# PHYSIOLOGICAL RESPONSES TO SELECTION
# FOR GREASY FLEECEWEIGHT
# IN ROMNEY SHEEP

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## ABSTRACT

The study was undertaken to identify physiological differences between the Control (C) and Fleeceweight-selected (FW) lines of Romney sheep at Massey University. These differences were examined with a view to determining the mechanisms by which sheep of high genetic merit attain their superior fleece production, and to identification of potential markers of genetic merit.

Three experiments were conducted using rams and ram hoggets from the selection lines. The first (E1) was a preliminary investigation in which 12 C and 12 FW rams (aged 14 months) were fed freshly cut pasture *ad libitum* and blood sampled by jugular venipuncture. In the second (E2) 9 ram hoggets from each line were fed a lucerne-based diet (at fixed intakes irrespective of liveweight) during a stabilisation period and a subsequent treatment period in which they received the basal diet plus one of 3 levels (0, 30, or 60g) of formaldehyde-protected casein. In the third experiment (E3) rams received an intravenous infusion of saline or saline plus 1.5g/day methionine in a switchback design. During this experiment rams were fed 110% maintenance (lucerne chaff) in a regimen designed to maintain steady state conditions.

In contrast to literature reports, no difference was found between the lines in plasma levels of either aspartate or alanine amino-transferase activities (E2). FW rams did maintain greater concentrations of reduced glutathione (GSH) in erythrocytes but differences were not significant. Concentrations of GSH were substantially increased by methionine infusion (E3).

In all three experiments, C rams maintained greater concentrations of urea in the plasma than FW rams (by approximately 1mM). Supplementation of the diet with protected casein increased plasma urea concentration but this effect was additive with that of selection line (E2). Methionine infusion did not affect plasma urea in FW rams but reduced that of C rams so that the between-line difference was reduced by 50%. Control rams were also observed to have greater creatinine concentrations than FW rams (E3) but these were not influenced by methionine infusion. The reduced plasma urea concentration of FW rams may be due both to their lower rates of amino acid deamination and to an increased glomerular filtration rate.

Fleeceweight rams also exhibited lower concentrations of thyroxine than C rams in the two experiments in which they were examined (E2, E3). This result is consistent with those from Australian Merino selection lines but the physiological basis for the difference is unknown.

It is concluded that, in view of their consistent association with genetic merit for fleece production, and their ease of measurement, plasma concentrations of urea, creatinine and thyroxine may prove to be useful predictors of genetic merit for wool production.

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| | |
|---|---|
| cm | centimetres |
| °C | degrees celcius |
| d | day |
| d.f. | degrees of freedom |
| dl | decilitre |
| DM | dry matter |
| g | gram |
| h | hour |
| IU | international units |
| kg | kilogram |
| l | litre |
| ME | metabolisable energy |
| mg | milligram |
| MJ | megajoule |
| ml | millilitre |
| mm | millimetre |
| mM | millimolar |
| µg | microgram |
| S.E. | standard error |
| $U.L^{-1}$ | units per litre |

## Levels of Significance

| | |
|---|---|
| NS | $.10 < P$ |
| † | $.05 < P < .10$ |
| * | $.01 < P < .05$ |
| ** | $.001 < P < .01$ |
| *** | $P < .001$ |

# CHAPTER I

# INTRODUCTION

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# INTRODUCTION

## Establishment of Selection Lines and Responses to Selection.

Numerous selection flocks have been established to test the hypothesis that mass selection for characters such as fleeceweight results in genetic improvement (Table 1.1). Selection, in most cases, has been for increased or decreased fleeceweight or for high or low values of other skin or wool characters. Some of the flocks have been initiated with the additional purpose of extending knowledge of skin and wool biology (Turner *et al.*, 1970).

Single character selection for clean or greasy fleeceweight has been applied to a number of the flocks (experiments 1 to 5, Table 1.1). Other flocks have been subjected to multitrait selection, the major objective being to increase fleeceweight while taking account of additional characters through the use of independent culling levels. In so doing, wool quality has been maintained and/or faults such as excess skin wrinkle or face cover have been decreased (Turner, 1977). Also of interest is a flock selected for increased or decreased crimp frequency (experiment 10), as both the chemical composition and physical properties of the wool have been examined extensively in this flock. Management and selection criteria of these flocks have been summarised by McGuirk (1979).

The majority of selection experiments undertaken thus far have involved the Merino breed. Several such flocks have, however, been established in New Zealand using Romney sheep. Included amongst these

TABLE 1.1 : Summary of selection experiments for increased fleeceweight and crimp frequency.

| | Breed | Selection basis | Nos per generation Rams | Nos per generation Ewes | Year | Reference |
|---|---|---|---|---|---|---|
| Single-trait selection flocks | | | | | | |
| 1. | Australian | High clean FW | 5 | 100 | 1951 - | Pattie and Barlow (1974) |
| | Merino | Control | 10-25 | 100 | 1951 - | McGuirk (1980) |
| 2. | Australian | High clean FW | 1 | 32- 50 | 1954 - 1976 | Turner, Brooker and Dolling (1970) |
| | Merino | Control | 5-10 | 200-250 | 1948 - 1976 | |
| 3. | New Zealand | High greasy FW | 4 | 80 | 1958 - | Blair *et al.* (1985) |
| | Romney | Control | 4 | 80 | 1958 - | |
| 4. | New Zealand | High greasy FW | 5 | 150 | 1973 - | Clarke (1983) |
| | Romney | Control | 5 | 150 | 1973 - | |
| 5. | New Zealand | High greasy FW | 5 | 150 | 1961 - | Johnson (1985) |
| | Romney | Control | 5 | 150 | 1966 - | |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| Multi-trait selection flocks |  |  |  |  |  |  |
| 6. | Australian Merino | High clean FW/checks on crimp freq., skin wrinkle & face cover | 6 | 200 | 1947-72 | Dun and Eastoe (1970) |
|  |  | Control | 5 | 100 | 1951- |  |
| 7. | Australian Merino | High clean FW/checks on fibre diam. and skin wrinkle | 8-15 | 200-600 | 1950-76 | Turner, Brooker and Dolling (1968) |
|  |  | Control | 5-10 | 200-250 | 1948-76 |  |
| 8. | Australian Merino | High clean FW/checks on skin wrinkle & fibre diam.(1950-52) or crimp freq.(1961-76) | 5-8 | 100-250 | 1950-76 | Turner and Jackson (1978) |
|  |  | Control | 5-10 | 100-250 | 1948-76 |  |
| 9. | South African Merino | High clean FW/checks on fibre diam. & crimp freq. | 5 | 100 | 1969- | Heydenrych *et al.* (1977) |
|  |  | Control | 5 | 160 | 1969- |  |

Table 1.1 continued

| Breed | Selection Basis | Rams | Ewes | Year | Reference |
|---|---|---|---|---|---|
| Crimp frequency selection flock | | | | | |
| 10 Australian | High crimp freq. | 5 | 100 | 1951-1972 | Robards and Pattie (1967) |
| Merino | Low crimp freq. | 5 | 100 | 1951-1972 | |

is a flock established at Massey University in 1956 and used in the current investigation. A full description of the flock and data collection procedures has been given by Blair *et al.*(1984). Two similar flocks established in New Zealand have been described by Clarke (1983) and Johnson (1985).

Direct responses to selection have been well documented (Pattie and Barlow,1974; Turner, Brooker and Dolling,1970; Dun and Eastoe, 1970; Turner *et al.*, 1968; Turner and Jackson,1978; McGuirk, 1979; Blair *et al.*, 1985; Johnson,1985; Clarke,1983). In general, selection for increased fleeceweight has resulted in a direct response of 1 to 3% per year and correlated responses such as increases in follicle density, fibre diameter and fibre length.

Apart from lines which differ in fleeceweight as a result of direct selection, there exist other groups of sheep in which fleeceweight is known to vary according to genotype for specific (and simply inherited) traits. Of particular interest are genes determining erythrocyte potassium levels in both selected and unselected groups. Data suggest that wool quality and quantity may be associated with genotype for blood potassium and with actual red blood cell potassium concentration within genotypes (Taneja,1968; Taneja *et al.*,1969; Agar *et al.*,1972). Moreover selection for increased fleeceweight or decreased crimp frequency has been accompanied by an increase in mean erythrocyte potassium concentration and by an increase in the gene frequency of the Hk allele (Evans *et al.*,1973; Hopkins *et al.*, 1975). There is also limited evidence of an association between haemoglobin type and wool production (Watson and Khattab,1964; Evans *et al.*, 1973).

The establishment of selection flocks allows the generation of lines divergent in genetic merit for fleeceweight. Even though the genotype of each individual animal is not known, it is nevertheless possible to examine the physiological differences between groups of animals known to differ in average genetic merit. In other genetically distinct groups (e.g. $K^+$ types), phenotypes for fleeceweight may be associated with readily identifiable genotypes for some other trait. In such cases it becomes possible to identify physiological parameters which may be causally associated with a propensity for high fleeceweight.

Little is known of the physiological mechanisms by which sheep of high genetic merit for fleeceweight attain their superior levels of production. Identification of the underlying physiological and biochemical parameters responsible could have considerable impact on the industry in three major areas, namely:-

(a) Prediction of genetic merit for fleeceweight in young rams and ewes.

Study of the comparative physiology of animals differing in genetic merit may lead to the identification of traits closely associated with superior wool production. These traits may in turn be used as metabolic predictors of genetic merit. Such predictors or "markers" might then be used to identify genetically superior animals (for use as replacement stock) at 6 to 8 months of age. At present rams and ewes are primarily selected on the basis of their own performance and, to a lesser extent, that of relatives, or through progeny testing. Identification of the superior sires prior to hogget shearing holds the potential to increase the rate of genetic gain through a decrease in the generation interval. Improved accuracy of selection is also a promising possibility.

Providing their assay is manageable these markers, while possibly decreasing the costs of breeding programmes, would also be expected to increase their effectiveness. This could be realised through the ability to screen large numbers of animals of both sexes, and through allowing ewes to be separated into genetically superior flocks.

The application of "markers" would have particular relevance if combined with the use of such recently developed techniques in reproductive physiology as artificial insemination . The ability to widely use sires of high genetic merit from a young age would allow desirable genetic material to be more rapidly disseminated throughout the national flock.

(b) Development of exogenous agents which increase the level of wool growth.

The widest applications of exogenous agents for increasing animal production have undoubtedly been as growth promotants in the field of beef cattle and prime lamb production and as promotants of lactation. In most cases, however, the discovery of their growth- and lactational-promoting properties has been incidental to basic research aimed at identifying the mechanisms underlying production. The genetic progress made in selection experiments has allowed comparison of the divergent genotypes with a view to identification of the physiological and biochemical mechanisms responsible for superior wool production. Subsequent to their identification, it may be possible to utilize those factors recognised as having a stimulatory effect on wool growth, or their synthetic analogues, to increase efficiency in commercial flocks.

