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Fleece Phenotype Influences Susceptibility to Cortisol-induced Follicle Shutdown in Merino Sheep

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ABSTRACT : This experiment was conducted to determine the extent to which susceptibility to cortisol-induced follicle shutdown is influenced by fleece phentotype. Twenty Finewool (10 sheep low fibre diameter, low coefficient of fibre diameter-LL and 10 low fibre diameter, high coefficient of variation of fibre diameter-LH) and twenty Strongwool (10 low fibre diameter, low coefficient of variation of fibre diameter-HL and 10 high fibre diameter and high coefficient of variation of fibre diameter-HH) sheep of 9 months of age were individually penned in an animal house and were injected intramuscularly with an aqueous suspension of hydrocortisone acetate at a rate of 1.42 mg/kg body weight for a period of two weeks. Fibre diameter was measured from clipped tattooed patch wool samples. Follicle activity was measured by histological changes in skin biopsies taken weekly. Blood samples were collected at two-week intervals and plasma cortisol measured. Increased plasma cortisol concentration significantly ($p<0.05$) reduced clean wool production and mean fibre diameter dropped to its lowest level four weeks after commencement and two weeks after the cessation of cortisol injection. Elevation of plasma cortisol concentration significantly ($p<0.0001$) increased the percentage of inactive follicles two weeks after injection started. High fibre diameter groups (Strongwool sheep; i.e. HL+HH) had significantly ($p<0.0001$) higher percentage of follicle shutdown than low fibre diameter groups (Finewool sheep; i.e. LL+LH). Average percentage of shutdown follicles for Finewool (LL+LH) and Strongwool (HL+HH) Merino sheep was 9.8±0.9 and 13.5±0.9 respectively. Shutdown of primary follicles was more pronounced in Finewool than Strongwool sheep. There was no significant effect of coefficient of variation of fibre diameter on propensity to follicle shutdown induced by exogenous cortisol. It is concluded that elevation in plasma cortisol concentration is inhibitory to the normal activity of follicles in Strongwool sheep but that variation in fibre diameter has little or no effect. (**Key Words :** Merino, Strongwool, Finewool, Cortisol, Injection, Follicle Shutdown)

INTRODUCTION

The hormonal basis of follicle shutdown and fibre shedding has not yet been fully elucidated. High levels of follicle inactivity and fibre shedding have been induced under conditions of ACTH treatment (Lindner and Ferguson, 1956) or cortisol injection (Chapman and Bassett, 1970). Daily intramuscular injections of 40 IU of ACTH for ten weeks diminished wool growth progressively until it was completely arrested (Lindner and Ferguson, 1956). Successive increases in plasma cortisol level up to 30 ng/ml inhibited wool growth in sheep on a restricted plane of nutrition (Chapman and Bassett, 1970). Administration of 300 mg of hydrocortisone acetate three times weekly, significantly decreased wool production, fibre diameter and fibre length (Spurlock and Clegg, 1962). Ansari-Renani and Hynd (2001) reported that daily intramuscular injection of 5 groups of Merino sheep with 0.19 to 2.86 mg/kg of cortisol for 4 weeks caused more than 30% of follicles to stop activity. As a result of follicle shutdown, wool staple strength was significantly ($p<0.0001$) reduced. Not only there was a difference in the susceptibility to shutdown between primary and secondary follicles, but also considerable variation was evident between individual secondary follicles. This raises the question 'why do follicle types differ in susceptibility to shutdown'? This differential susceptibility may be related to the shape, size, depth, density and the ratio of secondary to primary follicles or to the surface area, arrangement of blood vessels, uptake of hormones from the blood and extracellular fluids, efficiency of utilisation of hormones within the follicle or a

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Table 1. Fibre diameter (FD, μm), coefficient of variation of fibre diameter (CV, %) of Merino sheep differing in fleece phenotype (LL, LH, HL and HH) before commencement of treatment period

Group	Average FD (μm)	Average %CV
LL*	16.2^{a}±0.25	18.4^{a}±0.18
LH**	16.7^{a}±0.17	23.1^{b}±0.19
HL***	24.0^{b}±0.36	20.7^{c}±0.23
HH****	23.6^{b}±0.22	26.4^{d}±0.36

Superscript within groups significantly differ at $p<0.05$.
* Low fibre diameter, low coefficient of variation of fibre diameter (LL). ** Low fibre diameter, high coefficient of variation of fibre diameter (LH). *** High fibre diameter, low coefficient of variation of fibre diameter (HL).
**** High fibre diameter, high coefficient of variation of fibre diameter (HH).

combination of these factors.

To test the hypothesis that sheep follicle types differ in susceptibility to cortisol-induced shutdown, 2 groups of Finewool (LL = Low fibre diameter, low coefficient of variation of fibre diameter and LH = Low fibre diameter, high coefficient of variation of fibre diameter) and 2 groups of Strongwool (HL = High fibre diameter, low coefficient of variation of fibre diameter and HH = High fibre diameter, high coefficient of variation of fibre diameter) Merino sheep were selected. All 4 groups of sheep were exposed to one dose of cortisol injection (1.42 mg/kg body weight per day) for a period of two weeks. Plasma cortisol concentration, wool growth rate and percentage of follicle shutdown were measured and compared between the fleece phenotype groups.

