SEASONAL VARIATION IN PLASMA HORMONE/METABOLITE CONCENTRATIONS IN FLEECEWEIGHT-SELECTED AND CONTROL ROMNEY RAMS

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LIST OF ABBREVIATIONS

C	control
CFW	clean fleeceweight
cm	centimetre
^{0}C	degrees Celsius
DM	dry matter
FW	fleeceweight
g	gram
GFW	greasy fleeceweight
h	hour
IU	international unit
kg	kilogram
l	litre
mg	milligrams
µg	micrograms
MJ ME	megajoules of metabolizable energy
ml	millilitres
mm	millimetre
n	number
SE	standard error
vs	versus
w/v	weight (of solute) per volume (of solvent)

Levels of Statistical Significance

NS	Not significant P>0.10
+	0.10>P>0.05
*	0.05>P>0.01
**	0.01>P>0.001
***	0.001>P

CHAPTER I

INTRODUCTION

INTRODUCTION

Fleeceweight Selection and the Role of Genetic Markers

In New Zealand, sheep related products provide a substantial contribution to the national economy. Traditionally, the national economy has been influenced by the fluctuating prices of both meat and wool in world markets. New Zealand is the second largest wool producer in the world and it is the dominant coarse wool producer, accounting for approximately 45 per cent of world production and 70 per cent of all internationally traded coarse wools (Anon, 1989).

Of the various wool characteristics which determine returns to the farmer, production per head is probably the most important (Wickham and McPherson, 1985). By world standards, the average production of wool by New Zealand sheep is high. The national clip average is 5.5 kg per head and the lambing percentage is just under 100 per cent (Anon, 1989) despite relatively low labour inputs into New Zealand flocks. It is important for each individual farmer, as well as New Zealand as a whole, to maintain a high position in the world market and, in order to do this, ways have to be found to increase the gains made from the production of wool.

One method by which per head production may be increased is through genetic selection. Currently in New Zealand, rams are selected as two tooths (i.e. approximately 15 months of age), using measurements such as their own greasy fleece weight, and possibly that of their relatives, as indicators of superior wool production. Total fleeceweight is calculated for each ram and is sometimes adjusted for

non-genetic factors such as date of birth, birth rank (i.e. single or twin), body weight and maternal age. Once these factors have been taken account of, those rams with the best adjusted fleeceweights are selected and used as sires. Those rams not chosen, due to their lower production figures, are culled.

One of the major limitations inherent in this method of selection is the increase in the generation interval. The generation interval is defined as the average age of the parents when the offspring are born. Thus the generation interval on the male side for most current sheep selection procedures is at least two years (i.e. the rams will be a minimum of two years old when their progeny are born). Generation interval is also at least two years on the female side but tends to be somewhat longer on average because ewes typically remain in the breeding flock until they are 5-6 years old.

The rate of genetic gain per year is equal to the product of heritability and selection differential divided by the generation interval. Thus, one way of maximising genetic gain per year is to minimise the generation interval. Moreover, since hogget selection does not occur until the animals are two-tooths, all the ram and ewe hoggets must be carried through their first winter for selection and first mating as two-tooths (autumn). Consequently, this prevents other stock (e.g. breeding ewes) from being wintered and also means that the breeder has to carry potentially less productive stock (e.g. hoggets). To be able to minimise the generation on the ram side, the breeder must be able to predict the genetic merit of rams at an earlier age (e.g based on lamb fleeceweight) and carry only rams

selected on this basis over the winter. However, the maternal influence (such as effects of birth rank and dam age) and other environmental influences on lamb wool growth are much greater than on the hogget fleeceweight resulting in a lower heritability for lamb fleeceweight compared with hogget fleeceweight.

The heritability of fleeceweight is 0.3-0.4 (Blair et al. 1985) indicating that there is also a large environmental component (e.g. nutrition, disease) to which the individuals are subjected. Thus the accuracy with which the animals can be ranked on genetic merit using one phenotypic fleece production record only (including that at the hogget stage) is relatively low. This constitutes another limitation to improving rates of genetic gain.

Due to the above limitations of current selection procedures, there has been considerable interest throughout the world in alternative methods of selection. The possibility of using genetic markers to increase the rate of genetic improvement in sheep and other domestic animals has received much attention in recent years. McCutcheon and MacKenzie (1989) defined genetic markers as "characteristics of an animal, other than its own production, which may be used to predict genetic merit for production traits." A genetic marker must fulfill a number of criteria before being able to be used successfully. Firstly, it must be easily measured, its assay being both inexpensive and manageable with respect to ease and time involved. Secondly, a marker must be repeatable under a variety of conditions and ideally be able to be measured in both females and males at a young age. Finally, and most importantly, a marker must be genetically correlated with the production trait in question and not

be adversely correlated with other important production traits.

Markers which permitted identification of superior rams and ewes at, or before puberty, could allow selection to be carried out at an earlier age (less than 6-8 months) thereby resulting in the progeny being born when the parents were one year old. By doing this, the generation interval would be effectively halved and the rate of genetic gain doubled, at least on the ram side (assuming that the markers were as accurate as selection on hogget fleeceweight).

Another major advantage of using genetic markers over the current selection procedure could be the ability to cull undesirable rams and ewes at an earlier age. This could result in fewer dry stock being carried over the winter, and so increased profits due to an increased proportion of the stock carried being breeding animals. The use of genetic markers to increase the rate of genetic gain in the sheep industry could also result in increased accuracy of prediction of genetic merit.

There are three main ways by which genetic markers can be found, through the use of selection lines, progeny testing and breed comparisons (Blair et al. 1990). Selection lines can be used to identify possible markers by studying differences in the physiology of sheep from lines of low vs high genetic merit. Lines have been established by selecting ewes of low or high merit (based on their own wool production) which are in turn mated to rams of low or high merit. Repeated matings of this type over a number of years maintain the lines and increase the average genetic differences between the groups.

The phenotypic differences between these lines can be assumed to be due primarily to genetic differences if the lines are selected in a common environment. However, selection lines only allow a comparison of groups differing in average genetic merit since the genetic merit of individual animals within each line is normally not known.

Due to the length of time associated with selection experiments, one of the most important considerations is the cost of establishing and maintaining the lines. For these reasons, selection lines are typically small in size (60 to 200 breeding females) (Blair, 1985). As a consequence of the small population size, it is possible that genetic drift can occur. Thus, physiological differences observed between lines may be due to the effects of genetic drift rather than being correlated responses to the selection (e.g. for fleeceweight). If so, they would not be useful genetic markers. Also, the small size of the selection lines may mean that they are not representative of the population as a whole, so that markers that are apparently effective in the lines may not be useful predictors of genetic merit in commercial flocks.

An alternative approach to the use of selection lines is to employ progeny tests in identifying genetic markers. The principle involved in progeny testing is the use of the average production of the unselected offspring of an individual to predict its breeding value. Sires are bred to a random group of dams, from which the progeny are obtained.

Progeny testing can be used to identify genetic markers as well as to select sires. For example, rams can be chosen for different levels of a potential genetic marker (e.g. plasma urea concentration), bred to a random group of ewes and the progeny obtained. Production characteristics of the progeny (e.g. hogget greasy fleeceweight) can then be measured and the correlation between these characteristics and the level of genetic marker in the sire can be found. The magnitude of these correlations can then be used to determine whether the marker is useful as a predictor of genetic merit for fleeceweight.

An advantage of progeny testing is that it can be used to determine the genetic merit of individual sires. This differs from selection experiments where only the average genetic merit of the line as a whole can be determined. As a result, the great advantage of progeny testing is that markers can be evaluated in rams whose genetic merit is known accurately.

Progeny testing can be used to select for more than one production trait at any one time (e.g. fleeceweight and ovulation rate). This differs from the use of selection lines where selection is generally on the basis of one trait. Likewise, progeny testing can be used to simultaneously evaluate a number of potential markers (measured in the sire) to create an index-based predictor of genetic merit.

The long generation interval is one of the disadvantages of using progeny testing to evaluate markers. The use of progeny testing has been questioned on the grounds that if one must wait to test

sires, the interval between identification of a potential marker and evaluation of its usefulness is lengthened. Therefore, progress is slower than it might be if selection lines were used. As a consequence of this, most research into detecting genetic markers has involved the use of selection experiments. An added disadvantage of progeny testing is its expense, although results can be accumulated over several years. Greater accuracy is obtained when markers are evaluated in large numbers of sires which are progeny tested. However, in practice, it is not possible to test large groups of sires due to the costs and time involved. Consequently, progeny testing schemes to evaluate markers will typically be small in size and results therefore may not apply to the wider population of sires.

Comparisons can be made between breeds to help detect genetic markers. In this situation, different breeds of animals are chosen which exhibit diverse production characteristics with the possibility of differences being due to variation in genetic merit. However, the breeds may be selected on different traits and possibly on more than one trait. Therefore, physiological differences could be a consequence of selection for traits other than the one of interest. Also, as the two groups of animals have not come from the same base population, it is difficult to conclude that their differences are solely genetically based and not the result of environmental variation. Therefore, this method of detecting genetic markers is used only as a guideline for the other two methods of selection stated above.

In general, progeny testing and selection experiments are the most common methods used to detect genetic markers. Progeny testing tends to be carried out initially, after which animals showing superior genetic merit are used in a selection flock for a particular trait of interest.

Selection Flocks Based on Fleeceweight and Related Characteristics

Flocks selected for increased fleeceweight have been established in a number of breeds. The flocks have been classified as either single character selection flocks, in which selection is solely for increased greasy or clean fleece weight, or flocks in which selection is primarily for increased fleece weight but where other characters are taken into account (McGuirk, 1983). Table 1 outlines the several selection experiments for increased fleeceweight which have been reported in the literature. Most of the selection experiments have been based in Australia using Merino sheep. Work has also been carried out in New Zealand using Romney sheep.

Table 1. Summary of selection experiments for increased fleece weight

Breed	Flocks	Size of flock Rams	Ewes	Duration of experiment	References
a) Single trait selection flocks:					
Australian Merino	High CFW[a]	5	100	1951-	1
	Control	10-25	100	1951-	2
Australian Merino	High CFW	1	32-50	1954-1976	3
	Control	5-10	200-250	1948-1976	
New Zealand Romney	High GFW[b]	4	80	1958-	4
	Control	4	80	1958-	
New Zealand Romney	High GFW	5	200	1967-	5
	Control	20	200	1967-	
New Zealand Romney	High GFW	10	200	1985-	6
	Control	10	100	1986-	
New Zealand Romney	High GFW	10	200	1984-	7
	Control	10-15	200	1984-	
b) Multi-trait selection flocks:					
Australian Merino	High CFW with limits on crimp frequency, skin wrinkle and face cover	6	200	1947-1972	8
	Control	10-25	100	1951-	9
Australian Merino	High CFW with limits on skin wrinkle and fibre diameter	5-15	200-600	1950-1978	10
	Control	5-10	200-250	1948-1976	11

Australian Merino	High CFW with limits on skin wrinkle and either fibre diameter or crimp frequency	5-8	100-250	1950-1979	12 13
	Control	5-10	200-250	1948-1976	14
South African Merino	Clean fleece mass with limits on fibre diameter and crimp frequency	5	160	1969-	15
	Control	16	160	1969-	

a=clean fleece weight
b=greasy fleece weight

References

1=Pattie and Barlow, 1974
2=McGuirk, 1980b
3=Turner and Dolling, 1970
4=Blair, 1981
5=Johnson and Dobbie, 1987
6=Hawker et al. 1988
7=Hawker and McEwan, 1986
8=Dun and Eastoe, 1970
9=McGuirk, 1980b
10=Turner et al. 1968
11=Turner and Jackson, 1978
12=Turner et al. 1968
13=Turner and Jackson, 1978
14=Turner and Jackson, 1978
15=Heydenrych et al. 1977

1) Trangie, Australia

Merino selection flocks were first established in Australia around 1950. The main aim of the experiments was to check the prediction that mass selection for characters such as fleeceweight was effective, and to improve the understanding of wool and skin biology. Multi-trait flocks were also established with the aim of not only increasing fleeceweight but also maintaining wool quality and reducing such faults as excessive skin folds (wrinkles) and face cover.

Pattie and Barlow (1974) described the establishment of selection flocks established at Trangie Agricultural Research Station in New South Wales, Australia. Two closed selection flocks were established in 1951, each consisting of 100 ewes mated to five rams. One flock was selected for high clean fleece weight (Fleece-plus) and the other for low clean fleece weight (Fleece-minus). A randomly selected control flock (100 ewes mated to 10-25 rams) was used to measure the response to selection in the Fleece-plus and Fleece-minus flocks over approximately five generations (approximately 15 years). All three flocks were selected from a base population consisting of 1700 ewes and 150 rams, and were maintained in the same environment.

Response to fleeceweight selection in the Fleece-plus flock 'plateaued' after the first two generations of selection. After the second generation, response practically ceased despite continued selection pressure. This was reflected in a realised heritability of 0.07 which was not significantly different from zero. An eventual

plateau may have been expected after several generations based on earlier experiments with laboratory animals and poultry, but cessation of response after the second generation was quite unusual. McGuirk et al. (1986) acknowledged the fact that gains in these flocks were below expectations.

Pattie and Barlow (1974) suggested that sampling variation and the possibility of directional drift in the control flock could be partly responsible for the unusual results. Genotype x environment interaction can also reduce selection responses. These interactions have been recorded in the Trangie flocks where large differences in clean fleeceweight between the Fleece-plus, Control and Fleece-minus flocks at high levels of nutrition were much reduced after low levels of feeding. Also, clean fleeceweight is a complex character with a number of readily measureable components. Therefore, many factors make up fleeceweight production which could also result in increased errors and unusual results in terms of response to selection for clean fleece weight.

2) CSIRO, Australia

At approximately the same time as the first experiments at Trangie began, selection flocks were established at Cunnamulla, Queensland, Australia by the Commonwealth Scientific and Industrial Research Organization (CSIRO). However, after a succession of poor seasons, the flocks were moved to more favourable conditions at Armidale, New South Wales, Australia.

Turner, Dolling and Kennedy (1968) and Turner, Brooker and Dolling (1970) have reported on experiments using the selection flocks of CSIRO. In 1947, breeding experiments with medium-wool Peppin Merinos began with the aim of estimating the heritability or genetic correlations of production characteristics and comparing two methods of sire selection (Turner et al. 1968). Two of the groups which began in 1947 were the S, in which sires were selected on their own performance plus that of their half-sibs, and the MS, with selection on the sires' own performance alone (Turner et al. 1968). Again, a random control group (C) was formed so that the results of selection could be measured.

Turner et al. (1970) studied direct and correlated responses under selection for high and low values of single characteristics : clean wool weight per head, clean wool weight per unit skin area, bodyweight, wrinkle score, fibre number per unit skin area, fibre diameter, staple length and percentage clean yield. It was found that all selection groups showed overall divergence in the character under selection, though response was not always symmetrical. In two cases (staple length and fibre number) there was no continuing response after the initial response to extreme selection of the base parents. Wool weight per unit area was shown to have a greater influence on clean wool weight than that of surface area, with staple length on this occasion being the most important component of wool weight per unit area (Turner et al. 1970).

Turner, Dolling and Kennedy (1968) worked with the two CSIRO selection flocks over two periods - 1950-59 and 1961-64. In the first period, both groups had selection against high average fibre diameter and high degree of skin wrinkle, but in one group (S) the performance of the ram's half-sibs was considered as well as his own, while in the other group (MS) mass selection was practiced for rams. Mass selection for ewes was practiced in both groups. In the second period, mass selection was used for both sexes in both groups, with selection against high fibre diameter and degree of wrinkle in one group (S), and against low crimp frequency and high degree of wrinkle in the other (MS) (Turner et al. 1968).

Responses in clean wool weight were similar in both the S and the MS groups in the first period for both rams and ewes. The annual rates of increase in clean wool weight for S and MS were 2.1 and 1.8% for rams and 2.4 and 2.7% for ewes. Because the rates of increase were similar between the two groups, it was concluded that the method of selection involving the examination of half-sibs (i.e. the S group) was not worthwhile .

Counter-selection against fibre diameter and wrinkle score was successful as there were negligible trends in these two characters. The increases in clean wool weight were largely due to the increase in fibre number per unit skin area. Greasy wool weight, percentage clean yield, body weight and staple length also showed increases with increasing wool weight in both selected groups (Turner et al. 1968).

Selection against crimp frequency in the MS group was effective with crimp number per unit staple length decreasing in both groups. This showed the strong negative genetic correlation between clean wool weight and crimp number. No further response was observed in the second time period.

Turner and Jackson (1978) reported on further work selecting for clean wool weight in the CSIRO Merino selection flocks over the period 1966-74. One group (S), had a ceiling on fibre diameter and degree of skin wrinkle, while the other (MS) had a lower limit on staple crimp frequency and a ceiling on skin wrinkle.

Results showed that the S group made more genetic progress in clean wool weight than the MS group. This was to be expected as clean wool weight and fibre diameter have a low but positive genetic correlation whereas clean wool weight and crimp frequency have a high negative correlation. These results show that selection on staple crimp frequency is an inefficient way of controlling wool quality because its use severely restricts the likely progress in wool quantity.

The suggestion of a 'plateau' was also found by Turner and Jackson (1978) in their study with these groups of Merinos. For animals born during 1961-64 there was a fall in superiority of the selected group over the control group. Response to selection was

recovered in the 1966-74 period which suggests that the loss of response was due to a plateau. It was thought that these inconsistent results in the earlier 1966-74 period were due to a genotype x environment interaction, the nature of which is still obscure.

3) Massey University, New Zealand

The work conducted by Blair et al.(1984) and Blair et al. (1985) provided information on the use of selection flocks for greasy fleeceweight in New Zealand. Two selection flocks (a face-cover group and a fleeceweight group) , and a control flock, derived from the same base Romney flock, were established at Massey University, Palmerston North, New Zealand in 1956.

Ewes were alloted randomly within age to each of the three flocks and rams from the local studs were used. In 1957, replacement 1.5 year old ewes were allocated to the three flocks at random, from the 1955 drop of lambs in the base flock (Blair et al. 1984). Replacement ewes and rams for the face-cover and fleece weight group were selected on the most open faces and the heaviest hogget greasy fleeceweights, respectively. Selection of replacements for the control flock was done at random. Every effort was made to maintain similar conditions across the groups and the flocks were managed as nearly as possible to commercial conditions.

Blair et al. (1984) and Blair et al. (1985) examined both the response to selection for open face and the response to selection for

hogget greasy fleece weight. The response to selection for open face increased in a linear fashion in both sexes. Blair (1981) concluded that selection for high face cover grades should result in a flock of open-face sheep in relatively few generations of selection, depending on the intensity of selection.

A similar linear increase in response to selection for greasy fleece weight was seen in both ewes and rams with the rate of accumulation being approximately 0.2 kg wool per year (Blair et al. 1985). There was no sign of any decline in the rate of response in later years.

Blair (1981) also found that the response in ram hogget greasy fleece weight was only about one-half of that which would have been predicted by equivalent ewe hogget heritability estimates. This difference between the two sexes could be of practical significance to commercial breeders especially as significant genetic gains could be made through more intense selection for greasy fleece weight in the ewe.

4) Ruakura, New Zealand

Johnson (1987) reported an experiment on fleeceweight selection flocks at Ruakura Animal Research Station, Hamilton, New Zealand. Three flocks were established in 1967- a randomly bred Romney flock, a Romney flock selected for hogget body weight and a Romney flock selected for hogget fleeceweight. It was shown that the average hogget fleeceweight over the season 1978/79 was 2.65, 2.71 and 3.17 kg

for the random, hogget body weight and the hogget fleeceweight flocks respectively. Thus, the cumulative response to direct selection for fleeceweight was approximately 0.5 kg over the period from commencement of the study to 1978/79.

5) Invermay, New Zealand

In the study by Hawker and Littlejohn (1986) fleeces from 28,000 Romney ewe hoggets in 14 mobs on 7 farms were weighed. Within each mob, the top 1% on hogget fleece weight (High) and a random 1% (Random) were identified. Of these, the top 0.6% on hogget fleece weight from each mob and a random 0.3% were transferred to Woodlands Research Station where they were managed as one flock. Selection of ewe hoggets on high greasy fleece weight has resulted in a flock with a large advantage in greasy fleece weight relative to the random flock. This advantage was mostly independent of liveweight. Other responses associated with high fleece weight were higher fibre diameter, staple length and staple strength.

