuses/lambs was not fulfilled. Similarly, Kelly *et al.* (1992) found that under a November-February joining/mating program, pregnancy/lactation occurs during a period

when feed availability is low (autumn/April). This causes lower staple strength, but could be improved by providing feed supplementation in autumn.

Supplementary feeding using lupins in the middle half of pregnancy was found to increase clean fleece weight and the results also showed that the greater level of lupin supplementation (up to 400 g/day), the higher clean fleece weight. An increase also occurred in SS from 27.4 N/ktex without supplementation to 36.5 N/ktex when 400 g/day lupin grain was provided (Kelly *et al*, 1992).

4.1.4 Effect of Parasites and Disease on SS

When sheep were infected with external parasites such as blowflies, SS was reduced from 27.0 to 12.8 N/ktex (Walkden-Brown *et al.,* 1999). In a previous study, it was found that *Lucilia cuprina* blow flies was the common cause of blowfly strike and during larvae invasion, sheep can suffer fever, anorexia and stress, which may cause further tenderness and 'break' in the fleece (Donald, 1979; Kelly et al, 1992). The reduction in fleece weight for struck sheep was around 0.29 kg or 5% and similarly the strike reduced tensile strength as judged by woolclassers. In severe cases of fly strike, the proportion of weak fleeces can reach 56% of the total clip (Gill and Graham, 1939).

The impact of internal parasites on wool growth is significant (Donald, 1979). For instance, uncontrolled nematode parasite infection resulted in clean fleece losses of 1.0 kg annually and FD reduction by 1.5 μm (Besier 1992). Effects of parasites on SS are equivocal; Brown *et al.* (1985) found no SS difference attributable to parasites in a 5 year study, in contrast to Besier (1992) who found that SS was significantly lower (6.0 N/ktex) in infected sheep groups and proportionally more tender wool was still found in the 2 subsequent years.

Text in this last paragraph is deleted

4.2 Impact of Genetics on SS

4.2.1 Differences in SS between breeds and strains

Evidence for a heritable component to SS comes from several sources. Bigham *et al.* (1978) reviewed the effect of breed on SS and concluded that under the same experimental conditions in New Zealand, there was a small difference in SS (measured as percentage of tender wool) between Romney, Coopworth and Perendale animals or between Romney, Corriedale and Dorset. Wuliji *et al.* (1990) found that SS was higher in Romneys and Coopworths than in their crossbreds with Texel (Texel x Romney or Texel x Coopworths). There were also SS differences between two crossbreds with Texel (Texel x Romney or Texel x Coopworth). There were also SS differences between three crossbreds, Border Leicester x Romney (BR), Poll Dorset x BR (PBR) and Suffolk x BR (SBR). The BR had the highest SS and SBR the lowest (Morris *et al.* 1994).

Within the same breed, there were significant differences in SS between Romney strains. The Ruakura high fertility strain produced wool of lower SS (32.6 N/ktex) than the Coopworth (40.2 N/ktex), BL x Romney (38.8 N/ktex) or Romney D strains (38.4 N/ktex) (Bigham *et al.* 1983). Similarly, within Merino strains, a SS difference was clearly shown. Finewool Merinos had a SS which was 6 to 8 N/ktex higher than Mediumwool Merinos (Gherardi and Masters, 1996) (Figure 1.6).

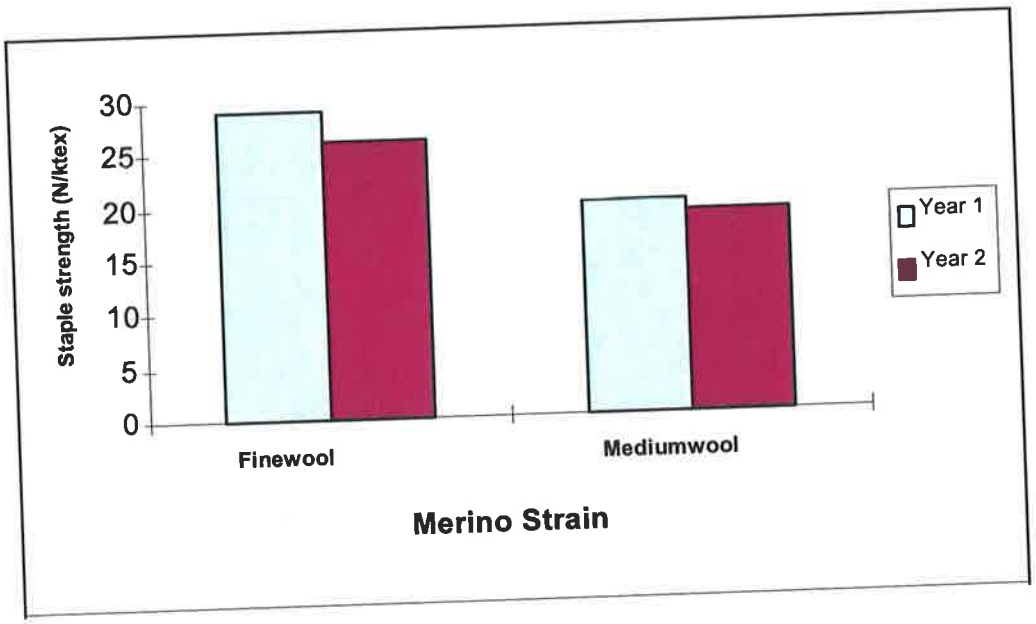


Figure 1.6 Staple strength of Finewool and Mediumwool Merino strains over 2 years in Western Australia (Gherardi and Masters, 1996).

In a Mediterranean environment, a higher staple strength (2.5-4.3 N/ktex) in Finewool Merinos (16.2 μm) than that of Strongwool Merino hoggets (20.1 μm) was also reported by Peterson *et al.* (1998). While a higher SS in Finewool and Strongwool appears to contradict the positive phenotypic and genetic correlation between FD and SS (Table 1.5), the conflict may be resolved by consideration of the impact of CVFD on SS. The Finewool sheep may have more uniform FD within staples than Strongwool sheep. Given the Strong negative correlation between CVFD and SS (Table 1.5) the lower CVFD in Finewool may override the impact of lower FD in Finewool per se on SS.

In contrast, Hughes (1995) showed that in South Australia Merinos (Strongwool), during the break of season (April 4th), the difference was not significant (19 and 21 N/ktex for Finewool and Strongwool Merinos respectively), however, there was a large variation in SS between animals, within and between genotypes (the range in SS was from 7.8 to

44.1 N/ktex). There is also evidence of a difference in SS between bloodlines within the Finewool Merino strain; SS was 20 % higher in the strongest bloodline compared to the weakest (Huson *et al.*, 2000[b]). Such variation within the same strain indicates that SS also varies between sheep under similar nutritional and environmental conditions (Reis, 1992; Greeff *et al.*, 1995). Approximately 65% of the variation in SS within a mob occurred between fleeces and the variation ranged from 5 to 80 N/ktex (mean 44 N/ktex) and 5 to 55 N/ktex (mean 21 N/ktex) between Finewool and Mediumwool Merino strains (Rottenbury *et al.*, 1981).

4.2.2 The heritability (h^2) of SS

The heritability (h^2) is a measure of the proportion of the variation in a trait likely to be passed on to the next generation (inheritance of a trait) (Turner and Young, 1969; Hohenboken, 1985). Estimates of the heritability of staple strength vary widely as shown in Table 1.4.

The heritabilities found in these studies were all categorised as moderate (between 0.15 and 0.30) to high (greater than 0.30), suggesting that staple strength may be improved by selection and breeding. Moreover, the finding that the heritability estimates of SS were reasonably similar between seasons (0.33 and 0.27 in Spring and Autumn) indicates that SS is a stable trait (Greeff, 2000). Indeed the potential for improving SS has been demonstrated by Bray *et al.* (1992) who selected for and against SS in New Zealand Romney sheep for three years (1986 - 1988) to produce low and high staple strength lines. They found that high SS lines were 25 % higher and low SS lines 15% lower in SS than a control group ($p<0.01$).

Table 1.4. Estimates of the heritability of SS

Reference & Description of Sheep Flock	Heritability Estimates (h^2)
Bigham *et al.* (1983): New Zealand Romney ewe hoggets	0.58
Rogan (1989): Merinos	0.17
Lewer and Ritchie (1992): WA medium wool Merino	
- High nutrition (Stud)	0.44
- Low nutrition (Commercial)	0.31
Ponzoni *et al.* (1994): South Australia Merino young rams	
- 10 month old	0.25
- 16 month old	0.47
Ritchie and Lewer (1994): Commercial and Stud flocks	0.31 ± 0.09 ; 0.44 ± & 43
Greeff *et al.* (1995):	
- GSARI Base (Merino ram hoggets)	0.51
- GSARI (mature ewes)	0.25
- Commercial	0.31
- Stud	0.40
- CSIRO	0.23
Wuliji *et al.* (1998): Romney hoggets	0.33
Dominik *et al.* (1999): WA Merino ewes:	
- High nutrition	0.32
- Low nutrition	0.37
Hill and Ponzoni (1999): SA Merinos	
- Male hoggets	0.45
- Female hoggets	0.42
- Female adult (ewes)	0.35
Li *et al.* (1999): Fine wool Merinos	0.34 ± 0.07
Rose and Pepper (2000): Queensland Merinos	
- Longreach	0.37
- Julia Creek	0.23

WA= Western Australia; GSARI = Great Southern Agricultural Institute (WA); SA = South Australia

4.2.3 Genetic correlations between SS and other traits

The potential to use SS as a selection criterion depends not only on its heritability and the degree of variance in SS, but also on the genetic correlation between SS and other economically important traits. For example, where selection for high SS to result in an

increase in fibre diameter, or a decrease in clean fleece growth, the incentive to attempt to increase SS would be reduced. Indeed there is evidence that the genetic correlation between SS and fibre diameter is positive, suggesting that an increase in FD will result from selection based on SS alone. Table 1.5 shows that in some cases the genetic correlations between SS and FD are very low such as in the W.A. Commercial flock (r_g = 0.07) or in Queensland Medium Peppin Merinos (r_g = 0 and 0.04). However, the studies generally showed that there are moderately high, positive genetic correlations between SS and diameter (r_g = 0.40-0.60) suggesting the possibility of increased coarser FD as the sole selection criterion. This is supported by Hygate and Scrivener (1999) who found that FD increased 0.1 μm as a result of response to selection after one generation using SS as the selection objective.

Genetic correlations between traits, however, can be beneficial in that they allow for indirect selection of the parameter of interest. The use of indirect selection parameter is for an efficiency including to avoid the cost of SS measurement as the parameter interest, so that inexpensive indirect selection criterion can be found. Moderate correlation between CFW and SS, for instance, means that selection for CFW will tend to increase SS. This is supported by the findings of Hawker *et al.* (1988) that the staple strength was higher in sheep selected for greasy fleece weight compared with a control group. Similarly when selection was based on SS alone, wool growth was also increased in selection line experiments (Bray *et al.,* 1992; Thompson, 1998). Perhaps the best example of the potential for indirect selection for SS is the use of FD variation measured as coefficient variation of fibre diameter (CVFD). CVFD is strongly related to SS (Table 1.5). High negative genetic correlations between SS and CVFD suggest that CVFD might be a useful indirect selection criterion for SS. This is particularly so because CVFD is

cheaper to measure than SS, as CVFD data are simultaneously obtained from the routine measurement of FD using Laserscan or OFDA.

Table 1.5 Genetic correlations between SS and other fleece traits from different sources/flocks

Reference & Flock	CFW	FD	CVFD	SL
Rogan (1989): Merino	-0.20	0.50		-0.10
Ritchie and Lewer (1994): WA medium wool Merinos)	0.26	0.41	-0.64	
Greeff *et al.* (1995)				
- W.A.Base flock (hoggets)	0.42	0.37	-0.62	0.31
- W.A. commercial	0.03	-0.07	-0.82	0.27
- W.A. studs	0.10	0.45	-0.66	0.24
- W.A. base flock (mature ewes)	-0.14	0.22	-0.86	0.80
- S.A. 10 month rams	0.15	-0.15	-0.27	-0.11
- S.A. 16 month rams	0.37	0.46	-0.46	0.08
- CSIRO fine wool	-0.19	0.27	-0.58	-0.31
Hill and Ponzoni (1999): S.A. Merinos				
- Male hoggets	0.44	0.50	-0.42	
- Female hoggets	0.09	0.43	-0.56	
- Female adult (ewes)	0.16	0.45	-0.52	
Rose and Pepper (2000): Queensland medium peppin Merino (15 month)				
- Longreach	0.19	0.00		0.01
- Julia Creek	-0.38	-0.04		-0.07

CFW= clean fleece weight; FD=Fibre diameter; CVFD=coefficient variation of FD; WA=Western Australia; SA=South Australia.

5. Follicle Shutdown: Its Significance, Seasonal Variation, Morphology and Determinants

As previously reviewed sudden changes in nutrient supply from dry to green herbage, produced by rain, occur at the break of season in autumn. For sheep shorn in Sptember, the proportion of mid-staple breaks is very high, coincides with the rainfall event in autumn, causing tender wool. At the follicular level, abnormal follicle morphology, follicle dysfunction and fibre growth cessation can also occur in Merino sheep. This phenomenon is called follicle shutdown (FS), which is defined as "the process whereby the wool follicle bulb and dermal papilla regress towards the skin surface and the follicle ceases to produce a fibre" (Hughes, 1995). Follicle shutdown has become an important issue since it was found to contribute to low staple strength (Schlink and Dollin, 1995). Follicle shutdown is also important because it is associated with fibre shedding as shown by the high regression coefficient between the numbers of shed fibres in the fleece and follicles classified as "shutdown" (Ansari-Renani, 1996). This section discusses in detail the significance of FS to the wool industry, the effects of season on FS, its morphological characteristics in relation to follicle function and the determinants of FS.

5.1 The Importance of Follicle Shutdown

It was, until recently, believed that follicle shutdown was of minor significance to the Merino wool industry. Recent evidence suggests that this is not the case, and that follicle shutdown can be a major contributor, not only to the production of weak staples, but also to fibre losses from inactive follicles that take some time to start producing a fibre again. In the worst case of follicle shutdown, at the point of break in non-supplemented pregnant/lactating Merino ewes, the incidence of shutdown follicles reduces staple strength significantly. There is a highly significant correlation ($r = 0.94$) between

shutdown incidence and SS (Schlink and Dollin, 1995). Under simulation of stress by injection of cortisol, the proportion of shutdown follicles and shed fibres was significantly correlated with staple strength (r = 0.66 and 0.62, respectively) (Ansari-Renani, 1996). The correlation between shed fibres and staple strength was also highly significant (r = 0.73) in a study of Merino sheep when data were pooled for sound and tender wool and with three levels of nutrition (Thompson, 1998).

The importance of staple strength and how it is measured is discussed in Section 2.1 and 2.2. Absent follicle sheaths (outer and inner parts) and absent fibres, as in the typical morphology of shutdown follicles can make the staple become thinner or smaller. This is worse when the shutdown/fibre shed occurs in the middle of the staple which relates to shearing time as discussed earlier.

Schlink (Pers. Comm) calculated the economic impact of follicle shutdown and fibre shedding based on some assumptions of the proportion of inactive follicles (8.3 %, or similar to 270 g of CFW loss annually) and fibre loss (3.45 % ~ 112 g of CFW loss). It was found that the total cost of inactive follicles and failure to re-grow to the wool industry was $3.06/head or $ 367 million/year, which is a major loss to the wool industry.

5.2 Seasonal Changes and FS

Although it was not termed follicle shutdown, the issue of follicle inactivity, follicle abnormality or fibre shedding has been studied since the 1960s, following the finding that there is a seasonal variation in wool production throughout the year. The pattern of follicle inactivity and wool growth in relation to seasonal changes seems different in Merinos compared to other breeds.

In Wiltshire sheep, a more primitive sheep breed, Ryder (1969) found that in Edinburgh, in the Northern Hemisphere, follicle inactivity was characterized by follicles

with brush end fibres and no medulla, beginning in September and continuing until March (autumn-winter). A similar pattern of inactivity was exhibited by Soay, Shetland and wild Mouflon sheep (Ryder, 1971a; Ryder, 1971b; Ryder, 1973) which generally had inactive wool follicles during winter. In the Southern Hemisphere, such as New Zealand or Australia, lower wool production in the short day length season, winter (June-July), was also demonstrated by other non-Merino breeds. Examples include New Zealand Dorset x Romney ewes (Reid and Sumner, 1991), Tasmanian Polwarths (Butler and Head, 1992) and Australian Wiltshire horn x Merino ewes (Maxwell *et al.* 1988). This shows that photoperiod does play a role in controlling wool growth/follicle inactivity.

In contrast, in South Australian Strongwool Merino ewes, Hutchinson (1962) found that a decrease in wool growth occurred in Summer (December-February) in spite of the longer hours of daylight, indicating that poor nutrition during a dry summer may reduce wool growth. Williams and Schinkel (1962) also presented results from different places in Australia (Roseworthy in South Australia, Canberra, Armidale and Muresk in Western Australia), showing that Merino sheep had low wool growth during summer-autumn. In terms of follicle inactivity, Lyne (1964) similarly showed that Australian Merino wethers had more fibre shedding in March-June (autumn), indicating that low wool growth occurred in the summer-autumn seasons.

In more recent studies, follicle shutdown in South Australian Merinos was reported after the break of season (autumn) around April-May, with the average incidence of shutdown around 10 %. In some individuals, up to 36 % of the follicles were characterized as shutdown; however other individuals appeared to be almost completely unaffected (2 % shutdown) (Hynd *et al.,* 1997). That there was a seasonal incidence of shutdown is evidenced by a low proportion of inactive follicles in summer and winter in this study (4% and 0.26%, respectively). Schlink and Dollin (1995) noted a similar

pattern of seasonal incidence; the proportion of abnormal follicles in spring, summer, autumn and winter were 3.6 %, 11.4 %, 13.6 % and 4.6 %, respectively.

The above discussion shows that, unlike in more primitive breeds of sheep, seasonal fibre shedding in Merinos is influenced more by nutrition than photoperiod or that any photoperiod effect is overridden by nutrition or perhaps some other component of the environment. This issue is discussed in detail in Chapter 2.

5.3 The Morphology of Follicle Shutdown and its Relation to the Hair Cycle and Follicle Function

In the early stages of shutdown, the follicle is characterized by an irregular connective tissue sheath, clustered (distally arranged) nuclei and a distorted follicle shape. In the worst case scenario, the fibre is absent and the follicle totally collapsed (Hynd *et al.*, 1997). In contrast, a normal healthy wool hair has a normal follicle shape, circular and regular fibre and random-scattered nuclei in the outer root sheath (ORS) (Figure 1.7).

Normal Follicle Follicle shutdown

a. Follicle Diagram (longitudinal)

Normal Follicle Shutdown Follicle

b. Transverse section

Figure 1.7 Diagram of a normal wool follicle and a shutdown follicle in longitudinal and trasverse section (adapted from Hynd *et al.,* 1997).

A diagram of a complete wool follicle is shown in Figure 1.8. The follicle can be divided into five zones (i) the follicle bulb as a mitotically active zone, (ii) the keratogenous zone where the keratin protein is synthesized to form the fibre and inner root sheath (IRS), (iii) the zone of final hardening, where the IRS is degraded, (iv) the zone of sloughing, where the degraded IRS sloughs into the pillary canal along with some ORS cells, and (v) the pilary canal, into which the sebaceous and sudiferous gland ducts open

(Chapman and Ward, 1979). The following discussion of follicle shutdown (FS) focuses on the active zone (zone of bulb) and the zone of final hardening, in both of which abnormal follicle morphology can be clearly seen.

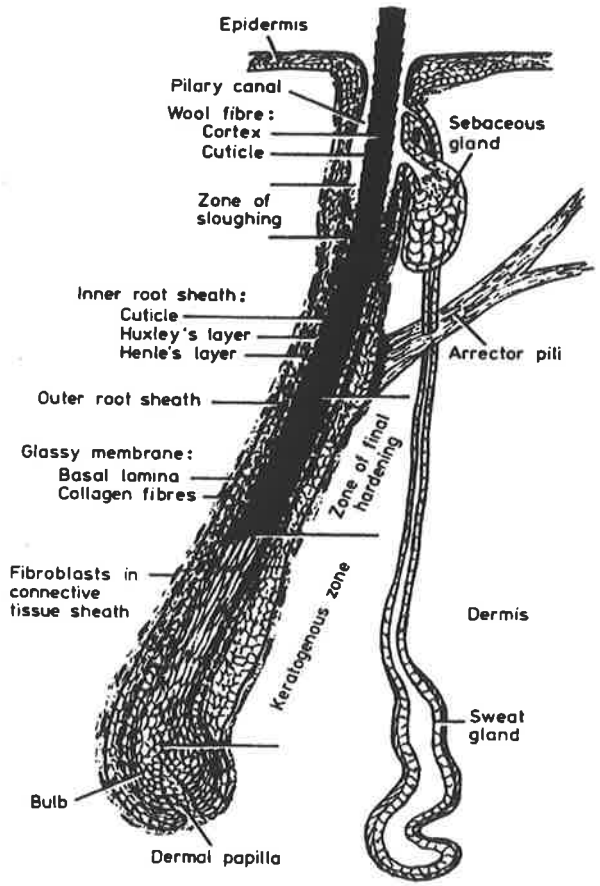
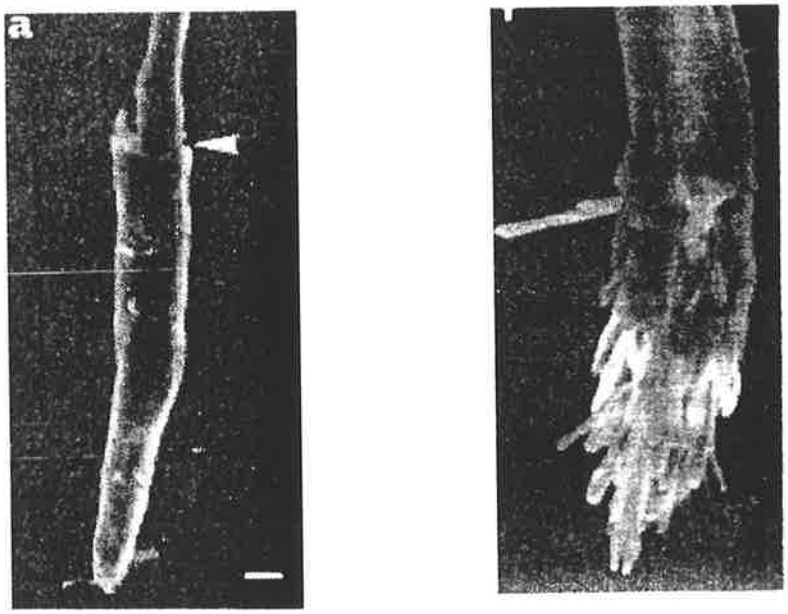


Figure 1.8 Diagram of a wool follicle with the division into five zones (Chapman and Ward, 1979)

Seasonal wool growth in more primitive sheep is in fact a part of normal cyclical hair production as described by Hardy (1993). The three phases of the hair cycle: anagen (growth period), catagen (regression period) and telogen (resting period) are described in this paper. Anagen is the stage when active formation and pigmentation of fibre occurs (Hardy, 1993). Highly specialized epithelial differentiation occurring in anagen produces a hair shaft, which contains tightly bound keratinized epithelial cells (Stenn *et al.*, 1998).

The catagen phase is the regression stage, which is characterized by cessation of mitotic activity in the bulb cells of the follicle, and formation of a brush end in the lower part of the follicle. The follicle regresses to less than half its length in the anagen phase (Hardy, 1993). The cessation of growth is first seen within the follicle bulb followed by atrophy and rounding up of cells in the papilla. Finally, there is regression of the follicular epithelium in the proximal parts of the follicle (Straile *et al.* 1961). Telogen is the resting phase, where the brush end-like fibre is present. The follicle then returns to anagen and the old fibre is dislodged, ready to form a new one (Hardy, 1993). Stenn *et al.* (1998) characterized this shedding stage as "exogen" as a part of hair cycle: in exogen a new hair shaft starts to regrow and the resting follicle from telogen stage shed, to ensure that "the animal is never naked". The new fibre developed in the exogen stage comes from cells in the middle of ORS in the catagen stage, which then transform into germ cells to become new bulb cells (Parakkal, 1969).

When photoperiod affects the wool growth of primitive breeds, the reduction in fibre growth relates to the telogen stage. In Merinos, however, this is not the case. Shutdown follicles have been characterized by a club end on the fibre, in contrast to the brush-like structure which forms in the fibre in the telogen stage (Schlink and Dollin, 1995) (Figure 1.9).



a. Club End Form b. Brush end Form

Figure 1.9 Follicle shutdown in club end and brush end morphology (adapted from Schlink and Dollin, 1995)

This different morphology of FS in Merinos may support the conclusion of a unique mechanism for shutdown in this breed of highly-selected sheep. It is postulated that nutrition may mediate the entry of Merino follicles into a quiescent (shutdown) state. The next section discusses all the possible factors affecting FS.

5.4 Factors Affecting Follicle Shutdown

As previously discussed, compared to more primitive breeds, follicle shutdown in the Merino is less affected by the photoperiod. In this breed seasonal follicle inactivity, follicle shutdown or fibre shedding may be more related to nutritional factors. In this section the effects of nutrition and some other non-nutritional factors including physiological status, follicle/fibre characteristics and breeds on follicle shutdown, are discussed.

5.4.1 Effect of Nutrition on FS

It was previously shown that follicle inactivity in Merinos occurs during the harsh summer-autumn season when poor nutrition is likely to be a problem, suggesting that nutrition plays an important role in inducing follicle shutdown. This conclusion is supported by a number of studies. The effect of adverse nutrition (roughage containing only 2.6 % crude protein, *ad lib.*) on follicle shutdown was clearly demonstrated by Lyne (1964) in which fibre shedding was a major reaction of the follicle population to the poor nutrition. The incidence of shed fibres was reduced to negligible levels when a higher protein feed was supplied (11.7 % vs 3.21 % of shed fibres for poor and better nutrition, respectively).

Similarly, Thompson (1998) showed that the incidence of shutdown follicles at the point of break was about 27% for sheep that received a poor quality ration (formulated to induce liveweight losses of approximately 100 g/day), compared to only 1% for the sheep fed maintenance diet (control). This study also showed similar results at the fibre level, in that there were 8.2% of follicles with club ends from sheep receiving the poor level of nutrition, in contrast to only 1% in sheep fed to maintain liveweight. Furthermore, Hynd *et al.* (1997) showed that a high stocking rate (>13 DSE/ha) produced a higher incidence of shutdown follicles compared to lower stocking rates (<8.0 DSE/ha). This suggests that the incidence of follicle shutdown is related to low availability of feed in the paddock at higher stocking rate.

These findings are also in agreement with results obtained when essential amino acids are fed as a supplement to reduce the incidence of follicle shutdown (the important role of amino acids for wool growth has been discussed earlier in Section 3.2.2.3. Schlink *et al.* (1992) showed that when maintenance feed (75% hay + 25% lupins) was supplemented with DL- Methionine or a mixture of L-valine, arginine, lysine and

threonine (VALT), the incidence of club formation decreased from 11.4% in control feed to 4.3% and 8.6% respectively. Similarly in a later study, Schlink and Dollin (1995) showed that the incidence of shed fibres was lower (3.5%) in methionine-supplemented pregnant /lactating ewes than non-supplemented sheep (9.0 %). Supplementation with VALT, in contrast, had no effect on the incidence of shed fibres (9.4 %).

The effect of poor nutrition was found to be greatest (measured as percentage of normal follicles) when the sheep came from a low staple strength selection line (Schlink *et al.,* 2000). This relates to the high correlation between follicle shutdown and SS, as previously discussed. In addition, Schlink and Dollin (1995) showed that the correlation (r) between follicle shutdown and SS reduced from 0.94 to 0.41 or 0.1, respectively for pregnant ewes that were non-supplemented, methionine supplemented or VALT supplemented. This indicates that nutrition has an important role in the correlation between FS and SS. Poor nutrition can increase the correlation between the two parameters.

This discussion shows that nutrition is an important factor to consider if we want to minimise the incidence of follicle shutdown and fibre shedding. In terms of practical strategies to avoid this problem, shearing time might be the key factor to consider, in relation to position of break, as discussed earlier (Section 4.2). However, other factors discussed in the next section also need to be taken into account.

5.4.2 Effect of physiological status, type of follicles, fibre diameter and staple strength on follicle shutdown

Pregnancy and lactation are physiological states that can induce follicle shutdown as shown by Schlink and Dollin (1995). Wethers (1.5 year old) had a significantly lower incidence of shed fibres (4.9%) than pregnant/lactating ewes (9.0%) under identical

nutritional regimes. Pregnancy and lactation may induce a reduced supply of nutrients to the follicle as a result of competition for nutrients between the foetus, mammary gland and follicle. Alternatively hormonal changes may induce shutdown.

At the follicle level, primary and secondary follicles differ in response to adverse nutrition, with secondary follicles suffering a 9.2% shedding incidence, and primary follicles only 1.4% incidence (Lyne, 1964). Similarly, the secondary follicles were more susceptible to follicle shutdown in response to cortisol injection in Merinos (16.4%, range 2.4 – 30.3%) than primary follicles (2.4%, range 0-6%), and this difference became more apparent as the cortisol concentration increased (Ansari-Renani, 1996). However, when nutrition is not limiting, the incidence of fibre shedding in Merinos is generally low and not different between primary and secondary follicles (Lyne, 1961; Ryder, 1962).

Wool traits are also associated with the incidence of follicle shutdown. Thompson (1998) showed that there was a strong relationship between fibre diameter measured at the point of break along the staple and the percentage of follicle shutdown ($r = 0.67$), with the lower fibre diameter group (<15 μm) tending to be more susceptible to follicle shutdown. This result seems to be not in agreement with that of Ansari-Renani and Hynd (1996), who showed that fibre diameter affected the incidence of follicle shutdown. The lower fibre diameter group (Finewool Merinos) was less susceptible to cortisol-induced follicle shutdown (9.8%) than the higher fibre diameter group (Strongwool Merinos) (13.5%). However, the measurement of lower FD group in previous work was at the point of break cannot be compared with the mean lower FD as occurred in Finewool Merinos versus Mediumwool sheep.

As previously discussed, follicle shutdown can reduce staple strength. Conversely, selection based on staple strength may influence the incidence of follicle shutdown. Schlink *et al.* (1996[a]) found that Merino wethers selected for low staple strength (24.6

N/Ktex) had higher follicle abnormalities (18.1%) after the break of the season (autumn), than a higher staple strength group (37.8 N/Ktex) which had only 13.6% of abnormal follicles. However, in a recent study, Schlink *et al.* (2000) found that the incidence of fibre shedding did not differ significantly between two staple strength lines, although the low staple strength line still had a higher proportion of shed fibres than the higher staple strength line (2.81 % vs 1.88 %). Thompson (1998) also suggested that selection for staple strength does not change the rate of fibre shedding.

5.4.3 Effects of hormones and growth factors on follicle shutdown (FS)

Although hormonal control of follicle activity is not a primary focus of the current study, this section provides a brief review in order to put nutrition and other potential mediators of shutdown in context.

5.4.3.1 Hormones and Wool Growth

Wool growth is markedly influenced by a number of hormones, which also directly or indirectly affect the process of follicle shutdown and fibre shedding. These hormones which are secreted by the pituitary gland, include adrenocorticotrophic hormones (ACTH), prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone and thyroid stimulating hormone (TSH).