MATERIALS AND METHODS

Selection of animals

The objective was to select 2 groups of sheep from each of a Finewool Merino flock (n = 150) and a Strongwool Merino flock (n = 150) such that 2 groups within each genotype differed phenotypically in coefficient of variation of fibre diameter. The sheep, 9 months of age at the time of selection, grazed at Turretfield Research Centre in South Australia. About 50 grams of wool was sampled by Oster small animal clippers (model 40 Oster Corp., Wisconsin, no.40 blades) from the midside area. Fibre diameter and coefficient of variation of fibre diameter was measured on 2,000 fibres on a Fibre Fineness Distribution Analyser (FFDA) (Lynch and Michie, 1973). A total of 40 sheep (20 from each flock) were selected and were divided into 4 groups (n = 10) based on fibre diameter and coefficient of variation of fibre diameter (Table 1) as follows: Finewool groups, similar low fibre diameter but low (LL) or high (LH) coefficient of variation of fibre diameter; Strongwool groups, similar high fibre diameter but low (HL) or high (HH) coefficient of variation of fibre diameter. These 40 sheep were shorn and brought to the Waite Institute Animal Biotechnology Centre of The University of Adelaide and kept in individual pens in an animal house. To alleviate any feed factor, all sheep were fed a maintenance level ration of sheep pellets and chopped hay (containing 15 g N kg^{-1} dry matter and 9.1 Metabolisable Energy, Australian Feed Services Pty. Ltd.) throughout the experiment. The diet was offered once a day at 0800 h. Animals were treated with an anthelmintic prior to the experiment. Water was available *ad libitum* for the entire experimental period. Once every other week prior to feeding in the morning after an overnight fast, sheep were weighed using electronic scales (Ruddweigh beef scales) and their rations adjusted according to live weight.

Design of the experiment

At the end of 10 weeks pretreatment period sheep were accustomed to diet and handling procedures. During the treatment period all sheep groups were injected with hydrocortisone acetate for two weeks to induce follicle shutdown and inhibition of wool growth. During the post-treatment period, cortisol injection was stopped and all groups continued to receive a maintenance level ration for a period of 14 weeks. Wool within the tattooed patch area and serial blood samples were taken every two weeks to measure clean wool weight and plasma cortisol concentration respectively. Skin biopsies were taken once a week for measurement of follicle activity and histological studies.

Cortisol hormone injection

An aqueous suspension of hydrocortisone acetate was adjusted at 1.42 mg/kg body weight was administered intramuscularly to all sheep on daily basis for a period of two weeks. Because cortisol is hydrophobic and is soluble in water only at a rate of 1.0 mg/100 ml, this hormone was suspended in a total volume of 3.0 ml of 80% ethanol.

Patch wool weight, fibre diameter

Immediately after being brought into the animal house, sheep were shorn and tattooed on the right midside region (Langlands and Wheeler, 1968). Every two weeks, wool within the delineated area was closely clipped with small animal clippers (Oster Corp., Wisconsin). The tattooed area was traced onto a transparency sheet and measured using an image analysis (Bioquant IV, R and M Biometrics, Tennessee). Clean patch wool weight, was determined by washing in Hexane and rinsed in hot water and dried.

Washed midside patch wool samples were reduced in length and the mean fibre diameter was measured by FFDA (Lynch and Michie, 1973).

Skin sampling and staining

At the end of pre-treatment period, skin samples (1.0 cm diameter circular trephine) were taken from the left midside region of each sheep one week before cortisol

administration, every week during the treatment period and every week after cessation of cortisol injection until week 14 of post-treatment period. The samples were then placed in individual cassettes (Tissue-Tek II, Miles laboratories, Inc. Naperville, Illinois) and dehydrated through a series of graded ethanols and cleared in Histoclear (Ajax Chem., Auburn, NSW) using a Citadel tissue processor (Shandon Southern Products, Ltd. England). Processed skin samples were embedded in paraffin using a Tissue-Tek II embedding centre (Miles laboratories, Inc.).

Embedded skin samples were sectioned in the transverse plane at 8 μm using a rotary microtome. Approximately 60 sections were cut per sample, but only every fifth section was retained. Before staining, all sections were deparaffinised and a special tetrachrome stain "Sacpic" (Auber, 1952) was used to demonstrate follicular tissue components.

Blood sampling

An indwelling catheter (1.00 mm i.d; 1.5 mm o.d.) was positioned in the jugular vein for the duration of experiment and blood samples were taken two weeks before cortisol injection started and two weeks after commencement and two weeks after cessation of cortisol injection. Serial blood samples were taken every 4 h at 0900 h until 2100 h. Another blood sample was collected at 0900 h the following day after an overnight fast. Blood samples collected in heparinized tubes were centrifuged immediately at 1,000 g and the plasma was stored at -20°C until assayed.