6) Woodlands, New Zealand

In 1984-85, a high fleeceweight Romney flock was established at Woodlands Research Station by screening ewe and ram hoggets with the highest differentials for hogget greasy fleeceweight (Hawker and McEwan, 1986). A control flock was also established consisting of 200 ewes and 10-15 rams, while the selection flock was made up of 200 ewes and 10 rams. Hawker and McEwan (1986) noted that fleeceweight-selected hoggets had higher mean fibre diameters and staple lengths

than control hoggets. Staple strength was also greater in the FW animals and there was a signficant positive relationship between staple strength and clean fleeceweight.

7) Tygerhoek, South Africa

In 1969, the Tygerhoek Merino flock was divided into five groups, two of which were selected on clean fleece mass at 18 months of age with limits on fibre diameter and crimp frequency (Heydenrych et al. 1977). Both of these groups contained 160 ewes and 5 rams. A control flock was also established consisting of 160 ewes and 16 rams.

After five years of selection, the clean wool production of the two fleece mass groups was 0.445 kg and 0.400 kg greater than the control groups (Heydenrych et al. 1977). This increase was attributed to the specific selection procedures carried out. It was concluded that the production of clean wool in Merino sheep could be increased substantially by direct selection for clean fleece mass at 18 months of age, in as short a period as five years, without adversely affecting wool quality (Heydenrych et al. 1977).

Correlated Responses

Selection experiments for clean fleeceweight are well established. Characters which are correlated with fleeceweight (e.g. fibre diameter, staple length, follicle density and skin wrinkle) are also of interest as they may identify possible mechanisms by which

selection can bring about an increase in wool production. Turner (1958) studied the components of fleeceweight and found that gains in fleeceweight could be initially attributed to increases in wool production/unit area of skin or to an increase in the wool growing surface area. Both of these components can be further subdivided into many factors and it is likely that these factors act together to cause the overall response to selection for clean fleece weight.

1) Wool production/unit area of skin

a) Fibre Diameter

In New Zealand, fibre diameter is one of the characters which brings about the increases in fleece production. In the study by Blair (1981) it was concluded that fibre diameter increased by about 1.5 microns during 21 years evaluation. However, Blair (1981) also notes that an increase in mean fibre diameter of this magnitude is unlikely to affect the value of Romney wool in New Zealand. Yearling data from the Massey University flocks were examined and several significant correlated responses were found. Mean fibre diameter showed positive correlated responses in both yearling sexes. The correlated response of +0.2% per year agrees with that obtained by Johnson (1981). The actual change in yearling mean fibre diameter was about 1.5 microns for both sexes which agrees with Blair's previous work in 1981.

For the Australian Merinos the contribution of fibre diameter to increasing wool weight is difficult to determine since, in order to retain the Merino's fine wool, a ceiling was placed on fibre diameter to prevent it from increasing. Both experiments by Turner, Dolling and Kennedy (1968) and Turner and Jackson (1978) had restrictions on fibre diameter. Turner et al. (1968) found that there were negligible trends in fibre diameter indicating that counter-selection had been effective in preventing an increase in this characteristic. Similarly, Turner and Jackson (1978) found a low, positive correlation between fibre diameter and clean wool weight.

b) Staple Length

In the study by Blair (1981), staple length was shown to have a correlated response of 0.38cm/kg GFW and 0.35cm/kg GFW for ewe and ram hoggets respectively. Even though this response was small, there is normally a moderately strong correlation between greasy fleece weight and staple length. This correlated response of 0.2% per year and an actual increase of 15mm in staple length was in agreement with Johnson (1981). In the study by Hawker and Littlejohn (1986) it was shown that the High hoggets had higher staple lengths (range +2 to +24mm) than the Random hoggets. The relative advantage of the High hoggets in staple length and fibre cross-sectional area (10 and 17% respectively) accounted for most of the 38% advantage in clean fleece weight.

From these results it can be concluded that selection for increased fleece weight will increase wool production per unit area of

skin, partly through the increases in staple length. These results are more apparent in the New Zealand Romneys compared with the Australian Merinos but there is evidence that staple length could play a role in increased fleece weight in the Merino.

c) Follicle Density

Follicle/fibre density can be considered as a product of the density of primary follicles and the ratio of secondary to primary follicles. Follicle density has been well researched in Australia as being part of the overall increase in fleeceweight, but it has not yet been examined in New Zealand research experiments. The increase in fibre density often seen in Australian selection experiments could possibly be enhanced by the counter-selection against increased fibre diameter, since there is a strong negative genetic correlation between fibre diameter and fibre density. Therefore, the increases in follicle density may not necessarily occur if there is no restriction on fibre diameter. However, as most Australian experiments do have a ceiling on fibre diameter, the increase in fleece weight is often due, in part, to an increase in follicle density.

Saville and Robards (1972) conducted experiments on the flocks at Trangie involving comparisons between different types of Merinos. A nucleus flock was maintained which had a selection programme based on both fleeceweight and crimp frequency. It was found in this flock that density was of great importance because simultaneous selection for crimp frequency prevented increased fleeceweight from being expressed in the diameter of the fibres. Therefore, even though the

fleeceweight of the Nucleus sheep was similar to that of the Fleece-plus group, the increase in fleeceweight of the Nucleus group resulted from a higher fibre density and surface area rather than increased fibre diameter (Saville and Robards, 1972).

Turner et al. (1968) found that in the CSIRO selection flocks the fibre number per unit skin area increased at 1.9% per annum with its sole source of increase being the ratio of secondary to primary follicles.

In the selection flocks at Trangie, it was found that fibre density was one of the most important components which contributed to the differences between the Fleece-plus and Fleece-minus flocks, accounting for 47% of the difference. Wiiliams (1973) also found that the density of follicles was 31% greater in the Fleece-plus ewes compared with the Fleece-minus ewes.

Turner, Brooker and Dolling (1970) found slightly unusual results with the CSIRO selection flocks when describing the selection for increased wool production per unit area. They state that selection for increased fibre density was less effective or even ineffective in increasing fleeceweight. This was also the view of Rendel and Nay (1978) who claimed that selection for increased density or secondary/primary follicle ratio did not increase fleece weight.

Heydenrych et al. (1977) studied the secondary to primary (S/P) wool follicle ratio in lambs from the Tygerhoek Merino flock produced from ewes selected for a wider S/P ratio. The S/P ratio has a high

positive genetic relationship (r=0.73) with follicle density (Jackson et al. 1975) which is one of the most important components of clean fleece mass. It was found that lambs selected for a wider S/P ratio had 3.369 and 3.432 more secondary wool follicles per primary follicles than the control lambs. Heydenrych et al. (1977) also noted that the correlated response obtained in clean fleece mass with selection for a wider S/P ratio showed a positive genetic correlation between the two traits. They concluded that selection for a wider S/P ratio at three months of age could substantially increase the production of clean wool in Merino sheep.

2) Wool growing area

a) Skin Wrinkle

The degree of skin wrinkle is associated with the wool growing area of the sheep as the more skin folds in the sheep the greater the surface area for wool production. This component is more apparent in the Merino breeds as wrinkling in Romneys is probably unimportant in contributing to greasy fleece weight.

Most experiments conducted in Australia based on selection for increased greasy fleeceweight have had a ceiling on skin wrinkle. However, in the study by Robards et al. (1976), 60 hogget ewes from flocks selected for 15 years for skin wrinkle (Folds-Plus), against skin wrinkle (Folds-Minus) and randomly selected control (Random) flocks were compared. It was found that selection for increased skin fold had little effect on wool production. Also, the increases in the wool bearing area resulting from increased skin fold in the selection

flock was offset by a significant decline in wool production per unit area. There was also a negative correlation between fibre length and skin folds as a result of which the Folds-Plus ewes had lower mean fibre length. Increased skin folds was also associated with lowered reproductive performance and increased mortality.

Because of these effects of increasing skin folds, most experiments have involved selection against wrinkling. In the study by Turner et al. (1968), selection for high clean fleeceweight was accompanied by upper limits for fibre diameter and skin wrinkle. There were negligible changes in either of these components showing that selection against these was successful. Turner and Jackson (1978) stated that there were positive genetic correlations between wrinkle score and crimp frequency or wrinkle score and fibre diameter. Restraint against increased diameter would reinforce selection against increasing wrinkle score whereas selection to keep a high crimp value would tend to raise the wrinkle score. Therefore, if both fibre diameter and high crimp frequency are selected against, the degree of skin wrinkle will also be kept at a minimum.

Seasonal Differences In Wool Growth Between Selection Lines

Several researchers have studied the seasonal pattern of wool growth in sheep (Coop, 1953; Story and Ross, 1960; Ebling and Hale, 1970; Bigham et al. 1978; Rougeot et al. 1984). All of these studies have shown a rhythmical pattern of production characterized by maximum production in summer or early autumn and reduced production in midwinter.

Story and Ross (1960) measured wool production, fibre diameter and fibre length on a monthly basis in sheep and found that the growth of wool was three times faster in summer than in late winter. The seasonal rhythm of wool growth showed a maximum occurring around February and a minimum around August.

Coop (1953) carried out extensive research into the seasonal rhythm of wool growth. Trials with maiden Corriedale ewes were conducted over a period of 2.25 years with different groups receiving various levels of feed. A pronounced seasonal rhythm of wool growth was found with a maximum occurring in February-March and a minimum in July-August. Maximum production was three times greater than the minimum production and this occurred in spite of relatively minor liveweight changes.

Doney (1966) studied the seasonal rhythm of wool growth in two contrasting breeds (Merino and Cheviot) on various levels of nutrition. It was found that Merino sheep seemed to be influenced mainly by nutritional variation rather than seasonal variation. Slee and Carter (1961) studied Merino and Wiltshire Horn sheep kept in a similar environment and under adequate planes of nutrition, and found much greater seasonal differences in the latter breed than in the former. Similarly, Williams and Schinkel (1962) suggested that, in grazing Merino sheep, the wool growth cycle was almost entirely determined by nutritional variation.

Differences in the seasonal pattern of wool growth, and in total wool growth, have been observed in the Massey University fleeceweight-selected and control lines. McClelland et al. (1987) studied the seasonal pattern of wool growth in 99 fleeceweight-selected and 60 control lambs from the Romney selection flocks. The proportional advantage of FW lambs over C rams was greatest in the winter. FW lambs showed a less marked winter depression in wool growth compared with the C lambs. FW lambs reached minimum wool growth in April while C lambs produced the least wool in June (McClelland et al. 1987).

Physiological Differences Between Selection Lines

1) Dietary intake

Individual animals vary both in their voluntary feed intake and in the efficiency with which feed is converted into body tissue or wool (Hamilton and Langlands, 1969). There have been some inconsistencies in studies investigating the correlation between intake and wool production. However, in general, results have shown wool production to be positively correlated with intake and efficiency under both ad libitum and restricted feeding levels. Dolling and Piper (1968) have suggested that the differences in wool production between the CSIRO high and low fleeceweight flocks are due to differences in efficiency of conversion of food to wool, rather than to differences in intake of food per unit body weight. They found

that 92% of the wool weight difference was accounted for by the difference in gross efficiency and that overall there was a negligible difference in voluntary food intake between the selected and control sheep. This viewpoint was also held by McGuirk (1983) who concluded that sheep from the Australian Merino high fleece weight flocks do not consume more feed, but are more efficient in converting feed to wool. Similarly, Saville and Robards (1972) found that in the sheep flocks at Trangie, selection increased both fleece weight and body weight but did not influence intake per unit body weight.

McClelland et al. (1987) examined the response in efficiency of wool production in the closed Romney flocks at Massey University. It was found that digestible dry matter intake per $kg^{0.75}$ did not differ between fleeceweight and control rams. However, differences in wool growth and wool growth efficiency were significant throughout most of the trial period. McClelland et al. (1987) concluded that selection for higher fleeceweight had produced animals capable of better utilization of nutrients for wool growth throughout the year.

Thomson et al. (1989) carried out an experiment using Romney sheep from the Massey University fleeceweight-selected and control lines. Two diets - medium quality (chaffed meadow hay) and high quality (lucerne chaff) - were fed under controlled steady state conditions to determine whether there were differences in the dry matter digestibility and nitrogen retention in sheep fed both diets. They found no significant difference in voluntary feed intake between the fleeceweight and control animals on the medium quality diet although control sheep had slightly higher intakes than fleeceweight

sheep. However, on the high quality diet, voluntary feed intake was higher in fleeceweight animals than in control sheep. Although these results are contrary to some other reports (Dolling and Piper, 1968; Saville and Robards, 1972; McGuirk, 1983), Thomson et al. (1989) suggested that the high intakes achieved on the lucerne chaff diet could be due to carryover effects from feeding the lower quality meadow hay diet.

2) Digestive ability

It is possible that the greater efficiency of high fleeceweight sheep in converting food to wool is due to an improved ability to digest dietary components. However, in the study by Piper and Dolling (1969), where Merino ewes selected for high clean wool weight per head and ewes from a random control flock were fed a high protein (H), medium protein (M) or low protein (L) diet, it was found that the gross energy intakes of groups H and M were almost identical. Also, the similarity of body weight changes indicates that their net energy intakes were similar. Piper and Dolling (1969) concluded that the differences between selection flocks in digestive ability were small, variable and in no instance significant.

McClelland et al. (1986) conducted two pen-feeding experiments with Romney rams from fleece weight-selected (FW) and control (C) flocks. In the first experiment, rams were fed cut ryegrass/clover pasture ad libitum for six weeks while, in the second experiment, rams were fed lucerne-based diets in three periods. In the first experiment, control rams showed a significantly greater dry matter

digestibility than FW rams. However, in the second experiment, no differences in liveweight, and no consistent trends in dry matter digestibility, were found. Thus it appears that selection for increased fleeceweight has not consistently changed the ability of animals to digest dietary nutrients.

Thomson et al. (1989) found in their study using the Massey University selection lines, that there was no difference in digestibility between the lines when fed a meadow hay diet although fleeceweight animals had a lower digestibility than control animals when fed lucerne chaff. This differs from results found by McClelland et al. (1986).

3) Metabolic rate

Animals which differ in wool production may also differ in metabolic rate. In the study by Graham (1968), two groups of rams chosen from a high wool production flock and a low wool production flock, respectively, were studied. Graham (1968) concluded that high wool producing sheep had higher metabolic rates than low producers although some of this difference was possibly due to the larger skeletal size of the high producing sheep.

Other researchers have found varying results. For example, Williams and Winston (1965) concluded from their results that there was no difference between the high fleeceweight and the low fleeceweight flocks in metabolic rate. Therefore, it is likely that

metabolic rate makes little significant contribution to genetic variation in wool production.

4) Utilization of amino acids

Wool growth is affected markedly by the availability of amino acids to the wool follicles (Reis, 1979). It is apparent that the quantity of amino acids absorbed by the sheep has a great influence on wool growth rate. Studies carried out by Reis (1969), where both protein and energy intake of sheep were varied, suggested that the energetic requirements for wool growth were largely satisfied on low intakes of energy and that the major requirement was then specifically for amino acids.

Wool proteins are characterized by a high overall content of sulfur, ranging from 2.7-4.2% (Reis, 1965). Most of this is present as cystine, with small amounts of cysteine and methionine. Glutamic acid, serine and glycine are also present in relatively large amounts.

The components of wool proteins have been studied extensively (Broad et al. 1970; Bradbury, 1973; Crewther, 1976) and can be classed into three main groups:

(1) Low-sulfur proteins, accounting for approximately 67% of the proteins in wool and containing all of the methionine and most of the lysine. These proteins are the main structural components of the microfibrils.

(2) High-sulfur proteins, accounting for large quantities of cystine, proline and serine. These proteins make up the major proteins of the matrix surrounding the microfibrils.

(3) High-tyrosine proteins which are rich in tyrosine and glycine and unusual in that they lack many amino acids. These proteins occur mainly in the matrix of the fibre cortex.

a) Cystine

Approximately 90% of the sulfur in wool occurs as disulphide bonds of cystine and 2% as the thiol groups of cysteine. Cystine and cysteine are readily interconverted in the mitochondria by oxidation and reduction or through thiol-disulphide exchange. An adequate supply of cystine for protein synthesis in the wool follicles is essential to maintain a high rate of wool growth. Marston (1935) was the first to suggest that the supply of cystine was likely to be the primary nutritional factor limiting wool growth. Although the addition of cystine to the diet had little effect on the wool growth rate, Marston (1935) did observe a 34% increase in wool growth rate in sheep given daily subcutaneous injections of 1g of L-cysteine. By supplementing the sheep in this way, both the cystine content and the sulfur content, of the wool increased. This was later confirmed by Reis and Schinkel (1963) who found that abomasal infusion of L-cysteine at a rate of 1.5-3.0 g/day substantially increased wool growth rate of sheep consuming moderate amounts of roughage.

Downes et al. (1970) found that large doses of L-cystine injected intraperitoneally act as depots from which the cystine is

released at a rate sufficient to increase wool growth. By avoiding administration of cystine via the diet, the cystine is not degraded in the rumen and can therefore be utilized for wool growth. Marston (1935) found that the dietary administration of cystine to sheep on a low protein diet was followed by a significant increase in growth of wool. However, this increase was trebled when cysteine was injected subcutaneously so as to avoid the destruction of the free amino acid by bacteria in the alimentary canal. In general, it can be concluded that dietary supplements of sulfur-amino acids are either ineffective, or inefficient, for stimulating wool growth unless the amino acids are protected from ruminal degradation.

b) Methionine

Methionine is an essential amino acid for sheep. Wool protein contains little methionine but dietary methionine can be converted to cysteine in the mitochondria, to be used for the synthesis of wool proteins. Only about 3% of the sulfur in wool is present as methionine. If methionine is given as a dietary supplement, it is extensively degraded in the rumen which consequently results in poor wool growth responses. However, methionine is degraded more slowly than cystine and it is therefore possible that relatively large dietary supplements of methionine may stimulate wool growth (Bird and Moir, 1972).

Attempts have been made to stimulate wool growth by giving large, infrequent doses of methionine. Langlands (1970) found that whereas daily abomasal supplements of methionine (1.0-1.5 g/day)

stimulated wool growth, the same total dose given only once or twice per week was either much less effective or was ineffective. These results suggest that methionine is rapidly metabolised and excreted.

It is thought that high levels of methionine can cause inhibitory effects on wool growth. Reis (1967) concluded that 9.84 g/day of DL-methionine usually inhibits wool growth. However, when Broad et al. (1970) infused large amounts (8-10 g/day) of DL-methionine into the abomasum of sheep, it was found that there was no suppression of the formation of proteins for wool growth. Also, the proteins produced under these conditions appeared to be normal, with an amino acid composition essentially identical to that of the proteins produced during infusion with lower levels of methionine (Broad et al. 1970).

The supply of amino acids available to the wool follicle can exert a considerable effect on the rate of wool growth (Reis, 1979). Also, differences have been observed between animals in the proportion of specific amino acids in the wool and this may be associated with differences in the capacity of the animal for wool production. Both a phenotypic and a genetic association between wool production and sulfur-amino acid content have been demonstrated in a number of studies (Reis and Williams 1965; Reis and Tunks 1968; Reis 1979;). It has been shown that high wool producers grow wool of a lower sulfur content. Williams (1973) found that wool clipped from Fleece Plus ewes had a lower sulfur content than that of Fleece Minus ewes and that this difference was independent of dietary intake. Reis et al. (1967) also found an inverse relationship between sulfur content of wool and wool production in two groups of Trangie Peppin Merino ewes

from flocks genetically different in wool production. The mean sulfur content of wool from the high producers was 3.13% (at grazing) and 3.06% (controlled feeding), whereas mean sulfur content for the low producers was 3.55% (at grazing) and 3.59% (controlled feeding).

It is possible that the greater wool production of fleeceweight-selected sheep compared with that of control sheep is due to a dilution of the same amount of sulfur in a greater quantity of low sulfur protein. However, differences in total sulfur output between selection lines have also been found. Williams et al. (1972a) found that the total sulfur output was greater from high fleeceweight animals especially where the availability of sulfur-amino acids was increased. Therefore, an increase in total sulfur output could be responsible for the greater wool production of the fleeceweight-selected sheep, even if the sulfur content of the wool was lower than that in the control sheep.