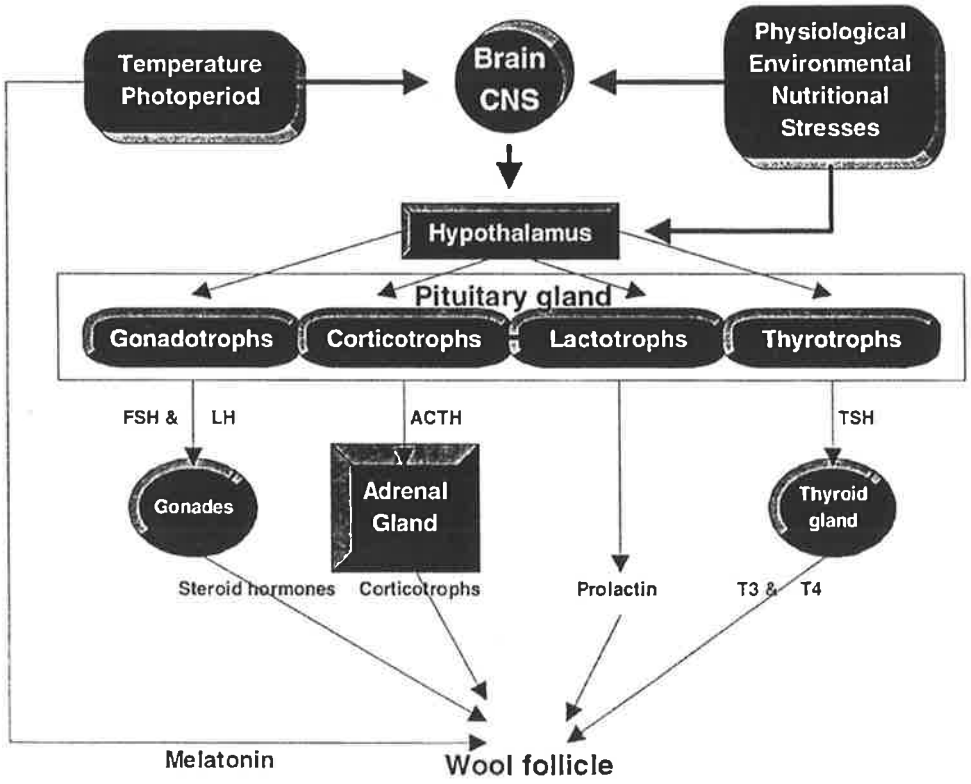


Figure 1.10 The mechanism of hormones involved in the control of coat moulting cycles (adapted from Gebbie *et al.*, 1994)

The secretion of hormones is controlled by highly specialized neuronal cells in the hypothalamus and is a response to physiological or nutritional state, stress or changes in temperature or photoperiod. These stimuli produce hypophysiotropic hormones, which reach the anterior pituitary via the hypophyseal-portal circulation and stimulate or inhibit the release of the trophic hormones. These mechanisms are reviewed in Gebbie *et al.* (1994) (see Figure 1.10).

Some work report that the hormones stimulating wool growth were thyroxine (Ferguson *et al.*, 1965; Hynd, 1989[b] ; Hynd, 1994[b]) and growth hormones (Ferguson *et al.*, 1965; Wallace, 1979); while adrenocorticotrophic hormone (Lindner and Ferguson, 1956; Chapman and Bassett, 1970; Ansari-Renani (1996); Chapman *et al.*, 1982;

Thwaites, 1972) and oestrogen (at high doses) (Slen and Connell, 1958) inhibit wool growth.

In relation to focus of this study, the hormonal mechanism in adrenocorticotrophic hormone may be more relevant to discuss. In detail, Ansari-Renani (1996) reported that the incidence of follicle shutdown was significantly increased from 2.4 % to 30.3 % by increasing cortisol dosage from 0 to 2.86 mg/kg body weight. Similar effects of cortisol inducing follicle shutdown/fibre shed have been reported in other studies (Chapman and Bassett, 1970); Chapman *et al.*, 1982; Thwaites, 1972). It was also found that cortisol stimulated wool growth at a concentration of 2 mg/100ml (Chapman and Bassett, 1970).

This review simply shows that the rate of wool growth is markedly hormone dependent through the endocrine system. The pituitary exerts both stimulating and inhibiting effects through the secreation of thyrotropic and adrenocorticotropic hormones, respectively. The effect of hormones on wool growth has indirect consequences to fibre or staple strength through follicle abnormality which are the subject of this thesis.

5.4.3.2 Growth Factors and Follicle Shutdown

A detailed review of the role of the growth factors in hair growth is presented by Moore *et al.* (1998); In this section a brief summary of this complex field of biology is presented in relation to FS.

Growth factors are a large group of polypeptides which are involved in regulating cell proliferation and DNA synthesis. Growth factors which are relevant to hair growth and skin cell development include: the epidermal growth factor (EGF) family, fibroblast growth factor (FGF), transforming growth factor (TGF), insulin-like growth factor (IGF), nerve growth factor, hepatocyte growth factor/scatter factor, vascular endothlial growth

factor and platelet-derived growth factor (Peus and Pettelkow, 1996). In this section, however, only those related to follicle inactivity/fibre shedding are discussed.

Firstly, growth factor that may be more related to the present study is epidermal growth factor (EGF) and is expressed in the sebaceous gland and outer root sheath of foetal sheep skin (Du Cros *et al.,* 1992) and in the epidermis of mouse skin (Green and Couchman, 1984). Moore *et al.* (1983) found that EGF had a function in stopping follicle development by delaying initiation of newborn mice hair follicles. Previously, Cohen and Elliott (1963) also found that high doses of EGF in mouse inhibited fibre production. Similar results were also found in a sheep experiment in which follicle abnormalities were induced by EGF treatment (Hollis *et al.,* 1983). EGF treatment reduces the rate of growth in hair length and diameter (Moore *et al.,* 1982). This is in agreement with studies in Merinos which show that EGF treatment inhibited wool growth and in many cases resulted in shedding of the entire wool fleece (Moore *et al.,* 1981; Moore *et al.,* 1982;). The characteristics of wool breaks caused by EGF treatment are similar to those of wool treated with ACTH (Lindner and Ferguson, 1956) or with hydrocortisone acetate (Chapman and Bassett, 1970). In contrast, Zshiesche and Eckert (1988) found that this growth factor increased the growth of the first coat of newborn mice. Furthermore, Philpott and Peus (1998) concluded that EGF stimulated proliferation of the epithelial cells of the hair germ that gives rise to the anagen hair follicle.

Although the findings are still equivocal, in general most of the work especially in sheep show that EGF has a role in inhibition of hair growth resulting follicle abnormalities and reduction of the wool growth.

Similarly, fibroblast growth factors (FGFs) which are expressed in the inner root sheath (FGF1), basement membrane of outer root sheath/matrix cells (FGF2), outer root sheath (FGF5) and dermal papilla (FGF7) (Rosenquist and Martin, 1996), play a role in

the inhibition of cell proliferation and regulation of cell survival/differentiation (Basilico and Moscatelli, 1992; Peus and Pettelkow, 1996).

TGF (transforming growth factor), which is expressed in inner root sheath (IRS), follicular papilla (TGFβ1) and IRS (TGFβ2 and 3) (Peus and Pettelkow, 1996), was found to inhibit hair growth in alopecia areata (Hoffmann *et al.,* 1996) and DNA synthesis in epidermal keratinocytes and hair follicle organoids (Peus and Pettelkow, 1996). In contrast TGF was reported to have a stimulating action on follicle morphogenesis in rabbit skin (Porras-Reyes *et al.,* 1993). In another member of the TGFβ superfamily, the BMP (bone morphogenic proteins), the mRNA encoding the BMP's are expressed in the mesenchyme during follicle initiation (Jones *et al.,* 1991) and stimulate hair growth (Jones *et al.,* 1991). Kratochwill *et al.* (1996) also showed that BMP acts indirectly on the hair follicle activating the secretion of lymphoid enhancer binding factor 1 (LEF-1), which stimulates follicle growth and also teeth development. In contrast Peus and Pettelkow (1996) also showed that BMP-2 and 4 inhibited all proliferation of epidermal and epithelial cells. But, most of the findings show that TGF including BMPs have a stronger more role in follicle inhibition than formation (Hynd, pers.comm).

Studies on some growth hormone in relation to wool growth, highlight the need for further investigation of the role of growth factors in follicle function, follicle abnormalities (follicle shutdown) and fibre shedding.

5.4.4 Genetic factors and follicle shutdown

While studies of environmental factors on follicle shutdown have been extensive, investigations into genetic factors and follicle shutdown are limited. Hynd *et al.* (1997) showed that under field experiment the incidence of follicle shutdown did not differ between genotypes of sheep (Finewool vs Strongwool Merino strain), whereas Ansari-

Renani and Hynd (1996) found that in a pen experiment, when cortisol was used to induce follicle shutdown, Finewool Merinos had less follicle shutdown than Strongwool Merinos (9.8 vs 13.5 %, respectively). These conflicting results make it difficult to conclude to what extent follicle shutdown is related to genetic or environmental factors. This question is tried to be elucidated in the further section discussing the mechanism of SS that is also determined by FS.

6. Speculation as to the genetic mechanism underlying variation in follicle shutdown and staple strength

On *a priori* grounds the most likely determinants of SS are factors influencing the minimum fibre diameter, the variation in diameter and length between fibres, the intrinsic strength of the fibres in the staple, and possibly the proportion of fibres that have ceased production in the course of staple formation.

While variations in the amount of material present at points within the staple are clearly going to influence the load-bearing capacity of the staple, the role of the inherent or intrinsic strength is defined as the load-bearing strength of a unit cross-sectioned area of keratin and keratin-related molecules comprising the fibre (Huson *et al.*, 2000[a]).

The inherent strength of fibre is genetically correlated with SS (Hunter *et al.*, 1983). IFS is influenced by fibre composition (ortho, meso and paracortical cells). Fibres with a high proportion of orthocortical cells have a greater SS (Orwin *et al.* 1985). They proposed that these cells have a higher content of macrofibrils, which then govern SS. However, Hansford and Kennedy (1988) dispute the findings by showing that paracortical cells were more related to cause sound wool. Hynd and Schlink (1992) support the latter work by speculating that the fact that Finewool Merinos have weaker fibres (IFS) might be due to a lower proportion of orthocortex in this sheep strain.

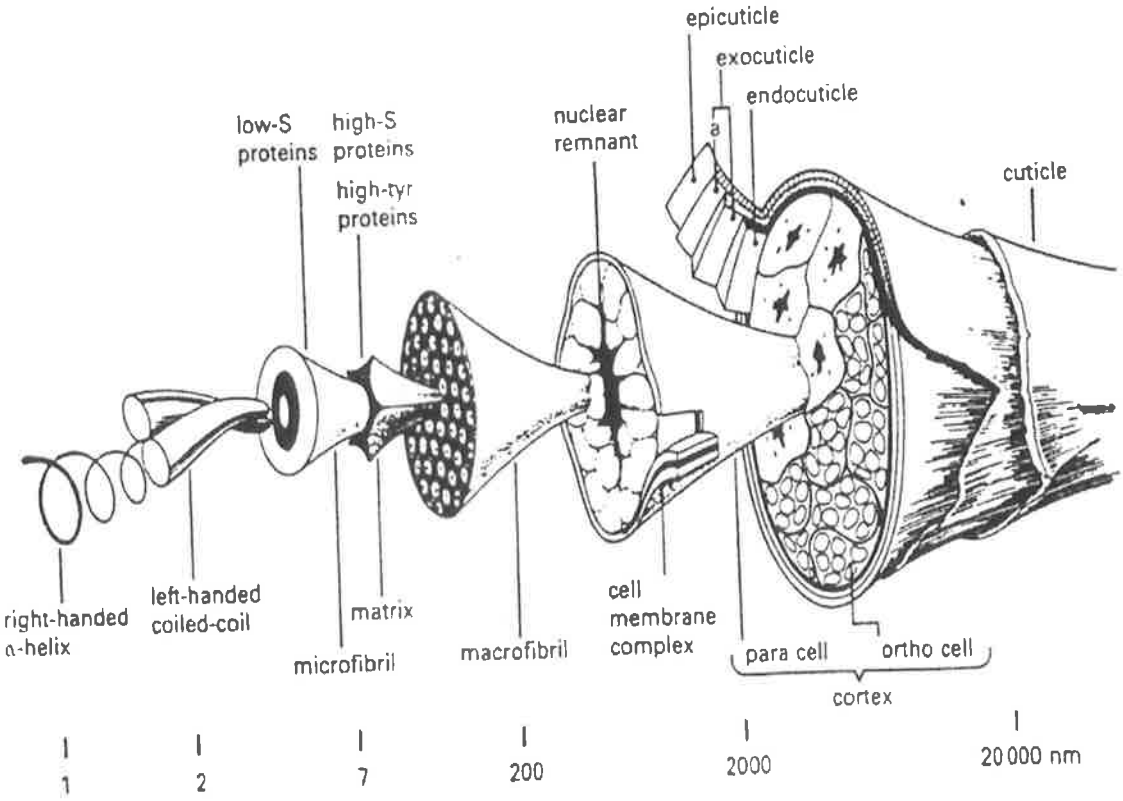


Figure 1.11 A diagram of Merino wool fibre showing the different cell types and their internal structure (adopted from MacLaren and Milligan, 1981)

Gillespie (1991) summarised inherent strength of the fibres materials includes the chemical composition or arrangement of cell types. There are three major classes of the keratin protein: (i) Low sulphur (LS) proteins with sulphur contents range 1.5-2.0%; (ii) High sulphur (HS) and Ultra-high sulphur (UHS) proteins with sulphur content 4.0-6.0% and (iii) High glysine tyrosine (HGT) proteins, which are rich in glycine and tyrosine. The LS proteins, categorised as the IF (Intermediate Fillament) super family of proteins, are mostly found just above the dermal papilla apex where the elongation zone begins. Then HGT keratin proteins (KAP 6, 7 and 8) are activated in the cells in the half of the

cortex, followed by the genes encoding the HS (KAP 1, 2 and 3) and UHS keratin proteins (KAP 4, 5, 9 ,10 and 12) at half of the cortex but they are first seen in between upper elongation zone and lower keratogenous zone. The upper parts of the follicle are composed of HGT and HS proteins that are produced in that area and specifically, LS gene are mostly expressed in orthocortical cells which contain IF's in a whorl-like arrangement (Gillespie, 1991). Whereas, HS and UHS gene expression was mostly found in the paracortical cells which are comprised of intermediate filaments (IF) arranged in a parallel array with intermediate filament associated proteins (IFAP) i.e the UHS and HS proteins interspersed between them (Lynch *et al.*, 1986; Powell *et al.,* 1991). The para and orthocortical cells mostly consist of filamentous bundles, called macrofibrils which have fibrous filaments called microfibrils embedded in a non-filamentous matrix (MacLaren and Milligan, 1981).

The strength of the matrix depends on the number of sulphide linkages present in the structure, and therefore sulphur amino acids might have related role in this mechanism. Huson *et al.* (1997) found that the cystine content of low IFS wool was 20% lower than in higher IFS wool. This is in agreement with Feughelman (1982) who suggested that the resistance of the alpha helices of the low–sulphur proteins (methionine and lysine) is primarily responsible for the longitudinal mechanical properties of the wool fibres. However, Thompson (1998) found that there were no significant relationships between the proportion of low sulphur proteins or high glycine-tyrosine proteins and IFS, and he also found a low negative correlation between high sulphur proteins (cyst(e)ine etc) and IFS. This area needs further investigation to elucidate the relationship.

IFS is associated with crimp frequency (the waviness of wool) (Smuts *et al.,* 1981). It is argued that 'kinks' and 'bends' present in highly-crimped wools may act to increase mechanical stresses causing lower fibre strength (Barach and Rainard (1950).

The amplitude and frequency of crimp is estimated to be associated with the proportion of the fibre occupied by ortho and paracortical cells. The expression of particular keratin genes and the subsequent protein composition of the fibre may reflect the different keratins present in the different cortical cell types. Such speculation awaits further study.

The effect of genetics on SS might be related to the mechanism of follicle initiation as proposed by Moore *et al.* (1998). This theory explains how genetics affects the production of primary and secondary follicles (S/P ratios). The S/P ratio difference between sheep genotypes may be related to different numbers of committed cells which are then recruited into primary follicles (Figure 1.11). In Fine-wooled Merino selection line (D+) which have high S/P ratio, fewer prepapilla cells migrated and were recruited into primary follicles. These cells are likely to divide and initiate original secondary follicles followed by further division to form several secondary branching follicles. In contrast, in the strong-wooled selection line which has much lower S/P ratios, these cells are likely to be recruited more to primary follicles than to divide and initiate secondary follicles, and as the consequences, there are no cells remaining for further division to form branching secondary follicles (Figure 1.12).

Differences in fibre diameter and length between primary and secondary follicles and variations within the two follicle types could be expected to influence SS as indicated previously.

All of the mechanism described, may reflect genetic differences in the physical and composition of fibres including sulphur content, ortho/paracortex proportion and distribution. These genetic component is focus of this study which is genetic determinants of the susceptibility of wool fibres and follicles in Merino to environment.

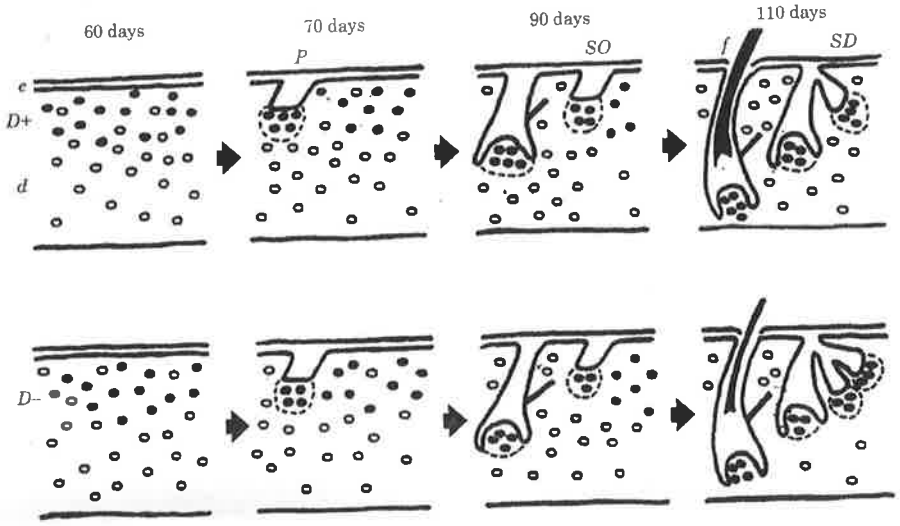


Figure 1.12 A schematic diagram of primary and secondary follicle initiation according to the Founder cell theory (Moore *et al.*, 1998).

7. Summary and Scope of Study

Staple strength (SS) is economically the second most important wool trait, closely related to the processing properties of wool and influencing the final profit to the wool industry. In Merino sheep, low SS has become a significant problem, particularly in Western Australia and South Australia where the Mediterranean climate imposes large variation in nutrient supply. Staple strength can be influenced by genetic factors and a number of physiological and environmental factors such as climate, parasites, stress, nutrition and pregnancy. In Merinos, nutritional factors play a dominant role in affecting wool growth and staple strength.

The determinants of SS include minimum FD (Hunter *et al.*, 1983), variability of FD (Hansford and Kennedy, 1988), fibre intrinsic strength (Hunter *et al.*, 1983) and follicle shutdown (Schlink and Dollin, 1995; Hynd *et al.*, 1997). Unlike the other determinants, follicle shutdown has not received intense attention until recently and it is

not clear whether there are genetic factors involved in this problem, as the results of genetic studies on FS are inconclusive. This is the subject of the first experiment described in the next Chapter.

CHAPTER 2

GENETIC AND ENVIRONMENTAL DETERMINANTS OF FOLLICLE SHUTDOWN IN MERINO SHEEP

1. Introduction

In Mediterranean environments in Southern Australia, seasonal follicle inactivity, also known as follicle shutdown (FS), occurs in Merino sheep. Several studies suggest that FS is a component of low staple strength, one of the key determinants of wool value, so it is important to identify the aetiology of FS. FS appears to be more influenced by nutrition in Merino sheep than by photoperiod (Williams and Schinckel, 1962; Lyne, 1964; Ryder, 1967; Chapman and Bassett, 1970; Thwaites, 1972; Schlink *et al.,* 1992; Ansari-Renani and Hynd, 1996; Thompson, 1998), and reaches a peak after the break of the season (autumn), but the precise cause of this phenomenon is the subject of speculation. Some suggest it is associated with the sudden change in nutrition induced by pasture germination with the opening rains. Others postulate that it is a stress-induced event.

Under similar environmental conditions (Autumn, similar stocking rate and sheep genotype), FS varies between individual sheep (range 2-36%) (Hynd *et al.,* 1997), suggesting that genetic factors may play a role. Investigation into this issue, however, has produced equivocal results. Hynd *et al.* (1997) showed that the occurrence of FS did not differ between strains of Merino (Finewool vs Strong-wool Merinos). In this study the variable most closely associated with FS was stocking rate, implying a nutritional effect. Stress as a key factor in FS was tested by Ansari-Renani and Hynd (1996). These authors showed that cortisol the 'stress' hormone induces FS but there was an interaction with genotype. Finewool Merinos had less follicle shutdown than Strongwool Merinos. These

conflicting results make it difficult to conclude to what extent follicle shutdown is related to genetic or environmental factors.

It was hypothesized that sheep with different genetic backgrounds have different propensities to follicle shutdown as a response to nutritional changes in the paddock. If there is a strong genetic component, the information could be used as a basis for selecting sheep that are more resistant to seasonal change, provided there are no antagonistic relationships with other fleece or body traits. The effects of other possible factors such as sex and type of birth and rearing were also investigated. Sex effects might be related to hormonal status, while type of birth and rearing could be associated with competition for nutrients between follicles from single, twin or triplet sheep, as might also occur in pregnant sheep. To test the hypothesis, the progeny of different Merino sires were studied to determine whether or not (the propensity to) follicle shutdown has a genetic (sire) component.

2. Hypotheses

The following specific hypotheses were tested

1. that follicle shutdown in Merino sheep occurs after the break of season,
2. that follicle shutdown has positive correlation with staple strength
3. that there is a 'sire' effect on follicle shutdown occurrence, indicating sheep with different genetic backgrounds have different propensities to FS as a response to nutritional changes in paddock
4. that sex of the animal has no effect on FS occurrence
5. that type of birth and rearing has an effect on FS,
6. that sire effects on follicle shutdown reflects differences in the fibre diameter and clean fleece weight.

3. Materials and Methods

3.1 Experimental Sheep

The sheep (Mediumwool Merinos) were from the Central Test Sire Evaluation (CTSE) Flock (Rosedale, Mt. Pleasant, S.A). Out of 424 progeny, a random sample of 210 sheep was selected (a mixture of ewe and wether weaners born in May 1996, which were the progeny of 14 different sires) (Table 2.1). The number of progeny per sire in the sample was 15 sheep. CTSE is a national sire evaluation scheme, in which a sire's genetic performance is assessed for a large number of traits of interest to sheep breeders (Cottle *et al.*, 1996).

In the year when the experiment was conducted (1997), the summer feed was augmented by periods of grazing on lupin and wheat stubbles before the opening rains in May. There was little "follow up" rain, by which time the feed was short and cold condition prevailed. However, the sheep were in good store condition at first shearing in September 1997 (Ponzoni *et al.*, 1996).

Table 2.1 Number of ewe and wether weaners in each sire

Sire no	Ewe weaners	Wether weaners	Total
1	7	8	15
2	8	7	15
3	7	8	15
4	8	7	15
5	7	8	15
6	7	8	15
7	8	7	15
8	8	7	15
9	12	3	15
10	7	8	15
11	8	7	15
12	8	7	15
13	7	8	15
14	8	7	15
Total	110	100	210

3.2 Biopsy Collection

As previous work showed that the majority of follicle shutdown occurred shortly after the break of season (Hynd *et al.*, 1997), biopsy samples were collected on 28 May 1997, four weeks after the season had broken (based on rainfall data). Figure 2.1 shows the rainfall data in 1997 (the year of the experiment) in Mount Pleasant (Rosebank) South Australia (site no: 023737; latitude: 34° 46 min 32 sec South; longitude: 139° 2 min 55 sec East; elevation 430 m) (Bureau of Meteorology).

Biopsies were carried out as follows. After wool was clipped from a small area on the left midside of each sheep, the skin area was anaesthetized by injecting Lignocaine (+2% adrenaline) subcutaneously with a 10 ml syringe fitted with a 25x5/8" needle until a small bleb appeared beneath the skin layer. Two trephine biopsy samples (1 cm in diameter) from each sheep, were placed in a vial containing 10 % buffered formalin (AR grade products from Ajax Lab. Pty., Ltd., St.Marys, NSW) and left overnight to allow fixation. The wounds were sprayed with antiseptic (Cetrigen, Virbac).

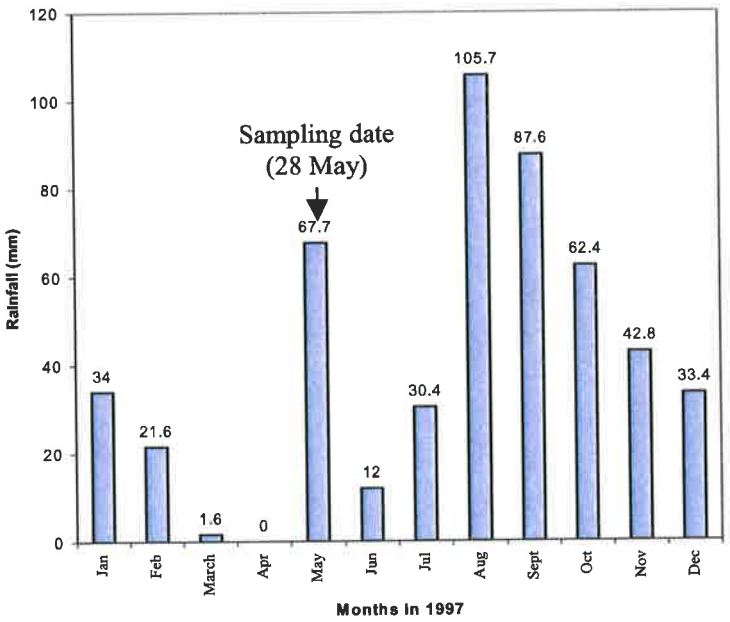


Figure 2.1. Rainfall data for Rosebank, Mount Pleasant, S.A. in 1997 (Bureau of Meteorology)

3.3 Sample Preparation

Samples were removed from formalin and trimmed of excess fibres by using two scalpel blades (size 22) and scissors, placed into embedding cassettes (Tissue-Tek II, Miles Laboratories, Inc., Naperville, Illinois) and retained in 70 % ethanol until processing.

Samples were dehydrated and impregnated with paraffin wax. The samples were then embedded using a Tissue-Tek II machine (Model 4604, Miles laboratories, Inc) to set the wax on the samples in the right orientation for transverse sectioning. Using a microtome, transverse sections were cut (8 μm) between the sebaceous gland and the bulb levels. Every fifth section was retained on a slide (previously treated with poly L-Lysine solution from Sigma Diagnostics St. Louis, Mo, (1:10) for 20 minutes) and dried overnight before staining.

The staining technique used in this experiment was a modification of SACPIC staining method (Nixon, 1993) that allows easy identification of follicles structures by staining structures with different colours. The slides consisting the dry samples were put in a slide rack and incubated there for 15 minutes prior to staining. The order and length of using the staining solutions are as follows: (1) histoclear (10 mins), (2) absolute ethanol (2 mins), (3) 80% ethanol (2 mins), (4) 30 % ethanol (2 mins), (5) RO water (2 min), (6) Lillee Mayer's haematoxylin (10 mins), (7) tap water (5 dips), (8) 70% ethanol (5 dips), (8) 70% ethanol (5 dips), (9) Winiwater's safranin (10 mins), (10) 70% ethanol (1-2 dips), (11) absolute ethanol (5 dips), (12) saturated picric acid in ethanol (20 dips), (13) tap water (short), (14) RO water (short), (15) Krause's picro-indigo carmine (2 mins), (16) 70% ethanol (short), (17) 80% ethanol (2 mins), (18) absolute ethanol (2 mins) and (19) histoclear (5 mins), with the total processing time around 1 hour.

Mounting fluid (DPX) was poured on the samples, a cover slip was placed on each slide and samples were left overnight to dry. In this method, the following tissues stain as indicated: keratinised fibre cortex (yellow), prekeratinised fibre cortex (red), inner root sheath (IRS) (red), outer root sheath (ORS) (green), ORS nuclei (purple), arrector pili muscle and sweat glands (green), sebaceous glands (purple) and collagen (green).

3.4 Assessing the Morphology of Follicles

Table 2.1 shows the morphology of follicle (fibre, ORS IRS and CT) in each class (class1 for normal to class 4 for complete shutdown).

Table 2.2 Classification used to quantify changes in follicle morphology (Hynd, *et al.*, 1997)

Morphological Class	Fibre	ORS	IRS	CT
1 (Normal)	circular picric yellow	large cytoplasm, cells arranged random	uniform	uniform
2 (slightly normal)	circular picric yellow	small, cuboidal nuclei distally arranged	uniform	irregular thickened
3 (early shutdown)	absent	distally arranged hyperplasia	irregular	very irregular thickened
4 (complete shutdown)	absent	complete collapse	absent	very irregular thickened

ORS=Outer root sheath; IRS= Inner root sheath; CT= Connective tissue.

The morphology of 200 follicles from each sample of 210 sheep was observed by light microscopy at 40x magnification and classified as one of four classes (Class 1, 2, 3 and 4), following the scoring scheme of Hynd *et al.* (1997). Class 1 follicles are

considered to be normal and actively producing a fibre; Class 2 follicles are still producing a fibre but some early signs of pathology are apparent; Class 3 follicles are inactive but in an early stage of shutdown; Class 4 follicles are inactive and completely shutdown (Table 2.1). Together, Classes 3 and 4 represent inactive (shutdown) follicles.

3.5 Statistical Analysis

Data were analysed either as total counts or as a percentage of follicle numbers in each class. The model fitted to the data included the following fixed effects: sire (1-14), sex (2, male & female) and type of birth and rearing (11= born as single and reared as single; 21= born as twin and reared as single; and 22= born as twin and reared as twin), as fixed. The data were analysed for each class of follicle shutdown either separately or in combination. Percentages were subjected to arcsine transformation prior to analysis to obtain a normal distribution of data (Steel and Torrie, 1987). The General Linear Models (GLM) procedure of the SAS (SAS Institute, 1990) was used to analyse the data.

Since fibre diameter (FD) and clean fleece weight (CFW) are continuous variables, they were correlated with data for each class of follicle shutdown (after arcsin transformation data) 'CORR' procedure to analyse the correlation and probability values. Data on FD and CFW were obtained from the September 1997, the first shearing for the flock.

4. Results

4.1 The Incidence of Follicle Shutdown during the Experiment

Regardless of the method of expression of the data (ratio, percentage or arcsine) the results were virtually identical, so only those for follicle number expressed as percentage are presented.

Table 2.3 Number of follicles (mean, standard deviation and range) in each class of morphology

Morphological Class	Mean of % of Follicles from total 200 follicle counts (no of sheep= 210)	Standard deviation (%)	Range (%)
1	97.03	3.07	73.5 – 100
2	1.59	1.59	0 – 9
3	0.99	1.52	0 – 11
4	0.39	0.98	0 – 10
3+4	1.38	2.27	0 – 21

Table 2.3 shows that most follicles were normal in morphology (class 1 = 97.03 %) and only 1.4% of them were classified as shutdown (classes 3+4). Figure 2.2 shows the morphology of follicles of one of the experimental sheep, in class 1, 2, 3 and 4 in a transverse section.