Plasma cortisol assay

Kodak Amerlax cortisol RIA kit (Kodak Clinical Diagnostics Ltd., Amersham, UK) was used to measure plasma cortisol concentration. The sensitivity of the Kodak Amerlex cortisol RIA kit is approximately 0.1 μg/100 ml. To verify the performance of the assay, controls were assayed in parallel with plasma samples. All tubes were mixed on a vortex mixer, heated for 60 minutes and centrifuged at 1,500 rpm at 37±2°C. Supernatant was removed by blotting and the amount of radioactivity was determined by counting on a gamma counter. Results were calculated using an RIA curve fit programme based on logit-log plotting. The percentage of bound (%B/B0) relative to the zero standard mean (B0) for each standard and sheep (B) i. e. (B/B0)×100 was calculated. The %B/B0 was plotted against standard concentration curve. The best fitted line through the mean of duplicate points was drawn and the cortisol concentration of sheep was read from the standard curve.

Follicle activity (morphology) and histology of shed fibres

The percentage of active and shutdown primary and secondary follicles was determined from the midside cross section of skin samples as described by Nixon (1993). To estimate the percentage of inactive follicles, approximately 300 follicles were counted per skin sample from 10 to 20 randomly selected follicular groups.

To examine histological changes of shed fibres and distorted end fibres, broken staples were selected and the tip portion of each staple was stained as described by Schlink and Dollin (1995) and mounted on a microscope slide.

Statistical analysis

Analysis of variance statistics was performed using the Super ANOVA Computer package (1989-1990, Abacus concepts, Inc. Berkeley, California) and the means and the standard errors of the means were generated with this program. The measurement of each characteristic was treated independently and Duncan's New Multiple Range Test was then used to compare the characteristics between groups. Super ANOVA was used to test the effects of time, genotype and cortisol dose on the clean wool weight, fibre diameter, follicle shutdown and plasma cortisol concentration. Results were considered significantly different when $p<0.05$.

RESULTS

The effect of cortisol injection on feed intake and live weight

There was no significant difference in the average live weight between sheep groups one month before and one month after the treatment period. During different periods of experiment, all animals consumed their ration and no significant loss of weight was recorded.

The effect of cortisol injection on plasma cortisol concentration

The average plasma cortisol concentration two weeks before commencement of cortisol injection in LL, LH, HL and HH groups was 1.83±0.20, 1.92±0.40, 2.78±0.76 and 2.75±1.17 μg/100 ml respectively. Plasma cortisol concentration was 9.77±1.03, 7.66±1.29, 10.20±2.14 and 6.26±0.94 μg/100 ml in LL, LH, HL and HH groups respectively two weeks after commencement of cortisol injection which was significantly ($p<0.05$) higher than the pre-treatment level (Figure 1). There was no significant interaction between week and group indicating that all 4 groups had a similar pattern of elevation in plasma cortisol concentration. Two weeks after cessation of cortisol injection, plasma cortisol concentration decreased to normal values and no significant difference was found between groups in this period.

Combined high coefficient of variation groups (i.e. LH and HH) had significantly ($p<0.05$) lower plasma cortisol

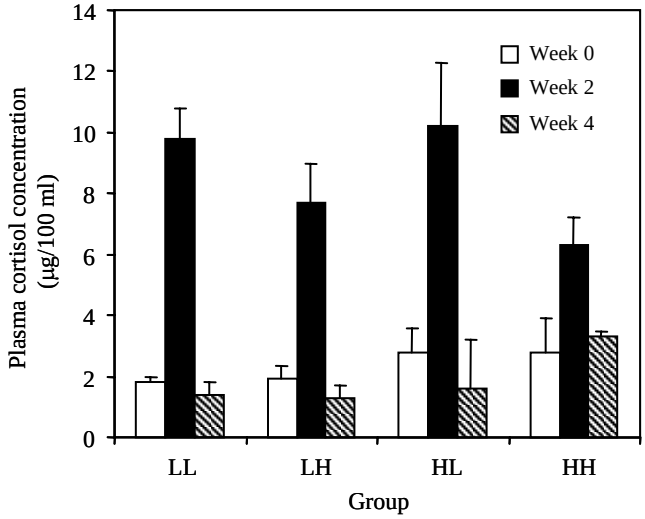

Figure 1. Plasma cortisol concentration (µg/100 ml) for the groups of sheep with different fleece phenotype (LL, LH, HL and HH), measured 2 weeks before (Designated as week 0), and 2 and 4 weeks after commencement of cortisol injection (Means with sem).

concentration (6.97±0.79 µg/100 ml) than the combined low coefficient of variation groups (i.e. LL and HL) (9.98±1.16 µg/100 ml) two weeks after commencement of cortisol injection.

The effect of cortisol injection on wool production and fibre diameter

Wool production : The average clean wool production two weeks before commencement of cortisol injection was 9.19±0.61, 9.63±0.52, 12.17±0.53 and 13.99±1.34 mg/cm^2/two weeks in LL, LH, HL and HH groups respectively (Figure 2). The increase in plasma cortisol concentration was associated with a significant ($p<0.05$) progressive decline in wool production in all groups of sheep. Wool production was 5.58±1.67, 6.61±0.67, 7.4±0.88 and 9.27±0.91 mg/cm^2/two weeks, in LL, LH, HL and HH groups 2 weeks after cessation of cortisol injection. Wool production gradually started to recover and took 8 to 10 weeks before it returned to pre-treatment levels. On average, Strongwool groups (HL+HH) produced about 1.4 times more wool than the Finewool groups (LL+LH) throughout the experiment (Table 2).