(c) Application of techniques in molecular biology to the task of improving wool production.

A study of the divergent flocks may ultimately lead to an identification of the genes responsible for high wool production. This will be necessary if gene manipulation is to be used in the longer term to produce animals capable of superior levels of wool production.

## Dietary Intake and Efficiency of Wool Production

Improved productivity in flocks selected for increased fleeceweight may result from an increase in feed intake and/or an increase in gross efficiency (defined as the ratio of wool production to feed intake). Efficiency of wool production has been examined both under conditions of *ad libitum* feeding (in which selection lines are permitted to express inherent differences in voluntary intake) and under restricted feeding.

An association between voluntary intake and fleeceweight has been demonstrated in comparisons between breeds (Weston, 1959) and between strains (Daly and Carter, 1955: Ahmed *et al.*, 1963). In these studies the animals with higher fleeceweights were also heavier. However, the responses in fleeceweight were proportionately greater than the responses in voluntary intake with the result that selected animals were more efficient.

Schinckel (1960) and Hutchinson (1961) each demonstrated that the variation in voluntary intake among animals within an unselected Merino flock made a significant contribution to the phenotypic variation in wool production. This aside, both emphasised that differences between individuals in wool production were largely determined by differences in gross conversion efficiency.

Hamilton and Langlands (1969) considered the efficiency of rams from flocks selected for and against fleeceweight. Three pastures differing in herbage availability were grazed *ad libitum*, and voluntary intake was measured indirectly using output of faecal organic matter.

Results showed that intake of high fleeceweight animals exceeded that of low fleeceweight animals only on the lower levels of pasture availability. At all levels of pasture intake, significant differences between the flocks in both efficiency and wool growth were observed. Thus, it was concluded that the increased wool production of the high fleeceweight flock was attributable mainly to an increase in efficiency, rather than a greater intake.

A limited number of studies compared the efficiency of selected flocks only under conditions of restricted feeding. Voluntary intake was also measured in these studies under the premise that although animals are offered equal quantities of feed, they may not eat similar amounts. Under these conditions, additional wool production was largely due to the superior conversion efficiency of selected sheep rather than to differences in feed intake. This applied in Merino sheep selected either for increased clean fleeceweight (Dolling and Moore, 1960; Williams and Miller, 1965; Williams and Winston, 1965) or for decreased crimp frequency (Robards *et al.*, 1974). There was, moreover, an indication of increasing efficiency with continued selection for either increased fleeceweight or decreased crimp frequency.

The majority of efficiency studies have considered the effect of both *ad libitum* and restricted feeding levels on unselected (Wodzicka- Tomaszewska, 1966) and selected flocks (Williams, 1966; Dolling and Piper, 1968; Piper and Dolling , 1969; Saville and Robards, 1972; McClelland *et al.*, 1986). Results from these studies were in close agreement with those from experiments involving one level of feeding.

Results in general have shown efficiency to be positively correlated with wool production under both *ad libitum* and restricted feeding levels. Moreover, the response in efficiency accounts for most of the variation in wool production between divergently selected flocks.

Many of these studies demonstrated a genotype by environment interaction in relation to level of nutrition. The difference between genotypes in wool production and efficiency was shown to increase with increasing levels of nutrition. Animals with a high potential for wool production possessed a greater ability to respond to a given increase in feed intake (Weston, 1959; Schinckel, 1960; Williams and Winston, 1965; Williams, 1966; McManus *et al.*, 1966; Hamilton and Langlands, 1969; Piper and Dolling, 1969; Saville and Robards, 1972).

It is clear, therefore, that efficiency of conversion of feed into wool is the major factor contributing to variation in wool production when comparisons are made between genotypes. Variation in wool production within flocks can also be explained in much the same way. Differences in voluntary feed intake do not appear to make a major contribution to the genetic variation in wool production.

## Factors Contributing to Variation in Efficiency

### 1. Diet selection

It may be possible for strains to obtain a productive advantage through a difference in their inherent ability to select a diet of superior quality. The degree to which these differences are expressed may depend on the environmental conditions to which animals are subjected (i.e. the opportunity to express differences in diet selection will be greatest when animals are offered pastures or other mixed diets *ad libitum* ).

A number of studies have therefore been conducted in both pen-feeding and grazing situations. Moore and Dolling (1961) found that the nutritive value of feed selected by individual Merino sheep under pen-feeding conditions had a coefficient of variation of approximately 10%. Weston (1959) estimated that the diet selected by strong-woolled Merinos was of a higher nutritive value than that of fine-woolled Merinos, but differences were small. Ahmed *et al.* (1963) conducted a pen trial in which a comparison was made between a control flock and flocks selected for either high or low fleeceweight. There was no evidence to suggest that the high fleeceweight flock had a greater preference for a high protein diet. All flocks selected the same average diet and, despite individual preferences, protein intake paralleled feed intake.

From the results obtained it can be concluded that variability in diet selection is not a major factor influencing the variation in efficiency of wool production between genotypes.

## 2. Digestive ability

The enhanced efficiency of feed conversion in high fleeceweight animals could also be attributable to an improved ability to digest dietary components. However, Dunlop *et al.* (1966) found that the difference between Merino strains in the digestibility of organic matter and protein was extremely small and non-significant. Similar results were obtained by Weston (1959). In that study strong-woolled sheep exhibited a greater digestibility of dietary nitrogen than fine-woolled sheep in some pen-feeding experiments but the differences were small and results were not consistent between experiments.

Comparisons between flocks divergently selected for fleeceweight have shown much the same results. Piper and Dolling (1969) found that the differences between selected flocks (high fleeceweight and control) in the ability to digest organic matter, dry matter, energy and nitrogen (of a range of pelleted and chaffed diets) was small, variable and in no instance significant. Williams (1979) showed that the concentrations and ratios of volatile fatty acids and the concentration of ammonia in the rumen fluid were similar in sheep selectively bred for high or low wool production. McClelland *et al.* (1986) obtained variable results from two pen-feeding trials using Romney sheep. When fed freshly cut pasture *ad libitum*, control rams showed a greater dry matter digestibility than fleeceweight rams. However the same differences were not observed when different levels of a lucerne-based diet were fed.

Relative to the coefficient of variation in digestibility of organic matter and nitrogen between sheep within strains, the variation between strains in efficiency is large (Weston, 1959). It appears, therefore, that the ability to digest nutrients, at least as measured by apparent digestibility, has not been altered by selection for increased fleeceweight. It follows that if two lines have similar feed intakes, they must also have similar intakes of digestible energy and nitrogen. These components do not then contribute to between-line variation in efficiency of wool growth.

The possibility that selection lines vary in their ability to convert digestible nutrients to metabolisable nutrients (i.e. that differences exist in gaseous and urinary losses of energy and nitrogen) does not appear to have been examined in detail. McClelland *et al.* (1986) have reported that fleeceweight - selected and control Romney rams do not differ in rates of urinary nitrogen excretion. This suggests that selection for increased fleeceweight is not accompanied by changes in the conversion of digestible to metabolisable nitrogen.

## 3. Metabolic rate

Animals of similar weight which differ in wool production may also differ in metabolic rate or maintenance requirement. This possibility has been examined in several studies using fleeceweight-selected flocks (Graham, 1968; Williams and Miller, 1965; Williams and Winston, 1965). While the last two studies found no difference in maintenance requirement between flocks, Graham (1968) showed some evidence of a greater metabolic rate (fasting heat production) in high fleeceweight compared to low fleeceweight rams. Some of this difference may have been due to the larger skeletal size of the high fleeceweight rams. However, since no systematic study of the effect of skeletal size on metabolic rate of adult sheep had been conducted, it was not possible to accurately adjust for the difference in skeletal size between the groups.

In summary, the efficiency of conversion has been shown to be a major factor contributing to variation in wool production between genotypes. It is apparent that differences in voluntary feed intake, feed selectivity, digestive ability and metabolic rate make no significant contribution to the genetic variation in wool production. Differences between genotypes in the efficiency of conversion of feed into wool must, therefore, be due primarily to differences in the post-absorptive utilisation of nutrients.

## Regulation of Wool Growth and Nutrient Utilisation in Selection Lines

### 1. Hormonal regulation of wool growth

The regulation of wool growth by various anabolic and catabolic hormones has been comprehensively reviewed by Ferguson *et al.* (1965) and Wallace (1979). During subsequent years few advances have been made and knowledge in this area has remained relatively static. Although methods for measuring the plasma concentrations of hormones have been available for some years, few attempts have been made to relate these to wool growth in selection lines or other genetically divergent groups of sheep.

Differences between sheep in plasma hormone concentrations may account for individual variation in wool growth. Furthermore selection for high fleeceweight might in turn influence levels of hormone secretion or alter the sensitivity of follicles to circulating hormones. There are several means by which hormones may exert an influence on wool growth. These include the alteration of either the mitotic rate in the follicle bulb or the growth rate of cells once beyond the mitotic zone. A further possibility is changes in the supply of specific limiting nutrients which in turn control the rate of synthetic activity in the follicle.

Several pituitary hormones appear to play an integral role in the regulation of wool growth. There may also be some as yet unidentified hormones released from this, or other glands, which in turn possess important regulatory functions. In general pituitary hormones act by stimulating the release of hormones from other endocrine glands. They will therefore be discussed in conjunction with those hormones whose release they stimulate.

(a) TSH and thyroid hormones

Thyroid stimulating hormone (TSH) acts on the thyroid gland stimulating the release of the biologically active triiodothyronine (T3) and thyroxine (T4) which is later converted to active T3 in the body tissues. The role of thyroid hormones in the growth and development of structures such as hair, feathers and wool has long been recognised and many workers have attempted to elucidate their mechanism of action.

Thyroid hormones are known to be required for the differentiation and growth of the wool fibre. Simpson (1924) found that thyroidectomy of lambs retarded wool growth and reduced fleeceweights by 50% at 13 months of age. Moreover, thyroidectomy of newborn lambs prevented the normal maturation of secondary wool follicles. Subsequent administration of L-thyroxine allowed normal follicle development to continue. Depression of follicle maturation was accompanied by a decrease in wool growth rate, an affect which appeared to be independent of the lowered follicle population (Ferguson *et al.*, 1956). Thyroxine requirements for normal follicle maturation and wool growth appeared to be greater than the requirements for general body growth (Ferguson *et al.* , 1956). Since much of the development of the wool follicle occurs before birth, Hopkins and Thorburn (1972) investigated the effects of foetal thyroidectomy. In the thyroidectomised foetus the development of wool follicles was markedly retarded, the degree of retardation depending upon the age of the foetus at the time of surgery. Thyroidectomy at 81 days gestation prevented differentiation of cells into fibres in both primary and secondary follicles. The same operation performed at 95 days gestation resulted in a birthcoat consisting mainly of primary fibres. Such wool fibres were significantly different in length, but not in diameter, from those of sham-operated twin foetuses. Development of the sebaceous glands was also retarded but there was no effect on the potential S:P. ratio.