Williams (1973) showed that the genetic difference in wool production was associated with a difference in utilization of cystine for wool growth. In that study, mature Merino ewes were chosen from the Trangie flocks selected for high and low clean fleece weight (Fleece Plus and Fleece Minus respectively) and were infused intravenously with radioactively labelled L-cystine for 12-14 hours. Wool was clipped subsequent to the infusions. It was found that the specific radioactivity of wool fibres was less in high fleeceweight ewes than in low fleeceweight ewes. However, during infusion of L-cystine the two flocks showed similar mean specific radioactivities in the plasma. These results were later confirmed by Williams (1976).

It is thought that the differences between the high producing and low producing sheep in the levels of labelled cystine in the wool is due to an intermediate pool of cystine causing the dilution of labelled cystine during its passage from plasma into the wool fibre. High fleeceweight sheep could possibly have a larger cystine pool, compared with low fleeceweight sheep, which dilutes the labelled cystine. This results in a lower cystine content in the wool fibres. The differential dilution may reflect differences in the size or turnover of an intermediate pool, or pools, of cystine (Williams, 1979).

The net entry rate of cystine gives a measure of the cystine availability to the animal. Under steady-state conditions, the entry of cystine into plasma is balanced by its removal from this pool (Williams *et al.* 1972). Williams (1976) investigated the entry rate of cystine in sheep administered an intravenous injection of L-cystine. It was found that the entry rate was greater in the low fleeceweight ewes than in the high fleeceweight ewes. Also, Williams *et al.* (1972) found that genotype for wool production had no effect on the entry rate of cystine when measured by a continuous infusion. Therefore, differences seen between high and low wool producing lines of sheep in the concentration of free cystine in plasma, when there is no difference in net entry rate, must represent an altered distribution space of cystine. It should also be noted that free cystine is not the only pool of cystine available in the blood, there

being considerable quantities of this amino acid incorporated into reduced glutathione in the red blood cells (Clark, 1987).

5) Hormonal differences

Research has shown that the endocrine system is actively involved in the regulation of wool and hair growth (Ebling and Hale, 1970). However, few attempts have been made to relate hormonal differences to wool growth in selection lines or other genetically divergent groups of sheep. The influence of hormones on growth of wool is complicated by the seasonal patterns of wool growth exhibited by sheep. The constantly changing hormonal status of the individual animal, from season to season and from week to week, results in a wide range of responses occurring between animals and, consequently, interpretation of results is made more difficult.

The endocrine involvement in the growth of wool includes the pituitary and thyroid glands, the adrenal cortex and the gonads. The anterior lobe of the pituitary gland secretes several important hormones which regulate the proper functioning of the thyroids, gonads, adrenal cortex and other endocrine organs.

a) Pituitary Hormones

(i) Growth hormone

Growth hormone (GH) controls protein metabolism and alters nutrient partitioning to promote processes such as growth and

lactation. GH has also been shown to have an effect on wool growth in sheep although results are inconsistent. A number of experiments have used bovine GH or ovine GH to determine their effect on wool production. In the study by Johnsson et al. (1985), daily subcutaneous injections of bovine GH were given from 8 to 20 weeks of age to crossbred female lambs. A highly significant increase in greasy fleeceweight was observed over the 12 weeks trial period in animals receiving bovine GH. Reklewska (1974) showed that purified bovine "somatotrophic hormone" (i.e. growth hormone) significantly increased wool production in 36 crossbred lambs. Treated lambs showed a 9% increase in greasy fleeceweight over control lambs when shorn at 11 months of age. However, immediately after cessation of GH treatment, the average wool production per unit of skin area was similar for both treated and control sheep.

The above results were not in agreement with those of Wheatley et al. (1966) who found that the wool growth of sheep treated with ovine GH decreased during treatment, but increased immediately after cessation of treatment and remained above control levels for at least 20 weeks. They stated that the maximum increase in wool growth above the control group was about 15% and was only reached in the tenth week after the last injection of ovine GH. In the study by Hough et al. (1987), concentrations of several metabolites and hormones were measured in the blood/plasma of Merino sheep selectively bred for high and low wool production. There was no significant difference between the fleeceweight-selected and control rams in plasma growth hormone.

Responses to GH administration have been varied. It has been suggested that the delayed wool growth response to GH administration could reflect an immune response to the hormone (Ferguson, 1954) although this is an unlikely explanation in more recent studies which have used purified hormone. Therefore, the effects of growth hormone on wool are still uncertain and further research is needed in this area.

(ii) Prolactin

Plasma prolactin is known to be positively associated with the annual rhythm of wool growth and appears to be related to the moulting process of wool in primitive breeds. In the study by Lincoln and Ebling (1985), Soay rams were given implants of melatonin to determine effects on the seasonal cycle in reproduction, on prolactin secretion and on moulting. It was found that as the level of plasma prolactin increased, there was an earlier and gradual onset of the moulting of the body wool. Also, when prolactin levels decreased due to the administration of melatonin, the growth of the winter coat took place. Lincoln and Ebling (1985) also noted that administration of prolactin to mink could lead to moulting and development of the summer coat. Therefore, prolactin seems to have an effect on the annual rhythm of wool growth although this still remains poorly understood. To date, there has been no evidence for differences between selection lines in plasma prolactin or growth hormone concentration.

b) Gonadotrophic Hormones and Gonadal Steroids

Gonadotrophic hormones are hormones which have a stimulatory effect on the gonads. Two such hormones secreted by the anterior pituitary are follicle stimulating hormone (FSH) and luteinizing hormone (LH). Some work has been carried out on the effects of gonadotrophic hormones on pelage growth. Rust et al. (1965) administered various gonadotrophic preparations to hypophysectomized mink. It was found that the coat was not affected in any way except for the loss of some hair at the injection sites. Therefore, it was concluded that gonadotrophic hormones had little influence on seasonal pelage growth. However, FSH and LH influence the gonadal steroids (e.g. oestrogen and testosterone) and these may in turn affect wool growth.

(i) Oestrogens

Oestrogens have an effect on wool follicle activity as well as on the growth of wool. Specifically, it is thought that oestrogen causes the delay of follicular activity and reduces the rate of wool growth. Slen and Connell (1958) conducted an experiment in which oestradiol and diethylstilbestrol (a synthetic oestrogen) were administered to sheep and the effects on wool growth were monitored. Sheep treated with oestradiol showed a significant decrease in average fibre length and in the average weight of clean wool. There was no effect of diethylstilbestrol on wool growth during the 118 day period after the first implant of 12 mg was given. However, after a second implant of diethylstilbestrol (administered three months after the first), wool growth was depressed below the level of the control sheep for a period of 112 days. Diethylstilbestrol also caused a

significant reduction in the average fibre length of the treated sheep.

(ii) Testosterone

Slen and Connell (1958) administered testosterone to Corriedale yearlings (ewes and wethers) and found that it had a stimulatory effect on wool production. The testosterone-treated sheep showed an increase in thyroid gland weight which would suggest an increase in activity and involvement of the thyroid gland in wool growth. However, Johnston et al. (1956) reported results contrary to these in their experiments on adult ewes. They obtained a significant reduction in thyroid weights in ewes injected with 50 mg of testosterone twice weekly.

Southcott and Royal (1971) obtained similar results to those of Slen and Connell (1958) in their study with Merino wethers over a 5-year period. They found a trend towards greater wool production as the dose of testosterone propionate increased. The average percentage increase in greasy wool production obtained was 4%. They also concluded that testosterone acts to increase wool growth by stimulating thyroid function via the anterior pituitary.

c) Corticosteroids

It has been shown that corticosteroids can have some effect on follicular activity. ACTH (Ebling and Hale, 1970) and cortisone

(Ferguson et al. 1965) have been shown to reduce the diameter of fibres in sheep. Chapman et al. (1982) noted that administration of glucocorticoids to sheep caused shedding of some fibres and decreased the diameter and length growth rate of those fibres that continued to grow. Similarly, hair growth is stimulated by adrenalectomy in the rabbit and the mouse, and in sheep if given minimal replacement therapy (Ferguson et al. 1965).

Chapman et al. (1982) examined the effects of dexamethasone trimethylacetate (a synthetic glucocorticoid) on the fleece of Merino sheep. They found that in the most affected animals, the mitotic activity and size of the follicle bulbs decreased during the first five days of treatment. Subsequently, fibre and inner root sheath cells withdrew from around the dermal papillae and were replaced by cells, from the outer root sheath, which developed autophagic vacuoles. Regression of follicles proceeded until brush-ends formed. Therefore, it is likely that dexamethasone trimethylacetate has an inhibitory effect on wool follicles and fleece growth in sheep. However, further studies are required to ascertain whether this synthetic glucocorticoid has a direct effect or whether it acts indirectly.

d) Thyroid hormones

Thyroid function is controlled by thyroid-stimulating hormone (TSH) secreted by the anterior pituitary. TSH acts on the thyroid gland causing the release of the biologically active triiodothyronine (T_3) and thyroxine (T_4). T_4 is later converted to active T_3 in the

body tissues (e.g. liver, muscles). The secretion of TSH is in turn regulated in part by a direct inhibitory feedback of high circulating thyroid hormone levels on the pituitary.

Chapman et al. (1974) studied the effects of thyroxine administration and thyroidectomy on the development of skin and wool follicles of sheep foetuses. They stated that most primary follicles continued to develop after thyroidectomy although the rate of follicle maturation was retarded. Administration of thyroxine hastened the maturation of primary follicles. The initial stages of secondary follicle development were not affected by sub-normal concentrations of thyroxine and administration of thyroxine did not accelerate the development of the original secondary follicles.

In sheep, T_4 administration causes an increase in wool production. Lambourne (1964b) conducted experiments with Merino wethers to study the effects of T_4 implants on wool growth. It was concluded that T_4 increased the growth of wool by increasing the length of the wool fibres but had little effect on the fibre diameter. Lambourne (1964b) also found that there was a decrease in the efficiency of annual wool production by about 20% after repeated T_4 implants. T_4 treatment caused an overall increase in food intake (20-25%) which was greater than the increase in wool production (7% greasy weight, 3-7% clean weight). Therefore, the efficiency of wool production was lower in T_4-treated sheep.

Hough et al. (1987) studied the difference in plasma T_4 concentration between Merino ewes selected for high and low

fleeceweight. Sheep selected for low fleeceweight maintained a significantly greater plasma concentration of T_4 than those selected for high fleeceweight. Clark et al. (1989) found similar results in their study, with fleeceweight-selected rams maintaining lower concentrations of T_4 than control rams. The differences between the lines suggest the possible use of T_4 as an indicator of genetic merit for fleece production although they bear little relationship to the known effects of exogenous T_4 on fleeceweight.

e) Melatonin

Melatonin is synthesized and released by the pineal gland and plays a key role in the growth of pelage fibre. It is thought that melatonin alters the secretion of melanocyte stimulating hormone (MSH) from the anterior pituitary gland which in turn affects the fibre growth cycle.

In the experiment by Rose et al. (1984), three groups of mink were exposed to natural changes in daylength (controls), reduced photoperiod or natural changes in daylength plus a melatonin implant. Mink receiving melatonin and those on reduced photoperiod moulted their summer pelage and grew winter pelages earlier than controls. Rose et al. (1984) concluded that short photoperiods stimulated moulting and growth of the winter pelage. Also, as the winter pelage occurred earlier in melatonin-treated mink, they suggested that the photoperiodic effects on fur growth of mink are mediated through the pineal gland via the secretion of melatonin. Therefore, melatonin is

one of the hormones that functions physiologically to relay effects of changing photoperiod, and exogenous melatonin induces the response by simulating the short photoperiods characteristic of winter.

The effects of melatonin are also apparent in sheep. Some studies have shown that a melatonin implant or exposure to short-days is associated with the growth of the winter fleece (Lincoln and Ebling, 1985). Again, melatonin plays a key role in mediating the effects of changing phtotperiod on wool growth. Therefore, a long duration of melatonin secretion signals short days and hence the induction of the winter coat (Lincoln and Ebling, 1985).

Harris et al. (1989) administered a pharmacological dose of sustained release melatonin over a month in early summer to both fleeceweight-selected and control rams from the Massey University Progeny Test lines. Although there was no evidence of a causal relationship between melatonin and seasonality of wool production, there was evidence that melatonin played a direct role in affecting reproductive fucntion of the male sheep (Harris et al. 1989). Melatonin treatment was sufficient to produce a change in reproductive characteristics (in this case testis diameter), but there were no significant effects on either greasy or clean fleeceweight.

f) Epidermal Growth Factor

Epidermal growth factor (EGF) has much potential as a defleecing agent in sheep. Thorburn et al. (1981) stated that there

was marked hypertrophy of cells in the sebaceous glands and follicle sheath of Merino foetuses after infusion of 500-800 µg/day over 3-5 days. There were also degenerative changes in primary wool follicles with many collapsed fibres, and a reduced primary to secondary ratio.

Moore et al. (1981) noted that partial or complete defleecing of sheep was possible depending on the dose and site of injection. They infused at least 12 mg of EGF into sheep which resulted in the loss of the whole fleece within 7 days. However, infusion of 3 mg of EGF interrupted the growth of some of the fibres and led to the formation of a partial break in the fleece. Intradermal injections were found to inhibit fibre growth but the effects were confined to the site of injection. Subcutaneous administration of EGF resulted in rapid shedding of the entire fleece (Moore et al. 1981).

6) Blood metabolites

a) Glucose

Hough et al. (1987) measured concentrations of several metabolites and hormones in blood/plasma of Merino ewes selectively bred for high or low wool production with a view to finding differences between the genotypes which might prove useful for identifying superior animals. No significant difference was found in plasma glucose concentration between the high and low fleeceweight lines. Similarly, Clark (1987) found that although fleeceweight-selected rams generally maintained higher plasma glucose

concentrations than control rams, the difference between the selection lines was not consistent.

b) Reduced Glutathione (GSH)

It is thought that the major function of reduced glutathione (GSH) in erythrocytes is the transport of cystine to body tissues. Interest in the study of GSH is mainly due to its implications in many physiological and biochemical processes and its possible relationship with productive traits such as growth rate, body size and wool growth (Rizzi et al. 1988).

Hopkins et al. (1975) studied the effects of GSH on Merino ewes drawn from the closed single selection flocks maintained at the Agriculture Research Station at Trangie, N.S.W. It was found that sheep selected for low clean fleeceweight showed a significant increase in erythrocyte GSH concentration. Similarly, sheep with a low mean GSH concentration maintained a significantly higher mean wool growth rate than sheep with a high mean GSH concentration.

Williams et al. (1972) noted that high fleeceweights in Merino sheep were associated with low plasma concentrations of free cystine. Therefore, the genetic effects on GSH concentration are similar to those on the plasma concentration of free cystine. However, little is

known about the relationship between the plasma concentration of free cystine and GSH concentration.

These results are contrary to those of Clark (1987) who noted that fleeceweight-selected and control rams showed no difference in erythrocyte GSH concentrations.

(c) Urea and Creatinine

Differences between fleeceweight-selected and control lines of Romney sheep in plasma urea concentrations have been investigated in three recent studies. McCutcheon et al. (1987) and Clark et al. (1989) found that the control rams maintained significantly higher plasma concentrations of urea and creatinine than fleeceweight-selected rams. However, this difference was dependent on the level of feeding and was apparent only under controlled feeding conditions.

Thomson et al. (1989) conducted a third experiment using the Massey University fleeceweight-selected and control Romney flocks. Animals were fed diets of meadow hay or lucerne chaff. It was found that fleeceweight-selected animals had approximately a 1mM lower plasma urea concentration than control animals on both diets.

In the study by Hough et al. (1987) plasma urea concentration was measured in Merino ewes selected for high or low wool production. No significant difference was seen between the high fleeceweight sheep and the low fleeceweight sheep. Urea and creatinine levels do not

appear to have been measured in other fleeceweight selection lines of sheep.

Genetically based differences in circulating urea and creatinine have also been observed in lines selected for lean tissue growth and milk production. Carter et al. (1989) examined ciculating metabolite concentrations in 10 rams from each of the Massey University High and Low Backfat Southdown selection lines. Low line rams exhibited reduced baseline concentrations of plasma urea during both fasting and refeeding.

Bremmers et al. (1988) conducted a trial using ram hoggets from the same backfat selection lines which were offered lucerne chaff at 0.7 and 1.2 times maintenance. They concluded that significant differences in plasma urea concentration were apparent only after 1.2 times maintenance feeding with concentrations being greater in the High Backfat line rams than in those from the Low Backfat line. Also, Low Backfat line rams exhibited greater concentrations of creatinine in plasma than High Backfat line rams, particularly at 0.7 times maintenance. It was concluded that Low Backfat line rams broke down muscle protein more readily during underfeeding, perhaps because they had larger muscle protein reserves, and this resulted in the higher creatinine concentration. These results are in contrast of those of McCutcheon et al. (1987) who found that when Romney rams were fasted, the creatinine concentration in fleeceweight-selected and control lines remained stable with the control line maintaining higher creatinine concentrations throughout.

Van Maanen et al. (1989) also found that High Backfat line rams maintained consistently greater plasma urea concentrations than Low Backfat rams throughout a 24 hour sampling period in which rams were fed every two hours and during fasting and refeeding. However, plasma creatinine concentrations were only slightly greater in High Backfat line rams compared with those from the Low Backfat line and this difference was not significant.

Differences in plasma urea concentration have also been observed in pig selection lines with low plasma urea concentrations being associated with selection for reduced fatness. In the study by Mersmann et al. (1984) 12 pigs were used, six from a line selected for maximal backfat thickness and six from a line selected for minimal backfat thickness Pigs were fed either a low or high fat diet. Maximal backfat thickness (obese) pigs had higher plasma urea nitrogen levels than the minimal backfat thickness (lean) pigs regardless of diet. This is in agreement with results obtained from studies with sheep in that the genetically superior animals for lean growth showed a lower plasma urea concentration than those animals of lesser genetic merit.

In studies conducted by Tilakaratne et al. (1980) and Sejrsen et al. (1984) it was found that plasma urea concentration was negatively correlated with breeding value for milk production in cattle. Both of these trials involved fasting and refeeding periods. It was found that the differences in plasma urea between selection lines were greatest during fasting. For example, Sejrsen et al. (1984) blood sampled 15 bull calves of differing breeding values at

3.5 and 7 months of age. During this time, the calves were placed on a feeding regimen of two days of ad libitum feeding, five days of fasting and two days of refeeding. It was found that plasma urea concentrations during ad libitum feeding and early in the fast were similar in both groups. However,from the second day of fasting at 3.5 months, and the fourth day of fasting at 7 months, plasma urea was higher in the bulls with low breeding values (Sejrsen et al. 1984). This difference in plasma urea concentration was significant only at 3.5 months of age. Tilakaratne et al. (1980) found that the level of plasma urea was higher in the low breeding value calves than in the high breeding calves, particularly during a period of fasting.

MacKenzie et al. (1988) obtained different results in their experiments with young Friesian bulls. A fasting and refeeding regimen was adopted, with bulls being fasted for three days before being refed. Plasma urea concentration initially rose during the fast and then remained stable. Following refeeding, plasma urea concentration rose again and the two lines diverged. During the refeeding period the plasma urea concentration was significantly greater in the high breeding index bulls compared with the low breeding index bulls. MacKenzie et al. (1988) suggested that this difference in plasma urea concentration may reflect differences in the rate of entry of urea from ruminal degradation of dietary amino acids, rather than genetic variation in utilization of absorbed amino acids for production.

7) Mechanisms which may be responsible for between-line differences in circulating urea and creatinine

Evidence of differences in plasma urea and creatinine concentrations between animals of high and low genetic merit has been seen in a number of different selection lines. Generally, genetically superior animals have lower levels of urea compared with genetically inferior animals and, in the Massey University fleeceweight selection lines, there is a parallel difference in creatinine concentration. These consistent differences between selection lines would suggest that plasma urea, and to a lesser extent plasma creatinine concentration, may prove to be useful markers of genetic merit for wool, milk and lean meat production. It is therefore important to understand the mechanisms which cause these differences in plasma urea and creatinine concentrations. Plasma urea arises from two main sources. Firstly, it is produced from ammonia generated in the rumen by the action of microbes on dietary protein and non-protein nitrogen. The ammonia is absorbed across the rumen epithelium and, because it is toxic, must be converted to urea by the liver. Secondly, urea is produced through the deamination of amino acids which are excess to requirements for protein synthesis or whose carbon-skeletons are required for gluconeogenesis. Creatinine arises as a byproduct of protein breakdown in muscle. Therefore, there are three main ways by which differences between selection lines in circulating urea and creatinine could arise - through differences in metabolite space or distribution, in the rate of entry of urea/creatinine and in excretion rate of these metabolites from plasma.