Fibre diameter : Selection of sheep resulted in a significant difference ($p<0.05$) in pretreatment fibre diameter (16.21±0.25 to 24.02±0.35 µm) between groups two weeks before cortisol injection commencement. Fibre diameter declined to 15.72±0.42 to 21.29±0.37 µm four weeks after commencement of cortisol injection (Figure 3). Fibre diameter underwent varying degrees of change in

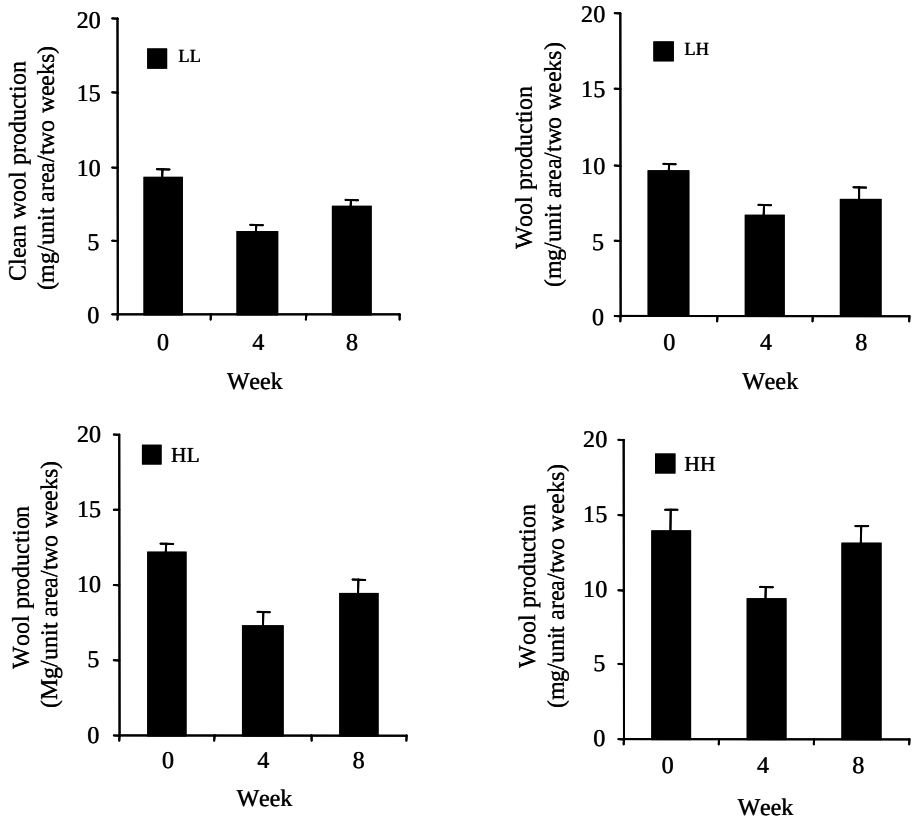

Figure 2. Clean wool production per unit area of skin (mg/unit area/two weeks) for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH), measured 2 weeks before (Designated as week 0) and 2 and 4 weeks after commencement of cortisol injection (Means with sem).

Table 2. Clean wool production per unit area of skin (mg/unit area/two weeks) for Finewool (LL+LH) and Strongwool (HL+HH) Merino sheep, measured 2 weeks before (Designated as week 0) and 4 and 8 weeks after commencement of cortisol injection (Means with sem)

Group	Week		
	0	4	8
Finewool	9.4±0.40[a]	6.1±0.43[a]	7.5±0.41[a]
Strongwool	13.1±0.73[b]	8.3±0.65[b]	11.2±0.80[b]

Superscripts within group comparisons significantly differ at $p<0.05$.

Finewool and Strongwool groups. While Strongwool groups (HL×HH) on average had a decline of 3.2 μm, the decline in Finewool groups (LL×LH) was only 0.28 μm two weeks after cessation of cortisol injection (Table 3).

Table 3. Fibre diameter (μm) of Finewool (LL+LH) and Strongwool (HL+HH) Merino sheep measured at 2 weeks before (Designated as week 0) and 4 and 8 weeks after commencement of cortisol injection

Group	Week		
	0	4	8
Finewool	16.4±0.15[a]	16.1±0.28[a]	17.8±0.25[a]
Strongwool	23.8±0.27[b]	20.6±0.41[b]	23.4±0.40[b]

Superscripts within group comparisons significantly differ at $p<0.05$.

The effect of cortisol injection on the formation of wool break and fibre shedding

Coinciding with the resumption of normal wool growth a few weeks after cessation of cortisol injection, a clear 'break' appeared in the wool of both Finewool and Strongwool sheep. The magnitude of the wool break was similar in all groups of sheep but differences were found between sheep within and between groups. At the site of formation of the wool break, the density of fibres had substantially dropped as a result of fibre shedding. Finewool and Strongwool sheep shed fibres all over the body, but it was more pronounced on the rump and belly areas of sheep. In severe cases complete local wool casting occurred particularly at the rump site.