Although it is now widely recognised that thyroid hormones are necessary for the continuation of normal wool growth and follicle development, their mode of action remains unclear.

Ferguson *et al.*(1965), using hypophysectomised adult sheep, showed that daily injections of T4 restored wool growth to pre-operative levels, while increasing the amount injected from approximately 60 to 100 µg per day over a two year period failed to increase wool growth further. The wool growth response to a variety of pituitary extracts has also been investigated (Ferguson *et al.*, 1965). Injection of a crude TSH preparation in hypophysectomised sheep restored wool growth to pre-operative levels but, again, there was no further increase with increasing hormone dose. Ferguson *et al.*(1965) found that the wool growth response to TSH and T4 was dependent upon a maintenance dose of cortisone. Increasing this dose from 10 to 20 mg per day decreased the wool growth response to T4 and TSH preparations.

Like young lambs, thyroidectomised ewes on a constant feed intake show a 40 to 60% reduction in wool growth rate relative to a control group (Simpson, 1924 ; Ferguson *et al.*, 1965; Maddocks *et al.*, 1985). If thyroidectomy was incomplete (i.e. if fragments of the gland remained after surgery) wool growth remained normal. Thyroid hormones may therefore have a permissive rather than a regulatory role in the growth of wool. Daily subcutaneous injections providing either 30% of the normal plasma T4 concentrations (Maddocks *et al.*,1985) or complete replacement (Ferguson *et al.*, 1965; Maddocks *et al.*, 1985) restored wool growth to pre-operative levels. It is also possible to increase wool growth above control levels by maintaining supranormal concentrations (1.5 to 3 times normal concentrations) of T4 in thyroidectomised sheep (Ferguson *et al.*, 1965; Maddocks *et al.*, 1985). This is in direct contrast to observations in hypophysectomised sheep in which additional T4 fails to stimulate wool growth above the control levels. An additional pituitary factor must therefore be necessary for thyroid hormones to express their full physiological potential. The possibility that TSH possesses an additional, as yet uncharacterised function must also be considered. Hence the reduction in wool growth following hypophysectomy cannot be accounted for solely by the loss of T3 and T4.

Intact ewes showed no wool growth response until T4 was administered in doses equivalent to 1.5 to 3 times the endogenous levels. Thyroidectomised ewes showed similar increases in wool production when maintained on the same regime. Ten weeks after cessation of the treatment, wool growth returned to normal in the intact ewes and, in thyroidectomised animals, to the level existing prior to commencement of replacement therapy (Ferguson *et al.*, 1965). Ferguson (1958) and Ross and Lewis (1958) also administered T4 to intact sheep and showed similar responses in wool growth but noted a decrease in body weight.

Whereas intradermal injections of T4 caused local increases in fibre diameter (Downes and Wallace, 1965), the changes in wool growth induced by replacement therapy following thyroidectomy or T4 administration to intact animals appeared to be due exclusively to an increase in fibre length growth rate while fibre diameter remained unchanged (Theriez and Rougeout, 1962; Labban, 1957; Ross and Lewis, 1958). As variation in the plane of nutrition changes both the length growth rate and fibre diameter, it is unlikely that the effect of T4 on wool growth is due to induced changes in nutrient availability at the follicular level.

Overall consideration of the data on the effect of thyroid hormones on wool growth has suggested that T4 levels in normal grazing animals are unlikely to be of major consequence in affecting the individual variation in wool growth. A study by Hough *et al.* (1986) using Merino ewes selected for high and low fleeceweight, however, provides some evidence of a difference between the selection lines in plasma T4 concentration. Sheep selected for low fleeceweight maintained a significantly greater plasma concentration of T4 than those selected for high fleeceweight ($P < .01$). Hence further consideration of the role of T4 in determining individual variability in wool growth is clearly warranted.

### (b) ACTH and adrenal corticosteroids

The physiological action of adrenocorticotrophic hormone (ACTH) is to increase the secretion of steroids from the adrenal gland. The inhibitory action of administered ACTH and glucocorticoids on the function of skin and on the hair growth of several species has been well documented. However, in many of these studies, little attempt has been made to relate individual variation in wool growth to the circulating levels of glucocorticoids. In sheep, administration of ACTH and glucocorticoids causes depression of fibre growth (Linder and Ferguson, 1956; Ferguson *et al.*, 1965; Chapman and Basset, 1970; Panarretto *et al.*, 1975; Panarretto and Wallace, 1978a,b) and the depressive effect increases with hormone dose (Ferguson *et al.*, 1965). Fibre growth is completely suppressed when plasma cortisol concentration increases to 3 to 5 $\mu g.dl^{-1}$ (Ferguson *et al.*, 1965). When normal wool growth resumes after ACTH or glucocorticoid treatment a "break" in the fleece occurs due to diminished fibre diameter. Shedding of some fibres also occurs.

Cortisol is the major circulating glucocorticoid in sheep. Depression of wool growth in cortisol-treated animals is dose-responsive, but the length of time the plasma concentrations must be maintained in order to stop wool growth is unclear. Chapman and Bassett (1970) injected sheep with cortisol, increasing the dose every 3 weeks. After 12 weeks wool growth had ceased at a plasma cortisol concentration of 2.7 to 5.1 $\mu g.dl^{-1}$. Variable effects on the dimensions of the fibre were recorded. At low cortisol concentrations fibre diameter decreased and length growth rate remained unchanged. As the concentration increased the major effect was a reduction in fibre length growth rate. When plasma cortisol concentration remained above 3 $\mu g.dl^{-1}$ follicle inactivation occurred with cessation of fibre growth and the formation of brush ends on the fibres.

The synthetic analogues of glucocorticoid hormones, dexamethasone and flumethasone, have been found to possess many times the potency of natural hormones as inhibitors of wool growth. The effects of these and some dexamethasone derivatives on wool growth have been studied (Ferguson *et al.*,1965; Panaretto *et al.*, 1975; Panaretto and Wallace, 1978a,b). Ferguson *et al.* (1965), using a single intramuscular injection of dexamethasone, depressed wool growth in intact ewes for 10 weeks. This was followed by a rebound above the control level. As dexamethasone is relatively insoluble it would be expected to remain at the injection site and be removed more slowly from the circulation than cortisol. The subsequent stimulation of wool growth might be due to suppression of ACTH secretion resulting from negative feedback control of dexamethasone. However, such a route has not been adequately investigated. In general there was variation between individuals and between body regions in the wool growth response to synthetic analogues. These ranged from no obvious effect to prolonged wool growth depression or complete or partial shedding of the fleece (Panaretto *et al.*, 1975; Panaretto and Wallace, 1978a,b). Such a differential response would imply differences between the follicular cells in their sensitivity to the hormones and their analogues.

The mechanisms by which glucocorticoids depress wool growth are unclear. The hormones appear to have a direct effect on wool protein synthesis. Intradermal injections of cortisol or dexamethasone depress local uptake of L- [$^{35}$S] cystine into follicles (Wallace and Downs, 1979). It seems likely, therefore, that cortisol and its analogues act directly at the follicular level, but before any effects on wool growth are observed the plasma concentration of glucocorticoids must be elevated for several days. However, neither DNA nor protein synthesis in isolated wool follicles is affected by cortisol (Ward, 1979).

Changes in adrenal secretion do not account for the seasonality of wool growth as adrenalectomised sheep maintained on a constant dose of cortisone acetate continue to express seasonal periodicity (Ferguson *et al.*, 1965).

Differences in basal adrenal activity probably contribute little to the differences in wool growth between individual animals. The adrenal response to environmental stress may, however, alter wool growth.

### (c) Growth hormone

Until recent times growth hormone (GH) was thought to exert its physiological effects directly on target tissues. However, it is now apparent that at least some of the effects of GH are mediated via one or more peptide hormones, in particular the insulin-like growth factors (IGF or somatomedins).

The effect of GH on milk production and body growth has been the subject of many investigations but its role in the regulation of wool growth has received only limited attention. As one effect of GH is the promotion of protein synthesis in growing and lactating animals it is reasonable to assume that it might also influence wool growth. Wool growth is not initiated in hypophysectomised sheep, however, when GH alone is administered. Nor does GH influence wool growth when hypophysectomised sheep are maintained on T4 (Ferguson *et al.*, 1965). A number of studies have shown that the intramuscular or subcutaneous administration of crude pituitary extracts (Ferguson, 1951), purified bovine GH (bGH) (Ferguson *et al.*, 1965; Reklewska, 1974), and purified ovine GH (oGH) (Wheatley *et al.*, 1966; Wynn *et al.*, 1979) to intact sheep depresses wool growth during GH treatment. Following cessation of GH injections a sustained increase in wool growth, relative to pretreatment values, occurs for a period of many weeks. The size and duration of the response is variable and may depend upon the dietary supply of nutrients (Wynn, 1982) or on the GH preparation and dose. Conversely, other studies (Ferguson, 1954; Wynn, 1982; Johnsson *et al.*, 1985) found that the administration of either crude preparations or purified GH increased wool growth throughout the treatment period. The elevation in wool growth was sustained for a period of several weeks post-treatment.

In those studies showing a variable wool growth response to GH treatment (depression during and an increase following administration) the increase in wool growth appeared to be due primarily to an increase in fibre diameter. Thus, during GH treatment fibre diameter decreased while fibre length growth rate remained unchanged. Following cessation of treatment both fibre length growth rate and fibre diameter increased, the response being greater in the latter.

Numerous hypotheses have been advanced to explain the inconsistency between studies investigating the effects of exogenous GH. The variable and poorly documented nature of GH preparations makes comparisons between experiments difficult. It has been suggested that the delayed wool growth response to injections of bGH might reflect an immune response to the bovine hormone (Ferguson, 1954). The demonstration that oGH elicited a similar response (Ferguson *et al.*, 1965; Wheatley *et al.*, 1966) now makes this hypothesis unlikely.

As many studies do not use the same routes of GH administration, it is possible that this in itself affects the nature of the wool growth responses. In the light of studies by Mosely *et al.* (1982), Fronk *et al.* (1983) and McCutcheon and Bauman (1986) this seems unlikely. These studies examined the effects of variable patterns and routes of administration of GH in growing and lactating cattle. Although the widely different methods of administration produced equally different patterns of plasma GH concentration, response to the hormone was not influenced by route of administration. It therefore appears that the critical factor influencing the effects of GH on lactation and growth may be the average plasma concentration of GH over a 24 hour period rather than the actual route of administration or pattern in which plasma hormone concentrations are elevated.