(a) Urea/creatinine Space or Distribution

One possible mechanism for genetically-based differences in circulating urea/creatinine is that genetically superior animals could have a smaller urea/creatinine space or distribution. Therefore, if a load of urea or creatinine was administered to the animal (e.g. increased urea entry after feeding), the initial plasma concentration would be greater in the genetically superior animals because the metabolite would be distributed in a smaller space. Also, due to the smaller space or distribution, genetically superior animals would be able to eliminate the urea/creatinine more rapidly from the system (because a greater proportion of the space would be cleared by the kidney per unit time) so that plasma concentrations (particularly of urea) would be lower in genetically superior animals during the postprandial period. While this mechanism has received little direct attention with respect to selection line differences, the changes in plasma/urea concentrations which occur during and after feeding in fleeceweight-selected and control Romney rams (McCutcheon et al. 1987) are not consistent with the hypothesis of differences in urea/creatinine space or distribution. On the other hand, such differences in urea space might explain the patterns of blood urea seen in dairy cattle of high versus low genetic merit (MacKenzie et al. 1988).

(b) Rate of Entry of Urea and Creatinine

Another theory that could explain the differences in plasma urea concentrations between high and low genetic merit animals

involves the rate of entry of urea, from ruminal degradation of dietary amino acids or deamination of absorbed amino acids, and creatinine from the breakdown of muscle protein.

Differences in plasma urea concentrations between high and low genetic merit animals could occur as a result of more efficient use of amino acids for protein synthesis and consequent reduced requirement to deaminate amino acids in the high genetic merit line. This would mean reduced rates of urea formation as more amino acids were being utilized, instead of being "wasted" and their amino nitrogen being excreted as urea.

This mechanism is best illustrated in the study by Clark (1987) where fleeceweight-selected and control sheep were given a continuous intravenous infusion of L-methionine. The plasma urea concentration in the control sheep decreased to approximately the same level as that of the fleeceweight sheep and then returned to its original level once the administration of methionine ceased. This suggests an increased utilization of non-sulfur containing amino acids by the control sheep for wool production which consequently caused a decrease in urea entry rate and hence in plasma urea concentration, i.e. the methionine supplementation allowed a greater proportion of amino acids to be used for wool production and consequently there were fewer amino acids to be deaminated and excreted as urea.

Results suggesting differences in efficency of amino acid use for production have also been obtained from experiments involving

cattle. Sinnett-Smith et al. (1987) conducted a study using calves of high and low genetic merit for milk production and concluded that the difference in plasma urea concentration was due to a different amino acid utilization by the two lines. They suggested that animals of high dairy merit derived energy preferentially from fat stores and therefore did not need to catabolize amino acids to the same extent as animals of low genetic merit.

Mersmann et al. (1984) studied the difference between genetically obese and lean pigs in plasma urea concentration and found that urea nitrogen was greater in obese pigs regardless of diet. They suggested that the lesser muscle mass of obese pigs compared with lean pigs probably reduced the protein requirement of obese pigs. Consequently, amino acids were utilized as energy sources to a grester extent by obese pigs than lean pigs. This would have resulted in increased urea entry rates and greater plasma urea nitrogen concentrations in the obese pigs compared with the lean pigs. However, urea entry rates were not measured directly in this study or in the studies conducted with divergent lines of dairy cattle.

Differences between high and low genetic merit animals in plasma creatinine concentrations could be due to a difference in entry rate of creatinine into the plasma pool. However, changes in the entry rate of creatinine seem to occur mainly when the animals are fasted. Bremmers et al. (1988) observed a significant increase in plasma creatinine levels when rams were underfed, presumably because of increased breakdown of muscle protein. This was the only study which found a higher concentration of creatinine in high genetic merit

animals compared with low genetic merit animals and this only occurred at the 0.7 times maintenance feeding level.

McCutcheon et al. (1987) found that plasma creatinine concentration was elevated after feeding in Romney sheep. This was thought to be due to a decrease in plasma volume since entry rates of creatinine into plasma would be expected to decline immediately after feeding. The difference between the fleeceweight-selected and control sheep in plasma creatinine was not affected by feeding and is unlikely to reflect differences in creatinine pool size or entry rate. Studies investigating the entry rate of creatinine have not yet been carried out in dairy/beef cattle or pigs.

(c) Urea Excretion and Glomerular Filtration Rate

Another possible mechanism for the differences between lines in plasma urea concentration is the urea excretion rate. It is possible that high genetic merit animals clear urea more rapidly than low genetic merit animals so that their plasma urea concentrations are lower. As the level of urea in the plasma decreases it will eventually counterbalance the increased rate of excretion of urea. Eventually the entry rate of urea will equal the level of excretion of urea and the plasma urea concentration will remain constant. Thus, animals genetically predisposed to high urea clearance rates could be expected to attain urea levels in plasma which are consistently lower than those of animals with low urea clearance rates.

Clark et al. (1989) suggested that between-line differences in plasma creatinine concentration may also reflect differences in the glomerular filtration rate (i.e. in clearance rate from plasma by the kidneys). As plasma creatinine is neither actively secreted nor reabsorbed in the nephron, changes in glomerular filtration rate can be related to changes in plasma creatinine. As fleeceweight-selected sheep have low concentrations of both urea and creatinine (compared with controls) it is likely that differences in glomerular filtration rate between these lines contribute to the differences in circulating levels of both metabolites.

Clark et al. (1989) found that although plasma urea concentration declined in the control rams during methionine infusion, there was no effect of methionine treatment on the difference in plasma creatinine levels between the two lines. This implies that the mechanism which caused the decrease in plasma urea concentration of control rams did not affect their creatinine levels. Clark et al. (1989) concluded that the results implied variation between the lines in glomerular filtration rate, the higher glomerular filtration rate of FW sheep being responsible for their lower circulating creatinine levels and partially responsible for their lower urea levels. The decrease in plasma urea concentration in control rams during the methionine infusion was therefore thought to be due to an alteration in the utilization of amino acids rather than a change in kidney function.

McCutcheon et al. (1987) found in their study with fleeceweight-selected and control Romney rams that high creatinine

clearance rates were associated with low plasma urea concentrations. Animals with high glomerular filtration rates would be expected to have low plasma concentrations of urea and creatinine since, if the glomerular filtration rate is high, more of these substances will be filtered and removed from plasma (McCutcheon et al. 1987).

Thomson et al. (1989) found that in sheep fed on two different diets (i.e. meadow hay and chaffed lucerne), FW animals had a higher creatinine clearance rate when fed the meadow hay diet than C animals. It was concluded that the level of nutrition at which measurements were made had a major influence on the differences expressed between two selection lines in postabsorptive utilization of nutrients.

It is therefore likely that differences seen between the fleeceweight-selected and control lines in plasma creatinine concentration are due to variation in glomerular filtration rate, while differences in plasma urea concentration may be a reflection both of differences in glomerular filtration rate and of differences in the efficiency with which amino acids are used for wool production.

Purpose and Scope of the Investigation

Genetic selection for fleeceweight is one method by which per head wool production may be increased. A limitation of this method is that the generation interval cannot be lowered to its minimum due to poor accuracy of selection prior to the first breeding season. This in turn limits the rate of genetic gain. Also, the low heritability of fleeceweight indicates that there is also a large environmental component to which individuals are subjected.

Due to the limitations of current selection methods for improving genetic gain, there has been much interest in the use of genetic markers to increase the rate of genetic improvement. Markers would allow identification of superior animals at an earlier age and would therefore decrease the generation interval and increase the rate of genetic gain.

Previous research has shown that plasma urea/creatinine concentrations and plasma thyroxine concentration could potentially be used as genetic markers for fleeceweight. It is already apparent that differences in these hormones/metabolites exist between fleeceweight selection lines, with low fleeceweight animals maintaining higher concentrations of urea/creatinine and thyroxine compared with high fleeceweight animals.

The mechanisms by which differences in plasma urea and creatinine arise are still unclear. A number of mechanisms have been suggested, as previously discussed. Of these, differences in urea/creatinine space or distribution have received little attention and it is unlikely that this mechanism plays a major role in the differences between fleeceweight selection lines. The rate of urea entry (a function of differences in utilization of amino acids) and the excretion rate of urea (a function of the glomerular filtration rate) are more promising mechanisms for study in terms of differences between fleeceweight-selected and control lines.

Seasonal variation in wool growth is well known in sheep and seasonality of wool growth (or a lack of it) may be an important

selection criterion in future. The ability to reduce the winter decline in wool growth could result in greater average wool production throughout the year and in wool of higher tensile strength. Since the Massey University selection lines differ in seasonal pattern of wool growth, as well as in total wool growth, it is imperative that potential genetic markers be examined at different times of the year to determine whether genotype by season interactions exist in physiological parameters as well as in wool growth.

The purpose of this study was therefore to examine the seasonal variation in plasma hormone/metabolite concentrations in the Massey University fleeceweight-selected (FW) and control (C) lines. Of particular interest was seasonal variation in between-line differences in plasma concentrations of urea, creatinine and thyroxine. If the seasonal pattern of wool growth in these lines was paralleled by a seasonality of between-line differences in hormone/metabolite concentrations, this would provide some clue as to the possible causal nature of the relationship between potential markers and fleece production. It would also help to identify the optimum times when animals should be blood sampled to provide measures of potential genetic markers (i.e. when differences between the lines in potential markers were greatest).

CHAPTER II

SEASONAL VARIATION IN PLASMA HORMONE AND METABOLITE CONCENTRATIONS IN FLEECEWEIGHT-SELECTED AND CONTROL ROMNEY RAMS

Seasonal Variation in Plasma Hormone and Metabolite Concentrations in Fleeceweight-selected and Control Romney Rams

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Abstract

The magnitude of between-line variation in plasma urea, creatinine, triiodothyronine (T_3) and thyroxine (T_4) concentrations was examined in rams from the Massey University fleeceweight-selected (n=16) and control (n=16) lines of Romney sheep at three nominal ages (5,9 and 13 months) which corresponded to three different seasons (summer, winter and spring respectively). The objective of the study was to examine whether the between-line difference in levels of these hormones/metabolites depended on age/season and to determine the physiological mechanisms responsible for the between-line differences in urea and creatinine.

The animals were housed in metabolism crates for two weeks (10-day adjustment period, 4-day experimental period) and were fed lucerne chaff at a rate of 1.3 times maintenance. Rams were subjected to natural photoperiod and water was available ad libitum. Before the commencement of the experimental period, jugular cannulae were

inserted to allow serial blood sampling to take place. Basal plasma concentrations of urea, creatinine, T_3 and T_4 were taken at the three ages after which rams were subjected to a urea/creatinine challenge (120 mg/kg urea; 3 mg/kg creatinine).

Fleeceweight-selected (FW) rams maintained higher rates of greasy and clean wool growth throughout the year compared with control (C) rams. Significant differences were found between the lines in basal plasma urea and creatinine concentrations only at 9 months (winter) with FW rams maintaining consistently lower concentrations than C rams. No signficant differences were found in plasma T_3 or T_4 concentrations. Plasma urea concentrations rose sharply at the beginning of the challenge with C rams maintaining higher concentrations than FW rams. Plasma creatinine concentrations showed little between-line variation during the challenge. Estimates of urea and creatinine spaces and clearance rates showed no significant difference between lines across the three ages/seasons but a significant difference in urea space and clearance between lines at 9 months of age with FW rams having higher values than C rams.

Differences in urea and creatinine concentrations could be due to a difference in space, which in turn could be related to variation between the lines in body composition at 9 months of age, and/or due to clearance rates which reflect kidney function and the blood flow through the kidneys. However, this does not explain the lack of variation in creatinine concentrations during the challenge.

It was suggested that if selection for fleeceweight was based on plasma urea and creatinine concentrations, the optimum time of the year for selection would be in winter (i.e. at 9 months of age) when the greatest difference between the lines is apparent. However, this would mean that selected rams could not be mated until they were 18 months old, as a result of which the generation interval could not be reduced by use of these genetic markers.

Introduction

In many wool production systems, selection of rams is based on their greasy or clean fleeceweights at about one year of age. The major limitation of this method is the longer generation interval, compared with selection at puberty, which in turn reduces the rate of genetic gain. Moreover, the heritability of yearling fleeceweight is 0.3-0.4 (Blair et al. 1985) so that accuracy of selection is relatively low.

The use of "genetic markers" as selection criteria at six months of age could potentially overcome limitations of current selection systems (Blair et al. 1990). These are physiological traits which are correlated with genetic merit for production traits (e.g. fleeceweight). Genetic markers should be measurable in young animals, preferably under field conditions, and should not be antagonistically correlated with other important production traits. To date, attempts to identify potential genetic markers for fleeceweight and other traits have primarily involved the study of physiological differences

between lines of animals divergently selected on the basis of production traits (Blair et al. 1990).

Plasma urea and creatinine concentrations could potentially be used as genetic markers for fleeceweight. A number of studies have shown that sheep from the Massey University fleeceweight-selected line exhibit significantly lower plasma concentrations of urea and creatinine than those from the control line (McCutcheon et al. 1987; Clark et al. 1989; Thomson et al. 1989). Sheep of high genetic merit for fleeceweight have also been found to have low circulating thyroxine concentrations, both in the Massey University lines (Clark et al. 1989) and in the Trangie lines (Hough et al. 1987).

Single-trait selection for greasy fleeceweight in the Massey University fleeceweight-selected lines has altered the seasonal pattern of wool growth as well as total wool growth (McClelland et al. 1987). Thus the possibility exists that the magnitude of between-line differences in circulating hormone and metabolite concentrations may also vary with season. The objective of this study was therefore to examine the seasonal variation in plasma hormone/metabolite concentrations in the Massey University fleeceweight-selected and control lines with a view to determining optimum sampling times for the use of these parameters as genetic markers for fleeceweight.

Materials and Methods

Animals

Thirty-two Romney rams, 16 each from the Massey University Fleeceweight-selected (FW) and Control (C) lines, were used in the study. Details of these lines have been provided by Blair et al. (1984). Briefly, they have been developed by single-trait selection for greasy fleeceweight (FW line) or random breeding (C line) since 1956. Each flock is maintained at 70-80 ewes per year, with ewes being culled at 5.5 years of age and rams being replaced every year. Four 1.5-year-old rams have been used for mating in each flock since 1958. Each year ewes within each flock are randomized, within age group, into the four sire groups. Shearing takes place at lambing to ensure a constant wool growth period to yearling shearing. The flocks are grazed together and are managed as closely as possible to commercial conditions.

Yearling ewe greasy fleece weight in the FW flock increased by about 1kg over 9.6 generations of selection (Blair, 1986). This corresponds to a realized heritability of about 0.2 which is lower than the 0.3 typically assumed (Blair et al. 1985). There was no evidence of a decline in the rate of response to selection (Blair, 1986).

Housing and Feeding

The rams were randomly divided into two blocks of 16, for ease of management, with eight FW and eight C rams in each block.

Experiments were carried out at three nominal ages (5,9 and 13 months), with the following procedure being the same at each nominal age. The animals were housed in metabolism crates in the Massey University Animal Physiology Unit and were weighed in order to permit calculation of feed requirements. Natural lighting (via large doors and windows at either end of the barn) was used to maintain normal photoperiod. Rams were allowed a 10-day adjustment period before the experiment started. Chaffed lucerne hay was fed at rates equivalent to 1.3 times maintenance. Maintenance requirement was calculated as 0.7 MJ ME /$Kg^{0.75}$ (Rattray, 1986) and the feed was assumed to contain 9.5 MJ ME /Kg DM (Coop, 1986). On alternate days, 2g of a mineral supplement (94% sodium chloride, 6% sodium molybdate) was fed with the chaffed lucerne hay to counteract possible copper toxicity. Fresh water was available ad libitum. Refusals were weighed once daily (at approximately 1600 h). At the end of the adjustment period, rams were reweighed so that the feed allowance could be adjusted according to the new liveweights.

Cannulation Procedure

Jugular cannulae were inserted on day 11 (where day 1 = entry of sheep into the metabolism crates). The cannulation site was clipped and then cleaned using 70% ethanol. Local anaesthetic (10% Xylocaine, Astra Pharmaceuticals Ltd., N.S.W., Australia) was sprayed over the jugular vein at the cannulation site. Once the anaesthetic had taken effect, an Angiocath intravenous placement unit (14G Argyle Medicut "T" sleeve, Sherwood Medical Brunswick Company, St Louis, Mo., United States) was inserted through the skin and into the jugular

vein. Upon obtaining blood from the vein, the needle was withdrawn from within the sleeve. A sterile cannula (Internal diameter 1.0mm, external diameter 1.5mm, polyethylene tube-Dural Plastics and Engineering, Dural, N.S.W., Australia) was inserted through the sleeve and 100mm into the jugular vein. Blood was withdrawn to ensure flow through the cannula. The sleeve was then withdrawn, the cannula flushed with heparinized saline, and a plug secured on the end of the cannula. The cannula was fixed in place by wrapping at the wound site with Sleek tape (Smith and Nephew Medical Limited, Hull, England), tying suture material firmly around the tape and passing a single suture through the skin. Aureomycin powder (Cyanamid of New Zealand Ltd., Papakura, New Zealand) was applied to the site of cannulation to prevent infections. The cannula was then positioned behind the neck for easy access and held in place with 75mm elastic adhesive bandage (Elastoplast). Systenet (International Surgical Netting S.P.A., Via Luigi Einaudi 12-10024 Monralieri (TO), Italy) was placed over the bandage to prevent the cannula from being caught or pulled out. Patency of cannulae was maintained with sterile physiological saline containing 100 IU/ml sodium heparin (New Zealand Pharmaceuticals Limited, Palmerston North) and 0.4ml/l oxytetracycline (Pfizer Laboratories Ltd., Wiri, Manukau City). Rectal temperatures were recorded on the day of cannulation and penicillin (3-4 mls, Streptopen, Glaxo New Zealand Limited, Palmerston North), was administered to prevent infection. On day 15, cannulae were removed and Aureomycin powder was applied to the site of cannulation. Penicillin was also administered to each animal and rectal temperatures were taken on days 14 and 15 to ensure that no infection occurred at cannula withdrawal.

At the conclusion of the experimental period, rams were placed in a holding paddock for two days so that they could be monitored, and were then returned to the main flock (grazing mixed perennial ryegrass/white clover pasture), until the next time period.

Experimental Procedure

The experiment was conducted in two periods at each nominal age.

Period 1: Basal Hormone/metabolite Concentrations

On day 11, rams were reweighed and fed 1.3 times maintenance based on their new liveweights. At 1600 h on day 12, rams were fed 58% of their new ration. On day 13, 8.3% of the feed was offered every two hours commencing at 0600 h and concluding at 2200 h. At 2400 h, 67% of the ration was given, thus bringing the total amount of feed offered on days 12 and 13 to 1.3 times maintenance/day. On day 13, at 0600, 0800 and 1000 h, cannulae were checked to ensure that they were patent and were flushed with heparinized saline. Blood samples (10ml) were withdrawn via the cannulae every two hours commencing at 1200h and concluding at 2400h, in order to obtain basal plasma hormone and metabolite concentrations. Rams were fasted from 2400 h on day 13 until the end of the experimental period (i.e. 1200 h on day 15).

Period 2: Urea and Creatinine Challenge

On day 14, rams were given an intravenous injection of urea (120 mg/kg bodyweight) and creatinine (3 mg/kg bodyweight) which was injected, over a period of two minutes, through the jugular cannula in approximately 4ml of saline. Blood sampling commenced at 0900 h with three samples being taken before the challenge (at -60, -40 and -20 minutes) and the challenge being administered at 1000 h. Blood samples were then collected at 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300, 360 and 420 minutes after the challenge.