Histological examinations revealed that shed fibres of Finewool and Strongwool sheep were malformed or degraded, some having holes and cracks. A small number of fibres exhibited swelling containing darkly-stained bodies. A small number of fibres exhibited a region of swelling containing darkly-stained bodies just preceding the point of shedding. Shed fibres carried inner root sheath forming fibre ends with different degrees of distortion. The distorted end of shed fibres fell into four categories depending upon the structure of the fibre end as follows:

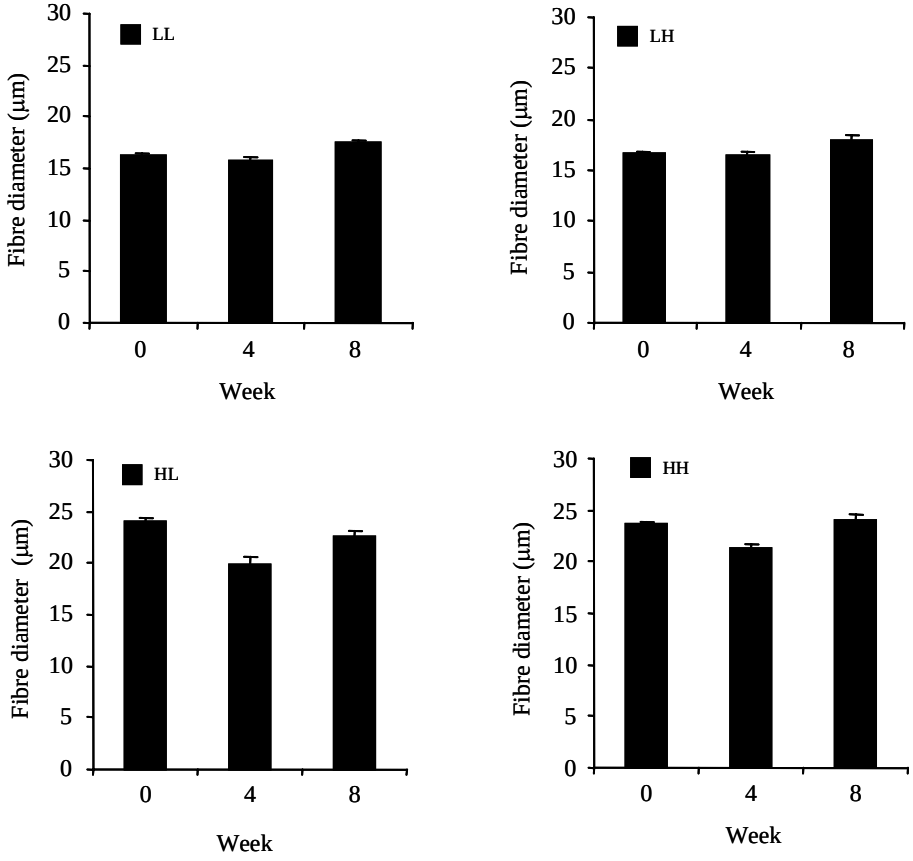

Figure 3. Fibre diameter (μm) for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH); measured 2 weeks before (Designated as week 0) and 4 and 8 weeks after commencement of cortisol injection (Mean±SEM).

i) Club end fibres. On the ends of these fibres, a thick column of stained inner root sheath was carried by shed fibre forming a club like structure (Plate 1).

ii) Brush end fibres. Several thin or sometimes two thick columns of stained inner root sheath were carried by shed fibres, forming a brush like structure (Plate 2).

iii) Step end fibres. A thin stepwise layer of stained inner root sheath was carried by shed fibres forming a step like structure (Plate 3).

iv) Tapered end fibres. The end of such shed fibres was reduced in diameter and a thin column of stained inner root sheath was carried by shed fibre (Plate 4).

The effect of cortisol injection on follicle shutdown

Follicle shutdown was significantly (p = 0.0012) dependent on fibre diameter while coefficient of variation of fibre diameter had no significant effect (p = 0.17) on follicle shutdown. There was no significant difference in the percentage of inactive follicles between groups before initiation of cortisol injection (Figure 4). Elevation of plasma cortisol concentration was associated with a significant (p<0.0001) increase in the percentage of inactive follicles at the end of treatment period. At week 4 when maximum number of follicles had shutdown between 3 to 60 percent of follicles lost activity in different sheep.

While there was no significant difference in the maximum level of follicle shutdown (17.30±6.06 to 21.40±5.26) on average high fibre diameter groups (Strongwool sheep; HL+HH) had significantly (p<0.0001) higher percentage of follicle shutdown (13.5±0.9) than low fibre diameter groups (9.8±0.9) (Finewool sheep; LL+LH) (Table 4).

Finewool (LL+LH) sheep had a higher shutdown primary follicles than strongwool (HL+HH) sheep. 1% of primaries and 17% of secondaries stopped activity in Strongwool sheep, while in Finewool sheep 7% of primaries and 11% of secondaries regressed (Table 5).

Table 4. Percentage of shutdown follicles averaged over time (weeks 1 to 8) for Finewool (LL+LH) and Strongwool (HL+HH) Merino sheep (Means with sem)

Group	Percentage of follicle shutdown
Finewool	9.8^{a} ±0.9
Strongwool	13.5^{b}±0.9

Superscripts between group comparisons significantly differ at p<0.0001.