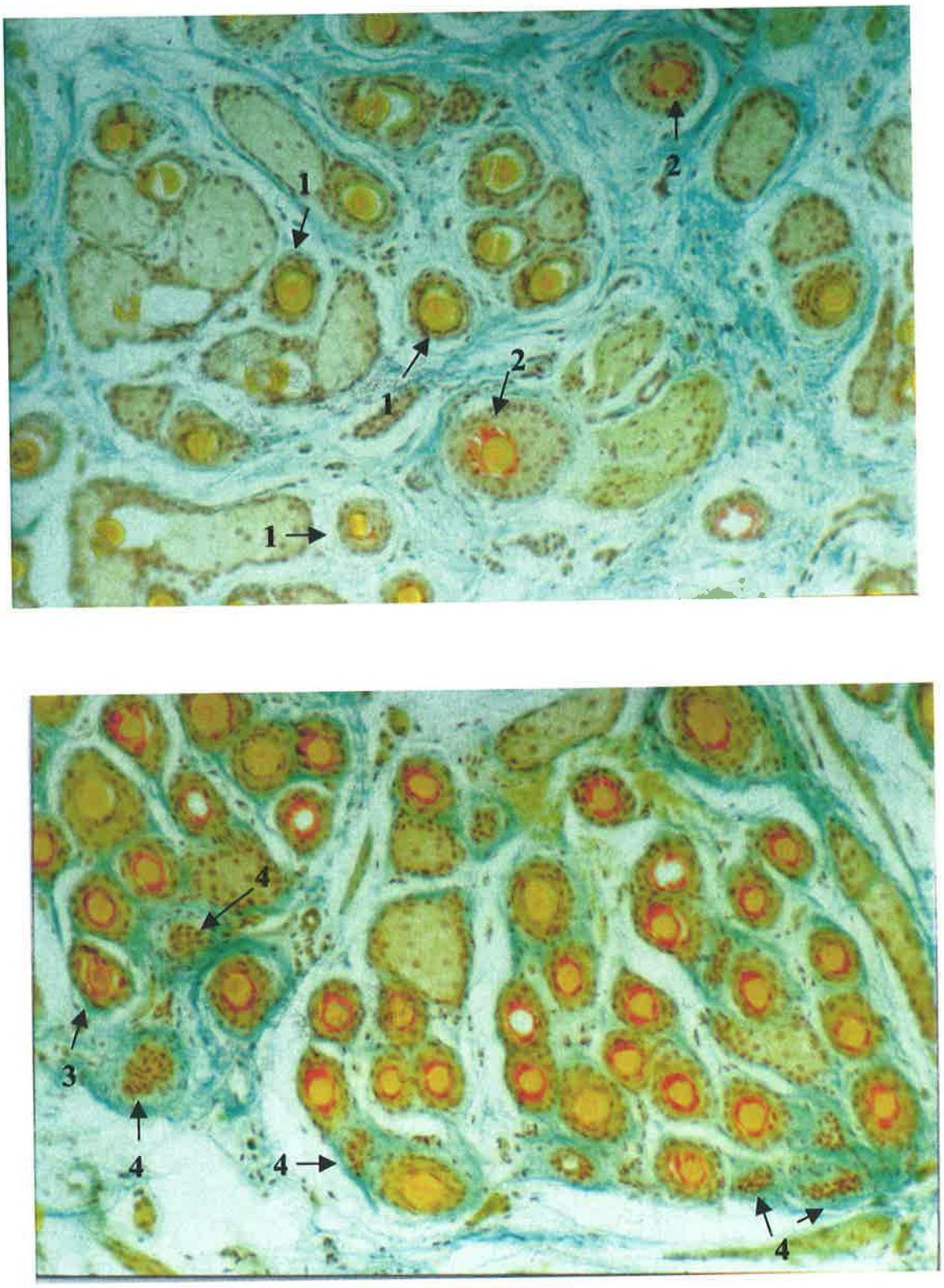


Figure 2.2 Morphology of wool follicles in class 1, 2, 3 and 4 in a transverse section

4.2 Effects of Sire, Sex and Type of Birth and Rearing on Follicle Shutdown and the Associations between FD, CFW and FS

The probability values of each fixed effect on number of follicle in each morphological class are shown in Table 2.4. Sire had no significant effect on the incidence of follicle shutdown (P=0.37 and 0.11 for class 3 and 4 respectively or P=0.24 for a combination of both classes). Similarly, the effect of sex of the animal on the number of follicle in all classes was not significant (P ranged between 0.11 to 0.56).

Table 2.4 Probability (P) values for fixed effects of sire, sex of animal and type of birth and rearing on number of follicles in each class

Morphological Class	Sire	Sex	TOB_R*
1	0.60	0.11	0.0001
2	0.90	0.16	0.0001
3	0.37	0.18	0.0052
4	0.11	0.56	0.0029
3+4	0.24	0.51	0.0013

*(TOB_R = type of birth and rearing)

When fibre diameter and clean fleece weight data were correlated with each class of follicle morphology, it was found that there were poor correlation between FD or CFW and percentage of follicle shutdown in class 4 or in class 3+4, although the correlation between FD and percentage of class 4 follicles approached significance (p=0.070). Correlation between CFW and class 2 follicles was significant although the value was not too high (r=0.154, P=0.027) (Table 2.5).

Table 2.5 Correlation (r) and Probability (P) values between FD or CFW and number of follicles in each class

Morphological Class	Fibre Diameter (FD) r value (P value)	Clean Fleece Weight (CFW) r value (P value)
1	-0.013 (0.852)	-0.112 (0.107)
2	0.021 (0.766)	0.154 (0.027)
3	0.029 (0.680)	0.055 (0.432)
4	0.1326 (0.070)	0.0004 (0.996)
3+4	0.073 (0.29)	0.036 (0.59)

In contrast and perhaps somewhat unexpectedly, type of birth and rearing had a significant effect on the ratio of follicles (in percent) in each class (1-4) ($p<0.01$). Sheep born and raised as singles were more susceptible to shutdown than those born as twins and raised as single or twins (Figure 2.3), suggesting that birth type was a key factor determining susceptibility to shutdown in this trial.

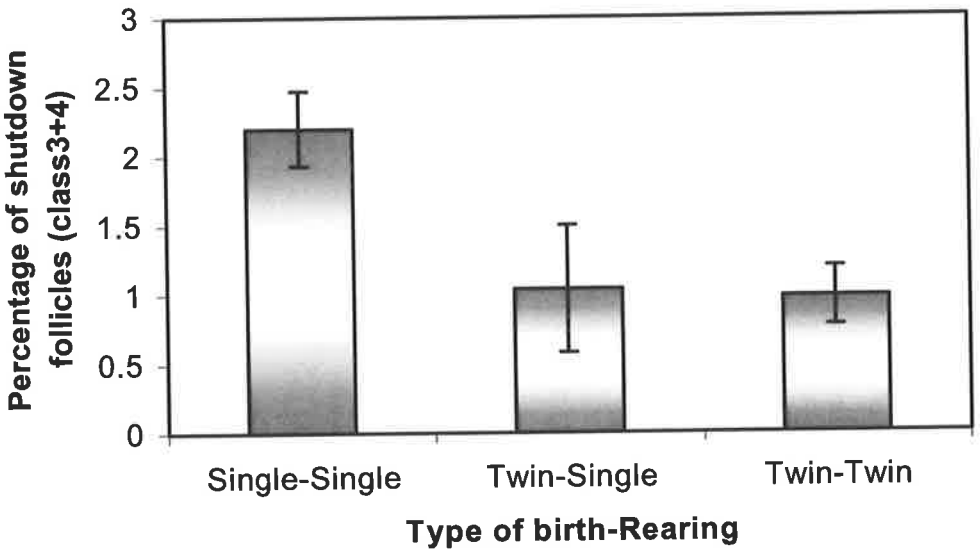


Figure 2.3. Effect of Type of birth and rearing on follicle shutdown (class 3 + 4) (means ± se)

5. Discussion

The incidence of follicle shutdown in this study (class 3+4) was lower than in a previous study which examined seasonal changes in the morphology of wool follicles using the same criteria as were used presently (Hynd *et al.,* 1997) (means of class 3+4 = 1.38 % vs 10 %, respectively). This may be related to either the feed quality, the genotype of the sheep or some undetermined feature of the environment. In the current experiment, close attention was paid to ensuring the animals received high quality feed as shown previously (Section 3.1), in contrast to the previous experiment where stocking rate was generally higher and changes in feed quality probably greater. If, as discussed below, follicle shutdown is an outcome of competition between follicles for nutrients, these differences in nutrient availability might be relevant.

Effect of FS on SS is not discussed in this chapter, as FS incidence is very low in this experiment, therefore SS data were not measured to calculate the correlation between FS and SS.

Another possible environmental factor is "the break" of the season which did not occur exactly in May as assumed in this work, but it was present later in August as reported by Ponzoni *et al.* (1996). Figure 2.1 shows the false break of the season in May 1997, which triggered the skin sampling protocol.

A previous study (Ansari-Renani and Hynd, 1996) suggested a difference in shutdown between Merino strains in response to exogenous cortisol, whereas Hynd *et al.,* 1997) reported no difference in shutdown incidence between sheep strains grazing pastures. The present results are in accord with the latter, there being no significant effect of sire on shutdown incidence, although the P values in class 4 or 3+4 was lower than in class 1,2 or 3 (Table 2.4). This tendency may indicate possible effect of genetic (indicated from sire) on follicle shutdown. However conclusions regarding genetic influences on

susceptibility to shutdown must be tempered by the low overall incidence of shutdown which occurred in the current experiment. A higher frequency of shutdown follicles may have allowed greater expression of genetic differences. Such speculation awaits confirmation.

There was no significant difference between ewes and wethers in follicle shutdown in this experiment, despite an observation by Ryder (1971[b]) that Soay sheep ram were less susceptible to shutdown than females of the same breed. However age was confounded with sex in Ryder's trial and this breed of sheep is highly responsive to photoperiod. The present the evidence suggesting little effect of sex of the animal on susceptibility to shutdown, therefore, can not be possibly comparable to Ryder's work.

Perhaps surprisingly, type of birth and rearing had a significant effect on follicle shutdown with single-born animals displaying a greater tendency to shutdown (2.2%) than twins (1.0%) (Table 2.4 and Figure 2.3). The following explanation is advanced to account for this result. Single-born animals, as a consequence of greater nutrient availability, initiate a greater density of follicles in the skin during fetal and early post-natal development than twins (Corbett, 1979). The greater density is a consequence largely of increased initiation of secondary follicles resulting in a higher ratio of secondary to primary follicles (S/P ratio) (Fayez *et al.*1976; Tsenkova, 1990). The higher follicle density as a result of more secondary follicles may result in greater competition between follicles when nutrient supply becomes limiting at the break of the season. Reduced nutrient supply per follicle then causes a premature entry of that follicle into catagen and telogen. While this is purely speculative, it is in accord with the finding of Hynd *et al.* (1997) that stocking pressure had a significant effect on follicle shutdown. As stocking pressure increased in that study, the incidence of inactive follicles increased, in line with a presumed reduction in nutrient supply to each follicle. However if nutrient

supply to each follicle is a prime determinant of susceptibility to shutdown, one would anticipate that sheep with a low mean fibre diameter and/or high clean fleece weight, both of which are genetically associated with high follicle density, to have a greater shutdown incidence. This was not the case in this experiment, nor in that of Hynd *et al.* (1997). Without actual measurements of follicle density in the current experiment, conclusions regarding follicle competition for nutrients and follicle shutdown, remain speculative.

In relation to fibre diameter, the present results show that the correlation between fibre diameter and follicle shutdown incidence in class 4 was close to significance ($r= 0.1326$, $P=0.07$). This might indicate that sheep with higher average fibre diameter tend to be more susceptible to follicle shutdown in response to the break of season. If this is true, this results support a previous report that Mediumwool Merinos are more responsive to the environmental changes than Finewool sheep (Ansari-Renani and Hynd, 1996). Similarly Jackson and Downes (1979) showed that sheep from the high fibre diameter line were more responsive to the environmental changes than those from low fibre diameter line.

Further studies of the susceptibility of defined genotypes to nutrient limitation at the break of the season are required. In particular the possibility that sheep of high clean fleece weight and low fibre diameter might be more prone to follicle collapse at the break of the season, requires investigation, as this has obvious implications for enterprises selecting such animals.

6. Conclusion

The question has been raised as to whether we can select sheep based on their susceptibility to follicle shutdown ie is there a genetic component to shutdown. It was

hypothesised that progeny of sires would differ in incidence of shutdown, but the results show that there was no difference in shutdown between sires. This conclusion, however, must be tempered by the fact that, somewhat unexpectedly, the overall incidence of follicle shutdown was low (<1.5%).

There were significant effects of type of birth and rearing on follicle shutdown. In relation to the mechanism speculated, it might be proposed that the S/P ratio trait can be used as an indirect selection criterion for selecting sheep in terms of their susceptibility to follicle shutdown.

As previously explained, the planned study on the involvement of growth factors in FS and the in vitro study of FS could not be conducted, because of the nonsignificant number of shutdown follicles found. However, topics related to susceptibility to environmental changes were still investigated, using the parameter of variation of fibre diameter (FD) along the staple instead of abnormality of follicles (follicle shutdown). This topics are discussed in next Chapter.

CHAPTER 3

GENETIC AND ENVIRONMENTAL DETERMINANTS OF THE VARIATION OF FIBRE DIAMETER ALONG THE STAPLE IN MERINO SHEEP

1. Introduction

Staple strength (SS) is an important economic character in the wool industry, as it affects processing in areas such as carding, combing and spinning (Rottenbury *et al.* 1986; Whiteley 1987). It has therefore become the second most important wool trait after fibre diameter in economic terms (Couchman *et al.,* 1992). One of the important determinants of SS is fibre diameter variation, which reflects the combination of both between fibre diameter and along fibre diameter variation. Coefficient of variation of fibre diameter (CVFD), is obtained simultaneously with FD measurement on a minicored mid-side sample. CVFD reflects the variation both between and along wool fibres and has been identified as a potential indirect selection criterion for SS because of the high genetic correlation between the two traits (r_g = -0.42 to –0.86) (Greeff *et al.,* 1995; Hill and Ponzoni, 1999) and the low measurement cost.

It is surmised that the relationship between CVFD and SS could be a result of variation in FD along the fibre, as it is well-established that either minimum FD or the rate of diameter change is positively related to SS (r = 0.691 and r = -0.774, respectively), (Hansford and Kennedy 1990). The along staple variation in FD can be quantified as the coefficient of variation of FD along staple (ACVFD). This trait represents the change in FD throughout the year, and in the case of sheep in a Mediterranean environment is largely a reflection of seasonal changes in nutrient supply. It is therefore thought that selection on the basis of FD "stability" in response to environmental changes is an

important issue. Jackson and Downes (1979) showed that high FD sheep lines had higher coefficient of variation of FD along the staple (8.34%) than low FD lines (6.44%). These authors also found that higher FD lines were more susceptible to environmental changes than lower FD lines in terms of ACVFD. This may show that genetics is involved in influencing FD changes along the staple; however, the heritability of this trait has yet to be calculated .

The following study was designed to test the hypothesis that ACVFD is heritable. The heritability of this trait and its phenotypic and genetic correlation with other fleece traits were determined by using a large number of sheep (650 ewe progeny) from a well recorded resource flock. To ensure an accurate estimate of the genetic parameters, the progeny of 47 different sires were examined. The measures of FD variation along the staple were ACVFD, difference between maximum and minimum FD, rate of FD changes and adjacent difference (FD difference between adjacent snippets). These traits indicate the changes of FD along the staple. The precise detail of how the traits were measured is explained in Table 1. Phenotypic and genetic correlations between these and other important fleece traits were calculated to determine the possible impact of selection based on ACVFD. In particular, this study compared the merits of these measures as predictors of staple strength with these of the coefficient of variation of fibre diameter measured in mid-side samples.

2. Hypotheses

The hypotheses tested in this study were that all measures of fibre diameter variation along and between fibres are heritable and are genetic correlated with other wool traits especially staple strcngth .

More specifically, the following hypothesis were tested:

1. that fibre diameter varies throughout the year with minimum fibre diameter occurs after the break of season,
2. that type of birth and rearing and age of dam do not have effects on fibre diameter, fibre diameter maximum and minimum and variance of fibre diameter along the staple,
3. that the progeny in different studs and sires differ in susceptibility to environmental changes in fibre diameter,
4. that heritability of traits of fibre diameter variation along staple are medium to high,
5. that traits of fibre diameter variation along the staple have medium to high phenotypic correlations with staple strength,
6. that traits of fibre diameter along the staple have correlations to traits in mid-side samples.

3. Materials and Methods

3.1 Location and Sheep

A resource flock of 2000 South Australian Merino strain ewes representative of the Bungaree and Collinsville "family" groups was established at the Turretfield Research Centre (South Australian Research and Development Institute) in 1988 (Gifford and Ponzoni, 1993). Each "family group" was represented by 2 studs. The results presented in

this paper are from 593 ewe progeny of this flock, born in 1992 and the offspring of 47 sires. Details of the number of progeny in each stud are presented in Table 3.1.

Table 3.1. Number of sires and ewe progeny in each stud

STUD	TOTAL SIRES	TOTAL EWE PROGENY (HEAD)
A	12	155
B	12	149
L	12	148
X	11	141
TOTAL	47	593

Data of rainfall from September 1995 to September 1996, which was the period of wool staple taken in this experiment were: 33.4, 43.3, 8.4, 7.8, 23.8, 18.4, 40, 18, 11.6, 93.4, 96.6, 83.4 and 57.8 mm, respectively (Bureau of Meteorology).

3.2 Measurements

The wool samples used in this study were taken from the mid-side of each fleece during the September 1996 shearing when the ewes were aged 4.5 years and had 12 months wool.

3.2.1 Mid-side Samples

The mid-side wool samples (approximately 50g) were used to measure average fibre diameter (FD), coefficient of variation of fibre diameter (CVFD) and staple strength (SS) (about 4g of 15 wool staples). FD and CVFD were measured using the Fibre Finess Distribution Analyzer (FFDA) (Information Electronic Limited, under license from CSIRO). Staple strength was measured on 15 staples per mid-side, using the CSIRO-

developed ATLAS (Automatic Testing of Length and Strength) instrument at Australian Wool Testing Authority (AWTA). The analysis of FD, CVFD and SS was conducted at Turretfield Research Centre, Rosedale, South Australia.

3.2.2 Along the Staple

Following measurement techniques are modification of Hansford's methods (Hansford, 1992). One staple was taken at random from each mid-side wool sample, and 10 snippets (2 mm in length) were cut out from equidistant points along the staple using a snippet profiler (CSIRO, 1991). The remaining pieces of wool between the snippets (Figure 3.1), as well as the extremities (the tip and the base of the staple) were discarded. Each bundle of snippets (numbers 1 to 10) corresponded to approximately 10 different times of the year, and thus wool samples were estimated to represent 12 months of wool growth (shorn in September 1996 = the base, Figure 3.1). The snippets were washed with tetrachloroethylene and dried with a snippet blaster. The fibre diameter of 1000 wool fibres in each snippet was then measured using a Sirolan Laserscan (CSIRO, 1991). This instrument allows the measurement of the small snippet sample used in this study. The fibre diameter measurements for each snippet were used to calculate the along staple traits outlined in Table 3.2.

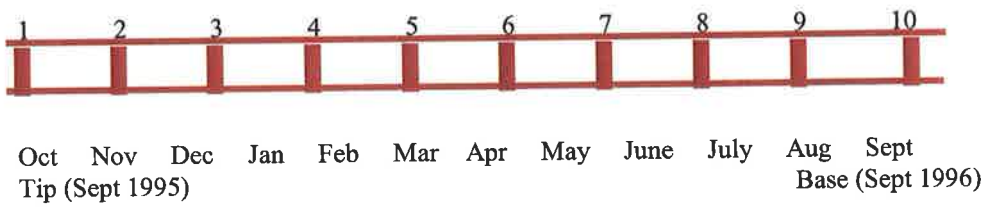


Figure 3.1. Diagram of measuring FD along wool staple. Each bundle of snippets (numbers 1 to 10) corresponded to approximately 10 different times of the year, and thus wool samples were estimated to represent 12 months of wool growth (shorn in September 1996 = the base)

3.3 Statistical Analysis

3.3.1 Effect of fixed effects on fibre diameter traits along the staple

Fixed effects (stud, type of birth and rearing and age of dams) on all traits of fibre diameter along the staple (Table 3.2) and sire as a random effect were fitted using Proc Mixed (SAS Institute, 1990).

3.3.2 Effects of stud and sire on susceptibility to environmental changes in FD

The susceptibility of FD to the environmental changes in different studs and strains was analysed based on the procedure of Jackson and Downes (1979). In brief, as described in Figure 3.1, the ten segment diameters for each staple were assumed to represent the same 10 wool growth environments for each staple and each sheep. The nature of the difference in susceptibility is described from the regression on environment partitions genotype by environment interaction. Genotype effects measured here were studs and sires and environment was FD in segments.

The slope of the regression line is a measure of the 'susceptibility' of each individual to diameter changes with environment. The significant differences between slopes were determined by the P values of interactions of stud by environment or sire by environment. The interactions were basically between individual stud/sire mean FD and overall mean FD for each snippet.

3.3.3 Heritability of traits in mid-side samples and FD traits along the staple and phenotypic correlations between the traits

Heritabilities and phenotypic correlations were estimated using ASREML (Gilmour *et al.*, 1998). An animal model was fitted to the data, including the fixed effects of stud (4), age of dam (2-4 years), type of ewe birth and rearing class (single raised as single, twin raised as single, twin raised as twin, triplet raised as single and triplet raised

as twin) and type of lamb birth and rearing (single died, single raised as single, multiple died, multiple raised as single and multiple raised as twin).

Estimates of Heritability (h^2) were calculated using this equation:

$$h^2 \text{ (adj)} = \sigma^2_A / \sigma^2_P = 4\sigma^2_S / (\sigma^2_S + \sigma^2_E)$$

where, σ^2_A = additive genetic variance

σ^2_S = between sire genetic variance

σ^2_P = total phenotypic variance

σ^2_E = residual variance

Phenotypic correlations were calculated using this equation:

$$r_P = cov_{P(x,y)} / \sqrt{(\sigma^2_{P(x)} \cdot \sigma^2_{P(y)}}$$

where, $cov_{P(x,y)} = (cov_{S(x,y)} + cov_{E(x,y)})$ and $\sigma^2_P = \sigma^2_S + \sigma^2_E$

$cov_{S(x,y)}$ = the sire covariance between trait x and trait y

$cov_{E(x,y)}$ = the residual covariance between trait x and trait y

σ^2_S = between sire variance

σ^2_E = residual variance

The heritability values were classified into 'low' (h^2 <0.15), 'moderate' (h^2 =0.15-0.30) and 'high' heritability (h^2 >0.30). While the classification of phenotypic correlation values were 'very low' ($r_P \leq 0.2$), 'low' ($0.2 < r_P \leq 0.4$), 'moderate' ($0.4 < r_P \leq 0.6$), 'high' ($0.6 < r_P \leq 0.8$) and 'very high' correlation ($0.80 \leq r_P \leq 1.00$) (Ponzoni, pers.comm).

Table 3.2. Traits measured in mid-side sample and along the staple

Trait (Abbreviation) (Units)	Measurement Procedure *)
1. **Mid-side sample**	
a. Mean FD (FD) (µm)	FFDA
b. Coefficient of variation of FD (CVFD) (%)	FFDA
c. Staple strength (SS) (N/ktex)	ATLAS at AWTA
2.**Along the staple**	
a. Mean, Max and Min FD	**Sirolan Laserscan**
- Mean FD (FDMEAN) (µm)	FDMEAN=mean(of fd1-fd10)
- Maximum FD (FDMAX) (µm)	as the highest FD along the staple; FDMAX=max(of fd1-fd10)
- Minimum FD (FDMIN) (µm)	as the lowest FD along the staple; FDMIN=min(of fd1-fd10)
- Difference of FD max and min (MAX-MIN) (µm)	MAX-MIN=FDMAX-FDMIN
b. Variation of FD	**Sirolan Laserscan**
- Coefficient of variation of Fibre Diameter (ACVFD) (%)	ACVFD = (stdfd / c)*100 ; stdfd = varfd**0.5; varfd = (a-b)/9 $a = fd1^2 + fd2^2 + fd3^2 + fd4^2 + fd5^2 + fd6^2 + fd7^2 + fd8^2 + fd9^2 + fd10^2$ $b = (fd1 + fd2 + fd3 + fd4 + fd5 + fd6 + fd7 + fd8 + fd9 + fd10)^2 /10$
- Adjacent Differences Difference between FDMIN and FDMAX (ADJDIFF) (μm^2)	as the sum of the squared difference between adjacent fibre diameter measurements along the staple, divided by the number of snippets along the staple minus one $AdjDiff = ((fd1-fd2)^2+(fd2-fd3)^2+(fd3-fd4)^2+(fd4-fd5)^2 +(fd5-fd6)^2+(fd6-fd7)^2+ (fd7- fd8)^2+ (fd8-fd9)^2 +(fd9-fd10)^2)/9$
- Rate of Fibre Diameter Change (RATE) (µm/snippet)	as the difference between FDMAX and FDMIN, divided by the difference between the corresponding snippet numbers at which FDMAX and FDMIN occurred. RATE = (FDMAX-FDMIN)/(A-B)
c. Coefficient of Variation of FD within each snippet (CV1-10) (%)	CV1-10=mean(of cv1-cv10)

*) number 1-10 behind the parameters (fd, CV, cv) refers to snippet number, with 1 = snippet 1,…,and 10 = snippet 10.

4. Results

4.1. Effect of Fixed Effects on Fibre Diameter Traits along the Staple

The analysis of varianceor results (P values) for the effects of studs, time of birth and rearing (TOB_R) and age of dam (AOD) on FD traits along the staple (FDMEAN, FDMAX, FDMIN, MAXMIN, ACVFD, Adjacent difference (ADJDIFF) and CV1-10) are presented in Table 3.3.

Table 3.3 Probability values for fixed effects (stud, type of birth and rearing, age of dam)

FIXED EFFECTS	FDMEAN	FDMAX	FDMIN	MAXMIN	ACVFD	ADJDIFF	CV1-10
STUD	**0.0001**	**0.0001**	**0.0001**	0.119	**0.0001**	0.051	0.822
TOB-R[1]	0.090	0.207	0.627	0.777	0.873	0.719	0.487
AOD[2]	0.971	0.768	0.773	0.212	0.436	0.443	**0.009**

[1] TOB-R = Type of birth and rearing [2] AOD = age of dam

The stud from which the animals derived significantly affected FDMEAN, FDMAX, FDMIN, ACVFD ($p<0.01$). These are discussed in detail in the next section. In contrast, type of birth and rearing did not affect any parameters measured ($p>0.05$). Non-significant effects were also found in age of dam on the parameters, with the exception of CV mean in all segments (CV1-10).

In relation to focus of this study, it was found that fibre diameter varied significantly along the staple. Season and animal variation generated a very wide range of snippet fibre diameters (14.2 - 35.2 μm). Snippet 7 (representing wool grown in May/June) had wool with the lowest mean fibre diameter whereas the highest mean FD (FDMAX) was in snippet 2 (representing approximately November) (Figure 3.2).

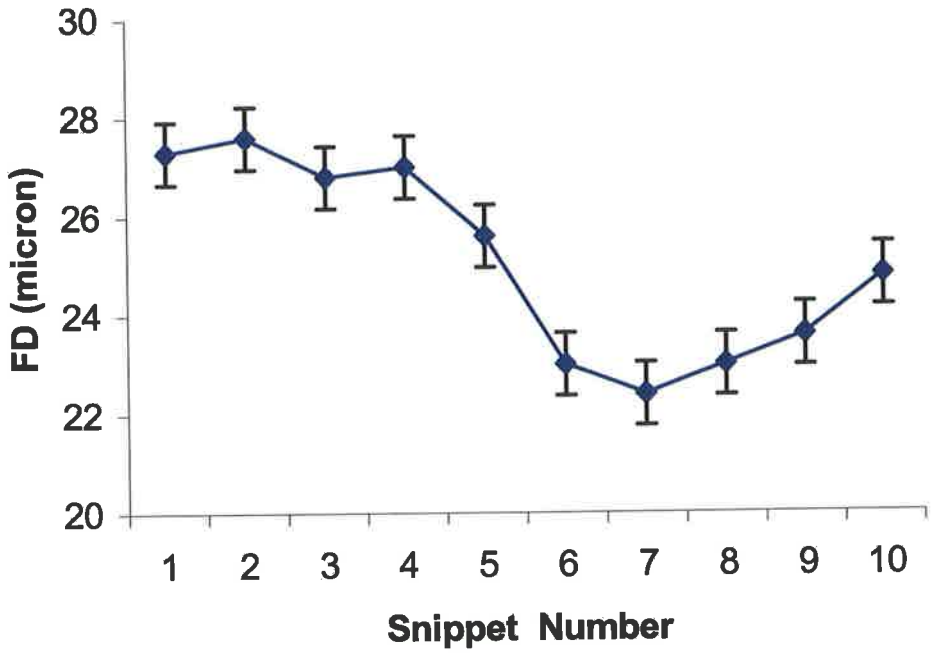


Figure 3.2 Fibre diameter variation along the staple (FDMEAN ± STD in each snippet).

4.2 Studs and Sires effects on Susceptibility to Environmental Changes in FD

When data were analysed to determine the effect of "stud", it was found that FDMEAN were different between the studs (p=0.0001). Similarly, coefficient of variation of FD along the staple (ACVFD) were statistically different between studs (p=0.0001) (Table 3.3).

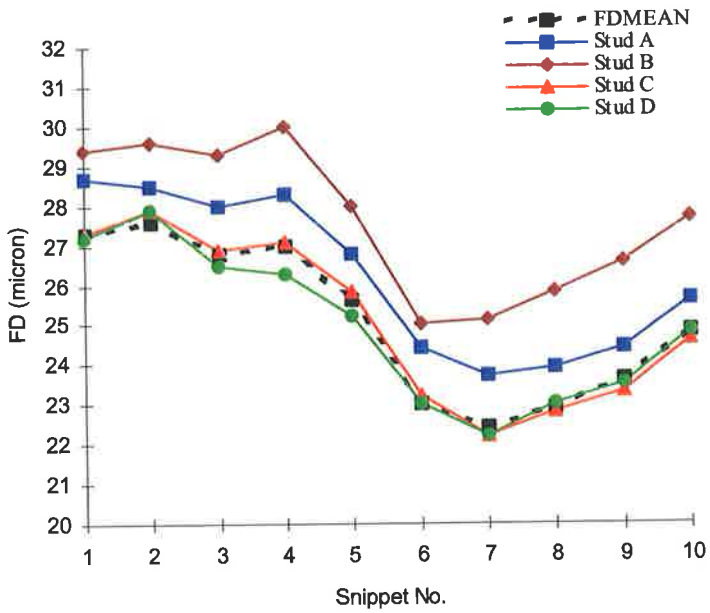


Figure 3.3 FD changes in different studs throughout the year which is represented by snippet number, averaged across all staples in the experiment.

The results of the analysis were based on the data of mean fibre diameter of the sheep from the four studs in each snippet representing different times throughout the year (Figure 3.3). This shows that the mean FD was different between studs in each snippet. For example, in snippet 1, the mean fibre diameter ranged between 27.2 µm (in stud D) and 29.4 µm (stud B) or in snippet 10, the ranged was between 24.8µm (stud D and 27.7 µ m (stud B). In all snippets, the average fibre diameter was also significantly different between studs. Sheep from stud B had significantly higher fibre diameter (27.6 ± 0.5 µm) at all times of the year than sheep from stud A (26.2 ± 0.5 µm), stud C (24.8 ± 0.6 µm) or stud D (25.1 ± 0.6 µm) ($p<0.01$). FD of Sheep from stud A was also significantly higher than those in stud C or D ($p<0.01$), however the difference in mean FD was not significant between sheep from stud C and D ($p>0.05$).

To confirm the different responsiveness trend, fibre diameter values sheep from the studs and 'environment' (snippet number) were plotted to estimate a linear relationship and the slopes of the regression were analysed. The results of the slope

analysis shows no difference in susceptibility to FD variation between studs, although the P value was close to significance (p=0.065). This is shown by the similar trendline and slopes of FD response between studs (Figure 3.4). The slope (b) values for the studs were 0.89 ± 1.040 (stud A), 0.93 ± 0.068 (stud B), 0.97 ± 0.048 (stud C) and 1.06 ± 0.029 (stud D).