Collection and Processing of Blood

Blood samples were withdrawn from the cannulae and were placed in centrifuge tubes containing 0.1ml of 50% (w/v) sodium citrate as the anticoagulant. Plasma was separated by centrifugation (2500g, 4°C, 20 minutes) and pipetted off into duplicate vials. These were then stored at -20°C for later analysis.

Chemical Analyses

Urea and creatinine concentrations in individual plasma samples were measured using the autoanalyser methods of Marsh et al. (1965) and Chasson et al. (1961), respectively. Triiodothyronine (T_3) and thyroxine (T_4) concentrations in plasma samples (pooled across sampling times) were measured using 'Coat-a-Count' solid-phase

radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA 90045) previously validated for ovine samples (Clark et al. 1989). Intra- and inter- assay coefficients of variation were: urea, 1.26%, 2.26%; creatinine, 1.87%, 5.67%; triiodothyronine, 5.35%, 7.93%; and thyroxine, 3.58%, 6.17%.

Midside Wool Growth Rate

Wool growth was measured over three time intervals, namely 5-9 months of age, 9-13 months of age and 13-17 months of age. On day 15 of the 5 month experimental period, a midside patch of wool measuring approximately 100 x 100 mm was cleared by clipping to within 1 mm of the skin surface. The same patch was clipped, and the wool collected, on day 15 of the 9 and 13 month experimental periods and again 4 months later. Recordings were taken of the length of the sides and the diagonal of the cleared patch. The greasy weight of the wool samples was measured and, after scouring, the clean weight was also determined. The midside wool growth rate of each ram in each period was calculated by dividing the weight of wool by the area of the midside patch and was expressed as mg wool grown per cm^2 patch area per day.

Statistical Analyses

Liveweight, intake and midside wool growth data were subjected to multivariate analysis of variance (MANOVA) to test the effects of line, block, nominal age and their interactions. Block effects were rarely significant and have therefore not been reported.

Statistical analyses of plasma hormone and metabolite concentrations arising from sequential blood sampling were carried out as follows. Basal concentrations of urea and creatinine were initially subjected to repeated measures analysis across sampling times within each nominal age. Since these analyses showed no significant line x sampling time effects (i.e. line differences were constant across times), mean concentrations within each age were then generated for each animal. MANOVA was then used to test line effects and line x nominal age interactions in mean urea or creatinine concentration.

Data arising from the urea/creatinine challenge were analysed as follows. First, the mean of the three pre-challenge (baseline) concentrations was subtracted from the concentration at each post-challenge sampling time. The baseline-corrected post-challenge data were then fitted to a single-pool exponential decay model (Bartle et al. 1988) of the form:

$$Y_t = Y_0 e^{-kt} \quad ,$$

where: Y_t = concentration (urea or creatinine) at time t,

Y_0 = concentration at time zero (by back extrapolation),

k = disappearance constant, and

e = base of natural logarithms.

This model was fitted over the interval 15-240 minutes post-challenge for urea and 15-120 minutes for creatinine to eliminate

post-challenge samples whose concentrations were below the pre-challenge mean baseline levels. Urea and creatinine spaces (% liveweight) were calculated as the ratio of metabolite dose administered to the zero-time rise in concentration above baseline (i.e. Y_0 expressed in units of concentration as for the dose). Estimates of space and disappearance constant were subjected to MANOVA to test the effects of line, nominal age and their interaction.

Plasma T_3 and T_4 concentrations of pooled samples were also subjected to multivariate analysis of variance to test for significance of line, block and their interaction with nominal age.

All statistical analyses were performed using the statistical package 'REG' (Gilmour, 1985).

Results

Liveweight, intake and age

Table 2 shows the liveweights, intakes and actual ages of the fleeceweight-selected (FW) and control (C) rams at the three nominal ages. The liveweights of the rams increased with age, this effect being highly significant ($P<0.001$). FW and C rams gained an average of 10 and 10.5 kg (respectively) between the first and second nominal ages and an average of 5.3 and 5.5 kg (respectively) between the second and third nominal ages. There was no significant difference in weight between the selection lines over the three nominal ages nor any nominal age x line interaction in liveweight.

Table 2. Liveweight, intake and actual age of fleeceweight-selected (FW) and control (C) rams at three nominal ages

Nominal age (months)	Variable	Selection line (n = 16) Control (C)	Fleeceweight (FW)
5	Liveweight (kg)	27.1 ± 1.2[a]	28.8 ± 0.8
	Intake (kg DM/day)[b]	0.9 ± 0.05	0.9 ± 0.03
	Age (days)	156.3 ± 2.3	164.3 ± 2.0
9	Liveweight (kg)	37.1 ± 1.5	39.3 ± 1.0
	Intake (kg DM/day)	1.2 ± 0.05	1.3 ± 0.04
	Age (days)	283.3 ± 2.3	291.3 ± 2.0
13	Liveweight (kg)	42.4 ± 1.7	44.8 ± 1.0
	Intake (kg DM/day)	1.5 ± 0.06	1.5 ± 0.05
	Age (days)	396.3 ± 2.3	404.3 ± 2.0

	Significance		
	Nominal Age	Line	Age x Line
Liveweight	***	NS	NS
Intake	***	NS	NS
Age	N/A	**	N/A

a Values are means ± SE
b Over the last five days of the adjustment period
NS = Not significant ** P<0.01 *** P<0.001.
N/A = not applicable

Table 2 also presents the average dry matter intakes of the FW and C rams over the last five days of the adjustment period at each age. Highly significant ($P<0.001$) differences were seen between the nominal ages, with intakes increasing by about 0.3 kg DM/day between each age. However, there was no difference between the two selection lines in intakes and the effects of line were additive with those of age.

A significant ($P<0.01$) difference between the selection lines in actual age was apparent with the FW rams being an average of 8 days older than the C rams. This reflects the fact that birthdates of FW lambs are generally earlier than those of C lambs (Blair et al. 1985).

Midside Wool Growth Rate

The mean rates of wool growth on the midside patch (greasy and clean) over the three sampling periods are shown in Fig.1.

There was a significant ($P<0.01$) difference between the lines in greasy wool growth rate (GWGR) but no significant line x time interaction. FW rams maintained higher GWGR than C rams over all sampling periods and maximum rates of wool growth were obtained during time period 2 (spring) in both selection lines.

The clean wool growth rate (CWGR) measurements also showed a significant ($P<0.01$) line effect but no significant line x time interaction. FW rams again showed consistently greater CWGR than C rams. There was a slight decline in clean wool growth rate in time

period 2 leading to maximum clean wool growth in time period 3 (summer).

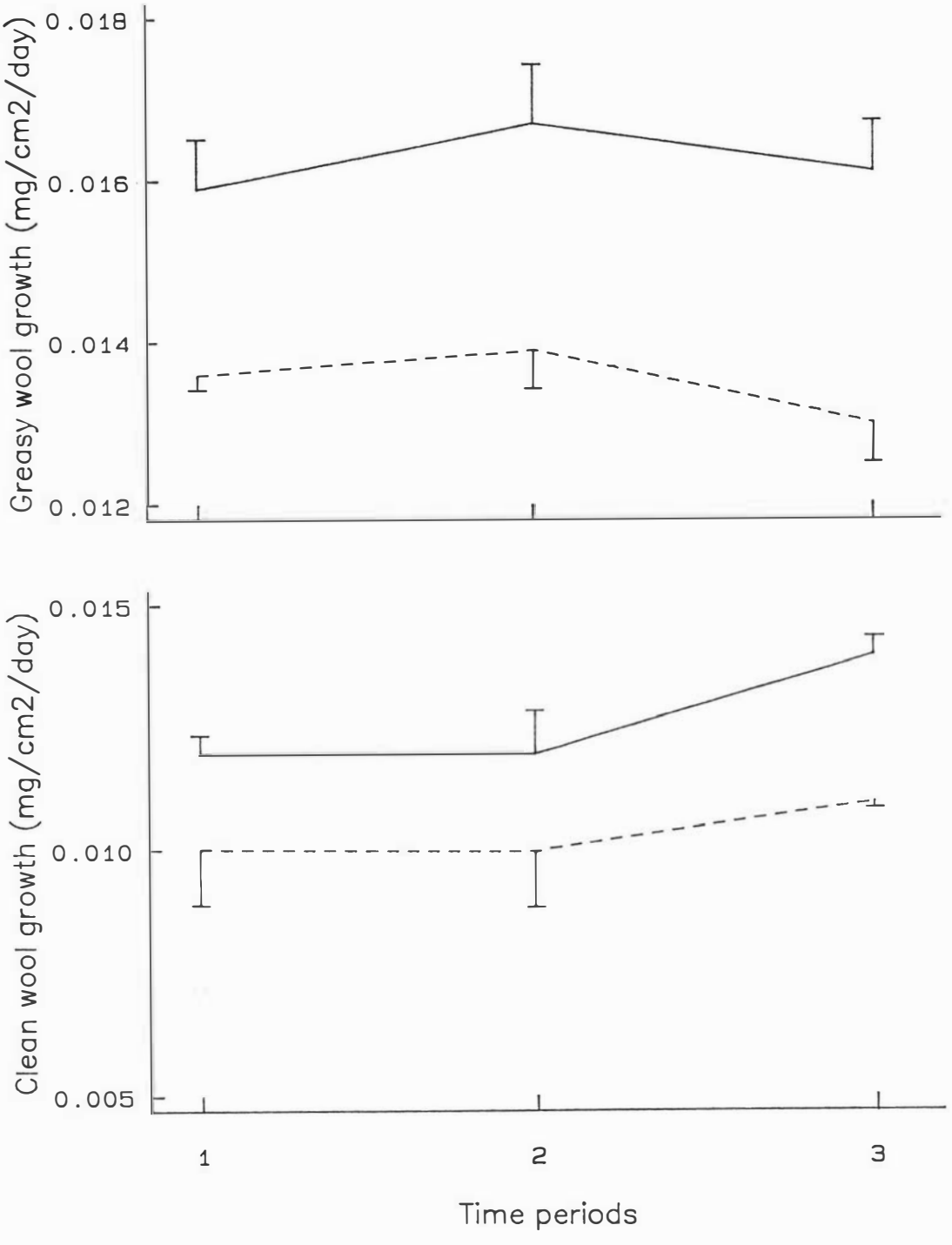

Fig. 1. Midside greasy wool growth rate (GWGR, upper panel), and clean wool growth rate (CWGR, lower panel), over three time periods (5-9, 9-13 and 13-17 months of age) in 16 control (- - -) and 16 fleeceweight-selected (——) rams. Vertical bars represent standard errors about the mean.

Plasma Urea Concentration

Basal plasma urea concentrations over the 12 hour sampling period at nominal ages of 5, 9 and 13 months are shown in Fig.2. At 5 months of age C rams maintained concentrations of urea which were consistently greater than those of the FW rams but the line effect was nonsignificant.

A significant ($P<0.01$) difference was seen between the selection lines at a nominal age of 9 months (i.e. June) but the difference was again nonsignificant at 13 months of age (Table 3). At all ages, there was a significant effect of sampling time, urea concentration tending to decline throughout the day despite the fact that rams were fed equal amounts of lucerne chaff every two hours. All line x sampling time interactions were nonsignificant (Table 3).

Analysis of mean urea concentrations (Fig. 3.) showed that the line effect was significant ($P<0.01$), as was the nominal age effect ($P<0.001$). The line x nominal age interaction was also significant ($P<0.05$), reflecting the greater difference between the lines at 9 months of age (i.e. June) than at other ages.

Plasma Creatinine Concentration

Fig. 4. shows the basal plasma creatinine concentrations over the 12 hour sampling period at nominal ages of 5, 9 and 13 months. No line effect or line x sampling time interaction was seen at 5 or 13 months of age (Table 4). The C rams generally had higher plasma

creatinine concentrations than the FW rams although there was some crossing over of the lines at the first age (5 months). At 9 months of age there was a highly significant ($P<0.001$) difference between the lines but again, no line x sampling time interaction (Table 4). At all ages there was a significant effect of sampling time, with this difference being greatest at 9 months of age (winter).

Mean plasma creatinine concentrations (Fig. 5.) showed a nonsignificant line effect and a marginally significant ($P<0.1$) nominal age effect. There was a greater difference between the lines at 9 months of age than at other ages which was reflected in a significant ($P<0.05$) line x age interaction.

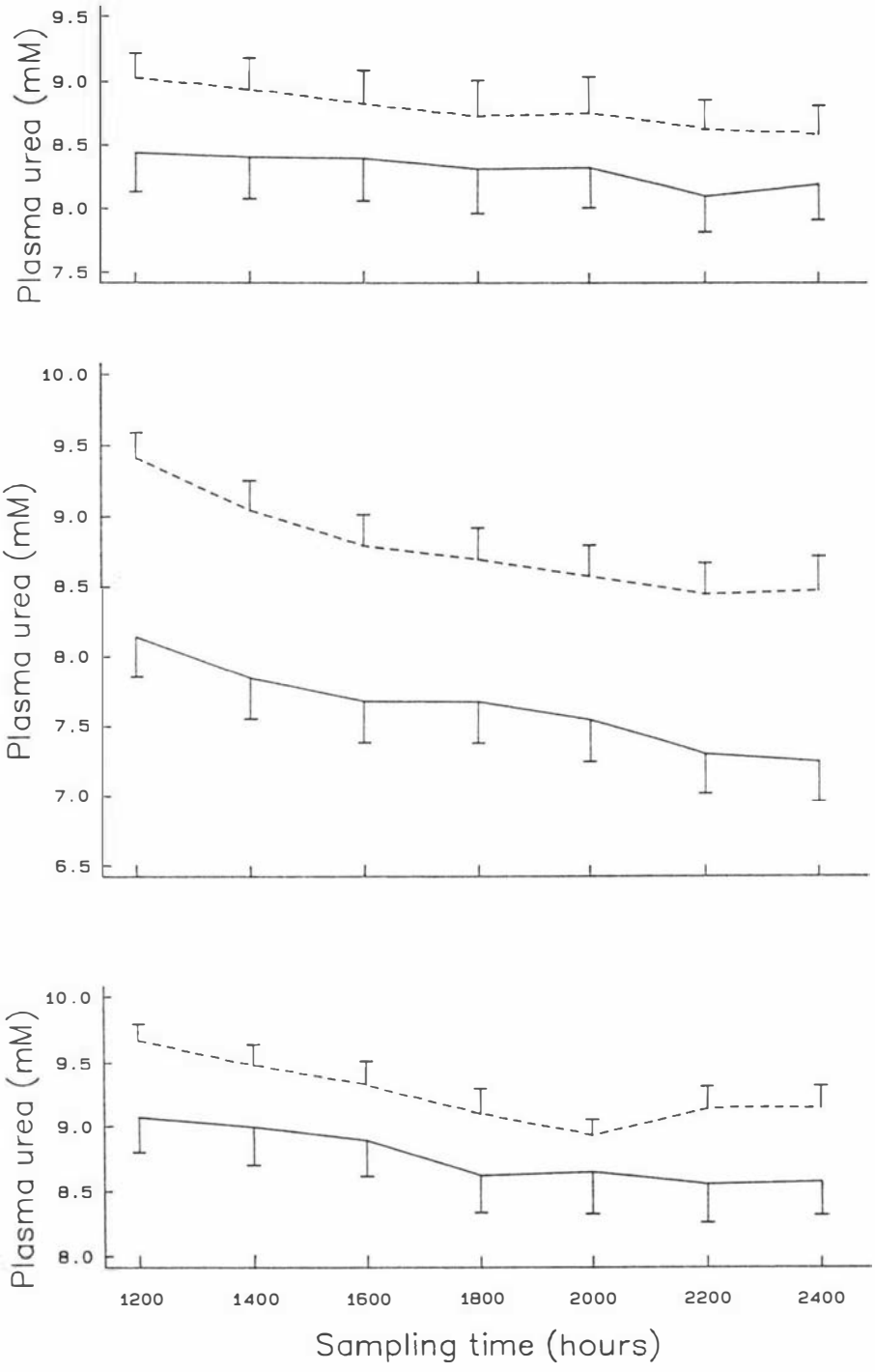

Fig. 2. Plasma urea concentrations at each sampling time in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5 months (upper panel), 9 months (middle panel) and 13 months (lower panel). Vertical bars represent standard errors about the mean.

Table 3. Significance of effects of line, sampling time and line x sampling time interaction on plasma urea concentration (separate MANOVA analysis at each nominal age).

Nominal Age (months)	Line (L)	Sampling Time (T)	Interaction (L x T)
5	NS	**	NS
9	**	***	NS
13	NS	***	NS

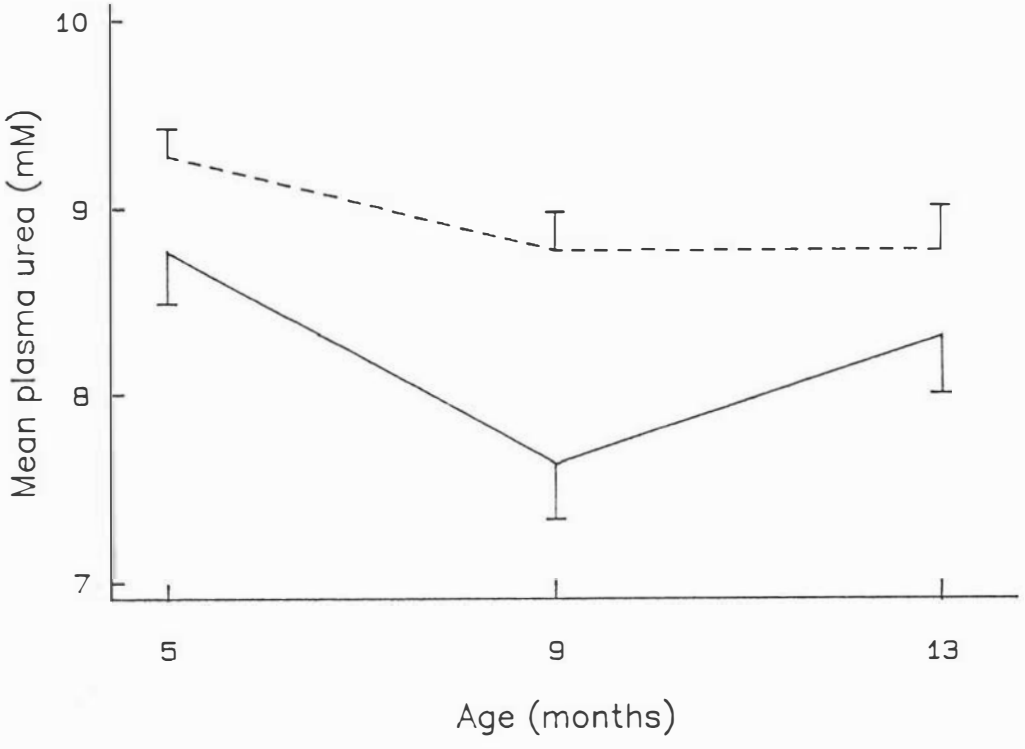

Fig. 3. Mean plasma urea concentration in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5, 9 and 13 months. Vertical bars represent standard errors about the mean.