Table 5. Average number and percentage of primary and secondary follicle shutdown for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH)

Group	Weeks 2 to 8					
	Primary			Secondary		
	Total	FSD*		Total	FSD	
		No	%		No	%
LL	1,050	45	4.3	19,950	2,132	10.7
LH	1,050	101	9.6	19,950	2,194	11.0
HL	2,100	5	0.2	18,900	2,782	14.7
HH	2,100	3	0.1	18,900	3,483	18.4
Total	6,300	154	2.4	77,700	10,591	13.6

* FSD: follicle shutdown.

DISCUSSION

Choosing the dose and duration of cortisol injection

The choice of injection of 1.42 mg of cortisol/kg for all groups of sheep was on the basis of a previous experiment conducted by Ansari-Renani and Hynd (2001). They indicated that at a dose of 1.42 mg/kg and higher dose of 2.86 mg/kg of cortisol per day, caused a significant (p<0.0001) elevation in plasma cortisol concentration. In the present experiment duration of cortisol injection was two weeks a period more likely to be experienced by sheep

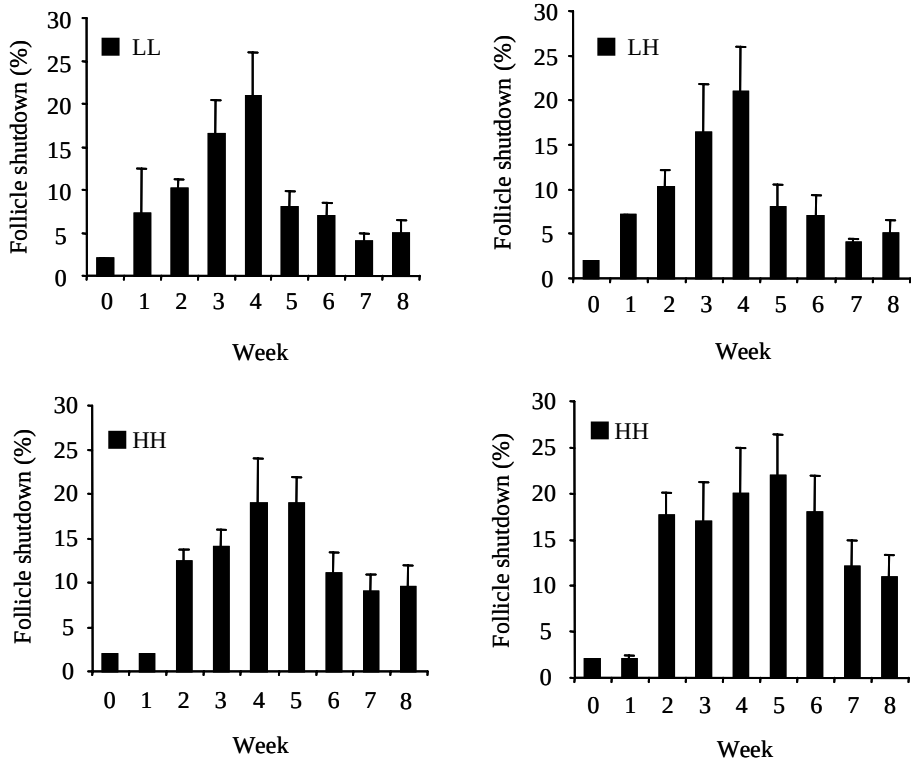

Figure 4. Percentage of shutdown follicles measured at 1 week before (Designated as week 0), during (Weeks 1 and 2) and after (Weeks 3, 4, 5, 6, 7 and 8) commencement of cortisol injection (Means with sem.); for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH).

subjected to environmental or disease stressors. The regime successfully induced significant ($p<0.0001$) follicles to shutdown and allowing weekend wool to be harvested by manually breaking the continuous fibres at the weakened zone. The use of cortisol as a defleecing agent offers a possible solution for avoiding time consuming and expensive mechanical harvesting.

The effect of cortisol injection on feed intake

There is evidence that the nutritional state of sheep influences the effect of cortisol on sheep. Thwaites (1972) showed that poorly-fed sheep had greater fibre breakage than well-fed sheep when cortisol was administered. Hynd et al. (1997) showed a strong effect of stocking rate on the percentage of follicle shutdown. The cause of this interaction between nutrition and response to cortisol administration is not clear. To eliminate the feed factor in the present experiment, all sheep groups were fed at a maintenance level. Bassett (1963) indicated that cortisol injection at a dose of 1.42 mg/kg (on average) for a duration of 12 weeks depressed feed intake. No information is available on the relative effect of a depilatory dose of cortisol injection on the feed intake of Finewool and Strongwool sheep. The results of the present experiment indicated that feed intake was not affected by cortisol injection and no significant loss of weight was recorded in either the Finewool and Strongwool sheep groups. Any difference between groups, then, must reflect effects of cortisol dose.