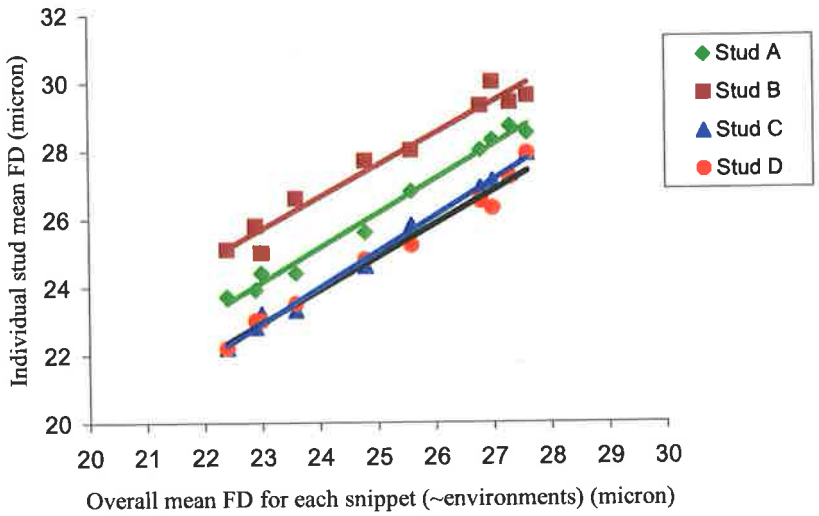


Figure 3.4. Regression of FD line means on the mean of all studs in each environment

Since there were no significant differences in susceptibility to season between studs, the effect of sires that is used to estimate heritability, were analysed with the same approach, the slope comparisons between the sires. Since the number of sires used were many (47 sires), the sire number was not shown in the figure to simplify the presentation of the results (Figure 3.5 and 3.6).

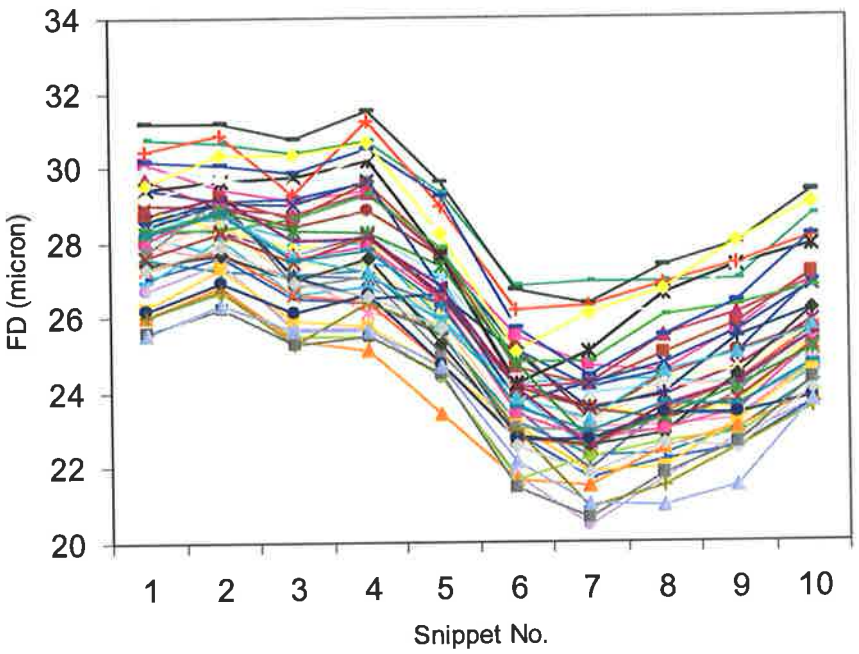


Figure 3.5. FD changes in different sires throughout the year which is represented by snippet number (legend for each sire was not presented as there are 47 sires which is too many to include in the figure).

Figure 3.5 shows the FDMEAN along the staple from the 47 sires. The results indicate that different FDMEAN between sires along the snippets, for example in snippet 1, the FDMEAN ranged from 25.6 μm to 31.2 μm and in snippet 10, the range was from 23.6 μm to 29.3 μm. The lowest FDMEAN was 23.7 μm and the highest FDMEAN was 29.2 μm. The difference of mean FD between the sires, however, were not significant, although the P value was close to significant (p=0.077) (Figure 3.5).

To confirm this, the analysis of the susceptibility of FD to environmental changes through the slope difference was also examined. The result shows that the slopes were not significantly different between sires (p=0.868) (Figure 3.6). The values of slope (b) of the 47 sires ranged from 0.75 ± 0.10 in sire 150 to 1.18 ± 0.04 for sire160.

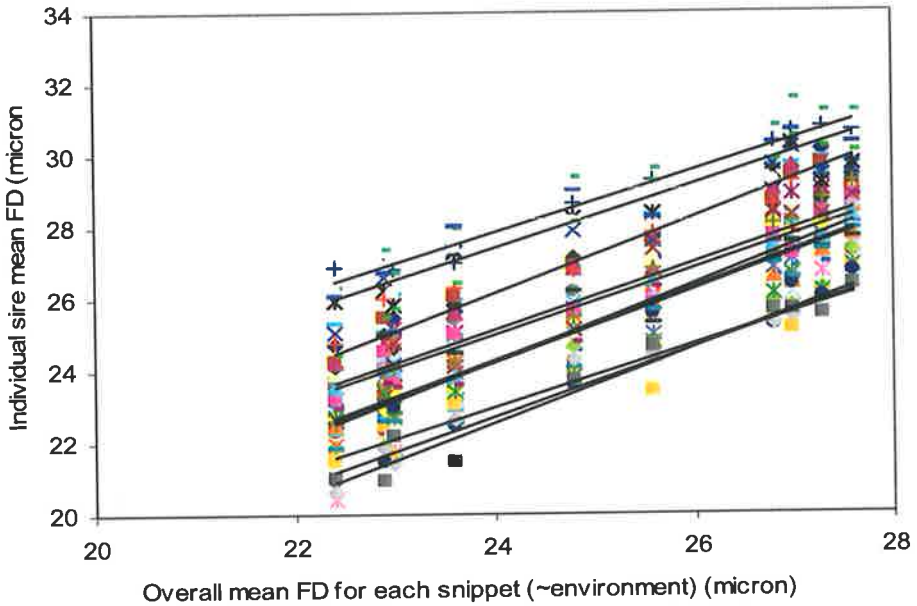


Figure 3.6. Regression of FD line means on the mean in some sires in each environment (To simplify the figure, the legend and many trendlines for each sire were not presented as there are 47 sires which is too many to include in the figure).

4.3. Heritability of Traits in Mid-side Samples and FD Traits along the Staple and Phenotypic Correlations between the Traits

4.3.1. Heritability of the traits

Table 3.4 shows the means, phenotypic standard deviations and heritabilities of each of the traits listed in Table 3.2. FD, CVFD, CV1-10, FDMEAN, FDMAX and FDMIN were all highly heritable. In contrast, the estimated heritabilities were moderate or low for the parameters that measured FD changes along the staple (ACVFD, ADJDIFF, MAX-MIN and RATE). Heritability of ACVFD and MAX-MIN were medium (h^2= 0.17 and 0.20, respectively), whereas the heritability estimate in ADJDIFF was low (h^2= 0.13), and RATE had a near zero heritability (h^2= 0.01).

Table 3.4. Means, phenotypic standard deviations (σ_P) and heritabilities [h^2 (s.e)] for the traits

TRAIT	Mean	σ_p	h^2 (s.e)
Mid-side sample			
FD	24.91	2.14	**0.60 (0.16)**
CVFD	22.55	2.5	**0.78 (0.17)**
SS	28.1	11.09	0.24 (0.11)
Along the staple			
ACVFD	9.36	2.73	0.17 (0.10)
ADJDIFF	3.46	2.23	0.13 (0.09)
FDMIN	21.49	2.53	**0.47 (0.15)**
FDMAX	28.11	2.36	**0.57 (0.16)**
FDMEAN	25.09	2.27	**0.55 (0.16)**
MAX-MIN	6.62	1.81	0.20 (0.10)
RATE	-1.49	0.95	0.01 (0.07)
CV1-10	17.83	2.7	**0.59 (0.16)**

4.3.2 Phenotypic correlations between traits

Phenotypic correlations among the traits are reported in Table 3.5. Of the variables measured along the staple, ACVFD, FDMIN, FDMEAN and MAX-MIN had the strongest correlations with SS. However, these correlations with SS were not significantly different ($p>0.05$) to the correlations obtained for CVFD (i.e. measures of along the staple variability were of similar value to that of a measure of variation in FD from the mid-side sample). The remaining phenotypic correlations were either low or very low, although, they were in the expected direction.

Table 3.5 Phenotypic correlations between FD, CVFD and SS of mid-side samples and the FD variability traits along the staple.

TRAIT	FD	CVFD	SS
Mid-side sample			
FD	1.00		
CVFD	-0.24	1.00	
SS	**0.32**	**-0.44**	1.00
Along the staple			
ACVFD	**-0.28**	**0.48**	**-0.43**
ADJDIFF	0.11	0.25	-0.24
FDMIN	**0.83**	**-0.30**	**0.47**
FDMAX	**0.84**	0.00	0.19
FDMEAN	**0.92**	-0.13	**0.41**
MAX-MIN	0.02	**0.41**	**-0.39**
RATE	-0.10	-0.11	0.15
CV1-10	**0.69**	**0.73**	**-0.30**

- Standard errors for the phenotypic correlations ranged from 0.01 to 0.04.
- Figures in bold are those considered to be of suffiecient magnitute to be important

Among the traits representing the change in FD along the staple, ACVFD had the highest negative correlation with staple strength (r = - 0.43) than other FD variability along staple traits, i.e. ADJDIFF, MAX-MIN and RATE (r=-0.24, -0.39 and 0.15, respectively for the three traits). The level of correlation between ACVFD and SS (r= -0.43) was similar to the correlation between CVFD (FD variation in mid-side sample) and SS (r= -0.44). The analysis of correlation also shows that the correlation between ACVFD and FD was negative (r = -0.28), and this is similar to the correlation between

CVFD and FD (r= -0.24) in mid-side measurement. In contrast, the correlations between ADJDIFF, MAX-MIN or RATE and FD were low (r= 0.11, 0.02 and –0.10, respectively).

In relation to SS at the point of break, as expected, the present results show that FDMIN had stronger correlation with SS (r= 0.47) than FDMAX and SS (r= 0.19). Similarly, the correlation between FDMIN and CVFD was stronger (r= -0.30) than between FDMAX and CVFD (r ~ 0). The result also shows that there was high correlation between CV1-10 and CVFD (r= 0.69).

5. Discussion

This experiment was designed to study possible genetic factors involved in FD variation along the staple which may indicate susceptibility to seasonally induced changes in nutrient supply. The objective was to determine whether it is possible to select sheep that are resistant to seasonal FD changes. It was hypothesised that FD variation along the staple (ACVFD) has heritable and genetically correlated with other important wool traits such as staple strength. The objective was to determine whether or not ACVFD may be a useful selection criteria for Merino sheep breeders on SS. The indirect selection criterion is to avoid the cost of SS measurement.

Variation in FD as well as wool growth throughout the year has been well-described particularly in more primitive sheep such as the Whiltshire, Shetland, Soay, and Wild Mouflon sheep. Lowest FD and wool growth occurs during autumn-winter and this is predominantly controlled/induced by photoperiod (Ryder, 1969; Ryder 1971a; Ryder 1971b; Ryder, 1973). Similarly, in the Southern hemisphere, variation in the FD of non-Merino breeds also occurs throughout the year, with a minimum FD frequently occurring during the period of shortday length (June, July) (Reid and Sumner, 1991; Butler and Head, 1992; Maxwell *et al.,* 1988). While modern Merinos are less affected by the effect

of photoperiod on wool growth (Hutchinson, 1962; Williams and Schinkel, 1962; Lyne, 1964), there is a tendency for higher fibre diameter genotypes to be affected by nutrition to a greater extent than Finewool sheep (Jackson and Downes, 1979; Hynd *et al.,* 1997). This has implications for interpretation of the results of the experiment described in this chapter. In more recent work, Hynd *et al.,* (1997) also reported a tendency for low FD to occur near the break of season (April-May). This may also relate to poor nutrition and the sudden stress of rainfall after the difficult season (summer).

The seasonal variation in FD apparent in the Merino sheep used in this experiment is consistent with that described for similar sheep in a Mediterranean environment (Hynd *et al.,* 1997) with a trough in Autumn and a peak in Spring. This seasonal pattern results from a combination of nutritional variation and photoperiod. In Mediterranean environments reduced daylength and low quantity and quality of feed availability coincide in Autumn. The relative effects of photoperiod and nutrition are impossible to discriminate although the present finding showing highest frequency of low FD around May-June is presumably a response to the poor nutritional conditions over Summer and the sudden change in nutrient supply and rumen function after germination of annual pasture plants.

Minimum FD coincides with the period of the year in which the phenomenon known as follicle shutdown occurs (Hynd *et al.,* 1997). While the initial experiment of this thesis found no genetic basis to shutdown (albeit in a year in which the incidence of shutdown was low), the possibility remains that there is a genetic component to the susceptibility of FD change to environmental change. Indeed shutdown may simply be the ultimate expression of FD reduction, since mean FD was not decreased by cortisol which caused substantial shutdown in the experiment of Ansari-Renani and Hynd (1996). The proposition then, that FD responsiveness to the environment has a genetic basis, was

tested in the current experiment by analyzing measured genetic components involved such as studs and sire.

The effect of four studs on the responsiveness used in this work was one of the factors considered because quality of sheep coming from different studs might be different due to selection or breeding program applied in the stud. Jackson and Downes (1979) reported that selection lines based on FD had different propensities for FD changes in response to the environmental changes. The present results show that mean FD was significantly different between studs. This should be further analyzed to investigate the possible effect of studs on the susceptibility to diameter change, given the fact that sheep with coarser wool are more succeptible to the environmental changes than Finewool sheep (Jackson and Downes, 1979; Ansari-Renani and Hynd, 1996). The current analysis, also found that the coefficient of variation of FD along the staple was statistically different between studs ($p=0.0001$) (Table 3.3). This might means that the responsiveness to the environment in changing fibre diameter was different between studs, with sheep from stud B were the least responsive among the studs.

To confirm these findings, a different method of quantifying the responsiveness to the environment based on a simple regression analysis of stud fibre diameter mean on mean FD, was then used. The slope of the line relating FD to 'environment' (snippet no) in order of mean FD was estimated and this can be a measure of how FD changes along the season differ between studs. This procedure was used by Jackson and Downes (1979) to quantify environmental sensitivity.

There are some possible weaknesses of this approach. The analysis was based on the assumption of the 10 segments diameters for each staple which were assumed to represent the same 10 wool growth environments for each staple and for each sheep. This assumption might introduce some objections, for instance the precision of the exact time

of the year represented by the snippet number and the effect of the sequential nature of the observations due to subsequent snippet/segment measurement. However, the possible effect of subsequent or repeated measurement has already adjusted with the Proc Mixed Asycov procedure with SAS program (SAS Institute, 1990). The advantage of this method is that the technique reduces the variance of diameter along the staple problem to a genotype by environment interaction problem, a more considerable method in relation to animal breeding theory. Moreover, this technique is relatively simple either in calculation or in understanding the results through the different slopes.

By using this approach, the results show that there was no significant difference in slope between studs (p=0.0646) (Figure 3.4). This means that the susceptibility to the environmental changes on FD change, is unlikely to differ between studs. In contrast, Jackson and Downes (1979) reported that High fibre diameter lines were more susceptible to the environmental changes than Low fibre diameter lines. There are some possible reasons why the current findings are different to these of Jackson and Downes (1979). Firstly, the mean difference of mean FD between studs in this study (2.8 μm, ranged from 24.8 and 27.6 μm) might not be sufficiently large to cause a slope difference in the studs as occurred in Jackson and Downes' work (1979) in which the FD differences between lines were around 7.2 μm (range from 16.18 and 23.37 μm). Secondly, the wool samples used in this work came from a larger number from resource flock (593 sheep) representative of 4 studs and 47 sires, whereas previous work only used a limited number of sheep (five sheep per group). Thirdly, although the relative large number of sheep used in this work, however, this might not still be enough for a field experiment requirement, as more factors may affect the sheep, such as temperature, humidity or pasture conditions.

Results of later work (Ansari-Renani and Hynd, 1996) also showed that Mediumwool Merinos had greater susceptibility to follicle shutdown at the break of

season. In relation to present result, the absence of a difference in slope or sensitivity to the environmental changes, might relate to different environmental condition in both studies. The "Environment" in the present experiment used was "the time of the year" represented by 10 snippets, while previous researchers used cortisol to artificially induce follicle shutdown rather than allowing the shutdown to occur naturally. Nutritional state and other environmental stressors, such as physiological stress, temperature or photoperiod, can influence the brain central nervous system to stimulate the pituitary gland, in the hypothalamus to produce corticotrophins, which can cause wool follicle dysfunction (Gebbie *et al.,* 1994). The cortisol application used in previous research may have a direct effect on the follicle, whereas nutritional stress may have a lesser effect because it is an indirect mechanism.

In relation to the P value from the analysis of slope difference between studs which was close to the significance (P=0.065), the results may indicate the tendency that the susceptibility to fibre changes might be different between studs in response to the environmental changes. Further analysis using more number of studs or other genetic parameters needs to elucidate this findings.

A second approach to determine genetic influences on susceptibility to FD change was conducted by comparing sires used in this experiment. The analysis of slope difference between the sires shows that FD variation with the environment was not significantly different between sires (P=0.868). In contrast, a significant sire effect on ACVFD was found in more recent work (Adams and Briegel, 2002) using simple Analysis of Variance, although the sire group accounted for only 16% of the variation along the staple ($p<0.001$, $R^2 = 0.16$).

On balance, we can conclude that in the present work, there is no strong evidence of genetic effects on the responsiveness to the environmental changes by using the

approach as discussed. Further analysis was required to confirm the genetic role in the responsiveness, i.e. by calculating heritability value for FD variation along the staple as well as other wool parameters either along the staple or mid-side sample.

The results show that high heritability values were found for FDMEAN, CVFD in both mid-side and along staple and FDMIN, FDMAX or CV1-10 (varied from 0.47 to 0.78) (Table 3.3). This results are in accordance with previous results showing high heritabilities in these traits (Hill and Ponzoni, 1999). This is why selection in wool industry has been based on wool traits such as FD and CVFD. These parameters have become standard measurement for wool traits because of the low cost, simple procedure and high genetic merit in wool industry as discussed earlier in the Literature Review. The high heritability results of FDMIN and FDMAX in this work and high phenotypic correlation between FDMAX and FDMEAN or between FDMIN and FDMEAN (0.94 and 0.88, respectively), however the impractical use of this traits for the selection criterion too, as this traits cannot be obtained easily with routine wool measurements such as FD and FD variation.

Moreover, in this work we focused on wool traits along the staple especially ACVFD. Unlike CVFD in mid-side sample, ACVFD had a moderate h^2 value, 0.17. Clearly CVFD and ACVFD are different traits. CVFD (in mid-side) or CV1-10 (average FD variation in segment 1-10) is actually a measure of FD between fibres as well as within fibres. Measurement of FD between fibres is basically a measure of a "static" condition of fibres such as FDMEAN, FDMIN or FDMAX which had also high h^2 in this work (0.55-0.60). In contrast ACVFD represents a "dynamic" response to season or environment (nutrition, climate etc), therefore the influence of environment might be very dominant to the dynamic trait. The results show that ACVFD had a greater phenotypic standard deviation than CVFD and CV1-10, but it only had a low heritability. The low

heritability of ACVFD was also supported by the results of other parameters measuring the changes of ACVFD. Adjacent difference in FD (ADJDIFF) and Rate of FD change (RATE) both also had very low h^2 (0.13 and 0.01, respectively). This findings suggest that a major component of the phenotypic variation in ACVFD is environmental rather than genetic in origin. Nutrition plays a major role in governing fibre diameter (William and Schinkel, 1962; Williams *et al.,* 1972; Hynd 1989[a]; Butler, 1994; Schlink *et al.,* 1996[a], Earl *et al.,* 1994) and variations in FD are often associated with seasonal fluctuations in pasture quality and availability (Ryder 1956; Hansford and Kennedy 1990). Poor nutrition can disrupt feed intake as well as the supply essential amino acids such methionine and cysteine required for normal follicle function including all cell division and protein synthesis leading to decreased wool growth, FD and probably enhancing wool follicle shutdown.

The heritabilities of fibre diameter, coefficient of variation of fibre diameter and staple strength in this study may be less accurate than those that have been estimated on larger populations. However, a large study (2170, 40 month old ewes that were the progeny of 155 sires) indicated that the heritabilities of FD, CVFD and SS were 0.68, 0.69 and 0.33 respectively (Hill and Ponzoni, 1999), which were similar to those found in this study (Table 3.4).

Since the heritability of ACVFD and other traits describing FD changes along the staple (ADJDIFF, MAX_MIN and RATE) were very low, correlations between the traits with staple strength is the next interest to investigate for possible indirect selection criteria for the staple strength. The results of this work show that phenotypic correlations among the measurements of FD variation indicate that the more common measure, CVFD, was strongly associated with CV1-10 (average FD variation in segment 1 to 10) but only moderately with ACVFD. This suggests that a greater proportion of the variation in

CVFD can be explained by variation within snippet (CV1-10) than along staple variation (ACVFD). This is what we expected as CV1-10 represents FD variation between and within fibres as applied to CVFD in mid-side, whereas ACVFD represents the variation along the staple which is also deal with variation between and within fibres, but it more relate to the later (within fibre). This might also imply to the present findings that higher and positive correlation between FD with CV1-10 (r=0.69) than with CVFD (r=-0.24) or ACVFD (r=-0.26). We would not expect that CV1-10 is similar to CVFD or ACVFD, as they are different traits. CV1-10 measures FD variation within the snippet (very short staple) which is more relate to between fibres than along fibres, whereas CVFD and ACVFD are the measurement of FD between and within the staple.

ACVFD and CVFD had similar phenotypic correlations with SS, but the correlation between CV1-10 and SS was weaker than between ACVFD or CVFD and SS. This might suggest that ACVFD in this environment is more important than CV1-10 in determining SS outcome. Biological explanation of this relationship might be related to the evenness of fibre diameter and length between fibres in wool. Higher FD variation might decrease the force to break the staple and so the staple strength. This mechanism is also shown in previous work that lower variation of FD between fibres creates higher uniformity of fibre length, which produce sound wool (Schlink *et al.*, 1996[b]). It is very interesting to further investigate why some sheep have more uniform wool fibres, less CVFD and therefore higher SS than the others. This might relate to the fibre structure and composition which is discussed in Chapter 4.

The similar correlation between ACVFD or CVFD and SS might indicate that ACVFD can be used as a standard of FD measurement in the wool industry. However, this result, combined with the estimated heritability values for these measurements, suggest that CVFD is a better indicator trait of SS than ACVFD and CV1-10. CVFD is

also more practical to use as this trait is easier and less expensive to measure than ACVFD.

The stronger phenotypic correlation between SS and FDMIN than between SS and FDMAX supports previous findings reported by Bigham *et al.* (1983) who suggest that wool fibres are most likely to break at the point of lowest fibre diameter within the staple. The positive correlation between SS and FDMIN indicates that to improve SS it would be necessary to select for an increased FDMIN or to manipulate nutrition to increase FDMIN. This would, however, result in wool with less desirable properties and consequently FDMIN is unlikely to be appealing as an indirect indicator of SS. The remainder of the fibre diameter variability measures had very low to low correlations with SS, which suggests that they would also be poor indicator traits for SS.

Since the correlations calculated in this study were phenotypic only (not genetic), recommendations regarding indirect selection criterion of SS based on the traits correlated should be treated with caution. The genetic and phenotypic correlation can be different as shown by Lewer and Ritchie (1992). For instance, the genetic and phenotypic correlations in some traits were very different (the difference was up to 3 or 5 times for the correlation between greasy fleece weight with SS or with CVFD, respectively). However, later work has shown that the difference between phenotypic and genetic correlations was not significant for some morphological characters (Koots *et al.*,1994; Roff, 1996) which indicates that phenotypic correlations may often be used as predictors of genetic correlations. In more recent work Hill and Ponzoni (1999) also show that the genetic and phenotypic correlations for all wool traits measured were similar. Therefore, data of phenotypic correlation can be used as predictor of genetic correlation, but genetic correlation needs to be calculated when a sheep breeding policy will be applied to ensure the validity of the calculation.

It is useful to discuss whether ACVFD trait is applicable to use in the wool industry. Recently the technique of measuring FD along the staple for wool measurement standard has been developed and the machine to facilitate the technique has been produced, namely OFDA2000 (Peterson *et al*, 2000). Peterson and Gherardi (2002) compared Sirolan Fleecescan (measuring mean fibre diameter in mid-side sample) and OFDA2000 (measuring mean fibre diameter along the staple) and concluded that the OFDA2000 had sufficient precision to be used as a tool for ranking sheep on mean FD. Ferguson *et al.* (2002) also recommended that OFDA2000 can be become an alternative for conventional certified core tests as there is good agreement between the two techniques (R^2=0.96). However, the low heritability demonstrated in this trial (Yamin *et al.,* 1999) suggest that its use may not be warranted in breeding programs. Adams and Briegel (2002), in the otherhand, agreed that the use of diameter profiling using the OFDA2000 is still recommended as a tool for managing the nutritional status (stocking rate) of wool-producing sheep.

Again, regardless of the possible importance of ACVFD measurement for national wool measurement standard, this present work was focussed on determining the genetic significance on the trait.

6. Conclusion

A concern has often been raised as to whether the measure of the coefficient of variation of fibre diameter in a mid-side wool sample is the best indicator trait for SS performance. It was thought that fibre diameter variation along the fibre could be an important contributor to lower SS, and therefore selection should concentrate on reducing ACVFD (variation along the staple), rather than CVFD (between and along fibres in mid-side). However, our results suggest that there would be no advantage in using variability

of FD along the staple when trying to predict SS. Since the sheep used in this work were 4.5 year old ewes, these results are applicable to mature ewes and may not necessarily be applied to hoggets, lambs or rams.

Since the heritability of ACVFD was moderate, further study on the relationship between fibre structure and along-staple variation in FD should not be undertaken. However, the issue of susceptibility to environmental changes was investigated in the next experiment, which focused on the traits FD, CFW and fibre structure characteristics (wool sulphur and paracortical cells ratio). The next study used different strains of sheep (Mid-side and Mediumwool Merinos) and different groups of sheep, which were selected, based on their phenotypic FD and wool sulphur content. This approach allows maximal response to the environmental changes, in this case, the infusion of cysteine, which is the first limiting amino acid for wool growth.

CHAPTER 4

EFFECTS OF SHEEP GENOTYPE ON THE RESPONSE OF WOOL GROWTH, FIBRE DIAMETER, FIBRE SULPHUR AND PARACORTEX RATIO TO NUTRITIONAL CHANGES

1. Introduction

Variation in the quality and quantity of feed on offer in Mediterranean environments undoubtedly influences staple strength, as a consequence it not only affects fibre diameter along the staple but possibly also by precipitating follicle shutdown especially at the break of the season. A decline in staple strength is associated with a significant loss in the wool industry as this trait is a major determinant of wool price. It has been thought that selection of sheep that are resistant to environmental changes in wool traits, may resolve this problem. This project was designed to study the extent to which there is a genetic component to the susceptibility of sheep to the fluctuation in the nutritional environment.

In Experiment 1 (Follicle shutdown, Chapter 3), there was no significant effect of sire on the incidence of follicle shutdown suggesting that there is little genetic component to shutdown. This conclusion, however, must be tempered by the fact that the incidence of shutdown in that trial was low (<1.5%). Although a further genetic study on follicle shutdown could not be conducted within the time constraints of this program, a possible genetic component to the susceptibility of FD to environment was postulated on the grounds that shutdown follicles may be related to lower FD at the break of season (Ansari-Renani, 1996). This issue then became the topic of the next study of FD variation along staple (ACVFD) in Mediumwool Merino (Chapter 3). Again, the results showed no 'stud' and 'sire' effects on the susceptibility to fibre variation along the staple and a

moderate heritability of this trait (h^2 = 0.17), indicating the variation was largely controlled by environment rather than sheep genes. However, while this conclusion may be true within a strain of sheep, the situation may differ across more disparate genotypes, such as Finewool vs Mediumwool Merinos. It was proposed to examine strains of Merino differing widely in mean FD on the basis of the previous work of Jackson and Downes (1979) who found that a low FD selection line was less susceptible to environmental changes than to high FD line. In more recent work, it has been also reported that Finewool Merino are less susceptible than Strongwool Merinos to environmental changes in terms of follicle shutdown at the break of season in Southern Australia (Hughes, 1995; Ansari-Renani, 1996; Hynd *et al.*, 1997).

That Finewool Merinos are less responsive to environmental changes is also supported by the findings that this strain has higher staple strength (SS) than Strongwool Merinos, although this seems to contradict the positive correlation between FD and SS (Table 1.5). However, as already discussed previously (Section 4.2.1), the conflict may be resolved by consideration of the negative correlation between CVFD and SS. Finewool sheep may have more uniform FD or less CVFD than Mediumwool Merinos, therefore Finewool sheep have higher SS.

It is proposed that the responsiveness of fibre diameter to the environment is genetically-determined and related to differences in the expression of high and ultra-high sulphur genes and to the type of cortical cells comprising the fibre. Paracortical cells comprise intermediate filaments (IF) arranged in a parallel array with intermediate filament associated proteins (IFAP) interspersed between them. The IFAP in the paracortical cells results from the expression of ultra-high sulphur genes, namely the KAP 4, 5, 9, 10 and 12 (Powell, 1996), while the IF contain the low sulphur proteins. Orthocortical cells, in contrast, contain IF in a whorl-like arrangement and differ from

paracortical cells in that the IF/IFAP ratio is higher. Differences in IF/IFAP ratio result in differences in the sulphur content of the wool such that fibres high in paracortical cells (low IF/IFAP) contain more sulphur than orthocortical cells (high IF/IFAP). The sulphur content of fibres, however, is significantly affected by the nutritional environment as the expression of UHS genes is increased by nutrient supply in general, and circulating cysteine in particular (Fratini *et al.*, 1994).

The chemical composition or arrangement of cell types can determine the intrinsic strength (inherent strength) of wool fibres (Chapman, 1965). IFS is defined as 'the tensile force required to break a sample of wool (more often cross sectional in single fibres), normalized by dividing the force by some measure of the amount of material being broken' (Huson, *et al.*, 2000[a]). Orwin *et al.* (1985) found that fibres with a high proportion of orthocortical cells had higher SS.

The cell types and composition are proposed to account for genetic differences in the susceptibility of fibre diameter between sheep to fluctuations in seasonal nutrient supply. It is proposed that Finewool Merinos are less responsive to cysteine infusion than Mediumwool Merinos and that sheep with a high wool sulphur content are also less responsive. It is proposed that this difference in responsiveness is controlled by the genes encoding the sulphur-containing keratin proteins.

If the results showed that the effect of cysteine infusion on wool sulphur, paracortex ratio, FD or wool growth is different between sheep strains and sulphur status, we can conclude that the susceptibility to environmental changes is controlled by the expression of the high sulphur genes. We may then use the sulphur content in wool fibres as a selection criterion to identify resistant sheep in terms of FD and wool production to the environmental changes. This could become a significant contribution to the wool industry, especially in Australia.

2. Hypotheses

The following overarching hyphothesis was tested: "That susceptibility to the environmental changes (cysteine infusion) in wool growth and FD is controlled by genes encoding high and low sulphur proteins".

Specifically, the following hypotheses were tested:

1. that Finewool Merinos have a higher proportion of paracortical cells in their wool,
2. that given paracortical cells contains more high/ultra high sulphur genes, Finewool Merinos have a higher wool sulphur content,
3. that in relation to Finewool Merinos, sheep with high wool sulphur content have lower fibre diameter but higher proportion of paracortical cells, wool sulphur and expression of KAP4.2, a ultra high sulphur gene,
4. that cysteine infusions increase wool growth, FD, paracortex ratio, wool sulphur and expressions of high/ultra-high sulphur encoding genes,
5. that as Finewool Merinos have higher SS, this strain of sheep are less responsive in terms of wool growth, fibre diameter, paracortex ratio, wool sulphur and high and ultra high genes expressions, to cysteine infusion than Mediumwool Merinos
6. that sheep with high wool sulphur content are also less responsive to cysteine infusion than low wool sulphur content sheep in both strains.