A further possibility is that the inconsistent patterns of wool growth in response to GH reflect variable patterns of nutrient partitioning between the wool follicle and body tissues. This could be due to differences in blood flow to the skin or to the actions of other hormones whose secretion is influenced by growth hormone.

Thus studies which have shown wool growth to decrease during and increase after GH administration may reflect a partitioning of nutrients into body tissues during treatment followed by a loss of the body weight advantage (relative to a control group) post-treatment. The increased availability of nutrients may then stimulate wool growth. Support for this theory comes from work using dairy heifers conducted by Brumby (1959) and Sandles (1985). It was shown that the increase in body weight during GH administration was not sustained once treatment was withdrawn and several weeks after treatment no difference existed between the treated and control groups in either case.

It is unclear why the biphasic effect on wool growth is not seen in all studies. A limited number have shown that administration of GH to sheep (~0.1 - 0.25 mg/kg) was accompanied by an increase in the plasma insulin concentration. Insulin is known to increase the uptake of specific amino acids by skeletal muscle (Prior and Smith, 1982). It is possible that the administration of low GH concentrations fails to elicit an insulin response whereas, at higher concentrations, insulin release may be stimulated and the aforementioned responses observed. However this hypothesis will remain equivocal until further research is conducted. This should include a comprehensive dose-response study noting, in particular, the effect of variable GH concentrations on the plasma insulin response and concurrent partitioning of nutrients between wool and body tissues.

### (d) Other hormones

The seasonal variation in wool growth is thought to be associated with various hormones but the capacity in which they act is unclear.

The annual rhythm of plasma prolactin is known to be positively associated with that of wool growth (Hutchinson, 1976). Several studies have shown that prolactin concentration increases with increasing daylength and that superior ganglionectomy (destroying innervation of the pineal gland) decreases or removes the photoperiodically-induced prolactin response. The actual role of prolactin in regulating the annual rhythm of wool growth, however, remains poorly understood. There is no evidence that elevated concentration in the blood affects wool growth. Injection of bovine prolactin into hypophysectomised sheep (Ferguson *et al.* , 1965), or into intact sheep (Wallace and Wheatley, 1979) has no effect on wool growth. Administration of bromocriptine (to suppress prolactin secretion) had no influence on wool growth when administered either separately or in combination with bGH (Johnsson *et al.*, 1985).

The involvement of the pineal gland (via melatonin) in the regulation of wool growth has received only limited attention, most being focused on its role in seasonal reproduction and onset of puberty. Pinealectomy or dennervation of the pineal gland renders sheep non-photoperiodic (Lincoln *et al.* , 1980; Williams, 1981). The administration of melatonin is known to decrease wool growth by blocking the effects of long days on wool growth (moulting of the old coat accompanied by increased wool growth). The effects of melatonin may be mediated via an alteration in the circulating levels of those hormones which affect wool growth. The question of which of these hormones is involved has yet to be adequately answered. It appears that the inhibitory effect on the thyroid gland (Relkin, 1983), is the most likely to influence seasonal wool growth. The adrenal gland and prolactin may also be involved, but this requires further clarification.

Although not widely studied, plasma insulin concentration has not been shown to influence wool growth. Furthermore, intradermal injection of insulin has no direct effect on the wool follicle (Wynn, 1982).

Gonadotrophic hormones stimulate the secretion of active steroids from the ovaries and testes. The effect of these hormones on wool growth was reviewed by Ferguson *et al.* (1965). Whereas unphysiologically large doses of oestrogen depress wool growth, similar doses of testosterone stimulate wool growth (Slen and Connell, 1958). However, there appears to be no further data to contradict the conclusion drawn by Ferguson *et al.* (1965) that oestrogens, androgens and progesterone are unlikely to affect wool growth, at least at physiological concentrations.

It has been suggested that catecholamines and the sympathetic nervous system are involved in the peripheral regulation of wool growth by virtue of their direct action on the skin or indirect action on blood vessels supplying the wool follicles. It is unlikely that the circulatory levels of catecholamines influence wool growth since adrenalectomy does not alter wool growth in sheep (Ferguson *et al.*,1965). However, Reklewska (1975) found a positive relationship between the rate of wool growth and concentrations of both adrenaline and noradrenaline in the blood of ram lambs. The same effect was not apparent in ewe lambs. The importance of catecholamines in the regulation of the annual rhythm of wool growth remains to be determined.

It is clear from the preceding discussion that numerous hormones play an integral role in the regulation of wool growth and its seasonal rhythm. As yet, however, few attempts have been made to determine the extent to which these hormones account for the variation in wool production between fleeceweight-selected lines.

## 2. Utilisation of amino acids for wool growth

Studies to further examine differences between selection lines in wool production and feed efficiency have focused on the availability and utilisation of amino acids. Particular attention has been paid to the sulphur-containing amino acids (sulphur-amino acids) cyst(e)ine and methionine, and the related changes in the sulphur content of wool.

Wool is a scleroprotein composed mainly of keratin. It has a high sulphur content ranging from 2.7 to 4.2% in individual wool samples (Reis, 1965a,b). Considerable variation exists between samples from sheep grazing the same pasture or fed similarly under pen conditions (Reis, 1979). Most of the sulphur in wool is present as cystine with smaller quantities of cysteine and approximately 3% as methionine (Reis and Schinckel, 1964). Wool also contains considerable quantities of glutamic acid, serine and glycine.

The composition of wool proteins has been comprehensively reviewed by Bradbury (1973) and Crewther(1976). Briefly, keratin is heterogeneous and the soluble proteins extracted from wool are commonly grouped as:

Low sulphur (comprising approximately 67% of the proteins in wool and containing all the methionine and most of the lysine);

High sulphur (which contain large quantities of cystine, proline and serine); and

High tyrosine proteins (rich in tyrosine and glycine and notable for the lack of many amino acids).

Within these groups considerable heterogeneity exists, there being many subfractions in the high sulphur and high tyrosine proteins. The three major groups are thought to be associated with various structural components of cortical cells within the wool fibre. Low sulphur proteins are concentrated in the microfibrils which form the central core of the fibre. High sulphur and high tyrosine proteins are concentrated in the surrounding non-fibrous matrix, although those components of the latter group which are rich in glycine are associated with the membranes of the cells which form the outer cortex of the fibre (Bradbury, 1973).

### (a) Metabolism of sulphur-containing amino acids

It is now widely recognised that, under many dietary regimes, the rate of wool growth and its sulphur content is limited by the availability of sulphur-amino acids. Many studies have demonstrated an increase in both wool growth rate and sulphur content in sheep receiving abomasal supplements of DL-methionine, L- and D-methionine, methionine hydroxy analogue (MHA), or L-cysteine (Reis, 1967; Williams *et al.*, 1972a; Reis *et al.*, 1973; Robards, 1971; Reis, 1970). Supplements of L-cyst(e)ine or L-methionine given via intravenous infusion (Reis *et al.*, 1973; Downes *et al.*, 1970; Dryden *et al.*, 1969) or intraperitoneal infusion (Downes *et al.*, 1970) also stimulated wool growth and increased sulphur content. Intraperitoneal or subcutaneous injections of DL-methionine given at one or two day intervals were similarly effective (Wickham, 1970; Barry *et al.* , 1973; Barry and Drew, 1978). The observed increase in sulphur content of wool during supplementation was found to be due to an increased yield of sulphur proteins and the altered ratio of the subfractions (Gillespie, 1962).

Studies of the synthesis of high sulphur proteins during the administration of sulphur-amino acids have shown the presence of additional components of these proteins which could not be detected in "control" wool from the same sheep (Gillespie *et al.*, 1969). These components correspond to the ultra-high sulphur proteins and may constitute up to 10% of the wool mass during sulphur-amino acid supplementation. Gillespie and Reis (1966) suggested that the synthesis of the ultra-high sulphur proteins (which are rich in cystine) might depend upon the availability of cyst(e)ine to the follicles.

Considerable differences have been observed between animals in the proportions of specific amino acids in the wool and hence in sulphur content. Furthermore, the variability between animals may be associated with differences in their capacity for wool production. A phenotypic association between wool production and sulphur content has been clearly demonstrated in a number of studies (Reis, 1965a,b; Reis and Williams, 1965; Gillespie and Reis, 1966; Reis and Tunks, 1968). In each case the higher wool producers were shown to grow wool of a lower sulphur content. This inverse relationship between sulphur content and propensity for wool production has also been observed in Merino sheep genetically different in wool production. The finding of such a genetic association provided the first evidence that the genetic variation in wool production was associated with a difference in the utilisation of amino acids (Piper and Dolling, 1966; Reis *et al.* , 1967). It was suggested by Piper and Dolling (1966) that some of the variation in the efficiency of fleeceweight-selected lines was associated with a difference between groups in the efficiency of utilisation of ingested sulphur at the follicular level. This was apparently due to the production of differing proportions of the high or low sulphur proteins that comprise wool keratin.

A similar relationship between sulphur content and wool production has been found in crimp frequency selection flocks. Wool from a flock selected for low crimp frequency (which has higher fleece weights) had a lower sulphur content than that of the high crimp frequency flock (McGuirk, 1980). An alteration in the amino acid composition also occurred in these lines. Selection for low crimp frequency reduced the percentage of cystine. It was also associated with a reduction in the content of those amino acids associated with the non-fibrous protein matrix within cortical cells (threonine, serine, proline and glycine), relative to those associated with the microfibrilar protein helix (aspartic and glutamic acids, alanine, leucine and lycine). A number of these changes have yet to be examined in flocks selected for high fleeceweight.

The greater quantity of wool produced by the fleeceweight-selected flocks is not due simply to a dilution of the same amount of sulphur in a greater quantity of low sulphur protein. Rather, the total sulphur ouput is, in general, greater from high fleeceweight animals especially when the availability of sulphur-amino acids is increased (Williams *et al.* , 1972a). In the same study it was shown that the sulphur content of wool from two groups of sheep genetically diverse in wool production was always increased by the abomasal infusion of sulphur-amino acids. However the response in the rate of wool growth of genetically high producers was significantly greater than in those selected for low production, even though wool from the latter group had a consistently higher sulphur content.

The study by Williams (1973) provided further evidence that the genetic difference in wool production was associated with a difference in the utilisation of cystine for wool growth. It was shown that following a continuous infusion of L-[$^{35}$S] cystine the specific radioactivity of wool fibres was less in high fleeceweight Merino ewes than in low producing ewes. During infusion, however, the two flocks exhibited similar mean specific radioactivities of cystine in the plasma. Similar results were obtained by Williams (1976) using single injection administration of L-[$^{35}$S] cystine. These studies indicate the occurrence of a greater dilution with non-labelled cystine during passage of the label from plasma to wool fibres in high producing sheep. This suggests the presence of an additional pool of cystine which is available for use by the wool fibre, but which does not equilibrate rapidly with the plasma cystine pool. It is possible that reduced glutathione provides such an additional pool through its capacity to incorporate cystine.