The effect of cortisol injection on plasma cortisol concentration

There was no significant difference in average plasma cortisol concentration between Finewool and Strongwool groups before commencement of cortisol injection. Plasma cortisol concentration was however significantly ($p<0.05$) higher in the low coefficient of variation of fibre diameter groups (LL+HL) than high coefficient of variation of fibre diameter groups (LH+HH) two weeks after commencement of cortisol injection. The difference in plasma cortisol level between groups of sheep may reflect the difference in adaptability of these groups to external stimuli. Wool growth in Scottish blackface sheep which is characterized by having variable fibre diameter was more sensitive to the depressing effect of cold exposure than in Merino×Cheviot sheep which is more uniform in fibre diameter (Slee and Ryder, 1967). In another study Greef et al. (2005) showed that a strong genetic relationship exists between cvFD and fat content in sheep. From the results of the present study it is possible to conclude that Merino sheep with low coefficient of variation of fibre diameter may be more adaptable to external stimuli. This result is confirmed by the

fact that Strongwool sheep with a high coefficient of variation of fibre diameter had significantly ($p<0.0001$) higher percentage of follicle shutdown. The difference in susceptibility to cortisol injection could be due to differences in systemic factors such as the rate of metabolism and utilization of cortisol.

The effect of cortisol injection on wool growth rate

The elevated plasma cortisol concentration two weeks after commencement of cortisol injection was inhibitory to wool growth in all groups of sheep. The major part of the decline in wool growth occurred two weeks after cessation of injection when wool growth was substantially reduced to almost 50% of the pretreatment level in Finewool and Strongwool groups. The reduction in clean wool production was due to the combined effect of reduction in fibre diameter and an increase in the percentage of inactive follicles and possibly a reduction in staple length (no measurement was made of staple length growth in present experiment). Given the hypothesis that cortisol prevents elongation of fibre cells and delays hardening of inner root heath cells (Chapman, 1989) staple length must have decreased in both Finewool and Strongwool sheep. Due to the fact that the level of decline in wool production was similar in both of these groups of sheep, despite a higher percentage of follicle shutdown and a greater reduction in fibre diameter in Strongwool sheep; Finewool sheep must have a greater reduction in staple length in order to have a similar level of reduction in wool production.

There was a significant ($p<0.0001$) interaction between week and group in effects on fibre diameter, indicating that fibre diameter underwent varying degrees of change in Finewool and Strongwool groups. While Strongwool groups (Groups 3 and 4) on average had a decline of about 3.2 μm, the decline in Finewool groups (Groups 1 and 2) on average was only 0.28 μm two weeks after cessation of cortisol injection. The key questions are: what systemic factor could account for the effect on fibre diameter; and what might be responsible for the differences in response to cortisol between genotypes? The decline in fibre diameter could not have been due to an elevation in plasma cortisol level, since the results of the effect of cortisol dose on follicle shutdown and staple strength of a previous experiment (Ansari-Renani and Hynd, 2001) indicated that cortisol reduces wool production without any change in fibre diameter. Chapman and Bassett (1970) also showed that the effect could be due to other systemic changes. It is highly possible that vasoconstriction induced by cortisol injection could have been responsible for this change.

Fibre diameter in Strongwool sheep was more sensitive to the depressant effects of cortisol than in the Finewool Merino sheep. It is possible that the genotype difference in response may arise from systemic factors. It has been demonstrated that there is a greater blood flow to the skin of Strongwool than Finewool sheep (Hocking Edwards and Hynd, 1991).

The effect of cortisol injection on formation of wool break and fibre shedding

After wool production returned to normal levels, a clear break appeared in the wool of sheep most susceptible to cortisol. The intensity of wool break in both Finewool and Strongwool groups was similar but variation was found between and within sheep of the groups. This difference in the intensity of wool break of individual sheep is related to differences in their susceptibility to cortisol injection.

The pattern of fibre shedding induced by cortisol injection was similar in Finewool and Strongwool groups. Shedding started from the hairy areas of the hind legs and belly region extending to the rump and the rest of the areas of the body. This pattern of shedding is in agreement with the finding that there is a presence of a dorso-ventral and anterior-posterior gradient in wool growth rate (Young and Chapman, 1958). The sequence of shedding in response to cortisol differs from the sequence of natural shedding. In primitive sheep, natural shedding follows a sequential, bilaterally-symmetrical pattern, commencing on the chest and shoulders and spreading to the back on rump (Slee, 1963). Casting of the fleece in the Soay sheep (Boyd et al. 1964) proceeds across the body in similar sequence to that observed by Slee (1959) in the Wiltshire. This is essentially the same as found in Mouflon (Ryder, 1960) and Soay sheep (Slee, 1959).

Histological examinations of shed fibres induced by cortisol injection

At the point of wool break, numerous distorted end structures (club end, brush end, step end and tapered end) were formed by shed fibres of Finewool and Strongwool sheep. The formation of distorted fibre ends is due of changes that take place in the keratogenous zone of the fibre. Due to delayed hardening of the inner root sheath cells in response to the injection of depilatory compounds, normal activity of these cells in the keratogenous zone is disrupted (Chapman and Hardy, 1988). This disruption is accompanied by gross dilation of the endoplasmic reticulum with both intra- and inter-cellular accumulation of fluid and flocculant material (Chapman and Hardy, 1988). As a result, inner root sheath cells slough into the follicle lumen and subsequently are carried by the shedding fibre. The structure of the end of shed fibre depends on the structure of the sloughed inner root sheath in the bulb. As a result of accumulation of fluid, the fibre diameter of small number of fibres increases and lateral enlargement of fibres occurs.