3. Materials and Methods

3.1 Animals and Wool Samples

The objective of the experiment was to generate extreme phenotypes in both fibre diameter and fibre sulphur content. This would provide a unique resource for investigating the independent and combined effects of FD and wool sulphur on environmental responsiveness.

Wool samples from the flock of 469 Finewool Merino female hoggets from Blackford Merinos, Kingston, South Australia (FD = 17.80 μ; CVFD = 22.51%); GWG = 3.01 kg; CFW = not available) and 323 Mediumwool female Merinos from Anama Holdings Pty Ltd, Clare, South Australia (FD = 20.51 μ; CVFD = 22.98%; GWG = 5.14 kg; CFW = 3.65 kg) were used in this experiment. One hundred wool samples from each strain were selected from the flock based on their FD data. To generate a large difference in FD between strains, the samples selected from Finewool Merinos were the lowest 100 FD in the flock, whereas from the Mediumwool sheep, they were the highest 100 FD within the flock. Wool samples from 100 sheep of each strain were taken from left midside of the sheep. The wool was kept in a plastic bag and was identified based on sheep number before being washed / scoured for next treatments. The wool samples were then analysed for their sulphur content (section 3.6) and were selected to identify six sheep having the highest fibre sulphur content and six with the lowest sulphur from each strain. This made four groups of sheep with six replications for each group as follows:

a. Six Finewool Merinos with low fibre sulphur content (FW-LS)

b. Six Finewool Merinos with high fibre sulphur content (FW-HS)

c. Six Mediumwool Merinos with low fibre sulphur content (SW-LS)

d. Six Mediumwool Merinos with high fibre sulphur content (SW-HS)

In such experimental design, possible confounding effect of FD within strain or sulphur group was already omitted. The possibility that strain might be confounded with that sire cannot be discussed as no information on sire was recorded.

3.2 Experimental Feed

The sheep ration used in the experiment was formulated to provide protein and energy at maintenance levels as recommended by the National Research Council (NRC, 1985), ie. 95 g of crude protein (CP) and 8.4 MJ of metabolizable energy (ME) for 50 kg of ewes. For other weights, the CP and ME level was adjusted based on the 50 kg requirement, as shown in Table 4.1.

The feed ingredients used were oaten chaff (9.14% CP; 8.1 MJ ME) and commercial sheep nut (13.4% CP; 11.9 MJ ME) with the ratio 70:30% in the ration. Feed composition for different sheep weight was shown in Table 4.1.

Table 4.1 Quantity and quality of feed offered to the experimental animal

Sheep weight (kg)	Sheep Nut (gr DM)	Chaff (gr, DM)	CP (g/kg)	ME (MJ)
40	190	650	86	7.56
50	210	730	95	8.40
60	230	800	104	9.24

% DM sheep nut = 93% ; chaff = 94%.

Feed had been given six weeks prior to the infusion to remove possible effects of previous nutrition status, and this feed was used until 21 days post infusion when the experiment finished.

3.3 Cysteine Infusion

After being kept in an individual pen for the feeding adjustment period (6 weeks prior to infusion), each sheep was kept in a metabolism crate one week prior to the infusion as an adaptation period and kept there until the infusion finished (three weeks)

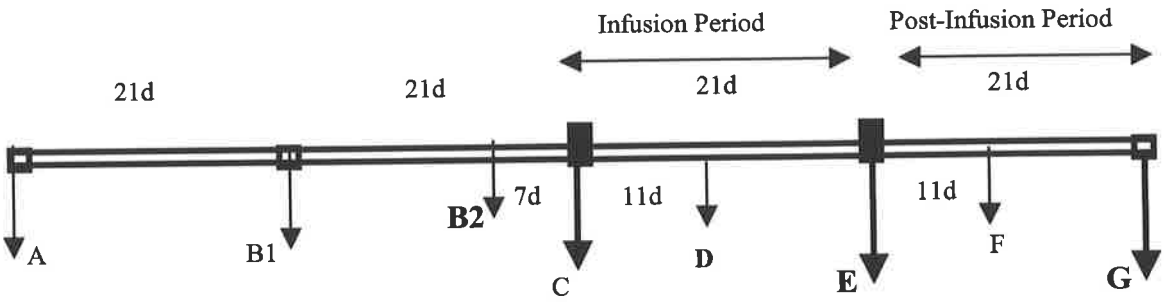
(Figure 4.1). Continuous infusion of cysteine on wool was conducted (over 21 days) by inserting a polyvinyl catheter (1 mm internal diameter) into the jugular vein of each sheep. Dissolved cysteine in 0.9% saline (concentration was 5.2 mg/ml) was infused continuously using a peristaltic pump and was changed before the solution finished. The sheep received ~4.0 g cysteine per day over the 21 day infusion period with a flow rate of 0.54 ml/min. This method was based on that used by Fratini *et al.* (1994) which successfully conducted a comprehensive examination of the levels of wool follicle mRNAs for the major IF and KAP gene families following cysteine infusion.

Materials used in setting up cysteine infusion were chemicals (L-cysteine (Sigma), 0.9% Saline (Baxter), ethanol 70%, Zeparine (1:100) and Heparin) and some equipment (catheter needles + catheter tubing about 60 cm length, polyethylene tube 1 mm internal diameter, swab, sprayer, scissor, syringe 20 ml, clipper and leucoplast 5 cm width).

3.4 Wool Sample and Skin Biopsy Collections

3.4.1 Wool sample collection

Wool was clipped cleanly from a tattooed area of skin (approximately 10x10 cm^2) on the left midside. Timing of wool clipping and skin biopsies either for collection or for discarding purposes is shown in the timeline diagram (Figure 4.1).



A = Start maintenance diet, wool patching, weighing sheep
B1 = wool clip (discarded), weighing sheep
B2 = wool clip and collected, skin collection (BI samples), weighing sheep, sheep were put in metabolic crates
C = wool clipped and discarded and start Infusion
D = wool clip (discarded)
E = wool clip, skin collection & stop infusion (D21 samples)
F = wool clip (discarded)
G = wool clip, skin collection (PI samples)

Figure 4.1 Time line indicating timing of the experiment events.

To omit wool patches under previous nutrition regimes, the wool sample was collected and discarded at three weeks prior to infusion or three weeks after experimental feed was given (B1). Wool and skin sample were collected at seven days prior to infusion when the sheep were placed in metabolizable crates (B2). This samples were recorded as 'Before Infusion (BI)' for next fibre measurements. Prior to infusion (C), the wool grown was clipped and discarded. At 11 days after cysteine infusion begins, wool grown were clipped and discarded, to allow enough time for fibre formation in response to cysteine infusion (D). Wool and skin sample at 21 days infusion was collected and this is recorded as '21 days of infusion (D21)' wool sample. At 11 days after the infusion stopped, the wool grown was discarded, as it would contain wool grown in the last days of the infusion) (F). Lastly, a post infusion wool sample was taken from the clipped area 21 days after the cysteine infusion was stopped (G, Figure 4.1).

3.4.2 Skin biopsy collection

Two trephine samples from each sheep were taken from the left midside area one day before infusion, after 21 days of infusion and 21 days post infusion. These skin samples were used for measuring the ratio of paracortex in the fibre and the relative expression of keratin genes. The technique of biopsy collection for paracortex study was that as described previously in Chapter 2, section 3.2., while for gene expression study, skin samples taken from the midside were placed immediately in a vial and stored in liquid nitrogen during the skin collection process (1 hour at the longest), then all samples in vials were kept in a freezer at -70 ^{0}C until ready for the next process.

3.4.3 Paracortex ratio measurement

Sample preparation for making histology sections was similar to that as discussed in Chapter 2, section 3.3 (Follicle shutdown). In brief, cleaned and trimmed skin samples were embedded and sectioned (8 μ transversely) and then stained using the Loeffler's methylene blue (BDH Chemicals, U.K) protocol for orthocortex/paracortex differentiation. This method produces a deep blue staining of paracortical cells and light blue of mesocortical cells, while orthocortical cells and background tissue remain unstained. In brief, the protocol of staining the samples was as follows: (1) Histoclear (10mins), (2) Absolute ethanol (2mins), (3) 80% ethanol (2mins), (4) 30% ethanol (2mins), (5) RO water (2mins), (6) Performic acid (H2O2 : RO water : 90% formic acid = 10:65:25) (30mins), (7) RO water (10secs), (8) Loeffler's Methylene blue (30ml, 0.8% Methylene blue in absolute ethanol + 99ml RO water + 1ml 1% KOH in RO water) (5mins), (9) RO water (10secs), (10) Acid ethanol (1% concentrated HCl in 70%) (10secs), (11) 80% ethanol (10secs), (12) Absolute ethanol (4mins) and (13) Histoclear (4mins).

The area and percentage of fibre occupied by paracortex was measured with image chromatic analysis in a computer program. The areas of paracortex stained dark blue were traced at 1430x magnification using an image analysis system (Bioquant IV, R&M Biometrics, Tennessee, USA). Areas of intensely stained paracortex and more lightly stained mesocortex from around 30-40 fibres were combined and expressed as proportion of total paracortex area.

3.4.4 Gene expression analysis

Northern blot analysis (as detailed below) was used to study the level of the expression of m-RNA of the experimental sheep skin. The Northerns were probed with some genes that may have a relationship to wool sulphur, KAP2.12 (encoding a high sulphur protein in the cortex), KAP4.2 (encoding an ultra high sulphur protein in the cortex), K2.9 (encoding a low sulphur protein of the cortex) and Trichohyalin (as a control gene encoding an inner-root sheath protein). Molecular techniques used for the isolation of wool follicle RNA isolation and preparation of northern blots (Sambrook *et al.,* 1989) are described in the following section.

3.4.4.1 RNA Isolation

The technique of RNA isolation used in this experiment is based on the protocol using the reagent Trizol (Invitrogen, Carlsbad, California, U.S.A). The skin tissue was homogenised by using a tissue homogeniser (Ultra-Turrax; Janke und Kunkel KG, Staufen Breisgau) in 1 ml Trizol reagent per 100 mg of tissue. The homogenised samples were incubated for 5 minutes at 15-30^{0}C to permit complete dissociation of nucleoprotein complexes. Then 0.2 ml of chloroform was added (molecular biology reagent; Sigma, St. Louis, USA) per 1 ml of Trizol. The sample was mixed vigorously for 15 seconds and incubated at 15-30^{0}C for 2-3 minutes. The sample tubes were then centrifuged at 12,000

x g for 15 minutes at 2-8^{0}C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. RNA from the aqueous phase was precipitated by transfer to a fresh tube, addition of isopropyl alcohol (Sigma, St. Louis, USA) (0.5 ml per 1 ml Trizol) and inversion. The sample was incubated at 15 to 30^{0}C for 10 minutes, centrifuged at 12.000 g for 10 minutes at 2-8^{0}C and RNA forms a gel-like pellet on the side and bottom of the tube. To wash the RNA pellet, the supernatant was removed and at least 1 ml of 75% ethanol per 1 ml of Trizol was added. The sample was then mixed by vortexing and centrifuged at 7500 x g for 5 minutes at 2-8^{0}C (SpeedVac Concentrator, Savant). In the redissolving process, the RNA was dried in air for 5-10 minutes, dissolved in 40 µl of RNase-free water or 0.5% (w/v) SDS solution (Lauryl sulphate, sodium salt, molecular biology reagent; Sigma, St. Louis, USA), then incubated for 10 minutes at 55 to 60^{0}C and stored at -70^{0}C.

3.4.4.2 RNA Concentration Measurement

To test the concentration and purity of RNA, each sample was analysed by UV spectroscopy (UV-Visible Recording Spectrophotometer UV-160A; Shimadzu Corporation, Kyoto, Japan). This technique uses the assumption that an absorbance of 1.0 at 260 nm (A_{260nm}) equals 40 µg/ml RNA (Maniatis *et al.,* 1982) where absorbance of UV gives a measure of quantity and the ratio of A_{260nm} / A_{280nm} provides an indication of purity. An ideal ratio for a pure RNA is approximately 1.8-2.0. Samples stored at -70 ^{0}C were thawed, heated at 65 ^{0}C for 10 minutes, then vortexed to dissolve the RNA and a 5 µl aliquot was taken from each sample. This was diluted in 395µl MQ water, mixed thoroughly then the A_{260nm} measured with the spectrophotometer using water as the control. For running the gel used later on, the concentration of each sample was adjusted accordingly based on the amount of RNA that will be put per track on the gel, i.e. 15 µg

RNA. The formula to calculate the RNA concentration is as follows:

Concentration RNA (µg/ml) = A $_{260nm}$ x dilution factor x 40

(Note: 40 is the concentration of RNA when A $_{260nm}$ =1.0)

The quality of RNA was then checked on a 1% agarose minigel (agarose, molecular biology reagent; Sigma, St. Louis, USA. The RNA samples (4 µl each) were mixed with 2 µl of formamide loading buffer (2x) (80% formamide; 10 mM EDTA pH 8.0; 1 mg/ml xylene cyanol and 1 mg/ml bromophenol blue) in an eppendorf. DNA size markers (SPP-1)(Geneworks, Adelaide, SA) were loaded in non-denaturised load buffer alongside RNA samples. To run the gel, RNA samples were heated for 2 minutes at 75 ^{0}C and loaded onto the gel. Gels were run in 1x TAE buffer at 100 mA/123 V using a Pharmacia power supply (EPS, 500/400). The gel was then stained with 0.1% (w/v) Ethidium bromide (Sigma, St. Louis, USA). The Molecular analysis program (BioRad) was then used to photograph the gel to examine the quality of RNA.

3.4.4.3 Electrophoresis of RNA (Agarose-Formaldehyde for Northern Blot)

Electrophoresis of 15 µg samples of RNA in agarose-Formaldehyde gels for Northern transfer was carried out as described by Sambrook *et al.* (1989). To make 200 ml 1% agarose gel, a 75 ml solution of formaldehyde and 5x running buffer was firstly prepared by mixing 35.70 ml formaldehyde (aqueous solution, molecular biology reagent; Sigma, St. Loius, USA) with 39.30 ml of 5x formaldehyde running buffer (0.1 M MOPS pH 7.0; 40 mM sodium acetate and 5 M EDTA) in the fume hood. Two grams of agarose was melted in 125 ml MQ in the microwave for 2-3 minutes, mixed until all agarose was dissolved and cooled to 65 ^{0}C. The agarose was then mixed with the 75 ml solution of formaldehyde and 5x running buffer, and 10 µg/ml ethidium bromide was added before the gel was poured.

Wool follicle RNA samples were electrophoresed alongside 4.5 µg of RNA molecular weight marker (RNA ladder, 0.24-9.5 kb; Gibco BRL, Life Technologies Inc,

Gaithersburg, MD). All RNA samples were dried under vacuum for 10 minutes then dissolved in a solution including 4.5 µl MQ H_2O, 2.0 µl 5x formaldehyde running buffer, 3.5 µl Formaldehyde and 10 µl Formamide. The samples were then mixed, centrifuged, incubated for 15 minutes at 65 ^{0}C in the oven, chilled on ice then 2 µl of sterile DEPC treated formaldehyde gel loading buffer (50% glyserol, 1 mM EDTA (pH 8.0), 0.25% bromophenol blue, 0.25% xylene cyanol FF) was added to each prior to loading.

Gel electrophoresis was in 1x Formaldehyde running buffer at 100 V for 5 min (pre-electrophoresis) then at 25 V (EPS 500/400; Pharmacia Fine Chemicals, Sweden) for 16 hours, to allow sufficient separation of the 18S and 28S ribosomal bands. To maintain constant pH, the buffer in the tank was circulated via a peristaltic pump (Minipuls 2; Gilson, Villiers, France).

To record RNA migration distances prior to northern transfer, the gel was washed with DEPC water to remove excess ethidium bromide and then photographed with Image Quantitative Program (Gel Doc 1000, Bio Rad).

3.4.4.4 Northern Blotting

The Northern blot technique used in this work was conducted in a vacuum blotting system (Vacugene XL Vacuum Blotting System; Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, NSW) at 40 cm H_2O of pressure, for 2 hours. RNA was transferred to Zetaprobe nylon membrane (Zeta-Probe GT genomic tested membrane; Biorad Laboratories Pty. Ltd., North Ryde, NSW). The blotted membrane was then neutralised on Whatmann paper soaked with 2x SSC (Sodium citrate/ Sodium chloride buffer).

To confirm successful transfer of RNA from the gel to the membrane, the gel and membrane were examined briefly under UV light. Following this, cross-linking of RNA to the membrane was performed with a Stratalinker (UV Stratalinker 1800; Stratagene

Cloning System, La Jolla, C.A; autocrosslink, 1200 MJ). The membrane was then air dried, labelled and stored until hybridisation.

3.4.4.5 Oligo-Labelling of c-DNA Probes

Probing of Northern transfer membranes was carried out using the Hybaid apparatus (Hybaid) with 3-4 membranes in each Hybaid bottle. Stored membranes were soaked 10xSSPE for placement in bottles, then pre-hybridised with 10 ml of pre-hybridisation solution (50% Formamide, 0.25 M NaCl, 0.12 M Na_2HPO_4 [pH 7.2], 7% SDS (Sodium dodecyl suphate), 100 µg/ml SS-DNA [Salmon sperm DNA, single stranded] for 1 hour preheated to 42 ^{0}C.

Oligo-labelled cDNA probes for the wool keratins were prepared using Megaprime DNA labelling Kit according to the manufacturers instruction (Amersham, UK). Prior to the hybridisation, the cDNA probes were purified in a spin-column (glass beads overlaid with sepharose CL-6B (Pharmacia) equilibrated in TE (10 mM Tris HCl, pH 8.0). Radiolabelled DNA fragments were eluted from the spin-column by centrifugation at 1900 rpm for 2 minutes (Heraeus). Incorporated dCTP (Deoxy-Cytidine Tri-phosphate) was determined by scintillation counting in optiphase Hisafe scintillation fluid using a LKB scintillation counter (Beckman, U.S.A). For 50 ng of DNA fragment, 20-30 x 10^6 cpm of dCTP were typically incorporated.

Before hybridisation, the oligo-labelled probe was denatured by heating at 100 ^{0}C for 5 minutes then combined with fresh pre-hybridisation solution. After removal of initial pre-hybridisation solution, the denatured probe solution was then transferred to hybaid bottles containing pre-hybridised membranes. The membranes were hybridised overnight at 42 ^{0}C in the Hybaid minioven. Following hybridisation, membranes were washed to remove unbound probe molecules, first at room temperature in 2xSSC / 0.5% SDS, then in this solution at 65 ^{0}C for 30 minutes. Depending on hybridisation signal

strength, membranes were then washed further in 0.2xSSC / 0-5% SDS at 65 ^{0}C. Membranes were then briefly air-dried, bagged and placed in a Phosphorimager cassette overnight. The signals of hybridisation were scanned and quantified in the Phospo-Imager (Molecular Dynamics PhosporImager; Molecular Dynamics, Sunnyvale, CA) using the ImageQuant version 1.0 Analysis Programme.

The signals are arbitrary units, reflecting the signal strength of radioactivity in bands detected by the phosphor imaging densitometer. The measurement is the number of points within the area of the band with the signal strength higher than backgrounds signal strength. Before the next hybridisation and use of other cDNA probes, blots were stripped to ensure complete removal of the previous probe by two washes in 0.1xSSC + 0.1 %SDS solution at 100^{0}C.

3.5 Wool Scouring

The wool was washed to remove grease and dirt after being weighed previously as greasy wool. Wool was put in a muslin bag and washed several times at different temperatures and period by using glass beakers and water baths to control the temperature (i) Beaker 1: hot water (50^{0}C) was mixed with lissapol detergent (TN-50) to a concentration of 0.4%, washing time 5 minutes, (ii) Beaker 2: hot water (45^{0}C) was mixed with lissapol to be 0.13% in concentration, washing time 5 minutes, (iii) Beaker 3: warm water (30^{0}C) without lissapol, washing time 5 minutes, and (iv) Beaker 4: cold water without lissapol, washing time 10 minutes.

During washing wool was gently agitated and squeezed without felting the wool. The wool was then dried in the lab oven (50^{0}C) overnight and then put in a desiccator to cool down to be weighed and put in a dry plastic bag, ready to be used for next analysis, i.e. wool growth data, FD and wool sulphur content.

3.6 Wool Sulphur Analysis

Three grams of wool from each sample was weighed and cut into 1 cm length in a conditioning room with a constant temperature (20 ± 2 ^{0}C) and relative humidity (65%) and put in a glass vial. Wool sulphur content of the samples was analysed at the Waite Analysis Lab, Department of Plant Science, Adelaide University. The method of sulphur analysis used in the lab is a modification of the technique described by McQuaker *et al.* (1979), namely Inductively Coupled Plasma - atomic Emission Spectrometry (ICP/AES). In brief, wool samples were digested with 65% Nitric acid and 72% Perchloric acid The samples were homogenised and then digested in the presence of excessive Nitric acid (HNO_3; 65%) followed by Perchloric acid ($HClO_4$; 72%) until dense white fumes of the Perchloric acid appear. The digested samples were then analysed automatically by ICP/AES. This procedure had been checked and compared with reference wool samples of known fibre sulphur content, from New Zealand (Corson *et al.* 1998). The calibration showed that the results of sulphur analysis using ICPAES at Waite was not significantly different to the reference samples (2.99 % and 3.03% of sulphur content from 3 replicates, respectively for Waite and New- Zealand measurements).

3.7 Statistical Analysis

Data on wool growth, FD, wool sulphur content and paracortex ratio and the expression of all keratin genes used of sheep were analysed by 3-Way Analysis of Variance using the General Linear Models (GLM) procedure of the SAS program (SAS Institute, 1990). The effects analysed were Strain (Finewool and Mediumwool Merino), wool sulphur group (high and low sulphur group), infusion (before infusion = [BI], 21 day infusion [D21] and 21 day post infusion [PI] and their two- and 3-way interactions (Strain x S-group; Strain x Infusion; S-group x Infusion; Strain x S-group x infusion). The

parameters analysed were greasy fleece weight (GWG), fibre diameter (FD), proportion of paracortical cells (PARA), wool sulphur content (SULPHUR) and expressions of KAP4.2, KAP2.12, K2.9 and TRN. Responsiveness of the changes of the parameters in different strains and sulphur groups to the cysteine infusion was also analysed.

Due to the same sheep used in the infusion treatments (before, 21 d Infusion and 21d post-infusion), in separate analysis, possible effects of the repeated measurements were analysed by Proc Mixed analysis (SAS Institute, 1990), in which sheep were fitted as random effect. However, the results show that the between-sheep variance was very close to zero. Thus the original GLM analysis (2 and 3 way analysis of variance) was used and presented in the results.

To confirm the analysis of variance was a valid and sensitive statistical approach, a backward elimination of nonsignificant effect was also used. Since the results of the elimination analysis did not change significantly, the data presented in the results were based on the 2 and 3-way analysis of variance.

4. Results

4.1. Results of 3-Way Analysis of Variance

Table 4.2 shows the probability values for the experimental effects analysed by 3-way Analysis of Variance of dependent variables: GWG, FD, paracortex ratio, wool sulphur content and expression of some selected keratin genes (KAP4.2, KAP2.12, K2.9 and Trychohyalin).

Table 4.2 Probability values for effects of strain (Fine and Mediumwool Merinos), sulphur level (high and low), cysteine infusion (before, 21day infusion and post infusion) and their interactions on greasy fleece weight, FD, paracortex ratio, and expression of selected keratin genes

EFFECT	GWG	FD	PARA	SULPHUR	KAP4.2	KAP2.12	K2.9	TRN
Strain	0.08	**0.0001**	**0.0001**	**0.0001**	0.14	**0.0074**	**0.001**	0.56
S-group	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.62	0.143	0.77	0.34
Infusion	**0.0001**	0.24	**0.0001**	**0.0001**	**0.005**	**0.0004**	**0.03**	**0.047**
Strain*S-group	0.32	0.12	0.79	0.50	0.89	0.68	0.67	0.53
Strain*Infusion	**0.0009**	0.09	0.16	0.70	0.34	**0.042**	0.15	0.65
S-group*Infusion	**0.03**	0.21	**0.03**	**0.0006**	0.28	**0.0175**	0.33	0.94
Strain*S-group*In-fusion	0.87	0.91	0.78	0.70	0.71	0.84	0.94	0.77

The three variables (strain of sheep, sulphur group and infusion) all had significant effects on most of the measured parameters (Table 4.2). Strain had a significant effect on FD, the percentage of paracortex in the fibre, fibre sulphur content, and expression of KAP2.12 (high sulphur gene) and K2.9 (low sulphur gene) ($p<0.01$), but no effect on greasy wool growth, and expression of KAP4.2 or trichohyalin ($p>0.05$). There was a tendency for strain to affect greasy wool growth but this failed to reach statistical significance. Sulphur group significantly affected greasy wool growth, FD, paracortex ratio and fibre sulphur content, but not gene expression. Cysteine infusion significantly altered all measured traits with the notable and surprising with the exception of FD. There were also some significant interactions particularly between sulphur group and infusion. These effects are described in detail in the next section.

4.2. Strain Effects on Wool Growth, Wool Composition and Gene Expression

Finewool Merinos had a significantly (21%) lower FD, 14% higher paracortex and 8% higher sulphur content than the Mediumwool strain ($p<0.01$) (Table 4). In terms of keratin gene expression, it was found that only KAP2.12 and K2.9 expressions differed between strains, KAP2.12 and K2.9 expression was significantly higher (37% and 64% respectively) in the Mediumwool Merino than in the Finewool Merino ($p<0.01$) (Table 4.3).

Table 4.3 Effects of strain on greasy wool growth, FD, paracortex ratio, sulphur content and gene expression of some keratin genes (KAP4.2, KAP2.12, K2.9 and Trychohyalin)

PARAMETER	MERINO STRAIN	
	FINEWOOL	MEDIUMWOOL
GWG ± se * (mg/day/100cm2)	171 ± 7 ^{a}	189 ± 7 ^{a}
FD ± se * (µm)	17.3 ± 0.2 ^{A}	21.0 ± 0.2 ^{B}
Paracortex ratio ± se * (%)	28.2 ± 0.5 ^{A}	24.7 ± 0.5 ^{B}
Sulphur ± se * (%)	3.0 ± 0.02 ^{A}	2.7 ± 0.02 ^{B}
KAP4.2 ± se *	19.1 ± 1.9 ^{a}	14.9 ± 1.9 ^{a}
KAP2.12 ± se *	9.8 ± 0.9 ^{A}	13.45 ± 0.9 ^{B}
K2.9 ± se *	2.78 ± 0.3 ^{A}	4.6 ± 0.4 ^{B}
TRN ± se *	5.7 ± 0.9 ^{a}	6.4 ± 0.9 ^{a}

*Different superscript in upper-case and lower-case indicate significance level at the 1% ($p<0.01$) and 5% ($p<0.05$) level, respectively, whereas the same upper-case indicate non-significant level ($p>0.05$).
- Units of gene expression (KAP4.2, KAP2.12, K2.9 and TRN are arbitrary (see section 3.6.4.5, paragraph 4).

4.3. Sulphur Group Effects on Wool Growth, Wool Composition and Gene Expression

Table 4.4 shows that high sulphur sheep had significantly lower greasy wool growth and FD than low sulphur group ($p<0.01$), but paracortex ratio and fibre sulphur content were significantly higher in the high sulphur group than in the low sulphur one

($p<0.01$). In contrast, the expression of all keratin genes were similar in the sulphur groups ($p>0.5$) (Table 4.4)

Table 4.4. Effects of sulphur group on greasy wool growth, FD, paracortex ratio, sulphur content and gene expression of some keratin genes (KAP4.2, KAP2.12, K2.9 and Trychohyalin)

PARAMETER	SULPHUR GROUP	
	HIGH-SULPHUR	LOW-SULPHUR
GWG ± se * (mg/day/100cm2)	154 ± 7 ^{A}	206 ± 6 ^{B}
FD ± se * (µm)	18.3 ± 0.2 ^{A}	20.0 ± 0.2 ^{B}
Paracortex ratio ± se * (%)	28.8 ± 0.6 ^{A}	24.1 ± 0.5 ^{B}
Sulphur ± se * (%)	3.0 ± 0.02 ^{A}	2.8 ± 0.02 ^{B}
KAP4.2 ± se *	16.3 ± 2.0 ^{a}	17.7 ± 1.9 ^{a}
KAP2.12 ± se *	10.7 ± 1.0 ^{a}	12.6 ± 0.9 ^{a}
K2.9 ± se *	3.6 ± 0.4 ^{a}	3.8 ± 0.3 ^{a}
TRN ± se *	6.7 ± 0.9 ^{a}	5.4 ± 0.9 ^{a}

*Different superscript in upper-case and lower-case indicate significance level at the 1% ($p<0.01$) and 5% ($p<0.05$) level, respectively, whereas the same upper-case indicate non-significant level ($p>0.05$).
- Units of gene expression (KAP4.2, KAP2.12, K2.9 and TRN are arbitrary (see section 3.6.4.5, paragraph 4).

4.4. Effects of Cysteine Infusion on Wool Growth, Wool Composition and Gene Expression

Results of the effects of cysteine infusion on all fibre parameters measured are shown in Table 4.5. Cysteine infusion increased greasy wool growth by 41% ($p<0.01$). The growth decreased significantly again after the infusion was terminated (PI stage). In contrast FD was not significantly affected by cysteine infusion with FD increase around 3% ($p>0.05$).