Net entry rates of cystine (providing a measure of cystine availability to an animal) have been investigated in a limited number of studies. Radioisotopically labelled cystine has been administered using both single and continuous injection methods (Williams, 1973; Williams, 1976). However, neither study showed any indication that entry rates were higher in high fleeceweight sheep. Moreover, Williams (1976) found the entry rate to be 25% greater in low fleeceweight animals. Williams *et al.*, (1972a) administered an intravenous infusion of L-[$^{35}$S] cystine and found that entry rates were similar in both high- and low-fleeceweight selection flocks. Hence the greater wool production of the genetically superior sheep is not due to a greater entry rate of cystine into the plasma. This may indicate that, in high fleeceweight animals, a lower proportion of available sulphur-amino acids is broken down or used for purposes other than fleece production. However, it should 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.

The concentration of free cystine in the plasma has been found to be lower in Australian Merinos selected for increased fleeceweight compared to a flock selected for low fleeceweight (Williams *et al.*, 1972b; Williams, 1976). This genetic difference in cyst(e)ine concentration is evident from an early age (Williams, 1984). Thus it appears that low fleeceweight animals are not limited by the plasma concentration of sulphur-amino acids.

Neither the entry rate nor the lesser concentration of cystine in the plasma indicate that genetically high producing sheep have a greater availabilty of cystine at the follicular level. Despite this, and for reasons which remain unclear, they are able to incorporate a consistently greater total quantity of sulphur into their wool.

### (b) Reduced glutathione concentrations in the blood

The major function of reduced glutathione (GSH) in erythrocytes is thought to be the transport of cystine to body tissues. Many investigations have been conducted to examine variation in erythrocyte levels of GSH. These have primarily been concerned with the use of sheep erythrocytes as a model for the study of human red cell disorders. Nevertheless, limited attempts have been made to relate GSH-type to wool production in selected and unselected lines of sheep.

Saltykov (1956) showed a phenotypic association between fleece-weight and blood GSH levels, and suggested the possible use of GSH levels in the estimation of wool production. This is supported by the observation that GSH infusion (at a rate equivalent to 100 mg cysteine per day) increased wool production by approximately 30% in two sheep. (Hoey *et al.*, 1984).

It has also been shown that erythrocytes of genetically high producing sheep contain a lower concentration of GSH than those of low producing sheep (Hopkins *et al.*, 1975; Kalla and Ghosh, 1975). Thus the genetic effects on erythocyte GSH concentration parallel those on the plasma concentration of free cystine. This implies possible relationships between cystine transport in the plasma and in GSH and genetic propensity for wool production. However, these relationships are yet to be examined in detail.

### (c) Plasma urea concentration

Studies conducted by Mersmann *et al.* (1984 ; using genetically lean vs obese lines of pigs), Sejrsen *et al.* (1984; using dairy bulls of high vs low genetic merit) and Carter *et al.* (1986; using genetically meaty vs fat lines of sheep) have provided evidence that a superior level of production is consistent with a lowered concentration of plasma urea. Given that this relationship consistently occurs in a number of species, it seems reasonable to assume the same might also be true of high vs low fleeceweight selection lines. These relationships could be explained by more efficient utilisation of amino acids for production, and hence reduced deamination of amino acids in the lines of high genetic merit (Mersmann *et al.* , 1984). Such relationships have not been examined in lines of sheep selected for high vs low fleece production.

## Purpose and Scope of the Investigation

Divergent selection for fleece weight in sheep has led to differences between selection lines in the efficiency of conversion of feed into wool. These differences cannot be adequately explained by alteration of gross digestive function and would therefore appear to be due to differences in the utilisation of absorbed nutrients. Investigation into the physiological effects of selection for increased fleeceweight has shown that a number of correlated responses occur. Principal among these are changes in amino acid metabolism, particularly the availability and utilisation of sulphur containing amino acids. Genetic differences in wool production may also be associated with altered levels of reduced glutathione in erythrocytes and urea in plasma, or with changes in hormone status.

The purpose of this study was to examine the physiological basis of the responses to selection for fleeceweight observed in the Massey University fleeceweight (FW) and control (C) lines. Identification and understanding of the mechanisms controlling the expression of genetic variation in wool growth is of considerable importance as the same factors may be useful predictors of genetic merit. This would then allow selection and mating (either naturally or by artificial insemination) of ram lambs of superior genetic merit at 6 to 8 months of age, thereby reducing the generation interval and increasing the rate of genetic gain.

The animals used in the following studies were drawn from the control (C) and fleeceweight-selected (FW) subflocks of the Massey University PT Romney flock. Management and selection procedures have been described in detail by Blair *et al.* (1984; 1985). Briefly, in

1956 mixed age ewes (1.5 to 4.5 years) from an interbreeding base flock were randomly allocated to three subflocks which were subsequently closed in 1958. Each subflock is maintained at 80 ewes per year (age 1.5 to 4.5 years at lambing) with four new rams being used each year for mating at 1.5 years of age. The two subflocks from which the present data are derived are the control flock, in which replacements are chosen at random, and the FW flock in which rising 2 year old replacements are selected on the basis of high yearling greasy fleeceweight. The third flock is selected for face cover and need not be considered further in the present context.

Lambs are born in August, weaned in November and shorn, as lambs, in December. This practice ensures a constant period of wool growth for all animals between weaning and hogget shearing in the following October, when replacements are also selected. Following more than 25 years of single trait selection yearling ewe greasy fleeceweight has increased approximately 1 kg, and average lifetime production approximately 20% relative to the C flock (Blair *et al.*, 1985).

Three studies were conducted using hoggets and rams from these lines. The first involved a pilot study which sought to examine the plasma urea levels exhibited by rams of the two genotypes. A difference between the selection lines became apparent, with C rams showing elevated levels of plasma urea, indicating the need for more detailed study. Accordingly two further experiments were conducted to investigate the effects of casein supplementation and continuous methionine infusion on differences between the genotypes in plasma urea concentration. These experiments also provided the opportunity to examine variation between the lines in their blood levels of reduced glutathione, amino acid transferases, glucose, thyroxine, and creatinine.

# CHAPTER II

# PRELIMINARY INVESTIGATION OF PLASMA UREA CONCENTRATION IN FLEECEWEIGHT-SELECTED AND CONTROL RAMS

# CHAPTER II

# PRELIMINARY INVESTIGATION OF PLASMA UREA CONCENTRATION IN FLEECEWEIGHT-SELECTED AND CONTROL RAMS

## Introduction

Through the comparison of selection lines it may be possible to identify the physiological mechanisms by which animals of superior genotype achieve their increased levels of production. In a limited number of studies differences in plasma urea concentration have been found to exist between genetically lean vs obese lines of pigs (Mersmann *et al.* , 1984), dairy bulls of high vs low genetic merit (Sejrsen *et al.* , 1984) and genetically meaty vs fat lines of sheep (Carter *et al.* , 1986). In each study the higher level of production was associated with a lowered plasma urea concentration. Furthermore, it has been suggested that animals of high genetic merit may utilise amino acids more efficiently for production, reducing deamination and thereby decreasing plasma urea concentration.

It has yet to be determined if the same relationship between plasma urea and level of production exists in fleeceweight-selected lines of sheep. The opportunity to investigate this was presented during a nutritional study involving animals from such lines (McClelland *et al.* , 1986). A preliminary investigation was therefore conducted to measure plasma urea concentrataion at different times after feeding in order to determine if genetically related differences exist between sheep with differing potential for wool growth.

Despite its limited availability to ruminant tissues, the considerable amounts of glucose in the blood and extracellular fluid forms a readily available energy source for wool growth (Chapman and Ward, 1979). The possibility therefore exists that the two selection lines differ in plasma glucose concentration. Accordingly, plasma glucose concentration was also investigated.

## Materials and Methods

The study involved 24 rams (12C and 12 FW) aged 14 months. Initial and final liveweights were taken after a 24 hour fasting period and liveweight gain thus calculated. Fleeceweight was measured at October shearing and wool growth rate over a 28 day period was determined by clipping a midside sample of approximately 100 $cm^2$. Liveweight and fleeceweight data can be found in Table 2.1. The animals were housed indoors in individual metabolism crates throughout the 6 week experimental period from the end of October through to mid-December. All animals were fed freshly cut mature ryegrass/clover pasture *ad libitum*. The feed was offered in equal amounts twice daily at 0830-0900 h and 1500-1600h. Dry matter digestibility of the pasture was approximately 61% and crude protein content approximately 15%.

During the sixth week indoors the rams were sampled on two consecutive days. On the first day samples were collected 1 hour after feeding from 0930 - 1030h.. On the following day samples were collected immediately prior to feeding (0830-0900h) and again 1 to 2 hours after feeding at 0930 - 1030h. All samples were collected in a random order. Blood samples (5 ml) were withdrawn by venipuncture from the jugular vein using heparinised vacutainers. Plasma was separated by centrifugation and stored frozen at -12°C for determination of plasma urea and glucose concentrations.

Urea concentration in the plasma was determined by the autoanalyser method of Marsh *et al.* (1965) using a "Technicon Autoanalyzer". Plasma glucose concentrations were determined using a "YSI Model 27 Industrial Analyzer" (Yellow Springs Instrument Company, Ohio, USA.)

Glucose and urea concentrations at each sampling time were used to determine a mean concentration over the sampling period. This was then subject to analysis of variance, to test effects of selection line, using the statistical package 'REG' (Gilmour, 1985). Other variables (liveweight, fleeceweight, etc) were similarly subjected to analysis of variance.

## Results and Discussion

The mean values and standard errors for liveweight, dry matter intakes and fleeceweight for FW and C rams are given in Table 2.1. Both genotypes were similar in initial liveweight. The greater liveweight gain of C rams was not enough to cause a significant difference in the final liveweight. Dry matter intake of FW and C rams was similar whether expressed in absolute terms or corrected for final liveweight.

Mean plasma glucose concentrations over the sampling period are shown in Table 2.2. Although FW rams generally maintained higher plasma glucose concentrations, the difference between the lines was not significant. This is consistent with the results of Hough *et al.* (1986), who found no difference in plasma glucose concentration in Merino sheep selectively bred for high or low fleeceweight.

Plasma urea concentrations (means ± S.E.) are shown in Table 2.3. Control rams maintained consistently greater concentrations of urea (mean ± S.E., 7.98 ± 0.28) than those of FW rams (7.23 ± 0.27). Urea concentration was significantly different between the two lines at the samples taken on day 2 immediately prior to feeding (U2, $P < .10$) and 1 to 2h after feeding (U3, $P < .10$). On day 1 the first sample (U1) was also taken 1h after feeding and although C rams maintained a higher plasma urea concentration than FW rams, the difference was not significant.