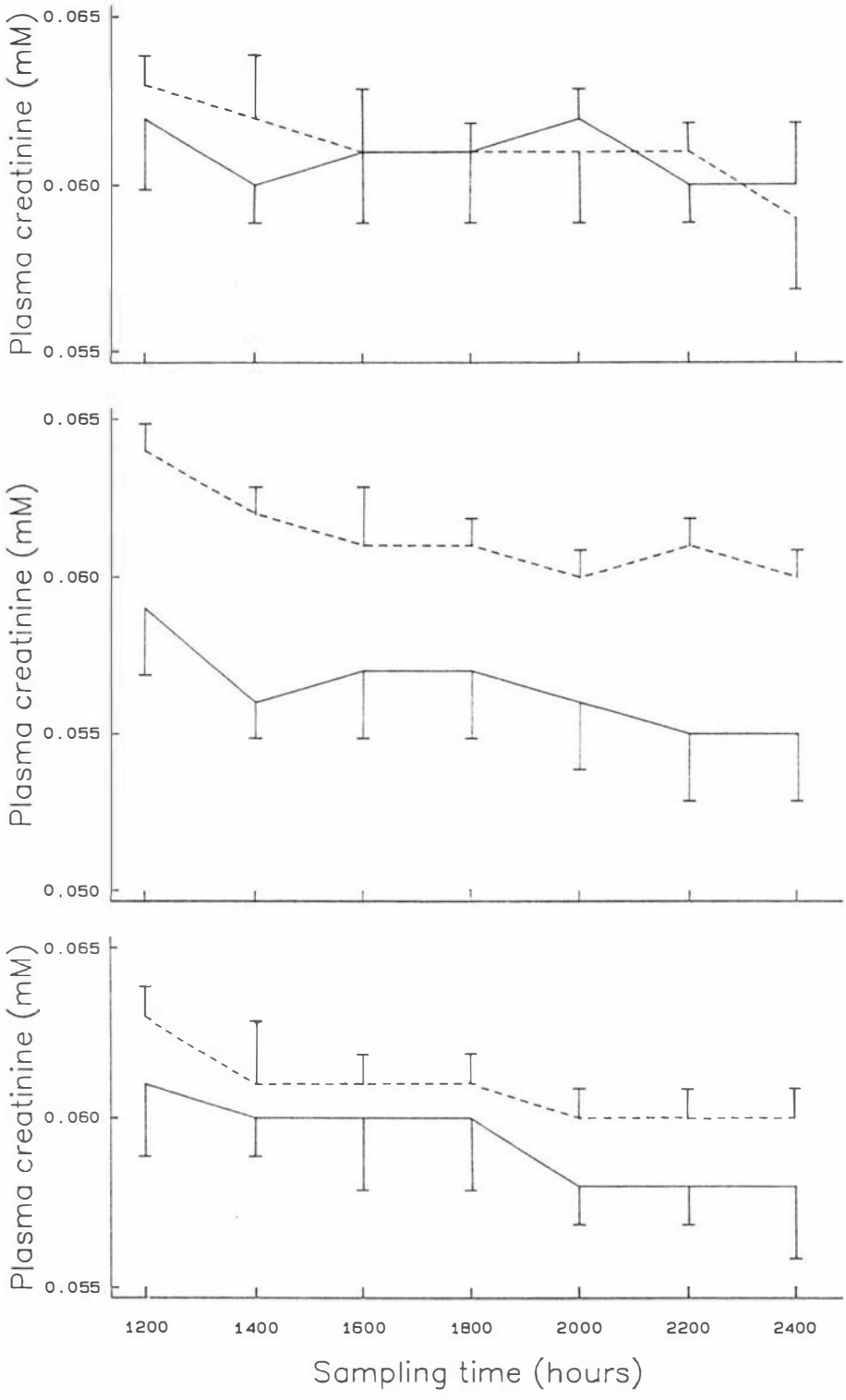

Fig. 4. Plasma creatinine concentrations at each sampling time in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5 months (upper panel), 9 months (middle panel) and 13 months (lower panel). Vertical bars represent standard errors about the mean.

Table 4. Significance of effects of line, sampling time and line x sampling time interaction on plasma creatinine concentration (separate MANOVA analysis at each nominal age).

Nominal Age (months)	Line (L)	Sampling Time (T)	Interaction (L x T)
5	NS	*	NS
9	***	***	NS
13	NS	**	NS

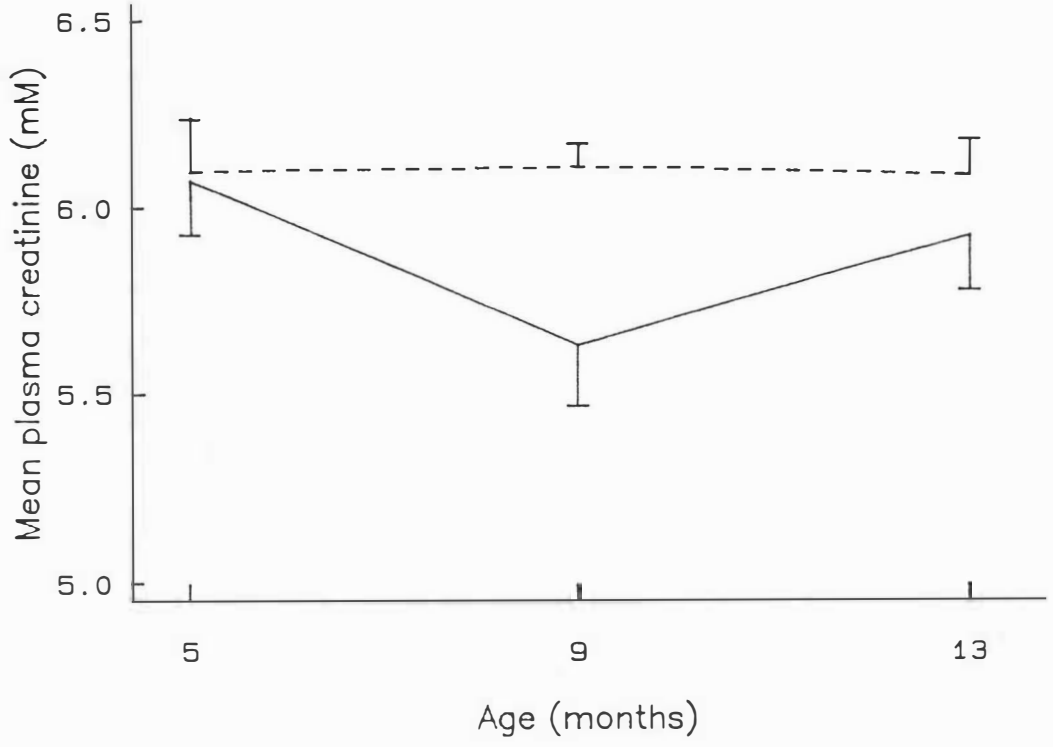

Fig. 5. Mean plasma creatinine concentration in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5, 9 and 13 months. Vertical bars represent standard errors about the mean.

Plasma T_3 and T_4 Concentrations

Plasma concentrations of T_3 and T_4 are shown in Fig.6. C rams maintained higher plasma T_3 concentrations than FW rams at the first and third nominal ages (5 and 13 months respectively) but FW rams showed higher levels at 9 months. Neither the line effect nor the line x age interaction was significant although the increase in plasma T_3 levels with age was significant ($P<0.01$).

Plasma T_4 concentrations showed a similar crossing-over between C and FW rams at the second age (9 months). There was no line or line x age effect in plasma T_4 concentration although there was a highly significant ($P<0.001$) age effect, plasma T_4 concentrations increasing as the rams aged.

Urea and Creatinine Challenge

Plasma concentrations of urea and creatinine in response to the challenge are shown in Figs.7 and 8 respectively. Plasma urea concentrations rose sharply at the onset of the challenge with C rams showing consistently greater concentrations of urea than FW rams both before and after the challenge.

Plasma creatinine concentrations showed little between-line difference throughout the entire sampling period with C rams maintaining marginally higher plasma creatinine concentrations in the first and third time periods but not during the second.

Urea and creatinine spaces and clearance rates derived from the challenge data are shown in Table 5. There was a highly significant ($P<0.001$) difference in urea space between ages but no line or line x age differences. The clearance rates of urea and creatinine were not influenced by line or age.

Although MANOVA analyses showed no overall effects of line or line x age interation in urea space or clearance, univariate analyses showed that both parameters were greater in FW than in C rams at 9 months. Thus the urea spaces were 50.47 ± 1.41% liveweight in C rams versus 54.68 ± 1.41% liveweight in FW rams (difference = +8.3%, $P<0.05$) while the urea clearance rates were -0.0035 ± 0.0002 min^{-1} (C) versus -0.0042 ± 0.0002 min^{-1} (FW) (difference = +20%, $P<0.05$).

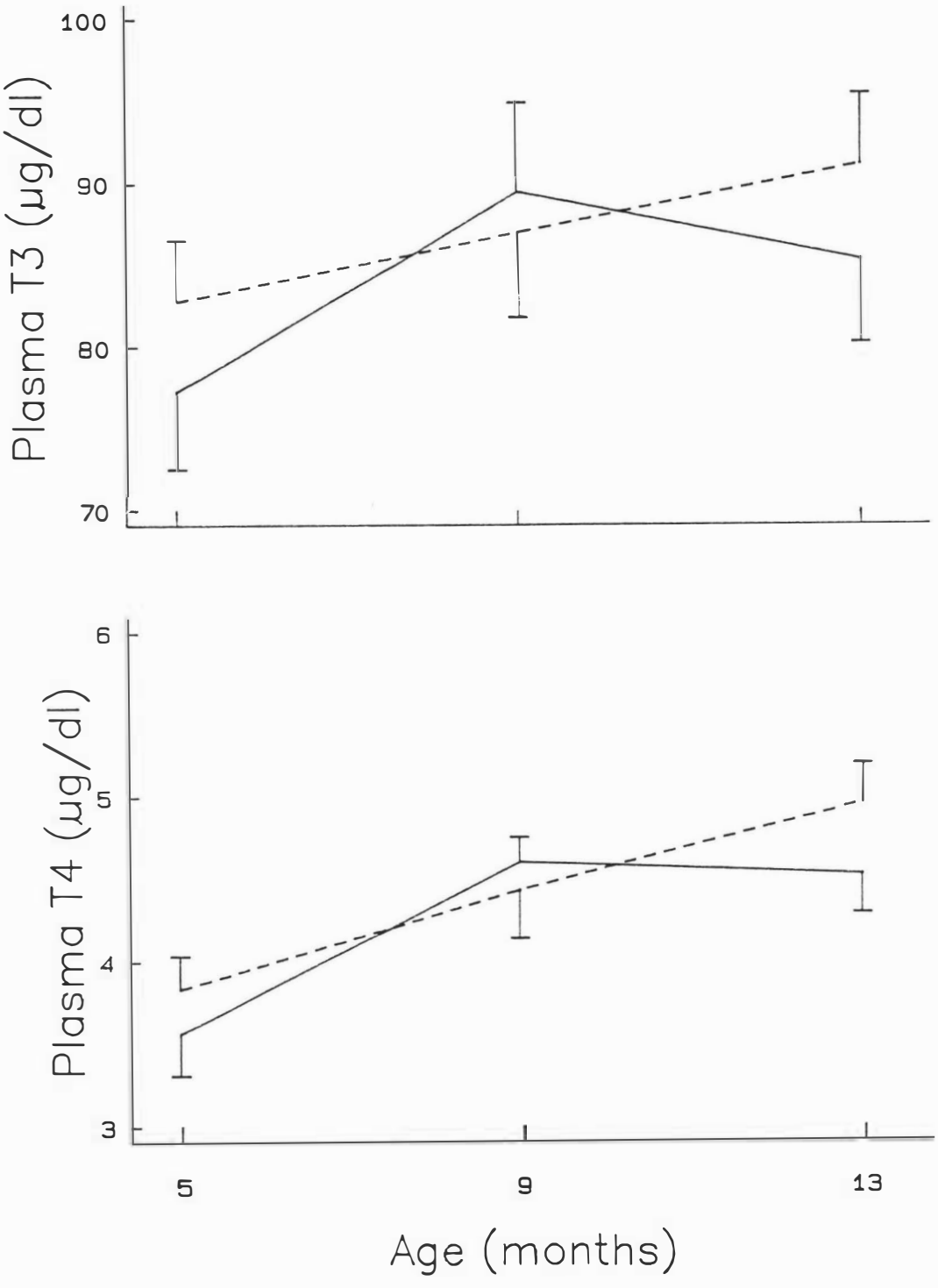

Fig. 6. Plasma triiodothyronine (T_3, upper panel) and thyroxine (T_4, lower panel) concentrations in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5, 9 and 13 months. Vertical bars represent standard errors about the mean.

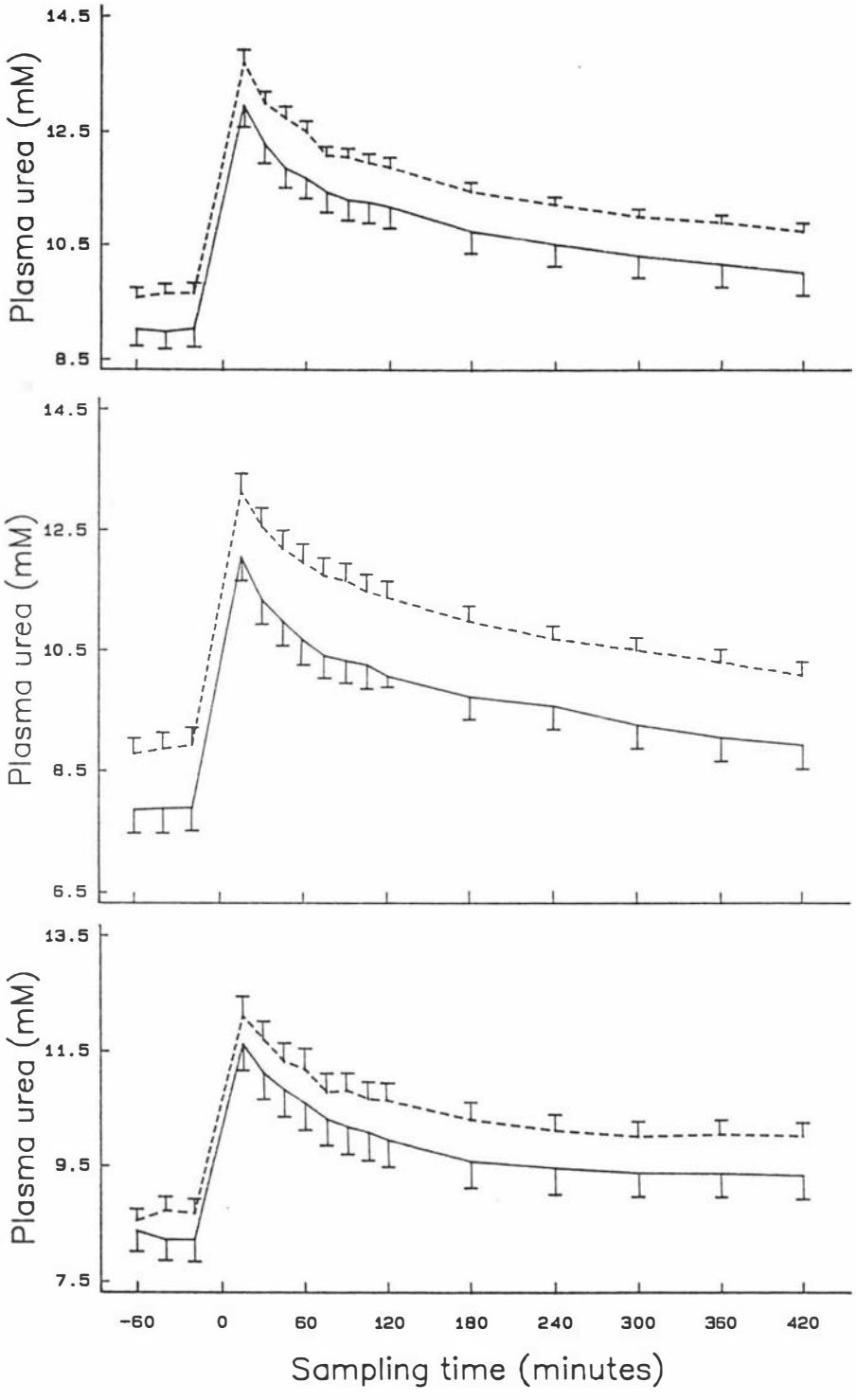

Fig. 7. Plasma urea concentrations at each sampling time in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5 months (upper panel), 9 months (middle panel) and 13 months (lower panel) during the urea challenge (120 mg/kg). Vertical bars represent standard errors about the mean.

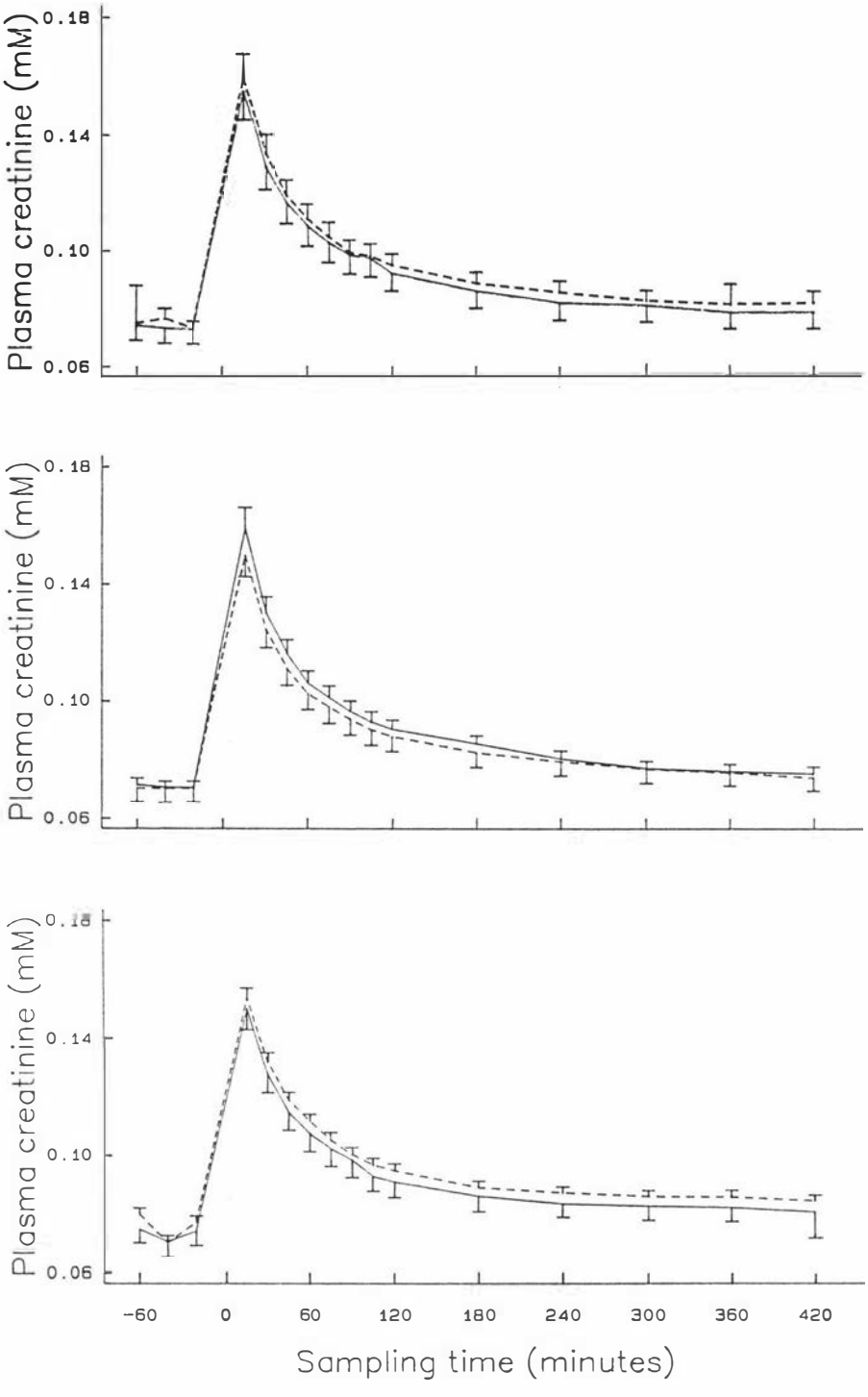

Fig. 8. Plasma creatinine concentrations at each sampling time in 16 control (- - -) and 16 fleeceweight-selected (——) rams at nominal ages of 5 months (upper panel), 9 months (middle panel) and 13 months (lower panel) during the creatinine challenge (3 mg/kg). Vertical bars represent standard errors about the mean.