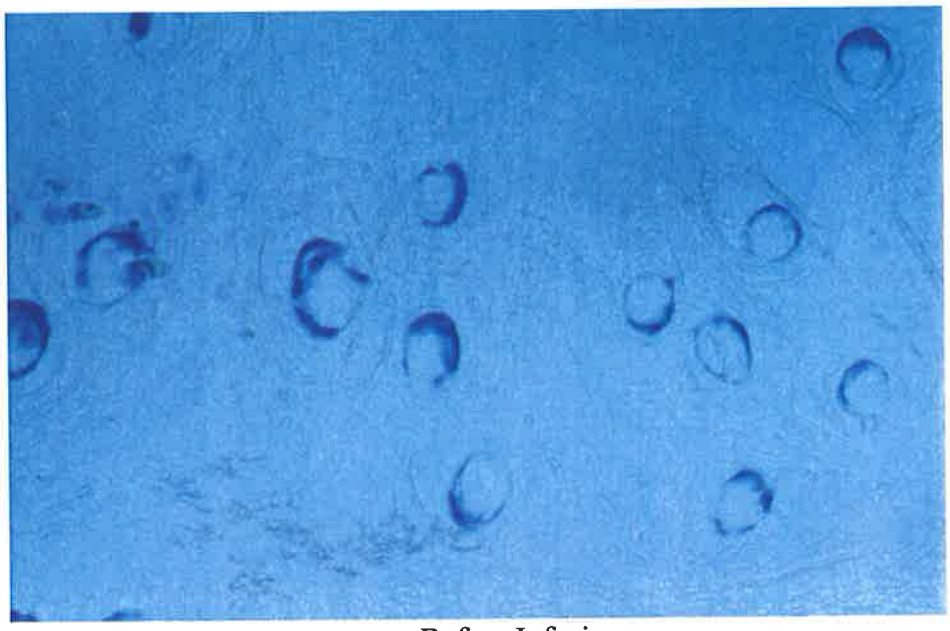
Table 4.5 Effect of cysteine infusion on fibre parameters (greasy wool growth, FD, paracortex ratio, sulphur content and gene expression of some keratin genes (KAP4.2, KAP2.12, K2.9 and Trychohyalin)

FIBRE PARAMETER	LOW CYSTEINE (BI)	HIGH CYSTEINE (D21)	LOW CYSTEINE (PI)	% CHANGE	
	Mean	Mean	Mean	D21-BI	PI-BI
GWG (mg/day/ 100cm2)	152 ± 8 ^{A}	215 ± 8 ^{B}	173 ± 9 ^{A}	41.1	13.9
FD (μm)	18.8 ± 0.3 ^{a}	19.4 ± 0.3 ^{a}	19.2 ± 0.3 ^{a}	3.2	1.8
Paracortex ratio (%)	19.3 ± 0.6 ^{A}	33.7 ± 0.6 ^{B}	26.3 ± 0.7 ^{C}	75.0	36.7
Sulphur Content (%)	2.7 ± 0.03 ^{A}	3.2 ± 0.03 ^{B}	2.7 ± 0.03 ^{C}	19.3	3.4
KAP4.2	12.9 ± 2.0 ^{A}	21.2 ± 2.0 ^{B}	n.a	64.9	n.a
KAP2.12	9.1 ± 0.9 ^{A}	14.2 ± 0.9 ^{B}	n.a	54.8	n.a
K2.9	3.1 ± 0.3 ^{a}	4.2 ± 0.3 ^{b}	n.a	35.2	n.a
TRN	4.7 ± 0.9 ^{a}	7.4 ± 0.9 ^{b}	n.a	55.3	n.a

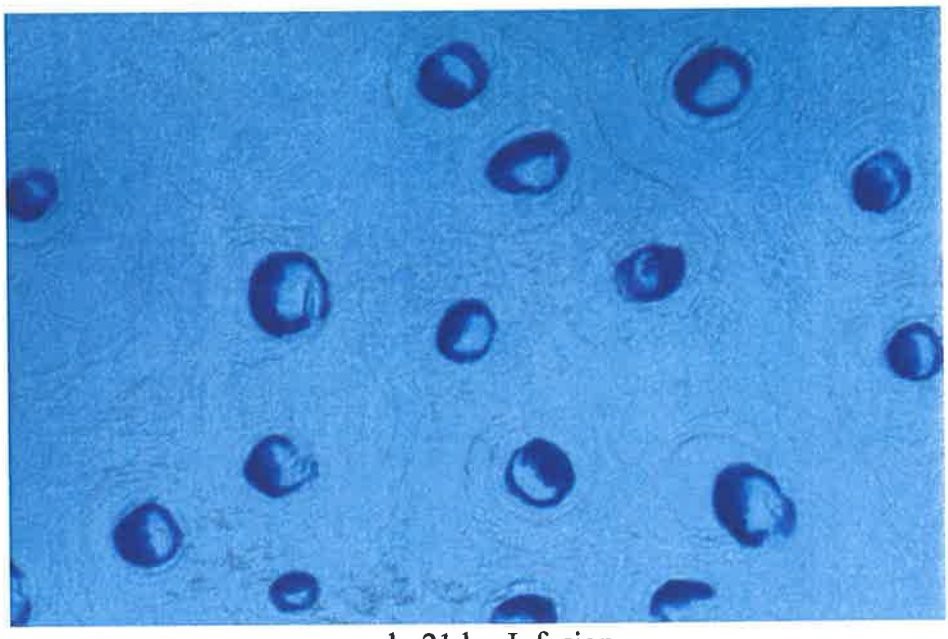
*Different superscript in upper-case and lower-case indicate significance level at the 1% ($p<0.01$) and 5% ($p<0.05$) level, respectively, whereas the same upper-case indicate non-significant level ($p>0.05$).
- Units of gene expression (KAP4.2, KAP2.12, K2.9 and TRN are arbitrary (see section 3.6.4.5), paragraph 4).

Cysteine infusion significantly increased the proportion of the fibre occupied by paracortical cells (75%, $P<0.01$) and the wool sulphur content by 19% ($p<0.01$) (Table 4.5). At the post infusion stage (PI), both paracortex ratio and wool sulphur content were still significantly higher than at the before infusion stage (BI) ($p<0.01$), however, the level of both parameters was significantly lower than when the high cysteine was induced (D21) because the level of paracortex and sulphur content decreased markedly after the infusion was stopped (PI) (Table 4.5).

The increase in paracortex % induced by infusion, is demonstrated clearly in Figure 4.2 for one of the Mediumwool Merino sheep. This animal was a strong responder but reflects the significant effect of cysteine infusion on fibre response.



a. Before Infusion



b. 21day Infusion

Figure 4.2 The effect of cysteine infusion on the increase in paracortex ratio (cells were in dark blue stain) in one of the experimental sheep (no.71594, Mediumwool Merino with a low fibre sulphur content) before infusion and 21day infusion (D21)

Gene expression was also significantly affected by cysteine infusion. Infusion increased KAP4.2 (65%), KAP2.12 (55%) m-RNA levels significantly ($p<0.01$), and K2.9 (35%) and trichohyalin (55%) ($p<0.05$) (Table 4.5).

4.5. The Responsiveness of GWG, FD, Paracortex, Sulfur and Keratin Gene Expression in Different Strains and Sulphur Groups to the Cysteine Infusion

4.5.1. Effect of strains on the responsiveness

Table 4.6 show the effect of cysteine infusion on all fibre parameters measured for two different of strain of Merinos. The results show that in Finewool Merinos, there was no significant effect of the infusion on greasy wool growth rate, although the P value was close to significance level ($p=0.055$). In Mediumwool Merinos, however, the infusion increased wool growth significantly ($p<0.01$). Mediumwool Merino sheep were more responsive in wool growth rate to the infusion than Finewool Merinos (61% vs 21% increase; respectively in both strains) (Table 4.6) and this responsiveness was statistically different ($p<0.01$) as shown from the analysis of the interaction between strain and infusion on the growth rate (Table 4.2).

In contrast, there was no effect of infusion on FD in Finewool Merinos ($p>0.05$), but the infusion significantly increased (around 5%) the FD in Mediumwool sheep ($p<0.05$) (Table 4.6).

Both paracortex ratio and sulphur content increased markedly in both Finewool and Mediumwool Merinos ($p<0.01$). In Finewool Merinos, the increase in paracortex ratio and sulphur content was 61% and 18%, respectively ($p<0.01$) and the increase was greater in Mediumwool sheep (92% and 21% for both parameters, respectively). From the analysis of the interaction, the difference of responsiveness to the infusion between strains was not statistically significant (Table 4.2).

Table 4.6 Effect of cysteine infusion on fibre parameters for the Finewool and mediumwool Merinos averaged over sulphur group.

STRAIN	FIBRE PARAMETER	LOW CYSTEINE (BI)		HIGH CYSTEINE (D21)		%	Signifi-Cance
		Mean	Se	Mean	Se	Change	
Finewool	GWG (mg/day/ 100cm2)	152.2	11.7	184	12	21.1	n.s (P=0.055)
	FD (μm)	17.0	0.4	17.2	0.4	0.8	n.s
	Paracortex ratio (%)	21.3	0.9	34.4	0.9	61.4	***
	Sulphur Content (%)	2.8	0.04	3.3	0.04	17.7	***
	KAP4.2	16.3	2.8	22.0	2.8	34.9	n.s
	KAP2.12	8.7	1.3	11.0	1.3	26.4	n.s
	K2.9	2.6	0.5	3.0	0.5	15.1	n.s
	TRN	4.7	1.3	6.7	1.3	43.6	n.s
Medium wool	GWG (mg/day/ 100cm2)	151.9	12	245	12	61.2	***
	FD (μm)	20.6	0.4	21.7	0.4	5.3	*
	Paracortex ratio (%)	17.2	0.9	33.0	0.9	91.9	***
	Sulphur Content (%)	2.5	0.04	3.1	0.04	21.0	***
	KAP4.2	9.4	2.8	20.4	2.8	116.8	***
	KAP2.12	9.6	1.3	17.3	1.3	80.4	***
	K2.9	3.7	0.5	5.5	0.5	49.5	*
	TRN	4.8	1.3	8.0	1.3	66.7	n.s (P=0.08)

With regards keratin gene expression in Finewool Merinos with cysteine infusion did not significantly increase the m-RNA expression of the keratin genes measured ($p>0.05$). However, in Mediumwool sheep the expression of KAP4.2 and KAP2.12 ($p<0.01$) and K2.9 ($p<0.05$) genes increased markedly. Although the expression of TRN did not increase statistically, the P value was close to significance ($p=0.08$) (Table 4.6). However, again based on the interaction analysis, the responsiveness between the two

strains on gene expression, was similar except for KAP2.12 ($p<0.05$) (Table 4.2).

The increase of expression in KAP4.2 and other keratin gene used, from Northern Blot results is shown in Figure 4.3.

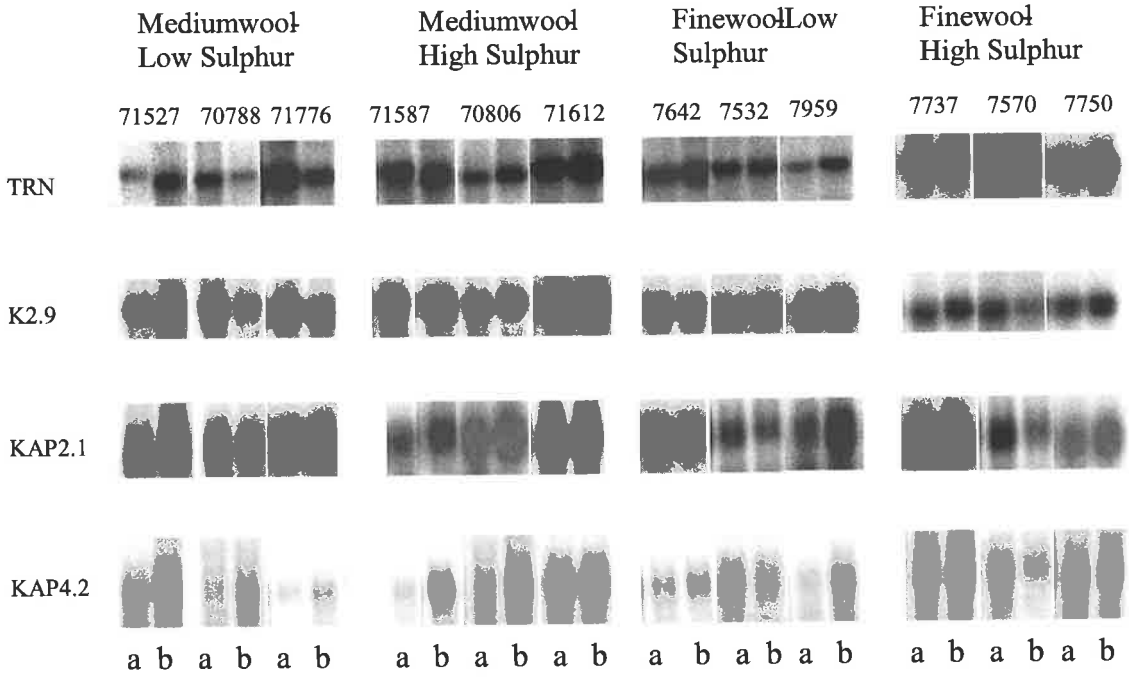


Figure 4.3 Scanned gels showing increases in changes mRNA levels induced by cysteine infusion in groups of 3 sheep selected from the 4 phenotypic extremes (a = before infusion and b = 21 days after infusion).

4.5.2. Effect of wool sulphur group on the responsiveness

The effect of cysteine infusion on thel fibre parameters measured for Merino wool sulphur groupings are shown in Table 4.7. The results show that in sheep with high sulphur wool, there was no effect of cysteine infusion on greasy wool growth, although the difference did approach significance ($p=0.06$). In the Low Sulphur group, however, the infusion increased wool growth significantly ($p<0.01$). The results also show that Low Sulphur sheep were more responsive in wool growth rate to the infusion than High Sulphur Merinos (56% vs 23% increase; respectively in both strains)(Table 4.7). The different responsiveness was statistically significant ($p<0.05$)(Table 4.2).

Table 4.7 Effect of cysteine infusion on fibre parameters for the High and Low sulphur group in Merinos averaged over strain of sheep

Sulphur Group	FIBRE PARAMETER	LOW CYSTEINE (BI)		HIGH CYSTEINE (D21)		% Change	Signifi-Cance
		Mean	Se	Mean	Se		
High Sulphur	GWG (mg/day/ 100cm2)	138	12	171	12	23.5	n.s (P=0.06)
	FD (µm)	18.2	0.4	18.2	0.4	0.3	n.s
	Paracortex ratio (%)	21.6	0.9	34.8	0.9	61.2	***
	Sulphur Content (%)	2.8	0.04	3.2	0.04	14.0	***
	KAP4.2	13.7	2.9	19.0	2.9	39.0	n.s
	KAP2.12	9.8	1.4	11.6	1.4	18.4	n.s
	K2.9	3.3	0.5	3.9	0.5	18.9	n.s
	TRN	5.4	1.3	7.9	1.3	46.8	n.s
Low Sulphur	GWG (mg/day/ 100cm2)	166	11	258	11	56.0	***
	FD (µm)	19.4	0.4	20.6	0.4	6.1	*
	Paracortex ratio (%)	17.0	0.9	32.6	0.9	92.6	***
	Sulphur Content (%)	2.5	0.04	3.1	0.04	25.1	***
	KAP4.2	12.0	2.7	23.4	2.7	94.3	***
	KAP2.12	8.5	1.2	16.7	1.2	96.6	***
	K2.9	3.0	0.5	4.5	0.5	53.3	*
	TRN	4.1	1.2	6.8	1.2	66.5	n.s

The infusion did not affect the FD in the High Sulphur group ($p>0.05$), but it increased the FD in the Low Sulphur sheep ($p<0.05$) (Table 4.7). Statistically the susceptibility was not statistically different between the sulphur groups ($p>0.05$) (Table 4.2).

Cysteine infusion did increase paracortex ratio and sulphur concentration in both High and Low sulphur groups of sheep ($p<0.01$). The responsiveness of paracortex and

sulphur changes was greater in the low-sulphur group than in the high sulphur group (93% vs 61% for paracortex increase ($p<0.05$); and 25.1 vs 14% for the increase in wool sulphur content ($p<0.01$), respectively in low and high-sulphur sheep) (Table 4.2 and 4.7).

In terms of expression of keratin genes measured, cysteine infusion did not significantly increase the m-RNA expression of all keratin genes ($p>0.05$) in High Sulphur sheep. However, in the Low sulphur group, the expression of KAP4.2 and KAP2.12 increased markedly ($p<0.01$) and K2.9 ($p<0.05$), but the expression of TRN did not change ($p>0.05$) (Table 4.7). In relation to difference in responsiveness, of the keratin and keratin related genes quantified, only the high sulphur IFAP-coding gene, KAP2.12, differed significantly between the sulphur groups ($p<0.05$) (Table 4.2).

4.5.3. Effect of strain and wool sulphur group interaction on the responsiveness

Table 4.8 shows the results of effect of cysteine infusion on fibre parameters for each group of strain and wool sulphur group interaction. The results show that in general, based on the percentage of the change of all fibre parameters as a result of infusion, the Finewool Merinos and High sulphur sheep were less responsive to the infusion than Mediumwool sheep and Low sulphur group. The range of increase in all fibre parameters in each group of sheep (strain x sulphur interaction) were -12.8–53%, 4.6–71.3%, 3.1-91.3% and 7.3-145%, respectively in Finewool-Highsulphur Finewool-Lowsulphur, Mediumwool-Highsulphur and Mediumwool-Lowsulphur sheep groups.

However, from the analysis of the interaction between strain x sulphur and S-groups x infusion, we can conclude that the responsiveness between 4 groups of sheep (Finewool-High Sulphur, Finewool-Low Sulphur, Mediumwool-High Sulphur and Mediumwool-Low Sulphur) to cysteine infusion on all fibre parameters was not significantly different ($p>0.05$)(table 4.2).

Table 4.8 Effect of cysteine infusion on fibre parameters for the interaction between Strain and Sulphur group in Merino sheep

Strainx Sulphur	FIBRE PARAMETER	LOW CYSTEINE (BI)		HIGH CYSTEINE (D21		% Change	Signifi-Cance
		Mean	se	Mean	se		
Fine-High	GWG (mg/day/ 100cm2)	140	16	148	16	5.7	n.s
	FD (μm)	16.6	0.5	16.1	0.5	-3.1	n.s
	Paracortex ratio (%)	23.2	1.3	35.5	1.3	53.0	***
	Sulphur Content (%)	2.9	0.1	3.3	0.1	12.0	***
	KAP4.2	17.4	3.9	19.1	3.9	9.3	n.s
	KAP2.12	9.2	1.8	8.0	1.8	-12.8	n.s
	K2.9	2.7	0.7	2.6	0.7	-4.7	n.s
	TRN	5.1	1.8	6.7	1.8	30.7	n.s
Fine-Low	GWG (mg/day/ 100cm2)	164	16	221	16	34.2	*
	FD (μm)	17.4	0.5	18.2	0.5	4.6	n.s
	Paracortex ratio (%)	19.5	1.3	33.4	1.3	71.3	***
	Sulphur Content (%)	2.6	0.1	3.2	0.1	24.2	***
	KAP4.2	15.1	3.9	24.9	3.9	64.3	n.s (P=0.09)
	KAP2.12	8.2	1.8	13.9	1.8	70.5	*
	K2.9	2.5	0.7	3.4	0.7	36.2	n.s
	TRN	4.2	1.8	6.7	1.8	59.1	n.s
Mediumx High	GWG (mg/day/ 100cm2)	137	18	194	18	41.7	*
	FD (μm)	19.7	0.6	20.3	0.6	3.1	n.s
	Paracortex ratio (%)	20.0	1.4	34.1	1.4	70.6	***
	Sulphur Content (%)	2.6	0.1	3.1	0.1	16.7	***
	KAP4.2	9.9	4.3	18.9	4.3	91.3	n.s
	KAP2.12	10.4	2.0	15.2	2.0	46.0	n.s (P=0.09)
	K2.9	3.9	0.8	5.3	0.8	35.1	n.s
	TRN	5.7	2.0	9.2	2.0	61.3	n.s
Mediumx Low	GWG (mg/day/ 100cm2)	167	15	296	15	77.3	***
	FD (μm)	21.5	0.5	23.1	0.5	7.3	*
	Paracortex ratio (%)	14.4	1.2	31.9	1.2	121.4	***
	Sulphur Content (%)	2.4	0.1	3.0	0.1	25.1	***
	KAP4.2	8.9	3.6	21.9	3.6	145.0	*
	KAP2.12	8.8	1.7	19.5	1.7	120.8	***
	K2.9	3.4	0.6	5.7	0.6	66.0	*
	TRN	3.9	1.7	6.9	1.7	75.0	n.s

5. Discussion

Previous studies have shown that individual sheep differ in the responsiveness of wool growth and wool traits such as fibre diameter, fibre length and staple strength to the prevailing environmental conditions. This variance in propensity to change extends to strains of Merino and to breeds of sheep. In general, more productive sheep (i.e. those with higher clean fleece weight and higher fibre diameter) are more responsive to the environment (Jackson and Downes, 1979; Williamson *et al.,* 1995; Ansari-Renani and Hynd, 1996; Miller *et al.,* 1998). Of the environmental factors, nutrition predominates in its effect on fleece phenotype. In particular it is the supply of sulphur amino acids cysteine and methionine that dictates the rate of fibre production and its composition (Reis and Schinkel, 1963; Williams *et al.,* 1972; Reis *et al.,* 1973; Fratini *et al.,* 1994; Sherlock *et al.,* 2001). What is not clear is why fleece genotypes differ in response to nutrient supply, although there is some suggestion that differential expression of keratin genes may be a significant factor. We hypothesized that differential wool growth (growth rate, fibre diameter) responses to nutrient supply are generated by differential keratin gene expression and resultant change in sulphur enrichment of wool. Low FD sheep are proposed to have more paracortical cells which contain more high/ultra high sulphur genes, resulting in wool of higher sulphur content. This is in turn would mean that the low FD sheep are more likely to switch on the high sulphur genes resulting in less cysteine available to increase wool growth and FD. In other words the Finewool Merinos respond to elevated cysteine supply by switching on high and ultrahigh sulphur genes producing wool of higher sulphur content rather than producing more wool of lower sulphur content. The experiment described in this chapter tested this possibility by examining the responsiveness of extreme fleece phenotypes to cysteine infusion.

5.1. Effect of Cysteine Infusion on Fibre Parameters

While it was intended that all wool growth data would be recorded as clean scoured wool, a technical problem resulted in some fibre loss during scouring. This occurred as a result of the small lengths of wool fibre obtained by clipping over short periods of time. Some short fibres passed through the muslin bag used to hold the wool during scouring. The ideal scouring procedure for small quantities of wool sample may be as described in Chapter 3 (Section 3.2.2). The wool is washed in tetrachloroethylene and dried with a snippet blaster. Although it was assumed that there was a positive and constant relationship between GWG and CFW, recent work (Sherlock et al, 2001) reported that the increase in wool growth measured as greasy fleece weight was not significantly different between treatments (high and low feed quality), but that the treatments significantly affected the response of CFW to cysteine infusion. The authors concluded that cysteine infusion significantly increased clean fibre yield relative to greasy materials (non-fibre matter). Therefore, results discussed in this section only relate to GWG. Given that FD was the trait of greatest interest, this unfortunate occurrence is of minor consequence in relation to the hypothesis tested.

The results of the current experiment show that cysteine infusion significantly increased GWG but produced only a small increase in fibre diameter. The cysteine infusion, as postulated, also increased paracortex ratio, wool sulphur content and expression of all keratin genes investigated. The level of increase in fibre diameter, paracortex, wool sulphur and KAP4.2 gene expression, however, was lower than that found in previous work (Fratini *et al.*, 1994). In this work the increases in fibre diameter 1.3-2.9 μm, paracortex percentage 123%, wool sulphur content 29% and KAP4.2 (77%), whereas in present work, they were 0.2-1.0 μm, 75%, 19.3% and 65%, respectively for the fibre parameters (Table 4.5).

The lower response found in the present work might be due to a better quality basal ration given to the animals. Fratini *et al.* (1994) used a basal diet containing 78 g crude protein and 7.4 MJ/kg of ME, whereas in the present work, a higher baseline plane of nutrition was used (95 g crude protein and 8.4 MJ of ME). The lower nutritional status of animals in Fratini's experiment would have allowed a greater response to the cysteine infusion for fibre growth. Alternatively, the different breeds of sheep used in the two experiments may be a factor contributing to the different responses. Corriedale and Merino cross breeds have larger follicles (Fratini *et al.,* 1994), and therefore a larger response to the cysteine infusion than Merinos used in the present work. Higher producing genotypes such as these have also been found to possess a greater blood supply (Hocking Edwards and Hynd, 1992) hence a greater supply of nutrients to the follicle for fibre growth.

Post infusion measurement was conducted in this experiment to ensure that cysteine infusion does affect the fibre parameters. All of the parameters decreased again at 21 days after the infusion was stopped, except keratin gene expression which was not recorded in this period. The level of all fibre parameters post infusion was higher than prior to infusion (ranged between 1.8 and 36.7% increase), but the level at post infusion was significantly lower than after 21 days cysteine infusion treatment. This shows clearly that cysteine infusion treatment does increase the fibre parameters and decrease when the infusion was stopped. This results are in accord to previous findings that wool sulphur content, KAP4 and 5 as well as proportion of paracortex decreased significantly when cysteine infusion was terminated (Fratini, *et al.*, 1994).

Similarly, in terms of study on keratin gene expression, to allow valid treatment effect of cysteine infusion on the expression of high or ultra high sulphur encoding genes (KAP2.12 and KAP4.2), we used the control genes, i.e K2.9 (Powell, 1996) and

Trichohyalin (TRN) (Bawden, *et al.*, 1998) that do not encode high and ultra high sulphur proteins . The results are as expected that there was nosignificant difference in the increase of the expression of K2.9 and trichohyalin genes in response to the cysteine infusion in both strains. The smaller increase in the expression of K2.9 and TRN in the present results, is very likely, as with the supply of amino acids for cell growth in the bulbs that enhance the cell differentiation towards the fibre as well as IRS where the two genes are normally found.

From the results in post infusion and 'control' keratin genes as described, we can then be sure that the changes of fibre parameters in response to cysteine infusion, is related to the treatment effect. Following discussion investigates on how and why the interactions between the change in fibre parameters (wool growth, fibre diameter, paracortical cells, wool sulphur content and the expression of ultrahigh sulphur encoding gene) resulted from the cysteine infusion.

The overarching hypothesis was that in response to cysteine infusion, sheep which changed very little wool growth or fibre diameter would increase the UHS gene expression in wool fibre to probably enrich in paracortex cells. Therefore, we would get the same growth rate and diameter, but richer in wool sulphur content as KAP4.2 increase. In contrast, sheep that change a lot in wool growth or fibre diameter, would not change much in KAP4.2. They would respond to the cysteine input for wool growth or increasing fibre diameter, not for the enrichment of fibre with sulphur which is expressed by the high KAP4.2 (Figure 4.4).

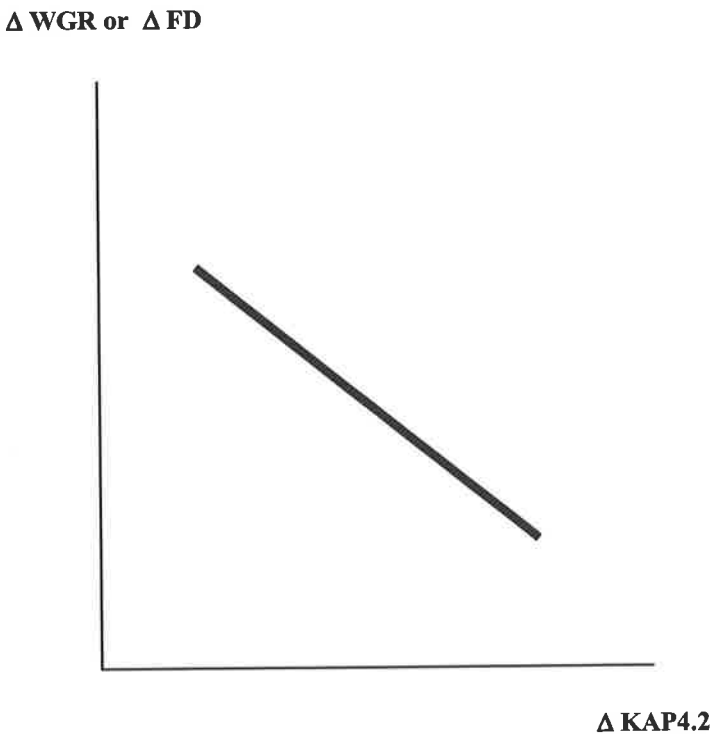
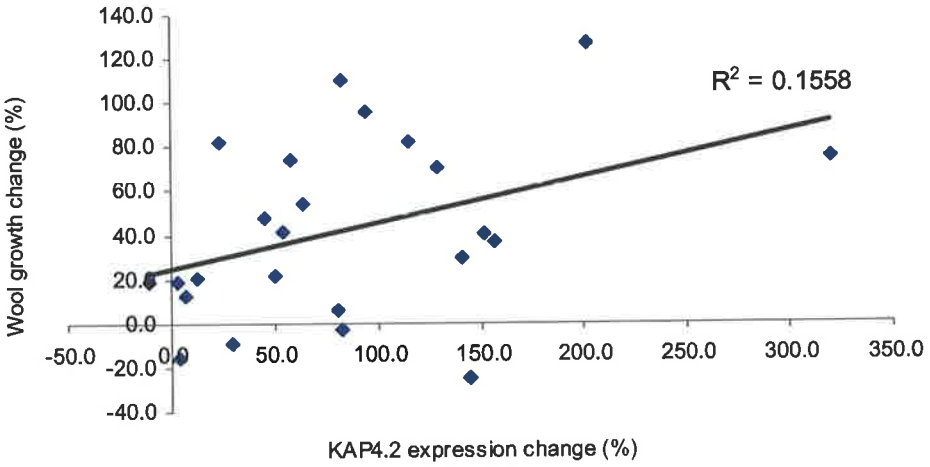
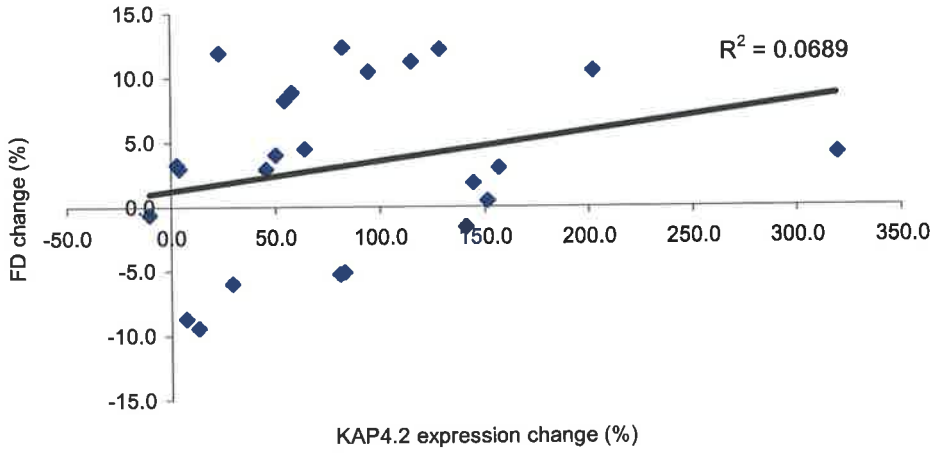


Figure 4.4 Relationship between the increase in wool growth rate (WGR) or fibre diameter (FD) and increase in KAP4.2 expression, as proposed in the hypothesis

The present results, however, did not confirm the negative relationship (Figure 4.5 a and b). In fact, sheep that changed very little in wool growth rate or fibre diameter also changed very little in UHS gene expression in the wool. While sheep that changed a lot in WGR or FD also changed a lot in UHS content. This may indicate that sheep utilised excess of cysteine for growth, FD increase and not for enrichment of wool sulphur content. This probably means that sheep utilise excess of cysteine for wool growth or increase fibre diameter as well as to enrich paracortical cells in the wool fibre with sulphur.



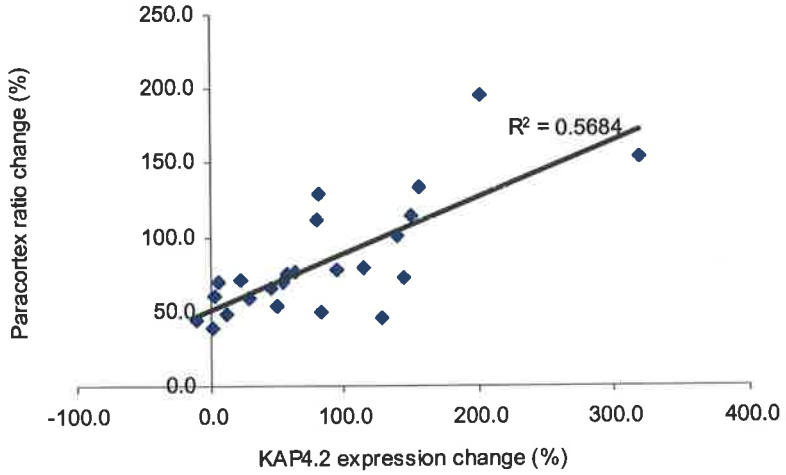
(a)



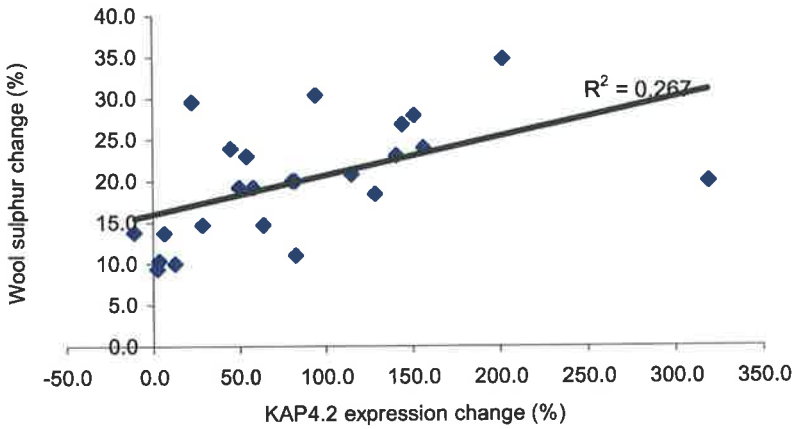
(b)

Figure 4.5 Relationship between change in wool growth rate (a) or fibre diameter (b) with KAP4.2 expression

The positive relationship between paracortex`ratio, wool sulphur content and KAP4.2 expression was in accord with the present results. Sheep with a large change in paracortex ratio or sulphur content would express more UHS gene and likewise sheep with a little change in paracortex ratio or sulphur content would express less UHS gene in the wool (Figure 4.6 a and b).