This represents the first indication of differences in plasma urea concentration between fleeceweight-selected lines of sheep. The results are not consistent with those of Hough *et al.*(1986) who

TABLE 2.1 : Liveweight, dry matter intake and fleeceweight of fleeceweight-selected and control rams.

| Variable | Classification<br>Control (C) | <br>Fleeceweight (FW) |
|---|---|---|
| Initial Liveweight (kg) | 44.3 ± 1.0[a] | 43.8 ± 1.3 |
| Final Liveweight (kg) | 47.5 ± 1.1 | 44.8 ± 1.4 |
| Fasted Liveweight gain ($g.d^{-1}$) | 74.2 ± 9.2 | 21.6 ± 16.7 |
| Dry matter intake ($g.d^{-1}$) | 1260.4 ± 64.2 | 1170.3 ± 88.1 |
| Fleeceweight (kg) | 3.5 ± 0.07 | 4.2 ± 0.15 |
| Greasy wool growth ($g.cm^{-2}.d^{-1}$) | 1.9 ± 0.07 | 2.1 ± 0.06 |

[a] mean ± S.E., n = 12

TABLE 2.1 : continued

| Analysis of Variance<br>Source of Variation | | d.f. | Mean square and significance | |
|---|---|---|---|---|
| Initial liveweight | Genotype | 1 | 1.08 | NS |
| | Error | 22 | 14.86 | |
| Final liveweight | Genotype | 1 | 45.10 | NS |
| | Error | 22 | 20.23 | |
| Fasted liveweight gain | Genotype | 1 | 0.17 | * |
| | Error | 22 | 0.002 | |
| Dry matter intake corrected for final liveweight | Genotype | 1 | 1.20 | NS |
| | Error | 22 | 17.55 | |
| Fleeceweight corrected for final liveweight | Genotype | 1 | 0.002 | *** |
| | Error | 22 | 0.0001 | |
| Greasy wool growth corrected for final liveweight | Genotype | 1 | 0.0002 | * |
| | Error | 22 | 0.00003 | |

TABLE 2.2 : Plasma glucose concentration in fleeceweight-selected and control rams

| Classification | Plasma glucose concentration ($mg \cdot dl^{-1}$) at each sample time[a] | | | |
|---|---|---|---|---|
| | G1[b] | G2 | G3 | G MEAN |
| Control (C) | 69.9 ± 1.5 | 65.4 ± 1.4 | 66.8 ± 1.9 | 67.0 ± 1.4 |
| Fleeceweight (FW) | 72.8 ± 2.3 | 66.5 ± 1.4 | 66.3 ± 1.2 | 68.5 ± 1.1 |

Analysis of Variance[c]

| Source of variation | df | Mean square and significance |
|---|---|---|
| Group | 1 | 14.0 NS |
| Error | 22 | 17.0 |

a Mean ± S.E., n = 12

b Sample times where G1 = sample collected 1h after feeding, day 1

G2 = sample collected immediately prior to feeding, day 2

G3 = sample collected 1-2h after feeding, day 2

G MEAN = mean plasma glucose concentration

c Analysis of variance based on mean glucose concentration over sampling period

TABLE 2.3 : Plasma urea concentration in fleeceweight-selected and control rams

| Classification | Plasma urea concentration (mM) at each sample time[a]<br>U1[b] | U2 | U3 | U MEAN |
|---|---|---|---|---|
| Control (C) | 6.2 ± 0.2 | 8.6 ± 0.4 | 8.9 ± 0.3 | 7.9 ± 0.3 |
| Fleeceweight (FW) | 6.0 ± 0.2 | 7.7 ± 0.3 | 7.9 ± 0.4 | 7.2 ± 0.3 |

Analysis of Variance[c]

| Source of variation | df | Mean square and significance |
|---|---|---|
| Group | 1 | 2.6 † |
| Error | 22 | 0.8 |

a Mean ± S.E., n = 12

b Sample times where U1 = sample collected 1h after feeding, day 1.

U2 = sample collected immediately prior to feeding, day 2

U3 = sample collected 1 - 2h after feeding, day 2

U MEAN = mean plasma urea concentration

c Analysis of variance based on mean urea concentration over sampling period.

showed no significant difference between Merino ewes selectively bred for fleeceweight. The difference in plasma urea concentration recorded in the present study is, however, in agreement with the results of Mersmann *et al.* (1984), Sejrsen *et al.* (1984) and Carter *et al.* (1986), all of whom found that animals selected for high productivity (increased lean meat or milk production) maintained lower levels of urea in the plasma.

While the results provide an indication of a possible difference in plasma urea concentration between fleeceweight-selected lines, the difference was not highly significant. There was also substantial variation in plasma urea between animals within groups. It is important to note that within-group variation may be influenced by a number of factors including the possible diurnal variation in urea concentration and the effect of sample time with respect to time elapsed after feeding. Hence in order to minimise any possible variation within groups, all subsequent studies were designed to include more extensive sampling regimens.

# CHAPTER III

# THE EFFECT OF CASEIN SUPPLEMENTATION ON PLASMA UREA CONCENTRATION

# CHAPTER III

## THE EFFECT OF CASEIN SUPPLEMENTATION ON PLASMA UREA CONCENTRATION

### Introduction

The comparison of a fleeceweight-selected line of sheep with a random bred control line, as described in Chapter II, indicated that selected animals may maintain a lower concentration of urea in their plasma. Mersmann *et al.* (1984) showed a similar difference in plasma urea concentration between two groups of pigs differing in genetic merit (lean vs obese lines). It was suggested that the higher plasma urea concentration in obese pigs was due to the propensity of these animals to utilise a greater proportion of dietary amino acids for energy. An increase in amino acid breakdown implies, in turn, an increase in the entry rate of urea into the plasma pool. The possibility that a similar relationship exists in fleeceweight-selected lines of sheep must also be considered. Sheep of high genetic merit may possess an increased ability to conserve nitrogen, thus retaining a greater proportion of dietary amino acids in wool and/or body tissues. This could in turn lead to a lower concentration of urea in the plasma.

The addition of formaldehyde-protected casein to the diet, by providing an increased amino acid source to the animal, may highlight a differential response in plasma urea concentration between the two lines. Thus an experiment was designed to investigate the effects of protected casein added at three different levels to a lucerne-based diet, on plasma urea concentration in fleeceweight-selected lines of sheep.

Thyroxine (T4) is known to be required for differentiation of follicle cells and the continuation of normal wool growth. Although the mode of action of this hormone remains unclear, it is not unreasonable to assume that differences might exist between sheep selectively bred for fleeceweight. It has been shown by Hough *et al* (1986) that Merino rams selected for low fleeceweight maintain significantly higher plasma T4 concentrations than high fleeceweight rams ($P < .01$). No study has been conducted to determine if a similar relationship exists in Romney fleeceweight-selected lines.

The experiment also provided the opportunity to investigate the possible relationship between genotype for fleeceweight and aminotransferase activity. Limited evidence in Soviet literature has already indicated a relationship between the activity of transamination enzymes and level of production in unselected lines of sheep (Perchikhin, 1980; Novikova, 1978). From results of a study using Lincoln ewes Perchikhin (1980) suggested **that** the activity of the enzymes aspartate-amino transferase (AST) and alanine-amino transferase (ALT) had a positive genetic correlation with fleeceweight. It was further suggested that selection based on aminotransferase activity might be more effective than direct selection as a means of improving fleeceweight. No investigations of the relationship between aminotransferase activity and fleeceweight have been conducted using fleeceweight selected lines of sheep.

## Materials and Methods

The study involved 18 ram hoggets (9 C and 9 FW) aged 9 months at the commencement of the trial. The animals were housed indoors in individual metabolism crates throughout the duration of the experiment (late April through to August). The experiment consisted of two periods, the first being a six week stabilisation period and the second a four week treatment period. Throughout the experiment each animal was fed a basal diet of 1000g/day protein-extracted lucerne meal (90.76% dry matter, 3.04% nitrogen on a DM basis) which was offered once daily at 1000h. During the treatment period, supplements of formaldehyde-protected casein were given a 3 different levels; 0g, 30g or 60g/day. Three rams from each group were randomly assigned to each casein level. Liveweights were measured (prior to feeding) at the commencement and conclusion of the experiment. Fleeceweight was measured at October shearing (the period of wool growth being December to October) and wool growth measured by clipping right midside patches and left and right shoulder patches (area approximately 100cm$^2$) at the end of both experimental periods. Fleeceweight and liveweight data can be found in Table 3.2. A full discussion on wool growth, digestibility and intake has been given by McClelland *et al.* (1986).

Blood samples were collected from each animal at the end of the 6 week stabilisation period and again at the conclusion of the casein treatment period. On both occasions all animals were sampled at three times: 1h before feeding (0900h), 3h after feeding (1300h) and 6h after feeding (1600h). All samples were taken in random order. Blood samples were collected into heparinised vacutainers via venipuncture from the jugular vein. The plasma was separated by centrifugation and either analysed immediately for enzyme activity or stored frozen at -12°C for analysis of plasma urea concentration.

Amino-transferase activity (AST and ALT) was analysed using Merkotest automatic analyser packages (E.Merk Darmstadt, Federal Republic of Germany). Plasma urea concentration was analysed as described in Chapter II.

Plasma concentration of T4 was assayed using Diagnostic Products Corporation (Los Angeles, U.S.A.) Coat-a-Count kits for T4 (TKT 41). These provide a quantitative measurement of total circulating T4 in serum and plasma. The procedure is a solid phase radioimmunoassay based on antibody-coated tubes and human serum calibrators. As such the kits required validation for measurement of T4 in the plasma of sheep. A summary of the validation procedure is as follows.
Parallelism of the assay was assessed by assaying two pooled ram samples (made up of samples representative of the animals and experiment as a whole) at three sample volumes. Results are tabulated below (Table 3.1). Although a certain amount of non-parallelism is indicated, the results are within the tolerances required to detect differences in T4 concentration between FW and C lines of sheep. The internal recovery of standards was assessed by assaying three pooled ram samples in a 50 : 50 ratio with a selected range of standards (4, 10, 16 $\mu g.dl^{-1}$). Recoveries ranged from 92 to 110% with a mean of 106%.

It was therefore concluded from the above validation trial that the assay of T4 by this method was entirely adequate for the purposes of the current research. Assays were conducted on samples

TABLE 3.1 : Assessment of parallelism in thyroxine (T4) assay.

| | T4 concentration ($\mu g.dl^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Ram Pool#1 | | | Ram Pool#2 | | |
| Sample volume ($\mu l$) | Observed | Expected | O/E | Observed | Expected | O/E |
| 25* | 3.99 | | | 5.02 | | |
| 50 | 8.42 | 7.98 | 1.05 | 10.48 | 10.04 | 1.04 |
| 100 | 16.04 | 15.96 | 1.01 | 17.59 | 20.08 | 0.88 |

* Standard assay sample volume. One sample from each of the two pools assayed in duplicate at each sample volume.

bulked volumetrically across the three sampling times within experimental periods.