Table 5. Urea and creatinine spaces and clearance rates in fleeceweight-selected (FW) and control (C) rams at three nominal ages

Nominal Age (months)	Variable	Selection line (n=16) Mean ± SE Control (C)	Fleeceweight (FW)
5	Urea space[a]	53.70 ± 1.70	57.13 ± 1.70
	Urea Clearance[b]	-.0043 ± .0005	-.0044 ± .0005
	Creatinine space[a]	32.40 ± .790	33.21 ± .790
	Creatinine Clearance[b]	-.0137 ± .0005	-.0132 ± .0005
9	Urea space	50.47 ± 1.41[c]	54.68 ± 1.41[d]
	Urea Clearance	-.0035 ± .0002[c]	-.0042 ± .0002[d]
	Creatinine space	32.69 ± 1.27	30.37 ± 1.27
	Creatinine Clearance	-.0140 ± .0007	-.0142 ± .0007
13	Urea space	63.28 ± 2.81	64.51 ± 2.81
	Urea Clearance	-.0042 ± .0007	-.0056 ± .0007
	Creatinine space	32.31 ± .910	32.58 ± .910
	Creatinine Clearance	-.0144 ± .0006	-.0144 ± .000

	Significance (MANOVA) Nominal Age	Line	Line x Age
Urea space[a]	***	NS	NS
Clearance (urea)[b]	NS	NS	NS
Creatinine space[a]	NS	NS	NS
Clearance (creatinine)[b]	NS	NS	NS

NS P>0.10 ** P<0.01 *** P<0.001
a Spaces expressed as % liveweight
b K values (min^{-1})
c,d Means with different superscripts are significantly (P<0.05) different by univariate analysis of variance

Discussion

The purpose of this study was to examine whether the magnitude of between-line variation in plasma urea, creatinine, T_3 and T_4 levels depends on age/season and to determine whether the urea and creatinine differences were due to a difference in space or clearance rate. It has already been established that differences exist between lines selected for high and low fleeceweight in plasma urea, creatinine and T_4 concentrations (McCutcheon et al. 1987; Clark et al. 1989; Thomson et al. 1989) but, prior to this study, the possibility of selection line x age/season interactions in these parameters had not been examined.

Comparisons of wool growth rates between selection lines showed that FW rams maintained a higher rate of greasy and clean wool growth throughout the year compared with C rams. There was no line x age/season interaction which, at least superficially, suggests that a seasonal difference was not apparent. However, when compared with values obtained by McClelland et al. (1987), averaged over the same time periods, the pattern of change in wool growth rates was similar. Therefore, the apparent absence of seasonal variation in wool growth rates in this study was likely to be caused by the long interval between samples (i.e. approximately 4 months). In any event, the wool sampling regimes were not designed to test for seasonalilty of wool growth in the two lines. Their only purpose was to demonstrate that, in addition to coming from genetically divergent lines, the sheep used in the study were also phenotypically different in midside clean and greasy wool growth rates.

No differences were found between the selection lines in intakes, which agrees with results obtained by Saville and Robards (1972), McClelland et al. (1986) and Clark et al. (1989). Thomson et al. (1989) found that although there were no intake differences between FW and C lines when fed meadow hay, there were differences when the sheep were fed a lucerne chaff diet. This difference was thought to be due to a carryover effect from feeding the lower quality meadow hay diet before the higher quality lucerne chaff diet (Thomson et al. 1989). Their explanation is not, however, very convincing since it is difficult to imagine why prior feeding on meadow hay should cause a divergence between the lines in voluntary intake of lucerne chaff (as opposed to a similar compensatory response in intake in all animals).

The intake of dietary nitrogen has an influence on the entry rate of urea originating from rumen ammonia. As discussed previously, this is one of the possible mechanisms by which FW and C animals might differ in their plasma urea concentrations. However, as there was no difference between the lines in intake during this study, present results would suggest that the difference between the FW and C animals in circulating urea did not reflect differences in nitrogen intake.

There were no consistent trends in plasma T_3 or T_4 concentrations between the selection lines although levels did increase with age. These results are contrary to those of Clark et al. (1989) and Hough et al. (1987) who found differences between Romney lines and Merino lines (respectively), with animals genetically

predisposed to high fleece production maintaining lower concentrations of T_3 and T_4 than control animals. However, Clark et al. (1989) found only small between-line differences in plasma T_4 concentrations of Romney rams aged 9-12 months but larger and significant differences in FW and C rams aged two years. Hough et al. (1987) used non-pregnant/non-lactating mature ewes. Age differences between these studies could have a bearing on the variation in levels of plasma T_3 and T_4 since results of Clark et al. (1989) suggest the possibility that the selection lines diverge in thyroid hormone levels as they age. The use of T_3 and T_4 as possible genetic markers is therefore still in doubt until such time as the possible existence of line x age interactions in these hormone levels is resolved.

C rams had consistently greater plasma urea concentrations than FW rams. These results are in agreement with those of McCutcheon et al. (1987), Clark et al. (1989) and Thomson et al. (1989) who all found that FW animals maintained lower plasma concentrations of urea than C animals. Similar results have been found with other animals of high versus low genetic merit including lines selected for lean versus fat growth in sheep (Bremmers et al. 1988; Carter et al. 1989) and pigs (Mersmann et al. 1984) and dairy selection lines of high versus low genetic merit for yield of milk or milk solids (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). However, Hough et al. (1987) found no differences in plasma urea concentration between genetically divergent lines of Merino ewes. It is possible that differences in plasma urea concentration are more apparent in younger animals (i.e. 6-12 months) than in the mature sheep used in the study by Hough et al. (1987). This can be seen from the present

study in which basal plasma urea concentrations showed no significant line effects at 5 or 13 months of age but a significant line effect at 9 months of age. This would suggest that, in order to maximize the chance of detecting differences between the selection lines in plasma urea concentration, the best time of the year to blood sample animals would be during the winter or when the animals were approximately 9 months of age. It should also be noted that, in the present study, the effects of age and season were completely confounded. Thus it is not possible to determine whether the greater difference in plasma urea seen at 9 months versus 5 or 13 months was an effect of age or an effect of season (e.g. photoperiod, ambient temperature).

Basal plasma creatinine concentrations showed similar results to those of plasma urea in that FW rams maintained lower concentrations of plasma creatinine compared with C rams. Results were consistent with those of previous studies (McCutcheon et al. 1987; Clark et al. 1989) but, as with urea, there were marked line x age/season interactions in plasma creatinine. Thus there were no line effects on plasma creatinine concentration at 5 or 13 months of age but a significant line effect at 9 months of age. This again would suggest that differentiation between fleeceweight selection lines in plasma creatinine concentration would be most apparent at approximately 9 months of age or in the winter period.

Urea and creatinine spaces and clearance rates were calculated from data obtained from the challenge periods. Although plasma urea and creatinine concentrations rose sharply after the challenge, there were no overall differences (i.e. across all three ages) between the

selection lines in their spaces or clearance rates. However, univariate analyses showed that there was a significant difference in urea space and clearance between the lines at 9 months of age, with FW rams having higher values than C rams. The 8% greater urea space of FW rams at this age would predispose them to lower circulating concentrations of urea, assuming equal urea entry rates. In addition, the greater urea clearance rate (+20%) would also lead to lower urea levels. Thus the fact that between-line differences in plasma urea concentration were maximised at 9 months of age may be at least partly explained by corresponding seasonal differences in urea space and clearance rate. The underlying physiological mechanisms responsible for these differences are, however, unclear. Urea space is related to total body water content (Bartle et al. 1988), suggesting the possibility of a difference between the lines in body composition at 9 months of age. Urea clearance rate reflects kidney function and may be related to blood flow through the kidneys. Whether this has a direct physiological relationship with between-line differences in wool growth is not known.

Creatinine space and clearance rates showed no significant difference between lines or between ages. However, basal creatinine concentrations followed a similar pattern to basal urea concentrations (i.e. maximum between-line differences at 9 months of age). This implies that there is a common element causing the between-line differences in basal plasma urea and creatinine levels. However, in the case of creatinine, the cause of this difference is still in doubt. The nonsignificant difference in creatinine space or clearance rate at this time would suggest that these mechanisms are not

responsible for the differences in basal creatinine concentrations. Further research is needed in this area.

In conclusion, results from this study have confirmed that differences exist between fleeceweight and control selection lines in plasma urea and creatinine concentrations. The seasonality aspect of this study has shown that discrimination between fleeceweight and control selection lines in levels of these metabolites is greatest during the winter. This would suggest that if selection is to be made on plasma urea and creatinine levels, the best time of the year for selection would be during the winter. However, selection at this time could create problems for the breeder as animals would need to be carried through to the winter before selection could take place. This would in turn extend the generation interval and decrease the rate of genetic gain, thus limiting the usefulness of these metabolites as genetic markers for fleeceweight in sheep.

CHAPTER III

GENERAL DISCUSSION

CHAPTER III: GENERAL DISCUSSION

The objective of this study was to determine whether the magnitude of differences between the FW and C lines in plasma urea, creatinine, T_3 and T_4 concentrations was influenced by an age/season effect and also to determine whether the differences seen in urea and creatinine concentrations were due to differences in space and/or clearance rates. This chapter will consider the wider implications of the results found in this study and discuss where research efforts should be directed in future.

Plasma thyroxine (T_4) and triiodothyronine (T_3) concentrations were measured at three ages/seasons to determine if there were differences between the lines. No significant difference was found between the lines in either T_3 or T_4 although both showed a significant increase with age. In previous studies (Hough et al. 1987; Clarke et al. 1989), differences between selection lines in plasma T_4 concentration have been significant. However, it is interesting to note that, in the study by Clark et al. (1989), significant differences were seen in the experiment dealing with 2 year old rams but not in the experiment involving 9-12 month old rams. Also, in the study by Hough et al. (1987), differences in T_4 concentrations were found in mature ewes.

The plasma T_4 concentrations observed in this study were 4.44 ± 0.25 µg/dl versus 4.61 ± 0.19 µg/dl for C and FW sheep (respectively) at 9 months of age, and 4.96 ± 0.28 µg/dl (C) versus

4.53 ± 0.20 µg/dl (FW) at 13 months of age. Clark et al. (1989) found values of 4.3 ± 0.5 µg/dl (C) versus 3.3 ± 0.5 µg/dl (FW) for sheep aged between 9-12 months and 5.9 ± 0.6 µg/dl (C) versus 4.6 ± 0.6 µg/dl (FW) for sheep aged 2 years. While comparison of hormone concentrations measured by radioimmunoassay in different studies is not always valid, this study and that of Clark et al. (1989) used the same lines of sheep and the same assay kits. As reported in Chapter 2, these kits have a low inter-assay coefficient of variation. Hence the above comparison is probably reasonable and would suggest increasing divergence between the lines with age.

The possibility of differences between selection lines in plasma T_4 being more apparent at older ages should be the basis for future studies in this area. An experiment should be conducted using FW and C sheep of various ages (e.g. a range from 6 months to 3 years) to test the differences between the lines and to determine when these differences become significant. An alternative approach would be to supplement older (e.g. 2 year) FW animals with T_4, so bringing their circulating levels to those of the C sheep, and to compare the fleece production of supplemented FW sheep with those having no supplementation and the controls. This would help establish whether the line differences in T_4 levels are directly related to differences in fleece production or whether they are unrelated "responses" to selection (e.g. as a consequence of genetic drift).

It is possible that differences in T_4 concentrations are not the best measure of differences in thyroid function between the selection lines. A number of studies have detected differences between high and low genetic merit cattle in the level of thyroid

activity and, in particular, thyroid hormone degradation rates. Joakimsen et al. (1971) found that the thyroxine degradation rate in 82 mature Finnish bulls was positively correlated with fat-corrected milk production of their daughters. Similarly, Sorensen et al. (1981) found that thyroxine degradation rate in Danish bulls had a positive genetic correlation with breeding value for butter-fat production. In neither case were circulating levels of thyroid hormones correlated with genetic merit.

Chai (1970) studied tha rate of uptake of I^{131} in mice as an indicator of thyroid activity, as it was easier to measure and had a greater repeatability than that of the actual thyroid hormones. After 10 generations, a 4-fold difference was found between mice selected for high levels of I^{131} uptake compared with those selected for low levels of uptake. Tilakaratne et al. (1981) obtained higher growth at a young age in mice selected for high plasma thyroxine levels although this was apparently due to improved maternal performance rather than increased individual growth.

Thyroid activity is also related to basal metabolic rate in animals which would suggest that animals from selection lines could have differing metabolic activities.

The use of T_4 as a genetic marker for fleeceweight is still in doubt. Experiments of the above nature could help to determine whether or not differences in T_4 concentrations would be useful as genetic markers. Further work is needed in this area, particularly to find differences between the sheep selection lines in thyroid activity

which may be evident before two years of age (Clark et al. 1989) and so have potential as genetic markers.

It is well established that differences in plasma urea and creatinine concentrations exist between the Massey University fleeceweight-selected and control lines of sheep (McCutcheon et al. 1987; Clark et al. 1989; Thomson et al. 1989). Similar differences have also been found in other selection lines including pigs (Mersmann et al. 1984), cattle (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987), and low and high backfat sheep (Bremmers et al. 1988; Carter et al. 1989). None of the abovementioned trials have studied animals as young as the ones in this trial although Bremmers et al. (1988) and Carter et al. (1989) did make comparisons of 7-month-old rams from the Massey University High and Low Backfat Southdown selection lines. They found that even at 7 months of age, High Line (i.e. high backfat) animals exhibited significantly greater baseline concentrations of urea than the Low Line animals.

The study reported here involved sampling at three nominal ages (5, 9 and 13 months) which corresponded to three seasons (summer, winter and spring respectively). Significant differences were found between the lines at 9 months of age (i.e. in the winter) in plasma urea and creatinine concentrations with C rams having higher values than FW rams. However, this study has not enabled us to distinguish between an age effect or a season effect. Therefore, it is possible that the differences seen between the lines in plasma urea and creatinine concentration could be due to a difference in either age or season.

Results obtained by McCutcheon et al. (1987) and Clark et al. (1989) would tend to suggest that the difference in plasma urea and creatinine concentrations are due to a seasonal effect rather than an age effect. Clark et al. (1989) found significant differences between the lines in plasma urea and creatinine concentrations in rams aged 9-12 months and 2 years, in trials conducted from April-July and July-August (respectively). McCutcheon et al. (1987) conducted a trial during September (rams aged just over two years) and found differences in plasma urea and creatinine concentrations between the selection lines. Therefore, even though there was a greater difference between the selection lines at 9 months of age in this study, differences are still apparent at other ages. This would suggest that the large difference seen at 9 months of age is caused by a seasonal effect rather than an age effect as differences also occur at other ages.

The difference between the lines in plasma urea and creatinine concentrations could be due to a combined age and season effect. It is possible that the levels of urea and creatinine diverge with age while at the same time, the seasonal effect causes a large winter difference and a smaller summer difference in levels of urea and creatinine. Therefore, a greater difference between the lines may be seen with age and this effect could be magnified at 9 months of age by a winter seasonal effect whcih also causes the levels of urea and creatinine to increase.

In order to distinguish between these two possible causes, experiments should be conducted involving artificial photoperiod. For example, animals of varying ages could be subjected to a photoperiod characteristic of the winter period to establish whether the

differences in plasma urea and creatinine concentrations are due to a change in photoperiod or to a change in age. Photoperiodic effects could also be simulated through the use of melatonin. It is thought that melatonin is one of the hormones that functions physiologically to relay effects of changing photoperiod. Exogenous melatonin induces responses in wool growth by simulating the short photoperiod characteristic of winter (Lincoln and Ebling, 1985). Therefore , if exogeneous melatonin was administered to animals, this would have the effect of simulating the winter situation, which could result in greater differences in plasma urea and creatinine concentrations between selection lines compared with those seen in the summer or spring. Another way to achieve this effect would be to hormonally induce out-of-season lambing. This could also be more practical and less costly than controlling photoperiod.

Alternatively, it is possible that differences seen between the lines are a function of changing temperatures throughout the year in which case a similar experiment could be carried out involving a constant temperature. Animals of varying ages could be subjected to natural photoperiod at a constant temperature. If no differences are seen between the ages with controlled temperature, it would suggest that differences in plasma urea and creatinine concentrations are not due to differences in age but instead are due to a difference in environmental factors such as temperature and photoperiod.

One of the main reasons for this study was to try to establish the mechanisms responsible for the differences in plasma urea and creatinine concentrations. Previous papers have suggested various mechanisms, including differences between the lines in urea and

creatinine entry rates, clearance rates and also in their space or distribution (McCutcheon et al. 1987; Bremmers et al. 1988; Clark et al. 1989; Carter et al. 1989). However, as yet, these mechanisms have not been firmly established.

It was noted in this study that although there was no overall significant difference between the lines in urea and creatinine space or clearance rates, there was a significant difference between the lines in urea space and clearance rate at 9 months of age. This suggests that the difference between the selection lines in plasma urea concentration at this time could be a result of differences in these mechanisms. It is possible that there is a difference between the lines in body composition at this age as urea space is known to be related to total body water content (Bartle et al. 1988). Also, as urea clearance rate is a reflection of kidney function, it is possible that there are differences between the lines in the ability of the kidneys to clear urea/creatinine and also possibly the blood flow through the kidneys. Certainly the suggestion of greater urea clearance rate at 9 months in this study supports the observations of McCutcheon et al. (1987) and Thomson et al. (1989).

The same inferences cannot be made for plasma creatinine concentration as there were no significant differences between the lines in creatinine space or clearance rates. However, the fact that basal plasma urea and creatinine concentrations followed a similar seasonal/age pattern suggests that there is some common factor causing the changes in these metabolites. For this reason, further work is needed in this area to determine the mechanisms responsible for the differences in plasma creatinine between selection lines.

There were some limitations with the techniques used in this study and future studies involving this type of work may need to use a different approach. One consideration which needs to be taken into account when trying to determine the urea and creatinine space or clearance rates is the failure of post-challenge concentrations to reach their original baseline values. It was found that either values dropped below the baseline concentrations or a new baseline was reached. Another limitation with this method was the inability to distinguish between exogenous and endogenous urea or creatinine when determining the concentrations of these metabolites in the animals. It may be possible to overcome these problems by administering radioactively-labelled urea or creatinine and monitoring their progress through the animal's system. This would help to more directly determine the differences between selection lines in space and clearance rates. The mechanisms responsible for the apparent line x age/season interaction in urea clearance and space are not known at the present time.

In conclusion, work is still needed in this area before plasma urea and creatinine can be recommended as genetic markers for fleeceweight although they do satisfy many of the requirements of markers outlined in Chapter 1. They are able to be measured at an early age in both males and females and their assays are relatively simple and cost efficient. By using genetic markers for selection, it is hoped that the generation interval can be decreased and, as a consequence of this, the rate of genetic gain can be increased. Results from this study have raised a potential problem associated with this as it has been found that in sheep aged between 5 and 13

months, the largest difference in plasma urea and creatinine concentration is at 9 months of age. If selection were to be made at this time, the generation interval would not be decreased and thus the rate of genetic gain would remain unchanged as selection could only take place during or after winter. However, once the mechanisms resulting in the urea/creatinine changes are understood, it may be that they will be useful as physiological predictors at an early age.

REFERENCES

References

Bartle, S. J., Turgeon, O. A., Preston, R. L., and Brink, D. R. (1988). Procedural and mathematical considerations in urea dilution estimation of body composition in lambs. J. Anim. Sci. **66**, 1920-27.

Bigham, M. L., Sumner, R. M. W., and Elliott, K. H. (1978). Seasonal wool production of Romney, Coopworth, Perendale, Cheviot and Corriedale wethers. N.Z. J. Agric. Res. **21**, 377-82.

Bird, P. R. and Moir, R. J. (1972). Sulphur metabolism and excretion studies in ruminants VIII. Methionine degradation and utilization in sheep when infused into the rumen or abomasum. Aust. J. Biol. Sci. **25**, 835-48.

Blair, H. T. (1981). Responses to selection for open face and greasy weight in Romney sheep. Ph.D. Thesis, Massey University, Palmerston North.

Blair, H. T. (1985). How can selection lines help us? 11th Workshop on Overfatness and Lean-meat Production from Sheep. pp. 13-16.

Blair, H. T. (1986). Response to 27 years of selection for yearling fleece weight. Proc., 3rd World Congr. on Genetics Applied To Livestock Production, Vol. 12, pp 215-20.

Blair H. T., Garrick, D. J., Rae, A. L., and Wickham, G. A. (1984). Selection response in New Zealand Romney sheep. I. Selection for wool-free faces. N.Z. J. Agric. Res. **27**, 329-36.

Blair, H, T., Garrick, D. J., Rae, A. L., and Wickham, G. A. (1985). Selection responses in New Zealand Romney sheep II. Selection for yearling greasy fleece weight. N.Z. J. Agric. Res. **28**, 257-64.

Blair, H. T., McCutcheon, S. N., and MacKenzie, D. D. S. (1990). Physiological predictors of genetic merit. Aust. Assoc. Anim. Breed. Gen. Proc. 8th Conf. pp. 133-42.