(a)



(b)

Figure 4.6 Relationship between paracortex ratio change (a) or wool sulphur content change (b) and KAP4.2 expression change

Wool growth increases in response to a nutrition supplementation, are usually accompanied by an increase in fibre diameter. Previous studies show that cysteine supplementation increases wool growth as well as fibre diameter (Reis *et al.*, 1973; Fratini *et al.*, 1994). This was not the case in the present work in which the wool growth increase due to cysteine infusion was not responded by an increase in fibre diameter. This is also

shown from lower regression value in relationship between KAP4.2 increase and FD than with wool growth rate (r^2 = 0.0689 vs 0.1558, respectively). This is similar to the previous report that 3 month continuous cysteine infusion increased wool growth, but not the fibre diameter (Sherlock *et al.*, 2001).

The mechanism to explain the results needs to be explored. There are probably two possibilities why cysteine infusion increased wool growth rate but with very little effect on fibre diameter, i.e. (i) increase in follicle/fibre density, or (ii) growth in fibre length.

To test the first possibility whether follicle density affect the wool growth, we calculate by using a formula to calculate wool growth introduced by Turner (1958) as follows:

$$\mathbf{W = S \times N \times A \times L \times K}$$

where W = whole body wool growth per sheep; S = wool growing surface area; N = follicle density; A = mean fibre cross-sectional area or mean FD; L = fibre length and K = a constant which reflects the specific gravity of wool (Q) = 1.4 (Liu *et al.*, 1994). While S and total follicle number (N) are probably constants except in extreme environmental conditions that cause, for example, fibre shedding (no evidence present in this work), the possibility that the specific gravity of wool (K) is still a constant with an increasing proportion of paracortex ratio needs examination. The paracortex ratio before infusion and after 21 days infusion were 0.22 and 0.32, respectively, making the orthocortex ratio 0.78 and 0.68, respectively. By multiplying the ratio of each cell type with its cell density per 100 nm^2 (1.22 for paracortex and 1.53 for orthocortex; Thompson, 1998) and summing them, we then obtain the total density of wool fibre. The density thus determined before and after 21 day infusion was 1.46 and 1.43, respectively.

This suggests that the infusion did not significantly change fibre density and since the FD was also not altered in this current work, it is concluded that the only factor contributing for the increase in wool growth was fibre length.

The following mechanism is proposed to explain the possible growth more in fibre length than in diameter. Figure 4.7 shows the proposed mechanism.

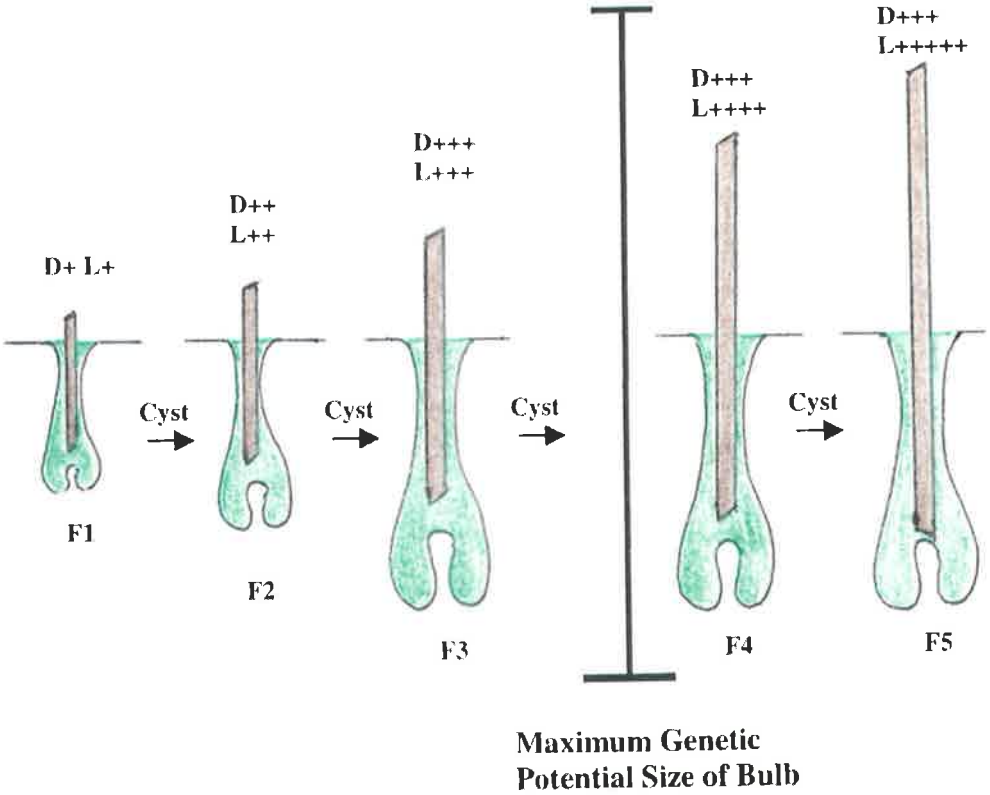


Figure 4.7 A schematic proposed mechanism of follicle growth due to cysteine infusion in relation to L/D ratio change. Before reacing maximum genetic line, the wool fibre diameter and length grow in a constant ratio (shown by the number of + at D and L). Afterwards, the growth in length keeps continued, while in diameter the fibre remains steady.

The nutrient supply such as cysteine infusion, in common case would be utilized to produce wool fibre through the increase in fibre length as well as fibre diameter in a constant ratio. This was shown with positive correlation between follicle bulb and fibre

diameter (Hynd, 1994[a]). This constant relationship between length and diameter of fibre would continue until the follicle bulb has reached the maximum genetic potential to increase, therefore also to a maximum growth potential of fibre diameter. In this situation, thc amino acid supplementation would increase the amount of keratin protein cell filling during keratinisation. The increased filling of cells would enhance the cortex cells to lengthen, hence cortical cell length increased making higher length growth rate (Figure 4.7).

This finding is 'an exciting breakthrough' in the wool industry. While it has been generally believed that selection to increase wool growth is also usually accompanied by increased fibre diameter, which is clearly unwanted, the present results show we may be possible to select sheep to be higher in wool production without making coarser wool.

5.2 The Responsiveness of Different Merino Strain and Wool Sulphur Group in Fibre Parameters in Response to Cysteine Infusion

The present work found that Finewool sheep were less responsive to cysteine infusion than Mediumwool sheep in terms of their wool growth response. This finding may support previous results that Finewool Merinos are less susceptible to the effects of the environment on follicle shutdown (Ansari-Renani, 1996).

However, the responsiveness was not different between the strain in paracortex and wool sulphur change. This is in contrast to previous work (Fratini *et al.,* 1994) who showed that there were significant differences in the responsiveness between breed of sheep used. Corriedale, a coarser breed, were more responsive in changing paracortex and wool sulphur than Merino-cross, a finer sheep breed (219% vs 27% of paracortex and 40% vs 18% of wool sulphur content, respectively for both sheep breed) as a result of cysteine infusion. Different genotypes in the categories of sheep which are referred to as

'Merino strain' in the present work and 'sheep breed' in previous studies, are now discussed. Sheep from different breeds may have more extreme differences, in this case in wool growth and wool quality, than in sheep that come from different strains. Generally, breeds may have more genetic variation in most production traits, including wool traits, than strains, because breeds probably have more extreme differences in their genotypes, while a strain is obtained from the selection based on certain traits, within a breed.

In relation to different responsiveness in terms of changing keratin gene expression, the higher response on KAP2.12 expression in Mediumwool Merinos, is as postulated. Unexpectedly, the response between sheep strain on KAP4.2 was not different. This may relate to the fact that differences in sulphur content between strains (0.3% difference) were not sufficient to produce a difference in the expression of the KAP4.2 gene. In contrast the different sulphur content between strains in previous work was 0.6% (Fratini *et al.,* 1994) and produced a significant increase in KAP4.2 expression.

Sheep with low wool sulphur content were more responsive in wool growth, paracortex ratio, wool sulphur content and KAP2.12 expression than those with high sulphur. This result indicates that sheep with lower wool sulphur content were able to increase the total wool sulphur output in response to cysteine. These sheep had 1.24 fold the wool sulphur content in response to cysteine infusion compared with a 1.1 fold response in high wool sulphur sheep. Furthermore, the total wool sulphur output estimated from wool growth and wool sulphur concentration (%), increased by 3.9 mg/day/100 cm2 and 1.7 mg/day/100cm2, respectively in sheep with low and high sulphur content or 2.3 times of the difference between the wool sulphur groups. These present results are in agreement with the work of Fratini *et al.* (1994), which showed a higher responsiveness to cysteine infusion in sheep with lower wool sulphur content (Corriedale) compared to sheep with higher wool sulphur content (Merino cross).

The question raised now why Mediumwool Merinos or sheep with lower sulphur content were more responsive than Finewool sheep or High sulphur group. The following mechanism is speculated to answer the question.

Suppose before infusion, the cysteine available for follicle growth is 1 g/d in the plasma and the capacity of blood vessel to the skin is 10%, so the cysteine transported to the follicle is 1 g/day x 10% = 0.1 g/day . When 4 g/day of cysteine is infused, the amount of cysteine in the follicles increased become 4 g/day x 10% = 0.4 g/day, or 4 times increase. In comparison to Mediumwool sheep with a higher capacity of blood flow (Hocking-Edwards and Hynd, 1992), let say, 20%, so the amount of cysteine transported to the follicles before and after infusion is 0.2 g/day and 0.8 g/day, respectively. The increase of cysteine amount in the follicles of Mediumwool in response to the infusionis is also 4 times, the same with Finewool Merinos. This clearly shows that the responseness is the same although the capacity of blood vessel is bigger in Mediumwool Merinos than in Finewool sheep.

It is then proposed that instead of larger capacity of blood flow, they might have bigger capacity to turn on the cysteine uptake, when there is a sudden change of environment such as the cysteine infusion. In relation to the simulation, for instance, the capacity of cysteine uptake of Mediumwool Merinos is 40% and Finewool is still 10%, so the cysteine amount in the follicles after the infusion become 4 g/day x 40% = 1.6 g/day, or increased in 8 times compared to before infusion (0.2 g/day) as calculated previously (Figure 4.12.c).

It might imply that Mediumwool sheep is possible to have the capacity to turn on mRNA to have higher affinity and/or higher capacity amino acid transporter to an increased nutritional level. Amino acid transporter is a diverse group of membrane proteins that facilitate the movement of water soluble molecules, in this case amino acids,

through the lipid bilayer of biological membranes (Wolfersberger, 1994). Through the permeability of the membrane, the essential molecules enter the cell by simple diffusion, while metabolic intermediates remain in the cell and waste compounds can leave the cell (Darnell *et al.*, 1990).

This speculation support previous postulation (Nattrass, 2000) that a cysteine transport gene might play a role in the binding ability. In the case of the current study, Mediumwool Merinos and the low sulphur group might have more efficient cysteine transport genes due to a possible mutation within a selection process for other traits. Cysteine transport genes may increase cysteine transport system, by increasing the binding ability of cysteine or by increasing the release of cysteine into the cells. Further investigations needs to be undertaken to study the mechanism of cysteine transport system in the wool follicle, with the aim to enable further genetic manipulation to increase wool quality, in this case, staple strength.

6. Conclusion

The main emphasis of this experiment was on genetic effects on the responsiveness, in terms of wool properties, to environmental changes in Merino sheep. It was postulated that Finewool and Mediumwool Merinos and those differing in initial wool sulphur content differentially change their wool properties in response to a cysteine infusion. The present results show that a cysteine infusion increased most of the traits measured (greasy wool growth, paracortex ratio, wool sulphur content and the expression of keratin genes), but surprisingly, not the FD which did not change consistently within strains and sulphur groups.

These results also showed that Finewool Merinos were less responsive in their increase in greasy wool growth and KAP2.12 expression than Mediumwool sheep, but

that the strains had similar responsiveness in other traits measured. With respect to the different sulphur groups, low sulphur sheep were more responsive to the cysteine infusion in terms of an increase in wool growth, paracortex ratio, wool sulphur content and KAP2.12 gene expression. It can therefore be concluded that different responsiveness of some wool traits to environmental changes between strains and sulphur groups may be genetically controlled. This result could be important as a breeding consideration in selection programs.

In contrast to most studies of Genotype x Environment interaction in which relative changes in phenotypic traits are quantified, the present study investigated the interaction at the level of gene expression and cell type. This approach is a powerful means of unraveling the mysteries of G x E interactions and could be applied to other animal industries. By postulating specific pathways are involved in the response of different genotypes to environmental change, one can then examine the relative responses of the candidate genes and even proteins. This then opens the possibility of manipulating genes in a beneficial way by knockout, antisense methods, gene insertion or directly manipulating the biochemical pathways. Alternatively one might determine whether polymorphisms exist in candidate genes, and thereby identify potential genetic markers to improve animal performance.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

There are several impediments to improving wool quality in general and fibre diameter in particular. Some are sociological in nature, such as failure to adopt genetic and reproductive technologies. Others relate to biological impediments, for example, variation in fibre diameter, a key determinant of staple strength, is induced by a combination of the environment and genetics. In Mediterranean climates, summer and autumn are seasons in which there is poor availability of feed. Merino sheep tend to be affected more by the nutritional changes during the year than by photoperiod. FD variation along the staple and follicle shutdown have been reported to occur as a result of variation in nutrition, and these biological changes can have a negative impact on staple strength causing tender wool due to the point of break occurring in the middle of the wool staple. Tender wool is a serious matter in the Australian wool industry, as staple strength is the second most important determinant of wool price after fibre diameter and can contribute up to 21% of wool price paid at auction.

While there are some management practices that can reduce or eliminate fibre diameter variation and its consequences (e.g. supplementary feeding, shearing at the point of minimum diameter or autumn shearing), a cheaper, more effective and permanent solution would be to select and breed sheep that are resistant to diameter change.

The present research was designed to study genetic factors that control the susceptibility or responsiveness of Merino sheep in the stability of wool traits in the presence of environmental variation. The issues of follicle shutdown, fibre diameter along the staple, wool composition (paracortex ratio and fibre sulphur content) and wool keratin gene expressions were investigated in this study.

It was initially hypothesised in experiment 1 (Chapter 2), that sheep with different genetic backgrounds have different susceptibility to follicle shutdown (FS) as a response to nutritional changes in the paddock. Investigation on FS is a significant study as this phenomenon is estimated to cause major loss to the wool industry due to inactive follicles and fibre loss, causing reduction in wool production. With the use of different sires in this experiment to investigate genetic factors within Medium-wool South Australian Merino sheep in the Rosedale flock it was found that there was no sire effect on FS incidence. The non-significant effect, however, must be tempered, as the incidence of FS in this experiment was low. A higher frequency of shutdown follicles as a result of increased summer impact, may have allowed greater expression of genetic differences.

Further investigation of the role of genetic factors in determining susceptibility to follicle shutdown in Merinos is warranted. If the hypothesis is true, sheep with finer fibre diameter would be less susceptible to the environmental changes than coarser sheep. By selecting this type of sheep, we can obtain three advantages (i) the selection line may have wool with finer fibre diameter, which has greater value, (ii) the flock of sheep selected may have stronger staple strength, as FS has a strong relationship with fibre diameter at point of break along the staple ($r^2 = 0.45$) (Thompson, 1998), and (iii) as a result of less shutdown follicles and possible fibre loss, the selected sheep flock can produce more wool.

In summary, the results obtained might have implications for the wool industry to generate higher wool production, finer fibre diameter and more sound wool. From the results, we might select a high-producing sheep (through the increase in fibre length) whilst maintaining low fibre diameter, and considering the possible biological mechanisms, the selection can be based on follicle characteristics including smaller bulb diameter, bulb area

and the length of cortical cells. However, further study to elucidate the mechanism of FS needs to be done before this approach is adopted.

The work on abnormality of follicles (follicle shutdown) needs to be continued in further studies that are less limited in terms of time and resources. In the present work, in experiment 2 (Chapter 3), the investigation focussed on susceptibility to environmental changes and the role of the genetic x environment interaction in controlling the trait of FS, using the parameters of fibre diameter along the staple in the experimental design. By profiling the wool staple by taking 2 mm segments at ten equidistant points, fibre diameter variation along the staple was proposed to be representative of wool samples of 12 months of wool growth.

Fibre diameter variation along the staple represented all annual conditions with maximum FD occurring in September/October and minimum diameter mostly present in May-June when the break of season usually occurs. This is similar to when FS occurred, as discussed previously (Hynd *et al.,* 1997). It can decrease SS for the wool shorn in spring, occurs at the point of minimum FD at the break of the season.

As previously discussed, instead of a management approach (such as shearing time, feed supplementation etc), investigation of genetic control on this trait might be more sustainable for solving the SS problem. While there was a significant effect of stud on the trait of FD variation along the staple, the susceptibility to environmental changes on FD was not significantly different between studs (P=0.065). However, the P values were close to significance, and therefore, may indicate a tendency for different studs to vary in their susceptibility to seasonal changes in FD. This could be an important result that can contribute to selection for this purpose based on certain studs. Sheep flocks with good

adaptability to environmental changes would have stronger staple strength, an important wool trait in wool industry.

This result, however, was not supported by the results on sire effects, that the susceptibility to FD change along the staple was not significantly different for sires (P=0.868). Also, the low heritability of FD variation along the staple (h^2 = 0.17), indicates that this trait is mostly being controlled largely by environment, especially nutrition. However, phenotypic correlation between FD variation along the staple and staple strength was quite high (r=-0.43), similar to what was found with FD variation in mid-side samples (r=-0.44). This may indicate that recommendation can be proposed for indirect selection criterion of SS based on this trait (ACVFD), besides the use of midside CVFD, despite there not being a genetic correlation, and that phenotypic correlations can be used as predictors of genetic correlation. In addition to this, the use of FDCV along the staple (ACVFD) can be recommended as a tool for managing the nutritional status (stocking rate) of wool-producing sheep.

Investigation of the role of genes in susceptibility of Merino sheep to the environmental changes was also a significant concern in experiment 3 (Chapter 4) which used more distinct genotypes such as Fine-wool vs Medium-wool Merinos, selected for divergence in wool sulphur content. Artificial nutrition treatment through cysteine infusion was also applied to mimic the environmental changes we are concerned with in this area of study, i.e. feed availabilit, climate changes occurring during the break of season.

The most significant findings in relation to the application in the wool industry was the cysteine infusion increased greasy wool growth, but it did not increase fibre diameter. It can be a breakthrough to what has been generally believed that by selecting sheep to increase wool growth would increase fibre diameter, which was not actually wanted. This findings

might have a significant contribution to Australian Merino breeding program, however further studies are needed to test the findings in a bigger number of sheep, at different environment.

Other significant results from this work were that Mediumwool Merinos and sheep with low sulphur content were genetically more responsive to the environmental changes (cysteine infusion) than high-sulphur sheep. Mediumwool sheep and lower wool sulphur content was also related to coarser FD, less proportion of paracortical cells in the follicles and less expression of KAP2.12 and KAP4.2, genes encoding ultra high sulphur and high sulphur proteins. Among the traits, it seems that wool sulphur content is more practical to measure for possible selection purposes than specific genes.

The issue of using wool sulphur content as a selection criterion has recently received greater attention from some scientists. As found in the present work, some showed that wool sulphur had an inverse association with high-producing sheep (Antram *et al.* 1991; Sun *et al.* 1991), implying selection based on low wool sulphur in lambs can be an approach to producing sheep with higher wool production. Others reported that this trait was also related to sheep immunity to disease (Williamson *et al.* 1995; Miller *et al.* 1998). It was hypothesised that the increased use of cysteine for wool production in high producing sheep might lead to a reduced availability of cysteine for other functions, for example the synthesis of glutathione required for active T-cell immunity.

From the overall discussion above, we can conclude that the main focus of the present research was basically on the resistance of wool traits to environmental changes that might refer to nutrition, disease, climate, or some management practice changes. The capability of animals to adapt to their environment becomes one of general concern in the sustainable animal production issue. There were some indications that the increase of certain growth

traits, such as daily gain, carcase percentage, leaner meat, feed efficiency etc, were unfortunately also followed by a reduction in some other important traits such as reproductive rate and resistance to disease.

In the case of sheep and in relation to the present work, the susceptibility of Merino sheep to environmental changes (pasture availability) might be also as a result of a long selection process in the breed to increase wool production and quality. In contrast, in more primitive breeds such as Shetland, Mouflon or Romney, wool growth is relatively uninfluenced by the variation of nutrition in the pasture, but predominantly controlled by photoperiod.

Significant change in animal performance can occurr when changing from an extensive management system to a more intensive system that undoubtedly increased animal production (e.g sheep, goat and cattle). However this new management system can change the indigenous animals, which originally have considerable advantages over exotic breeds, particularly in relation to disease resistance, heat tolerance and ability to utilise low-quality feed, to become 'weaker and spoiled animals'.

However, since we still need to improve animal production to 'feed the world', we might have to compromise in order to find a way to fulfil human and animal needs. We need to 'find the balance' to produce a sustainable animal production system for human requirements as well as animal welfare.

BIBLIOGRAPHY

Adams, N.R. and Briegel, J.R. (2002). Variation in fibre diameter along the wool staple in sire progeny groups. *Proceedings of Anim. Prod. Aust.* **24:** 5-8.

Allden, W.G. (1968). Undernutrition of the Merino sheep and its sequelae I. The growth and development of lambs following prolonged periods of nutritional stress. *Australian Journal of Agricultural Research,* **19**: 621-638

Allden, W. G. (1979). Feed intake, diet composition and wool growth. **In** : "Physiological and Environmental Limitation to Wool Growth (Eds. J. L. Black and P. J. Reis), pp. 68-69. The University of New England, N. S. W. Australia.

Allain, D., Thebanet, R. G., Rougeat, J, and Martinet, L. (1994). Biology of fibre growth in mammals producing fine fibre and fur in relation to control by day length: relation with other seasonal functions. *European fine-fibre network. Occasional Publication,* **2** : 23-39.

Ansari-Renani, H. R. (1996). Follicle shutdown and wool staple strength. Thesis (Phd). The Univ. of Adelaide, South Australia.

Ansari-Renani, H.R. and Hynd, P.I. (1996). Fine-wool sheep are less susceptible to cortisol-induced follicle shutdown than strong-wool sheep. *Proceedings of the Australian Society of Animal Production,* **21:** 483.

Antram, R.J., McCutcheon, S.N., Blair, H.T. and McClelland, L.A. (1991). Wool sulphur concentration and output in fleeceweight selected and control Romney rams. *Australian Journal of Agricultural Research,* **42:** 269-277.

Barach, J. L., and Rainard, L. W. (1950). Effect of crimp on fibre behaviour. Part II. Addition of crimp to wool fibres and its effects on fibre properties. *Textile Research Journal,* **20:** 308-316.

Basilico, C. and Moscatelli, D. (1992). The FGF family of growth factors and oncogenes. *Advanced Cancer Research,* **59:** 115-165.

Bawden, C.S., Powell, B.C., Walker, S.K. and Rogers, G.E. (1998). Expression of a wool intermidiate filament keratin transgene in sheep alters structure. *Transgenic Research,* **7**, 273-287.

Bellotti, B., Collins, B. and Moore, A. (1992). The Mediterranean environments. *Proceedings workshop on wool quality*, Western Australia.

Besier, R.B. (1992). The effects of nematode parasite of sheep on wool production and quality. **In** " Management for Wool Quality in Mediterranean Environments", (Eds. P.T. Doyle, J.A. Fortune and N.R. Adams), pp. 160-169. Dept of Agric., Perth, W.A.

Bigham, M.L., Sumner, R.M.W. and Dalton, D.C. (1978). Wool production of different breeds of mixed-age ewes on hill country. *New Zealand Journal of Agricultural Research*, **21**: 119-126

Bigham, M.L., Sumner, R.M.W., Hawker, H. and Fitzgerald, J.M. (1983) Fleece tenderness – a Review. *Proc. of the N.Z. Soc. of Anim. Prod.* **43:** 73-78.

Bottemley, G..A. (1979). Weather conditions and wool growth. **In** "Physiological and Environmental Limitations to Wool Growth", (Eds. J.L. Black and P.J. Reis), pp. 115-125. The University of New England Publishing Unit. N.S.W. Australia.

Bray, A.R., Smith, M.C., Woods, J.L. and Baird, D.B. (1992). Responses to selection for wool staple strength in Romney sheep. *Proc. of the NZ. Soc. of Anim. Prod.* **52:** 285-287.

Brown, T.H., Ford, G.E., Miller, D.W. and Beveridge, I. (1985). Effect of anthelmintic dosing and stocking rate on the productivity of weaner sheep in a Mediterranean climate environment. *Australian Journal of Agricultural Research,* 36: 845-855.

Butler, L. G. (1994). Factors affecting staple strength with particular reference to Australia. *Wool Technology and Sheep Breeding,* **42 (3) :** 213-230.

Butler, L. G and Head, G. M. (1993). Photo periodic rhythm of wool growth and its contribution to seasonal wool production by the merino, Polwarth and their reciprocal crosses in Southern Australia. *Aust. J. of Exp. Agric.,* **34 :** 311-317.

Butler, L. G. and Head, G. M. (1992). Seasonal wool growth and the staple strength of wool from nosie Tasmanian flocks. *Proc. Aust. Soc. Anim. Prod.,* **19 :** 128-130.

Chapman, R. E. and Bassett, J. M. (1970). The effects of prolonged administration of cortisol on the skin of sheep on different planes of nutrition. *J. of Endocrinology,* **48:** 649-663.

Chapman, R.E., Panaretto, B.A. and Frith, P.A. (1982). Changes in wool follicles of sheep following administration of dexamethasone trimethylacetae. *Journal of Cell Science,* **53:** 323-335.

Chapman, R.E. and Ward, K.A. (1979). Histological and biochemical features of the wool fibre and follicle. **In** "Physiological and Environmental Limitations to Wool Growth', (Eds. J.L. Black and P.J. Reis). pp. 193-208. University of New England, Armidale, NSW.

Cohen, S. and Elliot, G. A. (1963). The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. *Journal of investigative dermatology,* **40:** 1-5.

Corbett, J.L. (1979). Variation in wool growth with physiological state. **In** "Physiological and Environmental Limitations to Wool Growth", (Eds. J.L. Black and P.J. Reis), pp. 79-98. University of New England Publishing Unit: Armidale, NSW.

Corson, D.C., Martin, W. And Lee, J. (1998). The determination of sulphur in wool using near infrared relectance spectrometry. *Proc. of the NZ. Soc. of Animal Prod.*, **58:** 281-283.

Cottle, D., Hickson, J., Coelli, K., Casey, A. and Atkins, K. (1996). What is Central Test Sire Evaluation? **In:** "Merino Superior Sires; Central Test Sire Evaluation Results". The Australian Association of Stud Merino Breeders Limited. Australia.

Couchman, R.C., Hanson, P.J., Stott, K.J. and Vlastuin, C. (1992). Wool quality: implications for worsted processing, grower receipts and R&D. **In:** "Proc. of a National Workshop on Management for Wool Quality in Mediterranean Environments". (Eds: Doyle, P.T., Fortune, J.A. and Adams, N.R). pp: 1-23. Australia.

CSIRO (1991). Sirolan-Laserscan; Operator and technical manual, software version 2. CSIRO, Australia.

Darnell, J.E., Lodish, H. And Baltimore, D. (1990). Molecular cell Biology. Scientific American Books Inc. New York.

Dawes, K. (1975). Objective measurement of wool. 1st Edition. Pp. 49-56. New-South Wales university Press. Australia.

Dominik, S., Crook, B.J. and Kinghorn, B.P. (1999). Genotype x management interaction on wool production traits and body weight in Western Australian Merino sheep. *Proc. Assoc. Advmt. Anim. Breed. Genet.*, **13:** 98-101.

Donald, A.D. 1979. Effects of parasites and disease on wool growth. **In** " Physiological and Environmental Limitations to Wool Growth", (Eds. J.L. Black and P.J. Reis), pp. 99-114. University of New England Publishing Unit: Armidale, NSW.

Doney, J. M. 1966. Breed differences in response of wool growth to animal nutritional and climatic cycle. *Journal of Agric. Science, Cambridge*, **67:** 25-30.

Douglas, S.A.S (1988) TEAM project. Presentation of report on trials evaluating additional measurement 1981-1988. *Wool Tech. And Sheep Breeding*, **37:** 537-548.

Doyle, P. T., Peter, D. W., and Masters, D. G. (1994). Prevention of low staple strength by nutritional means. *Proceedings of the Australian Society of Animal Production*, **20:** 52-54.

Doyle, P. T., Plaisted, T. W. and Love, R. A. (1995). Supplementary feeding pattern and rate of live weight gain in winter-spring affect wool production of young merino sheep on the South Coast of Western Australia.

Du Cros, D. L., Isaac, S. K. and Moore, G. P. M. (1992). Localization of epidermal growth factor immuno reactivity in sheep skin during wool follicle development. *J. of Investigative Dermatology*, **98**: 109-115.

Earl, C.R., Stafford, J.E., Rowe, J.P. and Ross, R.A. (1994). The effect of stocking rate on fibre diameter, staple strength and wool weight in high and low fibre diameter wool sheep on clover based pastures. *Proc. Aust. Soc. Anim. Prod.*, **20:** 309-312.

Fayez, I., Marai, M. and Taha, A.H. (1976). Wool follicle characteristics in the Awassi fat-tailed sheep. *Acta Anat. Basell,* **96(1):** 55-69.

Ferguson, K. A. (1949). The effect of sympathectomy on wool growth. *Aust. J. of Scientific Research,* **B, 2** : 438-443.

Ferguson, K. A. (1972). The nutritional value of diets for wool growth. *Proceedings of the Australian Society of Animal Production,* **9** : 314-320.

Ferguson, M, Gloag, C., Behrendt, R. and Brien, F. (2002). Building lines of wool based on OFDA2000 fibre diameter results. *Proceedings Anim. Prod. Aust.,* **24:** 64.

Ferguson, K. A., Wallace, A. I. C and Lindner, H. R (1965). Hormonal regulation of wool growth. **In** : "Biology of the Skin and Hair Growth". Pp 655–677. (Eds : A. G. Lyne and B. F. Short). August and Robertson, Sydney.

Feughelman, M. (1982). The physical properties of α-keratin fibres. *Journal of the Society of Cosmetic Chemists,* **33:** 385-406.

Fratini, A., Powell, B.C., Hynd, P.I., Keough, R.A. and Rogers, G.E. (1994). Dietary cysteine regulates the levels of mRNAs encoding a family of cysteine-rich proteins of wool. *The Society for Investigative Dermatology,* **102(2):** 178-185.

Gardner, J. J., Doyle, P. T., Rowe, J. B., Hetherington, R., Spicer, P., McQuade, N, and Crowhurst, M. (1993). Supplementation of young Merino sheep grazing annual pastures with lupin, barley grain or silage. *Australian J. of Exp. Agric.* **33**: 4,403-409.

Gebbie, F. E., Forsythe, I. A. and Ardent, J (1994). Effects of melatonin, bromocriptine and altered light/temperature patterns on coat growth in dairy goats. *European fine fibre network, occasional publication,* **2 :** 97–105.

Gherardi, S. and Masters, D. (1996). Feeding for increased SS. **In** "Focus on Staple Strength", (Eds. Cooperative Research Centre for Premium Quality Wool), p. 98. Department of Primary Industries of S.A..