While the majority of such fibres exhibit regions of enlargement as it was found in present experiment, in a small number of these fibre enlargement is confined to only one side.

The effect of cortisol injection on follicle shutdown

The average incidence of shutdown follicles in the pre-treatment period was very low in both Finewool and Strongwool groups (approximately 2%). Through selection for increased production, Finewool and Strongwool Merinos have become 'non shedding' and only a small percentage of follicles remain in the inactive catagen or telogen phases of the hair cyle (Ryder, 1960).

Elevation in plasma cortisol concentration was inhibitory to the normal activity of follicles. A significant ($p<0.05$) difference was found in follicle shutdown averaged over time between Finewool and Strongwool groups. Strongwool sheep (HL+HH) had a significantly higher percentage of shutdown follicles than the Finewool sheep (LL+LH). It is possible to speculate that the greater susceptibility of Strongwool sheep to cortisol may be associated with a greater degree of follicle sensitivity to shutdown, greater rate of blood flow and cortisol presentation to the skin, or to the higher levels of downstream regulators of wool growth such as the number of EGF receptors in the follicle.

Substantial variation in follicle shutdown was found between sheep within Finewool and Strongwool groups. A possible reason for this variation is that individual sheep may metabolize or utilize cortisol at different rates. The rapidity with which cortisol is removed from the circulatory system or the efficiency with which cortisol is utilized within the animal may play an important role in determining the susceptibility of individual sheep to follicle shutdown.

REFERENCES

Ansari-Renani, H. R. and P. I. Hynd. 2001. Cortisol-induced follicle shutdown is related to staple strength in Merino sheep. Livest. Produc. Sci. 69:279-289.

Auber, L. 1952. The anatomy of follicles producing wool-fibres, with special reference to keratinization. Transcripts of the Royal Society of Edinburgh. 62:191-254.

Bassett, J. M. 1963. The influence of cortisol on food intake and glucose metabolism in sheep. J. Endocrinol. 26:539-553.

Boyd, J. M., J. M. Doney, R. G. Gunn and P. A. Jewell. 1964. The Soay on the Island of Hirta, St. Kilda. A study of a feral population. Proceeding of zoological society of London. 142:129-163.

Chapman, R. E. 1989. Follicular malfunctions and resultant effects on wool fibres. In; The biology of wool and hair (Ed. G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marshall). Chapman and Hall, London. pp. 243-257.

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

Chapman, R. E. and M. H. Hardy. 1988. Effect of intradermally injected and topically applied mouse epidermal growth factor on wool growth, skin and wool follicles of Merino sheep. Aust. J. Biol. Sci. 41:261-268.

Hocking Edwards, J. E. and P. I. Hynd. 1991. Blood flow through the skin of high and low-wool producers. Proceed. Nutr. Soc. Aust. 16:206.

Hynd, P. I., Hughes, A., Earl, C. R., and N. M. Penno. 1997. Seasonal changes in the morphology of wool follicles in Finewool and Strongwool Merino strains grazing at different stocking rates in southern Australia. Aust. J. Agric. Res. 48: 1089.

Langlands, J. P. and J. L. Wheeler. 1968. The dyebanding and tattooed patch procedures for estimating wool production and obtaining samples for the measurement of fibre diameter. Aust. J. Exper. Agric. Anim. Husb. 8:265-269.

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

Lynch, L. J. and N. A. Michie. 1973. Laser fibre fineness distribution analyser: A device for the rapid measurement of the mean and distribution of fibre diameter. Wool Technol. Sheep Breed. 20:23-27.

Nixon, A. J. 1993. A method for determining the activity state of hair follicles. Biotech. Histochem. 68:316-325.

Ryder, M. L. 1960. A study of the coat of the Mouflon Ovis Musimon with special reference to seasonal change. Proceed. Zool. Soc. London. 135:387-408.

Schlink, A. C. and A. E. Dolling. 1995. Abnormal shedding contributes to the reduced staple strength of Western Australian Merinos. Wool Technol. Sheep Breed. 43:268-284.

Slee. J. 1963. Birth coat shedding in Wiltshire Horn lambs. Anim. Produc. 5:301-316.

Slee, J. and M. L. Ryder. 1967. The effect of cold exposure on wool growth in Scottish Blackface and Merino×Cheviot sheep. J. Agric. Sci. Cambridge. 69:449-453.

Slee, J. 1959. Fleece shedding, Staple –length and fleece weights in experimental Wiltshire Horn-Scottish Blackface crosses. J. Agric. Sci. Cambridge. 53:209-233.

Spurlock, G. M. and M. T. Clegg. 1962. Effect of cortisone acetate on carcass composition and wool characteristics of weaned lambs. J. Anim. Sci. 21:494.

Thwaites, C, J. 1972. The effects of short-term undernutrtion and adrenocortical stimulation on wool growth. Anim. Produc. 15:39-46.

Young, S. S. and R. E. Champion. 1985. Fleece characteristics and their influence in wool production per unit area of skin in Merino sheep. Aust. J. Agric. Res. 9:363-372.