Bradbury, J. H. (1973). The structure and chemistry of keratin fibres. Adv. Protein. Chem. **27**, 111-21.

Bremmers, R. P. M., Morgan, P. F., McCutcheon, S. N., and Purchas, R. W. (1988). Effect of plane of nutrition on energy and nitrogen retention and on plasma urea concentrations in Southdown ram hoggets from high and low backfat selection lines. N.Z. J. Agric. Res. **31**, 1-7.

Broad, A., Gillespie, J. M., and Reis, P. J. (1970). The influence of sulphur containing amino acids on the biosynthesis of high-sulphur wool proteins. Aust. J. Biol. Sc. **23**, 149-64.

Carter, M. L., McCutcheon, S. N., and Purchas, R. W. (1989). Plasma metabolite and hormone concentrations as predictors of genetic merit for lean meat production in sheep: effects of metabolic challenges and fasting. N.Z. J. Agric. Res. **32**, 343-53.

Chai, C. K. (1970). Selective breeding for thyroid I^{131} uptake in mice. Genetics **64**, 29-40.

Chapman, R. E., Hopkins, P. S., and Thorburn, G. D. (1974). The effect of foetal thyroidectomy and thyroxine administration on the development of the skin and wool follicles of sheep foetuses. J. Anat. 117 (2), 419-32.

Chapman, R. E., Panaretto, B. A., and Frith, P. A. (1982). Changes in wool follicles in sheep following administration of dexamethasone trimethylacetate. J. Cell Sc. **53**, 323-35.

Chasson, A. L., Grady, A. J., and Stanley, M. A. (1961). Determination of creatinine by means of automatic chemical analysis. Am. J. Clin. Path. **35**,
83-8.

Clark, M. C. (1987). Physiological responses to selection for greasy fleeceweight in Romney sheep. M.Agr.Sc. Thesis, Massey University, Palmerston North.

Clark, C. M., MacKenzie, D. D. S., McCutcheon, S. N., and Blair, H. T. (1989). Physiological responses to selection for greasy fleeceweight in Romney sheep. N.Z. J. Agric. Res. **32**, 29-36.

Coop, I. E. (1953). Wool growth as affected by nutrition and by climatic factors. J. Agric. Sc. **43**, 456-71.

Coop, I. E. (1986). Pasture and crop production. In 'Sheep Production: Vol 2. Feeding, Growth and Health'. (Eds S. N. McCutcheon, M. F. McDonald and G. A. Wickham.) Ch.6. (New Zealand Institute of Agricultural Science.)

Crewther, W. G. (1976). In 'Proceedings of the 5th International Wool Textile Research Conference'. Aachen, 1975, **1**, 1.

Dolling, C. H. S., and Piper, L. R. (1968). Efficiency of conversion of food to wool. III. Wool production of ewes selected for high clean wool weight and of random control ewes on restricted and unrestricted food intakes in pens. Aust. J. Agric. Res. **19**, 1009-28.

Doney, J. M. (1966). Breed differences in response of wool growth to annual nutritional and climatic cycles. J. Agric. Sc., Cambridge **67**, 25- 30.

Downes, A. M., Reis, P. J., Sharry, L. F., and Tunks, D. A. (1970). Metabolic fate of parenterally administered sulphur-containing amino acids in sheep and effects on growth and composition of wool. Aust. J. Biol. Sc. **23**, 1077-88.

Dun, R. B., and Eastoe, R. D. (1970). Science and the Merino breeder. Sydney, N.S.W. Governmant Printer.

Ebling, F. J., and Hale, P. (1970). The control of the mammalian moult. In Memoirs of the Society of Endocrinology, No.18. 'Hormones and the Environment'. (Eds G.K. Benson and J.G. Phillips) Cambridge University Press. pp 215-37.

Ferguson, K. A. (1954). Prolonged stimulation of wool growth following injections of ox growth hormone. Nature **174**, 411.

Ferguson, K. A., Wallace, A. L. C., and Lindner, H. R. (1965). Hormonal regulation of wool growth. In 'Biology of Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short). pp. 655-77. (Angus and Robertson, Sydney).

Gilmour, A. R. (1985). REG : a generalised linear models programme. Misc. Bull. No.1., Division of Agricultural Services, N.S.W. Dept. of Agriculture, N.S.W., Australia.

Graham, N. McC. (1968). The metabolic rate of Merino rams bred for high or low wool production. Aust. J. Agric. Res. **19**, 821-24.

Hamilton, B. A., and Langlands, J. P. (1969). Efficiency of wool production of grazing sheep. I. Differences between Merino sheep selected for high and low fleece weight. Aust. J. Exp. Agric. and Anim. Husb. 9, 249-53.

Harris, P. M., Dellow, D. W., Xu, Z. Z., Blair, H. T., McCutcheon, S. N., and Cockrem, J. (1989). The effect of exogenous melatonin, administered in summer, on wool growth and testis diameter of Romneys. N.Z. Soc. Anim. Prod. (submitted).

Hawker, H., and Littlejohn, R. P. (1986). Liveweights, fleeceweights and wool characteristics of screened high fleece weight and randomly selected Romney ewe hoggets. Proc. N.Z. Soc. Anim. Prod. **46**, 219-23.

Hawker, H., and McEwan, J. C. (1986). Ministry of Agriculture and Fisheries Annual Reports. Agric. Res. Div. pp 139-40.

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 fleeceweight. Proc. N.Z. Soc. Anim. Prod. **48**, 207-212.

Heydenrych, H. J., Vosloo, L. P., and Meissenheimer, D. J. B. (1977). Selection response in South African Merino sheep selected either for high clean fleece mass or for a wider S/P ratio. Agroanimalia **9**, 67-73.

Hopkins, G. J., Roberts, J., and Evans, J. V. (1975). Red blood cell concentrations of reduced glutathione and potassium as biochemical markers of wool growth in Merino sheep. J. Agric. Sc., Cambridge. **84**, 481-86.

Hough, G. M., Williams, A. J., McDowell, G. H., and Annison, E. F. (1987). Plasma/blood metabolites in ewes selectively bred for high or low clean fleece weights : use for selection of superior animals. (submitted to Applied Ovine Sciences Symposium, Perth - cancelled).

Jackson, N., Nay, T., and Turner, H. N. (1975). Response to selection in Australian Merino sheep. VII. Phenotypic and genetic parameters for some wool follicle charactersitics and their correlation with wool and body traits. Aust. J. Agric. Res. **26**, 937-57 (cited by Heydenrych et al. 1977).

Joakimsen, O., Steenberg, K., Lien, H., and Theodorsen, L. (1971). Genetic relationship between thyroxine degradation and fat-corrected milk yield in cattle. Acta Agric. Scand. **21**, 121-124.

Johnson, D. L. (1987). Selection for production characters in Romney sheep under New Zealand hill country conditions. Proc. Aust. Assoc. Anim. Prod. 220-21.

Johnson, D. L. (1981). Selection for production characters in Romney sheep under New Zealand hill country conditions. Proc., 2nd Conf. Aust. Assoc. Anim. Breed. Gen. pp. 220-21.

Johnson, D. L., and Dobbie, J. L. (1987). Responses to selection for yearling liveweight and fleece weight in Romney sheep. Proc., 4th Anim. Sc. Congr. Asian-Australasian Assoc. Anim. Prod. Soc., Hamilton, New Zealand. p 376.

Johnsson, I. D., and Hart, I. C. (1985). The effects of exogenous bovine growth hormone and bromocriptine on growth, body development, fleece weight and plasma concentrations of growth hormone, insulin and prolactin in female lambs. Anim. Prod. **41**, 207-17.

Johnston, E. F., Hudson, F., Bogart R., Oliver, A. W., and McKenzie, F. F. (1956). The effects of ingested testosterone on adult ewes. J. Anim. Sc. **15**, 177-84.

Lambourne, L. J. (1964b). Stimulation of wool growth by thyroxine implantation. II. Feed intake of grazing Merino wethers treated repeatedly with thyroxine. Aust. J. Agric. Res. **15**, 676-97.

Langlands, J. P., and Hamilton, B. A. (1969). Differences between breeds and strains varying in age. Aust. J. Exp. Agric. and Anim. Husb. **9**, 254-57.

Langlands, J. P. (1970). Efficiency of wool production of grazing sheep. 3. The use of sulphur-containing amino acids to stimulate wool growth. Aust. J. Exp. Agric. Anim. Husb. **10**, 665-71.

Lincoln, G. A. and Ebling, F. J. P. (1985). Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. J. Reprod. Fert. **73**, 241-53.

McClelland, L. A., Blair, H. T., Wickham, G. A., and Brookes, I. M. (1986). A comparison of rams from fleece weight-selected and randomly bred Romney flocks for intake, wool growth and feed utilization. Proc. N.Z. Soc. Anim. Prod. **46**, 215-18.

McClelland, L. A., Wickham, G. A., and Blair, H. T. (1987). Efficiency of Romney hoggets from a fleece weight selected flock. Proc., 4th Aust. Assoc. Anim. Prod., Animal Science Congress, Hamilton, New Zealand. pp. 330.

McCutcheon, S. N., and MacKenzie, D. D. S. (1989). Genetic markers in dairying. Proc. of the World Jersey Bureau Conference, Hamilton, New Zealand.

McCutcheon, S. N., MacKenzie, D. D. S., and Blair, H. T. (1987). Nitrogen metabolism and plasma urea concentrations in fleeceweight-selected and control Romney rams. Aust. J. Agric. Res. **38**, 917-26.

McGuirk, B. J. (1980). The effect of selection for increased fleeceweight. Proc. Aust. Soc. Anim. Prod. **13**, 171-74.

McGuirk, B. J. (1983). Genetic selection for wool production. In 'Sheep Production'. (Ed W. Havesign) pp. 545-66. (Butterworths, London).

McGuirk, B. J., Atkins, K. D., and Thompson, R. (1986). Long term selection experiments with sheep. 3rd World Congr. Gen. Applied Livest. Prod. XII 181-95.

MacKenzie, D. D. S., Wilson, G. F., McCutcheon, S. N., and Peterson, S. W. (1988). Plasma metabolite and hormone concentrations as predictors of dairy merit in young Friesian bulls: effect of metabolic challenges and fasting. Amin. Prod. **47**, 1-10.

Marsh, V. H., Fingerhut, B., and Muller, H. (1965). Automated and manual direct methods for the determination of blood urea. Clin. Chem. **11**, 624-27.

Marston, H. R. (1935). Studies on the relationship between nutrition and wool production of Merino sheep. II. The effect of the administration of cystine, cystein, sulphur and of methionine on the growth of wool of a Merino ewe on a protein-poor ration. J. Agric. Sc. **25**, 113-30.

Mersmann, H. J., Pond, W. G., and Yen, J. T. (1984). Use of carbohydrate and fat as energy source by obese and lean swine. J. Anim. Sci. **58** (4), 894-902.

Moore, G. P. M., Panaretto, B. A., and Robertson, D. (1981). Epidermal growth factor causes shedding of the fleece of Merino sheep. Search **12**, 128-29.

Pattie, W. A., and Barlow, R. (1974). Selection for clean fleece weight in Merino sheep. I. Direct response to selection. Aust. J. Agric. Res. **25**, 643-55.

Piper, L. R., and Dolling, C. H. S. (1969). Efficiency of conversion of food to wool. IV. Comparison of sheep selected for high clean wool weight with sheep from a random control group at three levels of dietary protein. Aust. J. Agric. Res. **20**, 579-87.

Rattray, P. V. (1986). Feed requirements for maintenance, gain and production. In 'Sheep Production: Vol 2. Feeding, Growth and Health'. (Eds S. N. McCutcheon, M. F. McDonald and G. A. Wickham.) Ch.5. (New Zealand Institute of Agricultural Science).

Reis, P. J. (1965). Variation in sulphur content of wool. In 'Biology of Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short). pp. 365-75. (Angus and Robertson, Sydney).

Reis, P. J. (1967). The growth and composition of wool. IV. The differential response of growth and of sulphur content of wool to the level of sulphur-containing amino acids given abomasally. Aust. J. Biol. Sci. **20**, 809-25.

Reis, P. J. (1969). The growth and composition of wool V. Stimulation of wool growth by the abomasal administration of varying amounts of casein. Aust. J. Biol. Sci **22**, 745-59.

Reis, P. J. (1979). Effects of amino acids on the growth and properties of wool. In 'Physiological and Environmental Limitations to Wool Growth' (Eds J. L. Black and P. J. Reis). University of New England Publishing Unit. Armidale, N.S.W., Australia.

Reis, P. J., and Schinckel, P. G. (1963). Some effects of sulphur-containing amino acids on the growth and composition of wool. Aust. J. Biol. Sci. **16**, 218-30.

Reis P. J., and Tunks, D. A. (1968). Reproducibility of individual differences in the sulphur content of wool grown by Merino sheep. Proc. Aust. Soc. Anim. Prod. **7**, 402-6.

Reis, P. J., Tunks, D. A., Williams, O. B., and Williams, A. J. (1967). A relationship between sulphur content of wool and wool production by Merino sheep. Aust. J. Biol. Sci. **20**, 153-63.

Reis, P. J., and Williams, A. J. (1965). Variations in sulphur content of wool from Merino sheep in two semi-arid grasslands. Aust. J. Agric. Res. **16**, 1011-20.

Reklewska, B. (1974). A note on the effect of bovine somatotrophic hormone on wool production in growing lambs. Anim. Prod. **19**, 253-5.

Rizzi, R., Zanotti, M., Giuliani, M. G., and Rognoni, G. (1988). Heritability of erythrocyte reduced glutathione (GSH) in ''delle Langhe'' sheep. J. Anim. Breed. Gen. **105**, 384-88.

Robards, G. E., Davis, C. H., and Saville, D. G. (1976). Efficiency of conversion of food to wool. Aust. J. Exp. Agric. Anim. Husb. **16**, 361-66.

Rose, J., Stormshak, F., Oldfield, J., and Adair, J. (1984). Induction of winter fur growth in mink (Mustela vison) with melatonin. J. Anim. Sc. **58** (1), 57-61.

Rougeot, J., Thebault, R. G., and Allain, D. (1984). Role of the compound hair follicle in adaptive pelage changes. Acta Zool. Fennica 171, 19-21.

Rust, C. C., Shackelford, R. M., and Meyer, R. K. (1965). Hormonal control of pelage cycles in the mink. J. Mamm. **46** (4), 549-65.

Saville, D. G., and Robards, G. E. (1972). Efficiency of conversion of food to wool in selected and unselected Merino types. Aust. J. Agric. Res. **23**, 117-30.

Sejrsen, K., Larsen, F., and Andersen, B. B. (1984). Use of plasma hormone and metabolite levels to predict breeding value of young bulls for butterfat production. Anim. Prod. **39**, 335-44.

Sinnett-Smith, P. A., Slee, J., and Woolliams, J. A. (1987). Biochemical and physiological responses to metabolic stimuli in Friesian calves of differing genetic merit for milk production. Anim. Prod. **44**, 11-19.

Slee, J., and Carter, H. B. (1961). A comparative study of fleece growth in Tasmanian Fine Merino and Wiltshire Horn ewes. J. Agric. Sci. **57**, 11-19.

Slen, S. B., and Connell, R. (1958). Wool growth in sheep as affected by the administration of certain sex hormones. Can. J. Anim. Sc. **38**, 38-47.

Sorensen, M. T., Kruse, V., and Andersen, B. B. (1981). Thyroxine degradation rate in young bulls of Danish dual-purpose breeds. Genetic relationship to weight gain, feed conversion and breeding value for butterfat production. Live. Prod. Sci. **8**, 399-406.

Southcott, W. H., and Royal, W. M. (1971). Effect of implanted testosterone propionate on the growth and wool production of Merino wethers. Aust. J. Agric. Res. **22**, 271-82.

Story, L. F., and Ross, D. A. (1960). Effect of shearing time on wool. VI. The rate of growth of wool and its relation to time of shearing. N.Z. J. Agric. Res. **3**, 113-24.

Thomson, B. C., Dellow, D. W., and Barry, T. N. (1989). The effect of selection for fleece weight upon urea metabolism and digestive function in Romney sheep. Aust. J. Agric. Res. **40**, 1065-74.

Thorburn, G. D., Waters, M. J., Young, I. R., Dolling, M., Buntine, D., Asa, P. S., and Hopkins, P. S. (1981). Studies of the physiology of epidermal growth factor. Proc. Endo. Soc. Aust. **24**, S4.

Tilakaratne, N., Alliston, J. C., Carr, W. R., Land, R. B., and Osmond, T. J. (1980). Physiological attributes as possible selection criteria for milk production. I. Study of metabolites in Friesian calves of high or low genetic merit. Anim. Prod. **30**, 327-40.

Tilakaratne, N., Hill, W. G., and Land, R. B. (1981). Direct and correlated responses to selection for plasma thyroxine levels in mice. Gen. Res. **38**, 157-69.

Turner, H. N. (1958). Relationship amongst clean wool weight and its components. I. Changes in clean wool weight related to changes in its components. Aust. J. Agric. Res. **9**, 521-52.

Turner, H. N., and Dolling, C. H. S. (1970) - cited by McGuirk et al. (1986).

Turner, H. N., and Jackson, N. (1978). Response to selection in Australian Merino sheep. VIII. Further results on selection for high clean wool weight with attention to quality. Aust. J. Agric. Res. **29**, 615-29.

Turner, H. N., Brooker, M. G., and Dolling, C. H. S. (1970). Responses to selection in Australian Merino sheep. III. Single character selection for high and low values of wool weight and its components. Aust. J. Agric. Res. **21**, 955-84.

Turner, H. N., Dolling, C. H. S., and Kennedy, J. F. (1968). Responses to selection in Australian Merino sheep. I. Selection for high clean wool weight, with a ceiling on fibre diameter and degree of skin wrinkle, response in wool and body characteristics. Aust. J. Agric. Res. **19**, 79-112.

Van Maanen, M. C., McCutcheon, S. N., and Purchas, R. W. (1989). Plasma metabolite and hormone concentrations in Southdown ram hoggets from lines divergently selected on the basis of backfat thickness. N.Z. J. Agric. Res. **32**, 219-26.

Wheatley, I. S., Wallace, A. L. C., and Bassett, J. M. (1966). Metabolic effects of ovine growth hormone in sheep. J. Endo. **35**, 341-53.

Wickham, G. A., and McPherson, A. W. (1985). A review of the importance of wool traits as genetic improvement objectives and selection criteria for New Zealand Romney sheep. Proc., N.Z. Soc. Anim. Prod. **45**, 203-8.

Williams, A. J. (1973). Metabolism of cystine by Merino sheep genetically different in wool production. III. The incorporation of radioactivity into wool fibres during and after intravenous infusions of L-[^{35}S]cystine and its relationship to wool growth and efficiency of conversion of food into wool. Aust. J. Biol. Sci. **26**, 465-76.

Williams, A. J. (1976). Metabolism of cystine by Merino sheep genetically different in wool production. IV. Rates of entry of cystine into plasma, measured with a single intravenous injection of L-[^{35}S] cystine, and the subsequent incorporation of ^{35}S into wool fibres. Aust. J. Biol. Sci. **29**, 513-24.

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. 337-54. (University of New England Publishing Unit : Armidale, N.S.W.)

Williams, A. J., and Winston, R. J. (1965). Relative efficiencies of conversion of feed to wool at three levels of nutrition in flocks genetically different in wool production. Aust. J. Exp. Agric. Anim. Husb. **5**, 390-95.

Williams, A. J., Leng, R. A., and Stephenson, S. K. (1972). Metabolism of cystine by Merino sheep genetically different in wool production. I. Comparison of the entry rates of cystine in sheep from flocks selectively bred for high and low fleece weight. Aust. J. Biol. Sc. **25**, 1259-68.

Williams, A. J., Robards, G. E., and Saville, D. G. (1972). Metabolism of cystine by Merino sheep genetically different in wool production. II. The responses in wool growth to abomasal infusions of L-cystine or DL-methionine. Aust. J. Biol. Sci. **25**, 1269-76.

Williams, O. B., and Schinckel, P. G. (1962). Seasonal variation in wool growth and liveweight in several environments. Aust. Soc. Anim. Prod. Proc. IV. pp. 38-45.