Data were initially analysed by repeated measures analysis using the statistical package 'REG' (Gilmour,1985) to test the effects of selection line and the line x time interactions. As the interactions were generally nonsignificant, data for each sampling period were reduced to a single mean and subjected to analysis of variance.

## Results and Discussion

The mean values and standard errors for liveweight and fleeceweight of C and FW rams for the two experimental periods are shown in Table 3.2. There was no difference in either initial liveweight or liveweight gain between the two lines. FW rams grew a greater amount of wool that C rams in all periods, the differences being accentuated at the higher levels of casein supplementation (McClelland *et al.*, 1986). Although selected rams maintained higher fleeceweights than C rams, the difference was not significant. It is possible that this was due to errors induced by collection of midside and shoulder wool samples and clipping of the neck for blood sampling before shearing and subsequent fleeceweight measurement.

Plasma urea concentrations during both the stabilisation and casein treatment periods for FW and C rams are shown in Table 3.3. As in the previous experiment (Chapter II), C rams maintained consistently higher concentrations of plasma urea than FW rams during the stabilisation period ($\bar{x} \pm$ S.E., C: 7.17 ±0.26mM; FW:6.09 ± 0.39mM) and at all levels of casein supplementation (Figures 3.1 and 3.2). This experiment provides the first clear evidence of a difference in plasma urea concentration between fleeceweight-selected lines of sheep. The difference was maintained despite the rams being fed at a constant level irrespective of liveweight and samples being collected at widely spaced intervals. Furthermore, the consistency of these results suggests the possibility of distinguishing between genotypes on the basis of plasma urea concentration.

The provision of an additional source of amino acids to the

TABLE 3.2 : Liveweight and fleeceweight of Fleeceweight-selected and Control rams.

| | | Classification | |
|---|---|---|---|
| Variable | n | Control (C) | Fleeceweight (FW) |
| Initial liveweight (kg) | 9 | 30.11 ± 0.95[a] | 29.39 ± 1.43 |
| Final liveweight (kg) | 9 | 42.56 ± 0.76 | 41.49 ± 0.35 |
| Liveweight gain ($g.d^{-1}$) | 9 | 12.44 ± 0.48 | 12.10 ± 0.79 |
| Fleeceweight (kg) | 9 | 3.76 ± 0.14 | 3.94 ± 0.18 |

Analysis of Variance

| Source of Variation | | df | Mean Square and Significance |
|---|---|---|---|
| Initial liveweight | Genotype | 1 | 2.35 NS |
| | Error | 16 | 13.21 |
| Final liveweight | Genotype | 1 | 5.12 NS |
| | Error | 16 | 5.82 |
| Liveweight gain | Genotype | 1 | 0.53 NS |
| | Error | 16 | 3.88 |
| Fleeceweight | Genotype | 1 | 0.16 NS |
| | Error | 16 | 0.25 |

[a] Mean ± S.E.

TABLE 3.3 : Plasma urea concentration in Fleeceweight-selected and Control rams.

| Sample period[a] | Mean plasma urea concentration (mM) | |
|---|---|---|
| | Control (C) | Fleeceweight (FW) |
| Stablisation period (P1) | 7.17 ± 0.26 | 6.09 ± 0.39 |
| Casein Supplementation (P2) | | |
| 0 g | 6.12 ± 0.15 | 4.82 ± 0.23 |
| 30 g | 7.20 ± 0.36 | 6.44 ± 0.71 |
| 60 g | 8.92 ± 0.05 | 7.38 ± 1.13 |

Analysis of Variance[b]

| Source of variation | df | Mean square and Significance | |
|---|---|---|---|
| Stabilisation period (P1) | | | |
| Genotype | 1 | 5.2 | * |
| Error | 16 | 1.0 | |
| Casein Supplementation (P2) | | | |
| Genotype | 1 | 6.5 | * |
| Casein | 2 | 10.8 | ** |
| Genotype x Casein | 2 | 0.2 | NS |
| Error | 12 | 1.0 | |

[a] Experimental period where P1 = basal diet
P2 = basal diet plus casein supplements

[b] Analysis of variance based on mean urea concentrations over sampling period

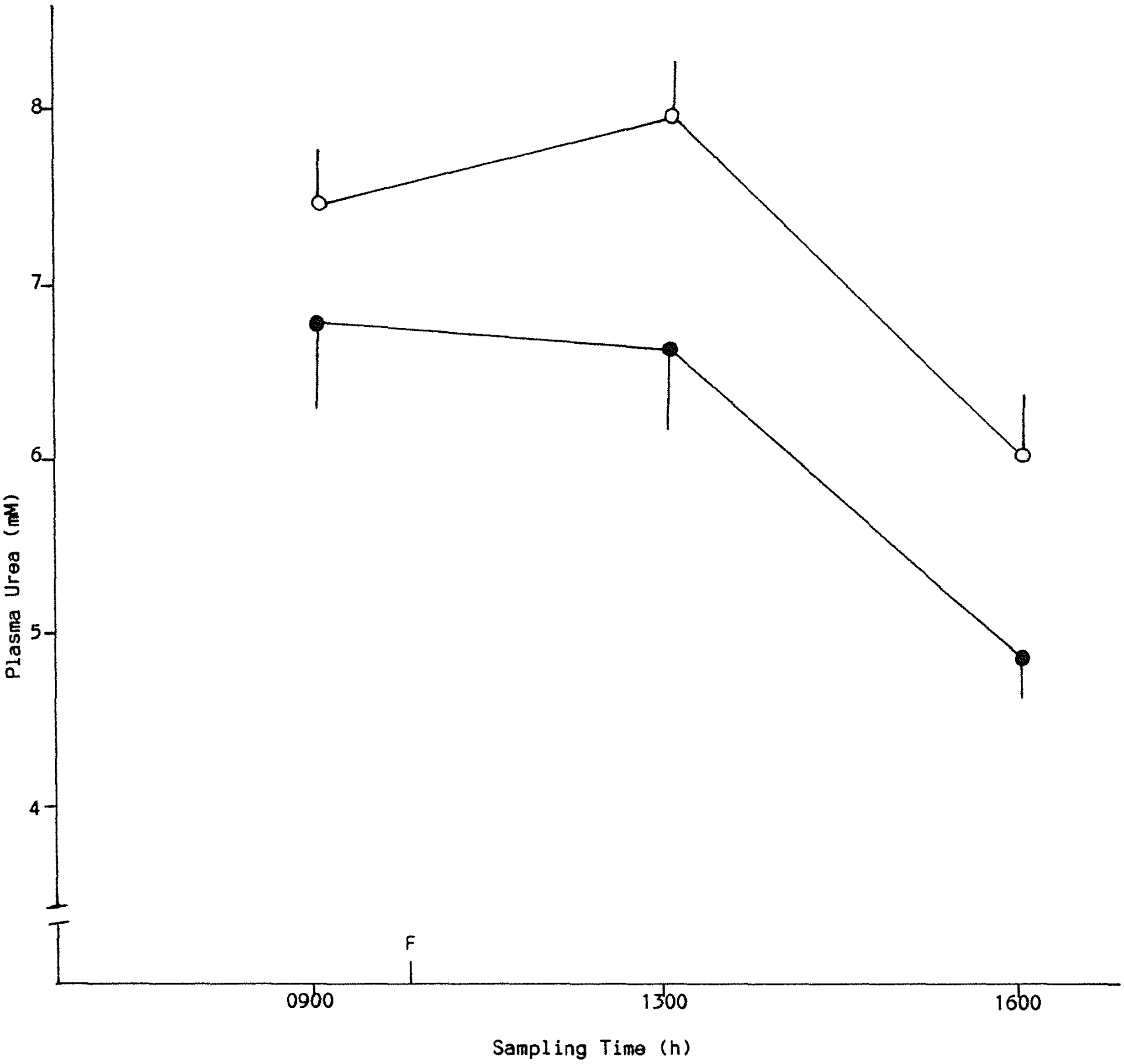

FIGURE 3.1 : Plasma urea concentrations during the stabilisation period in Control (o) and Fleeceweight-selected (●) rams. Bars indicate standard errors about the mean. Rams were fed at 1000h (F).