Gifford, D.R. and Ponzoni, R.W. (1993). The Turretfield Merino resource flock. *Proceedings of a National Workshop Turretfield Research Centre Rosedale, S.A.* (Eds. R. Ponzoni and D.R. Gifford). Pp. 15-20. Wool Research and Development Corporation and S.A. Research and Development Institute, S.A. Australia.

Gill, D.A. and Graham, N.P.H. (1939). Studies on fly-strike in merino sheep 3. The influence of fly-strike and conformation on body-weight and fleece-weight of Merino sheep at "Dungalear", New South Wales. *Journal of the Council for Scientific and Industrial Research,* **12**:319.

Gillespie, J.M. (1991). The structural proteins of hair: isolation, characterisation and regulation of biosynthesis. **In** "Biochemistry and Physiology of the Skin", 2nd Edition. (Ed. L.A. Goldsmith), Vol. 1, pp. 625-659. Oxford University Press: New York.

Gilmour, A.R., Cullis, B.R. Welham, S. And Thompson, R. (1998). ASREML reference manual. *NSW Agriculture Biometrics Bulletin,* **No. 3**. (Orange, NSW, Australia).

Gourdie, R.G., Orwin, D.F.G., Randford, S. And Ross, D.A. (1992). Wool fibre tenacity and its relationship to staple strength. *Australian Journal of Agricultural Research* **43:** 1759-1776.

Greeff, J.C. (2000) Staple strength is genetically the same trait in Autumn and Spring shorn wool. . *Proc. Of the 9th Congress of the Asian-Australasian Association of Animal Production Society and 23rd Biennial Conference of the Australian Soc. Of Anim. Prod.,* **13 (C) :** 234.

Greeff, J.C., Lewer, R.P., Ponzoni, R.W. and Purvis, I. (1995). Staple strength: Progress towards elucidating its place in Merino breeding. *Proc. Aust. Assoc. Anim. Breed. Genet.,* **11:** 595-601.

Green, M.R. and Couchman, J.R. (1984). Distribution of epidermal growth factor receptors in rat tissues during embryonic skin development, hair formation and the adult hair growth cycle. *Journal of Investigative Dermatology,* **83:** 118-123.

Hansford, K.A. 1989. The influence of nutrition and reproduction on the length, strength and position of break of Merino wool. Phd Thesis. (University of NSW, Australia).

Hansford, K.A. (1992). Fibre diameter distribution: Implications for wool production. *Wool Technology and Sheep Breeding,* **March-April**: 2-9.

Hansford, K.A. and Kennedy, J.P. (1990). The relationship between variation in fibre diameter along staple and staple strength. *Proceedings of the 9th International Wool Textile Research Conference, Christchurch, New Zealand,* **1:** 1-9.

Hansford, K.A. and Kennedy, J.P. (1988). Relationship between the rate of change in fibre diameter and staple strength. *Proceedings of the Australian Society of Animal Production,* **17:** 415.

Hardy, M. H. (1993). The secret life of the hair follicle. *Trends Genet.,* **8:** 55-61

Hart, D.S. (1955). The photoperiodic and hormone response of wool growth in sheep. *Proceedings of the N. Z. Society of Animal Production*, **15**: 57.

Hart, D. S., Bennett, J. W., Hutchinson, J. C. D., and Wadzicka-Tomaszewska, M. (1963). Reversed photoperiode genom and wool growth. *Nature,* **198 :** 310-311.

Hawker, H., Dodds, K.G., Andrews, R.N. and McEwan, J.C. (1988). Production and characteristics of wool from the hogget progeny of sheep intensively screened for fleece weight. *Proc. of the NZ. Soc. of Anim. Prod.,* **48:** 207-212.

Hill, J.A. and Ponzoni, R.W. (1999). Predicting genetic change in staple strength - How much gain can we expect. *Proc. Assoc. Advmt. Anim. Breed. Genet.,* **13:** 46-49.

Hocking-Edwards, J.E. and Hynd, P.I. (1992). Cellular characteristics of wool follicles and fibres in fine wool and strong wool Merinos. *Australian Journal of Agricultural Research,* **43:** 355-365.

Hoffmann, R., Eicheler, W., Huth, A., Wenzel, E. and Happle, R. (1996). Cytokines and growth factors influence hair growth in vitro; Possible implications for pathogenesis and treatment of Alopecia areata. *Dermatological Research,* **288(3):** 153-156.

Hohenboken, W.D. (1985). Heritability and repeatability. **In** "General Quantitative Genetics", (Ed. Chapman, A.B.), pp 77-111. (Elsevier Sci. Publishers B.V. Amsterdam-Oxford-NewYork-Tokyo).

Hollis, D. E., Chapman, R. E., Panaretto, B. A, and Moore, G. M. (1983). Morphological changes in the skin and wool fibres of Merino sheep infused with mouse epidermal growth factor. *Australian J. of Biological Sci.* **36 :** 419-434.

Hughes, A. (1995). The susceptibility of different genotypes to follicle shutdown induced by nutritional stress. Thesis (Honours). p. 60. University of Adelaide.

Hunter, L., Leeuwner, W., Smuts, S., and Strydom, M. A. (1983). The correlation between staple strength and single fibre strength for sound and tender wools. *SAWTRI Technical Report,* **No 514** :1-15.

Huson, M.G., Bedson, J.B., Phair, N.L. and Turner, P.S. (2000[a]). Intrinsic strength of wool fibres. *Proc. of the 9th Congress of the Asian-Australasian Association of Animal Production Society and 23rd Biennial Conference of the Australian Soc. of Anim. Prod.,* **13 (C):** 267.

Huson, M.G., Phair, N.L., Maxwell, J.M. and Turner, P.S. (2000[b]). Bundle strength and intrinsic fibre strength of Finewools from different bloodlines. *Proc. of the 9th Congress of the Asian-Australasian Association of Animal Production Society and 23rd Biennial Conference of the Australian Soc. of Anim. Prod.,* **13 (C):** 268.

Huson, M.G., Thompson, A.N., Ley, K.J. and Bedson, J.B. (1997). The intrinsic strength of wool in relation to its structure. *Proceedings of the 22nd Australasian Polymer Symposium, Auckland (2-5 February, 1997).*

Hutchinson, K. J. (1962). Climate Corrections to the seasonal wool growth rhythm of sheep grazing in a Southern Australia environment. *Proc. Australia. Soc. Animal Prod.*, **4:** 34-37.

Hygate, L and Scrivener, C. (1999). Indirect selection for staple strength in Merino sheep breeding programs. *Proc. Assoc. Advmt. Anim. Breed. Genet.,* **13:** 50-53.

Hynd, P. I. (1989[a]). Effects of nutrition on wool follicle cell kinetics in sheep differing in efficiency of wool production. *Australian Journal of Agricultural Research*, **40:** 409-417.

Hynd, P. I. (1989[b]) Factors influencing cellular events in the wool follicle. **In** : "The Biology of Wool and Hair". pp 169-184. (Eds, G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marshall). Chapman and Hall, London.

Hynd, P.I. (1994[a]). Follicular determinants of the length and diameter of wool fibres. 1. Comparison of sheep differing in fibre length / diameter ratio at two levels of nutrition. *Australian Journal of Agricultural Research,* **43:** 1759-1776.

Hynd, P. I. (1994[b]). Thyroxine deficiency induces follicle shutdown. *Proc. of. Aust. Soc. anim. Prod.* **20:** 20-452.

Hynd, P. I. (2000). The nutritional biochemistry of wool and hair follicles. *Animal Science*, **70:** 181-195.

Hynd, P.I., Hughes, A., Earl, C.R. and Penno, N.M. (1997) Seasonal changes in the morphology of wool follicles in Fine-wool and Strong-wool Merino strains grazing at different stocking rates in Southern Australia. *Australian Journal of Agricultural Research,* **48:** 1089-1097.

Hynd, P. I. and Nancarrow, M. J. (1996). Inhibition of polyamine synthesis alters hair follicle function and fiber composition. *The J of Investigative Dermatology*, **106(2)** : 249-253.

Hynd, P.I. and Schlink, A.C. (1992). Factors responsible for variations in the strength of wool fibres. *Proceedings of the Workshop on Management of Wool Quality in a Mediterranean Environments.* Eds. P.T. Doyle, W..A. Dept. of Agriculture.

Jackson, N. and Downes, A.M. (1979). The fibre diameter profile of wool staples from individual sheep. *Aust. J. Agric. Res.*, **30:** 163-171.

Jones, C. M., Lycons, K. M. and Hogan, B. L. M. (1991). Involvement of bone morphogenic protein-4 (BMP4-4) and Vgr-1 in morphogenesis and neurogenesis in mouse. *Dev.* **111**: 531.

Kelly, R.W., Speijers, E.J., Ralph, I.G., and Newnham, J.P. (1992). Lambing performances and wool production of maiden and adult Merino ewes fed different amounts of lupin seed in mid-pregnancy. *Australian Journal of Agricultural Research* **43**: 339-354.

Koots, K.R., Gibson, J.P. and Wilton, J.W. (1994). Analysis of published genetic parameter estimates for beef production traits. 2. Phenotypic and genetic correlations. *Animal Breeding Abstracts,* **62(11):** 825-853.

Kratochwil, K., Dull, M., Farinas, I., Galceran, J. and Grosschedl, R. (1996). LEF 1 expression in activated by BMP. 4 and regulates inductive tissue interactions in tooth and hair development. *Genes and Development*, **10:** 1382-1394.

Lewer, R.P. and Ritchie, A.J.M. (1992). Genetics for staple strength. **In** "Proceeding of a National Workshop on Management for Wool Quality in Mediterranean Environment", (Eds. P.T. Doyle, J.A. Fortune and N.R. Adams), pp 106-114. Department of Agric. W.A.

Lindner, H. R. and Ferguson, K. A. (1956). Influence of the adrenal cortex on wool growth and its relation to "break" and "tenderness" of the fleece. *Nature London,* **177:** 188-189.

Li, Y., Swan, A. and Purvis, I. (1999). Genetic variation in resistance to fleece rot in CSIRO's fine wool flock. *Proc. Assoc. Advmt. Anim. Breed. Genet.,* **13:** 524-527.

Liu, A.H., Wickham, G.A. and Blair, H.T. (1994). Effect of selection for greasy fleece weight on the components of fleece weight in New Zealand Romney sheep. *Proc. of the NZ. Soc. of Animal Prod.,* **54:** 243-246.

Lynch, M., O'Guin, W.M., Hardy, C., Mak, L. and Sun, T.T. (1986). Acidic and basic hair/nail ("hard") keratins: Their co-localisation in upper cortical and cuticle cells in the human hair follicle and their relationship to 'soft' keratins. *Journal of Cell Biology,* **103:** 2593-2606.

Lyne, A.G. (1961). The postnatal development of wool follicles, shedding and skin thickness in inbred Merino and Southdown Merino crossbred sheep. *Australian Journal of Biological Sciences,* **14:** 141.

Lyne, A. G. (1964). Effect of adverse nutrition on the skin and wool follicles in Merino sheep. *Aust. J. Agric. Res.,* **15:** 788-801.

Maclaren, J.A. and Milligan, B. (1981). Cross-linking. **In** "Wool Science, the Chemical Reactivity of the Wool Fibre", (Ed. J.A. MacLaren and B. Milligan), pp 181-217. Science Press: Marrackville, NSW.

Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular cloning; a laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

Masters, D. G. and Mata, G. (1998). Effects of reproduction and supplementary feeding on staple strength and other wool characteristics of grazing ewes.

Masters, D.G., Stewart, C.A. and Connell, P.J. (1993). Changes in plasma amino acid patterns and wool growth during late pregnancy and early lactation in the ewe. *Aust. J. Agric. Res.,* **44:** 945-957.

Masters, D.G., Stewart, C.A., Mata, G. and Adam, N.R. (1996). Responses in wool and live-weight when different sources of dietary protein are given to pregnant and lactating ewes. *Animal Science,* **62(3):** 497-506.

Maxwell, C. A., Scaramuzzi, R. J., Foldes, A. and Carter, N. B. (1988). The effects of long term exposure to continuous long or short days on conditioned clean wool weight in Wiltshire horn x Merino ewes. *Proc. Aust. Soc. Anim. Prod.*, **14 :** 246-249.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A. (1995). Animal nutrition. 5th Edition. Pp. 50-53. Longman Scientific & Technical. New York.

McKinley, A.H., Irvine, P.A., Roberts, E.M. and Andrew, M.W. (1976). The direct partitioning of variation in fibre diameter in tender wool. *Proceedings of the Australian Society of Animal Production,* **11:** 181-184.

McQuaker, N.R., Brown, D.F. and Kluckner, P.D. (1979). Digestion of environmental materials for analysis by inductively coupled plasma-atomic emission spectrometry. *Analytical Chemistry,* **51(7):** 1082-1084.

Miller, F.M., Blair, H.T., Reynold, G.W. and Revell, D.K. (1998). The role of cysteine in the increased parasite susceptibility of Romney sheep selected for hogget fleece weight. *Proc. NZ. Soc. Anim. Prod.,* **58:** 150-153.

Morcombe, P. W., Young, G. E and Boase, K. A. (1996). Grazing a saltbush (Atriplex-Maireana) stand by Merino weathers to fill the autumn feed – gap experienced in the Western Australian Wheat belt. Australian wheat belt. *Australian J. of Exp. Agric.,* **36 (6)** : 641-647.

Moore, G. P. M., Jackson, N., Isaacs, K. and Brown, G. (1998). Pattern and morphogenesis in skin. *J. Theor. Biology*, **191** : 87-94.

Moore, G. P. M., Panaretto, B. A. and Robertson, D. (1983). Epidermal growth factor delays the development of the epidermis and hair follicles of mice during growth of first coat. *Anatomy Rec.* **205** : 47-55.

Moore, G. P. M., Panaretto, B. A. and Robertson, D. (1981). Epidermal growth factor causes shedding of the fleece of Merino sheep. *Search, Sydney*, **12:** 128-129.

Moore, G. P. M., Panaretto, B. A. and Robertson, D. (1982). Inhibition of wool growth in Merino sheep following administration of mouse epidermal growth factor and a derivative. *Australian Journal of Biological Science*, **35** : 163-172.

Morris, S.T., Mc Cutcheon, S.N., Blair, H.T. and Parker, W.J. (1994) Effect of lambing policy and ewe breed cross on wool growth patterns and wool quality. *NZ. J. of Agric. Res.,* **37(1):** 65-78.

Nagorcka, N. N. (1979). The effect of photoperiod on wool growth. **In**: "Physiological and environmental limitations to wool growth". pp 127-137 (Eds. J. L. Black and P. J. Reis). University of New England Publishing Unit, Armidale.

National Research Council (NRC). (1985). Nutrient requirements of sheep. pp. 45-47. National Academy Press, Washington, U.S.A.

Nattrass, G.S. (2000). Molecular and functional characterization of a system ASC-like neutral amino acid transporter expressed in the wool follicle. Phd Thesis. Adelaide University, S.A.

Naylor, G.R.S. and Stanton, J. 2000. Managing time of shearing of Mediterranean wools fro improved fabric skin comfort. *Proceedings of the 9th Congress of the Asian-Australasian Association of Animal Production Societies (AAAP) and 23rd Biennial Conference of the Australian Society of Animal Production.* Eds. G.M. Stone, **Vol. C.** July 3-7 2000: pp. 255. (Univ. Of NSW Sydney, Australia).

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

Oldham, C. (1999). Impair discrimination against Mediterranean wools. *The Wool Press,* **6(2)** : 6.

Orwin, D.F.G., Woods, J.L. and Gourdie, R.G. (1985). Cortical cell types and wool strength. *Proceedings of the 7th International Wool Textile Research Conference, Tokyo, Japan.* **1:** 194-203.

Parakkal, P.F. (1969). Ultrastructural changes of the basal lamina during the hair growth cycle. *J. Cell Biol.,* **40(2):** 561-564.

Pearson, A.J. Parry, A.L., Ashby, M.G., Choy, V.J., Wildermoth, J.E. and Craven, A.J. (1996). Inhibitory effect of increased photoperiod on wool follicle growth. *J. Endocrinol.,* **148(1):** 157-166.

Peterson, A.D. and Gherardi. (2002). Comparison of the sirolan fleecescan and OFDA2000 for on-farm testing of fibre diameter. *Proc. Anim. Prod. Aust,* **24:** 173-176.

Peterson, A.D., Gherardi, S.G. and Doyle, P.T. (1998) Component of staple strength in fine and broad wool Merino hoggets run together in a Mediterranean environment. *Australian Journal of Agric. Res.,* **49(8):** 1181-1186.

Peterson, A.D., Gherardi, S.G. and Ellis, M.R. (2000). Managing the diameter profile leads to increased staple strength of young Merino sheep shorn in spring in South Western Australia. *Proc. Aust. J. Anim. Sci.,* **13(A):** 469-472.

Peus, D and Pettelkow, M. R. (1996). Growth factors in hair organ development and the hair growth cycle. *Dermatologic Clinics*, **14** : 4.

Philpott, M. and Peus, R. (1998). Principles of hair follicle morphogenesis. In : Molecular basis of Epithelial Appendage Morphogenesis. Ed : Cheng-Ming Chuong.

Plate, D.E.A., Robinson, G.A. and Rottenbury, R.A. (1987). The effect of staple strength and position of staple weakness of greasy wool on worsted spinning. *J. of the Textile Institute,* **4:** 269-279.

Ponzoni, R.W. and Gifford, D.R. (1993). The Turretfield Merino resource flock. In "Merino Genetic resource Flock in Australia". *Proceedings of a National Workshop Turretfield Research Centre, Rosedale, South Australia, 27-28 May 1993. Pp. 15-20. Wool Research and Development Corporation and South Australia Research and Development Institute, S.A..*

Ponzoni, R.W., Grimson, R.J., Kaylene, S.J., Smith, D.H., Gifford, D.R., Ancell, P.M.C., Walkley, J.R.W. and Hynd, P.I. (1994). The Turretfield sheep breeding project: Messages on phenotypic and genetic parameters for South Australian Merino sheep. *Proc. Aust. Assoc. Anim. Breed. Genet.,* **11:** 303-313.

Ponzoni, R.W., Kenyon, R.V., Partington, D.L. and Jaensch, K.S. (1996). South Australian Test Sire Evaluation Rosebank; 1995 Drop 1st Assessment. The S.A. Stud Merino Sheepbreeders' Association Inc. Australia.

Porras-Reyes, B. H., Ksander, G. and Weeks, P. M. (1993). Occurance and localization of transforming growth factor-β (TGF β1, β2) during rabbit skin development. *Tissue Res.*, **29**: 203.

Powell, B.C. (1996). The keratin proteins and genes of wool and hair. *Wool Tech. Sheep. Breed.,* **44(2):** 100-118.

Powell, B.C., Nesci, A. and Rogers, G.E. (1991). Regulation of keratine gene expression in hair follicle differentiation. *Annals of the New York Academy of Sciences,* **642:** 1-20.

Purser, D. B. (1979). Effects of minerals upon wool growth. **In** "Physiological and Environmental Limitations to Wool Growth. (Eds : J. L. Black and P. J. Reis). pp 243-255. The Univ of New England. Publishing Unit..

Quinnell, B., Whiteley, K.J. and Roberts, E.M. (1973). Variation in fibre diameter of wool fibres - a review. **In** "Objective Measurement of Wool in Australia", Technical Report, pp. 4.2-4.20. Australian Wool Corporation.

Ralph, I.G. (1986). Staple strength. *Journal of Agriculture, Western Australia,* **27**: 99-102.

Reid, T. C. and Sumner, R. M. W. (1991). Wool growth in autumn and spring lambing ewes. *Proc. of the NZ. Soc. of Anim. Prod.*, **51**: 383-387.

Reis, P. J. (1979). Effect of amino acids on the growth and properties of wool. **In** "Physiological and Environment Limitation to wool growth". (Eds : J. L Black and P. J. Reis). pp 223-242.

Reis, P. J. (1989). The influence of absorbed nutrients on wool growth. **In:** "The Biology of wool and hair". (Editors : G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marshall). pp 185-203. Chapman and Hall, New York.

Reis, P.J. (1992). Variations in the strength of wool fibres; A Review. *Australian J. of Agric. Res.,* **43:** 1-15.

Reis, P. J. and Colebrook, W. F. (1972). The utilization of abomasal supplements of proteins and amino acids by sheep units special reference to wool growth. *Aust. J. Biology Science.*, **25:** 1057-1071.

Reis, P.J. and Hynd, P.I. (1989). The influence of difluoromethylornithine on the activity of wool follicles. *Aust. J. of Animal Sci.*, **2:** 204-205.

Reis, P.J. and Schinkel, P.G. (1963). Some effects of sulphur-containing amino acids on the growth and composition of wool. *Aust. J. Biol. Sci.*, **16(1):** 218-230.

Reis, P. J. and Tunks, D. A. (1978). Effects of wool growth of the infusion of mixtures of amino acids into the abomasum of sheep. *Journal of Agricultural Science,* **90**: 173.

Reis, P.J., Tunks, D.A. and Downes, A.M. (1973). The influence of abomasal and intravenous supplements of sulphur-containing amino acids on wool growth rate. *Aust. J. biol. Sci.*, **26:** 249-258.

Ritchie, A.J.M. and Lewer, R.P. (1994). Genetic prevention of low staple strength . *Proc. Aust. Soc. Anim. Prod.* **20:** 51-52.

Roff, D.A. (1996). The evolution of genetic correlations: an analysis of patterns. *Evolution,* **50(4):** 1392-1403.

Rogan, I.M. (1989). Genetic variation and covariation in wool characteristics related to processing performance and their economic significance. *Wool Tech. And Sheep Breedin,* **Dec-Jan:** 126-135.

Rose, M. and Pepper, P.M. (2000). Genetic parameters for staple length and staple strength of Merino wool produced in Central and North West Queensland. *Proc. of the 9th Congress of the Asian-Australasian Association of Animal Production Society and 23rd Biennial Conference of the Australian Soc.of Anim.Prod.,* **13(B):** 87-90.

Rosenquist, T. A. and Martin, G. R. (1996). Fibroblast growth factor signalling in the hair growth cycle : Expression of the fibroblast growth factor receptor and ligand genes in the murine hair follicle. *Development Dynamics*, **205** : 379-386.

Ross, D.A. (1982). Staple length, staple strength, fibre length and processing. *Wool,* **7(4):** 12-15.

Rottenbury, R.A., Andrews, M.W., Bell, P.J.M. and Bownass, R. (1986). The effect of the strength properties of wool staples on worsted processing. Part 1: The level of staple strength. *J. Text. Inst.*, **3:** 179-190.

Rottenbury, R.A., Bow, M.R., Kavanagh, W.J. and Caffin, R.N. (1981). Staple strength variation in Merino flocks. *Wool Tech. and Sheep Breeding.* **29:** 143.

Ryder, M.L. (1956). Observations of nutritional and seasonal changes in the fleeces of some Masham sheep. *J. Agric. Sci., Camb.*, **47:** 187-190.

Ryder, M.L. (1962). Preliminary observations on seasonal changes in the fleeces of unshorn Merino sheep. *Proc. Aust. Soc. Anim. Prod.*, **4:** 46-48.

Ryder, M.L. (1967). Wool fibre shedding in some Merino sheep. *Austr. J. Agric. Res.*, **18:** 683-687.

Ryder, M. L. (1969). The development and structure of, and seasonal change in, the coat of some wilshire sheep. *Animal Production*, **11(4) :** 467-477.

Ryder, M. L. (1971[a]). Cycles of wool follicle activity in some Shetland sheep. *Animal Production*, **13:** 511-520.

Ryder, M. L. (1971[b]). Wool growth cycles in Soay sheep. *J. Agric. Sci., Lamb.*, **76:** 183-197.

Ryder, M. L. (1973). The structure of and growth cycles is, the coat of wild Mouflon sheep (*ovis musimon*) and their crosses. *Res. Vet. Sci.*, **15:** 186-196.

Sambrook, J., Fritsch, E.F. and Maniatis. (1989). Molecular cloning: A laboratory manual. 2nd Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor.

SAS Institute. (1990). SAS/STAT User's guide; GLM - VarComp. 4th Edition, Vol. 2. SAS Insitute, North Carolina, U.S.A.

Schlink, A.C. and Dollin, A.E. (1995) Abnormal shedding contributes to the reduced staple strength of tender wool in Western Australian Merinos. *Wool Technology and Sheep Breeding,*. **43:** 268-284.

Schlink, A. C., Lea, J., Ritchie, A. J. M and Sanders, M. (1996[a]) Impact of a Mediterranean environment on wool follicles and fibre growth in high and low staple strength Merino wethers. *Wool tecnology and Sheep Breeding,* **44 (2):** 81–82.

Schlink, T., Adams, N. and Peterson, A. (1996[b]). Component of staple strength. **In** : "Focus on Staple Strength". (Ed : Lucindale wool 2000 Commitee). pp 29-50. Cooperation Research Centre for Premium Quality Wool.

Schlink, A.C., Masters, D.G. and Dollin, A.E. (1992). Effects of amino acids on fibre shedding in reproducing ewes. *Proceedings of the Nutrition Society of Australia,* **17:** 80.

Schlink, A.C., Peterson, A.D., Huson, M. And Thompson, A.N. (2000). Components of staple strength. *Proceedings of the 9th Congress of the Asian-Australasian Association of Animal Production Societies (AAAP) and 23rd Biennial Conference of the Australian Society of Animal Production.* Eds. G.M. Stone, **Vol. C.** July 3-7 2000. Pp. 21-24. (Univ. Of NSW Sydney, Australia).

Scobie, D.R., Walls, R.J., Markham, L.J., Woods, J.L. and Bray, A.R. (1996). Wool fibre tenacity in Romney sheep genetically different in staple tenacity. *Australian Journal of Agricultural Research,* **47**; 1203-1212.

Sherlock, R.G., Harris, P.M., Lee, J., Wickham, G.A., Woods, J.L. and McCutcheon, S.N. (2001). Intake and long-term cysteine supplementation change wool characteristics of Romney sheep. *Aust. J. Agric. Res.*, **52:** 29-36.

Slen, S. B. and Connel, R. (1958). Wool growth in sheep affected by the administration of certain sex hormones. *Canadian J. Anim Sci.* **38**: 38.

Smith, V. G., Hacker, R. R., and Brown, R. G. (1977). Effects of alterations in ambient temperature on serum prolactic concentration in steers. *J. of Animal Sci.*, **44** : 645.

Smuts, S., Hunter, L., and Van Rensberg, H. L. J. (1981). Some typical single fibre tensile properties for wool produces in South Africa. *SAWTRI Technical Report*, **485:** 1-30.

Steel, R.G.D. and Torrie, J.H. (1987). Principles and procedures of statistics. Pp. 156-159. McGraw-Hill Book Company, Inc. New York-Toronto-London.

Stenn, K.S., Peus, R. and Filippi, M. (1998). Failure of topical estrogen receptor agonists and antagonists to alter murine hair follicle cycling. *J. Invest. Dermatol.*, **110(1):** 95.

Stewart, A. M., Moir, R. J. and Schinckel, P. G. (1961). Seasonal fluctuations in wool growth in South Western Australia. *Aust. J. Exp. Agric and Animal Husbandry*, **1**: 85-91.

Straile, W.E., Chase, H.B. and Arsenault, C. (1961). Growth and differentiation of hair follicles between periods of activity and quiescence. *Journal of Experimental Zoology* **148:** 205-221.

Sun, Y.X., Koolaard, J.P., Blair, H.T., Lee, J. and McCutcheon, S.N. (1991). Wool sulphur concentrations in fleece weight selected and control Romney hoggets. *Proceedings of New Zealand Society of Animal Production,* **51:** 395-400.

Sumner, R.M.W. and Wickham, G.A. (1969). Some effects of an increased stocking level on wool growth. *Proc. of the NZ Soc. Of Animal Production.* **29:** 208.

Thompson, A.N. 1998. Intrinsic strength of Merino wool fibres. Thesis (PhD). The University of Adelaide.

Thwaites, C. J. (1972). The effect of short term undernutrition and adrenocortical stimulating on wool growth. *Anim. Prod.*, **15:** 39-46.

Tsenkova, I. (1990). The effect of type of birth on wool production in Thrace Finewool sheep of the Stara Zagora type. *Zhivotnov'dni-Nauki,* **27(4):** 13-16.

Turner, H.N. (1958). Relationship among clean wool weight and its components. 1. Changes in clean weight related to changes in the components. *Aust. J. of Agric. Res.,* **9:** 521-552.

Turner, H.N. and Young, S.S.Y. (1969). Quantitative Genetics in sheep breeding. MacMillan of Australia. Australia.

Wallace, A. L. C. (1979). The effect of hormones on wool growth. **In**: "Physiological and environmental limitations to wool growth". (Eds : J. L. Black and P. J. Reis). pp 257-268. The Univ of New England Publishing Unit.

Walkden-Brown, S.W., Daly, B.L., Crook, B.J. and Colditz, I.G. (1999). Blowfly strike and wool growth. I. Role of anorexia in mediating effects on wool. *Recent Advances in Animal Nutrition in Australia.* **12**: 2A.

Wildman, A. B. (1957). Photoperiodicity and wool growth in Romney rams and wethers. *Nature*, **180**: 296-297.

Williams, A.J. (1979). Speculations on the biological mechanisms responsible for genetic variation in the rate of wool growth. **In** : "Physiological and environmental limitations to wool growth". (Eds : J. L. Black and P. J. Reis). pp 337-354. The Univ of New England Publishing Unit.

Williams, A. J., Robards, G. E., and Saville, D. G. (1972). Metabolism of cysteine by Merino sheep genetically different in wool production; II. The responses in wool growth to abnormal infusions on L-cysteine or DL-methionine. *Australian J. of Biological Science*, **25** : 1269-1276.

Williams, O. B. and Schinkel, P. G. (1962). Seasonal variations in wool growth and lineweight in several environments. *Proc. Aust. Social Animal Prod.*, **4 :** 38-45.

Williamson, J. F., Blair, H.T., Garrick, D.J. Pomroy, W.E., Douch, P.G.C., Green, R.S. (1995). Parasitological characteristics of fleeceweight selected and control sheep. *NZ. J. Agric. Res.*, **38:** 389-397.

White, C. L., Masters, D. G., Peter, D. W., Purser, D. B., Roe, S. P. and Barnes, M. J. (1992). A multielement supplement for grazing sheep. I. Intake, mineral status and production responses. *Australian J. of Agric. Res.*, **43:** 795-808.

Whiteley, K.J. (1987). Wool Processing. *Wool Tech. and Sheep Breed.*, **36(2):** 109-113.

Wolfersberger, M.G. (1994). Uniporters, symporters and antiporters. *Journal of Experimental Biology.* **196**: 5-6.

Woods, J.L., Orwin, D.F.G. and Nelson, W.G. (1990). Variations in the breaking stress of Romney wool fibres. *Proceedings of the 8th International Wool Textile Research Conference, Christchurch, New Zealand,* **1:** 557-568.

Woolmark. (2000). Staple strength. Website: http://melpub.wool.com/enews.nsf/.

Wuliji, T., Andrews, R.N., Davis, G.H. and Farquhar, P.A. (1990) Hogget fleece weight and fleece characteristics of Texel x Romney, Texel x Coopworth, Romney and Coopworth sheep. *Proc. of the N.Z. Soc. of Anim. Prod.*, **50:** 495-497.

Wuliji, T., Dodds, K.G., Andrews, R.N., Turner, P.R. and Wheeler, R. (1998) Responses to fleece weight selection and heritability estimates of wool characteristics in Romney sheep. *Wool Technology and Sheep Breeding,* **46(3):** 250-254.

Yamin, M., Hynd, P.I., Ponzoni, R.W., Hill, J.A., Pitchford, W.S. and Hansford, K.A. (1999). Is fibre diameter variation along the staple a good indirect selection criterion for staple strength? *Wool Technology and Sheep Breeding,* **47:** 151-158.

Zshiesche, W., and Eckert, K. (1988). Effects of anti EGF serum on newborn mice. *Experientia,* **44 :** 249-251.