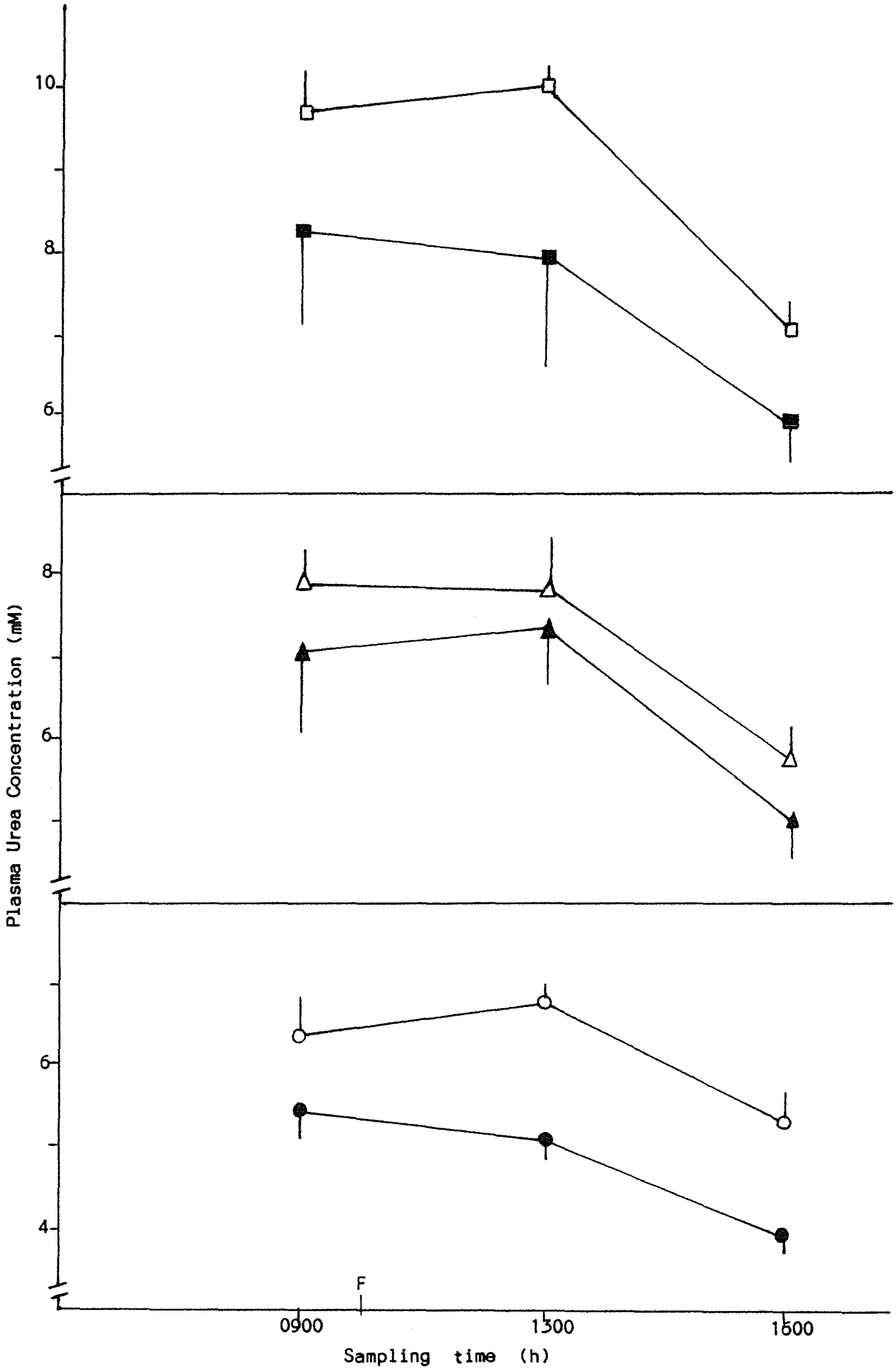

FIGURE 3.2 : Plasma urea concentrations during the casein treatment period in Control and Fleeceweight-selected rams. Bars indicate standard errors about the mean. Rams were fed at 1000h (F).

| | | C | FW |
|---|---|---|---|
| Casein level | 0 g | o | ● |
| | 30 g | Δ | ▲ |
| | 60 g | □ | ■ |

animal in the form of formaldehyde-protected casein, contrary to highlighting a differential response between the lines, resulted in a parallel increase in plasma urea concentration in both lines. This implies that the lower plasma urea concentration of FW rams is not governed simply by the ability of those animals to conserve increasing quantities of amino acid nitrogen in response to an increasing availability. In this case FW animals were also deaminating increasing amounts of amino acid nitrogen, though possibly at a lower rate than that of C rams.

Casein can be classed as a 'non-specific' nitrogen supplement providing an adequate source of many amino acids. It is, however, known to be deficient in the sulphur-containing amino acids cystine and methionine which are also limiting for wool growth. Supplying one or both of those limiting amino acids may differentially alter the plasma urea concentration between the selected lines consequent upon their ability to retain and utilise a greater proportion of non-sulphur containing amino acids. That casein was apparently not effective in inducing a difference in the rate of deamination between the two lines was shown by the absence of an interaction between casein level and selection line.

The mean concentration of T4 in the plasma is shown in Table 3.4. Control rams maintained greater plasma T4 concentrations than those of FW rams during the stabilisation period ($P < .1$) and the casein treatment period, although differences between the lines in the latter period were not significant ( Table 3.4). The results are generally in agreement with those of Hough *et al.* (1986) who recorded a significant difference in plasma T4 concentration between

TABLE 3.4 : Thyroxine (T4) concentration in plasma of Fleeceweight-selected and Control rams during stabilisation period and casein treatment.

| | | Mean T4 concentration ($\mu$g.dl$^{-1}$) | |
|---|---|---|---|
| Period[a] | n | Control (C) | Fleeceweight (FW) |
| Stabilisation period (P1) | 9 | 4.28 ± 0.38[b] | 3.28 ± 0.35 |
| Casein Supplementation (P2) | | | |
| 0 g | 3 | 3.65 ± 0.21 | 2.81 ± 0.32 |
| 30 g | 3 | 4.05 ± 1.18 | 3.46 ± 0.32 |
| 60 g | 3 | 3.75 ± 0.71 | 4.18 ± 0.32 |
| Mean | 9 | 3.82 ± 0.39 | 3.39 ± 0.26 |

Analysis of Variance

| Source of Variation | | df | Mean Square and Significance | |
|---|---|---|---|---|
| P1 | Genotype | 1 | 4.55 | † |
| | Error | 16 | 1.20 | |
| P2 | Genotype | 1 | 2.86 | NS |
| | Casein | 2 | 1.04 | NS |
| | Genotype x Casein | 2 | 0.11 | NS |
| | Error | 12 | 23.87 | |

[a] Experimental period where P1 = basal diet
P2 = basal diet plus 3 levels casein supplementation

[b] Mean ± S.E.

two lines of Merino sheep selectively bred for high and low fleeceweight. Ewes selected for low fleeceweight maintained higher T4 concentrations ($P < .01$). While the present results provide an indication of variation in plasma T4 levels between the fleeceweight-selected lines, the difference in this case was not highly significant. Plasma concentration of T4 increased with casein level in FW but not Control sheep. However, there was no significant interaction between selection line and level of casein in the diet. The consistent difference between the selected lines of sheep indicates the need for further study of plasma T4 concentration over an extended sampling period (Figure 3.3).

Mean ( ± S.E.) ALT and AST activities over the sampling periods are shown in Tables 3.5 and 3.6 respectively. In both periods there was no significant difference between FW and C rams in either ALT or AST activity. The level of casein fed had no effect on enzyme activity. This experiment represents the first occasion on which ALT and AST levels in the plasma have been measured in fleeceweight-selected lines of sheep. The results are in contrast with those of Perchikhin (1980) who found a positive genetic correlation between enzyme level and wool production and suggested that selection based on amino transferase activity was more effective than direct selection for increasing fleeceweight. The present study, however, provides no suggestion of a relationship between genotype for fleeceweight and activity of the enzymes ALT and AST.

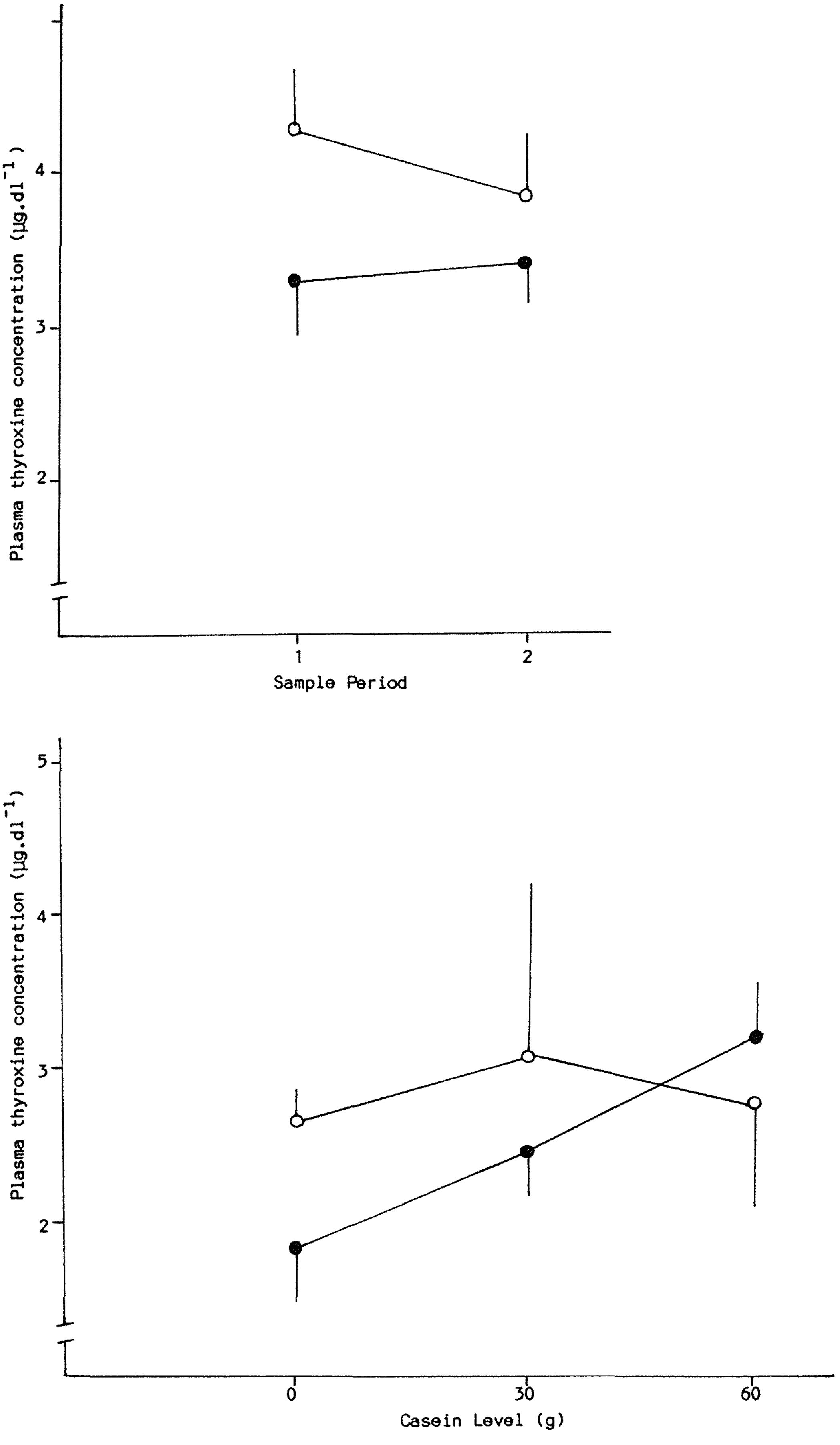

FIGURE 3.3 : Plasma thyroxine concentrations during the stabilisation period (upper panel) and casein treatment (lower panel) in Control (o) and Fleeceweight-selected (●) rams. Bars indicate standard errors about the mean.

TABLE 3.5 : Alanine-amino transferase (ALT) activity in plasma of Fleeceweight-selected and Control rams.

| | Mean ALT concentration ( $U.l^{-1}$ ) | |
|---|---|---|
| Sample period[a] | Control (C) | Fleeceweight (FW) |
| Stabilisation period (P1) | 27.9 ± 1.85[b] | 27.6 ± 1.87 |
| Casein Supplementation (P2) | | |
| 0 g | 30.0 ± 1.92 | 30.2 ± 2.71 |
| 30 g | 28.8 ± 4.08 | 31.8 ± 3.18 |
| 60 g | 31.2 ± 1.56 | 27.8 ± 1.70 |

Analysis of Variance[c]

| Source of variation | df | Mean Square and Significance | |
|---|---|---|---|
| Stabilisation period | | | |
| Genotype | 1 | 0.4 | NS |
| Error | 16 | 31.1 | |
| Casein Supplementation | | | |
| Genotype | 1 | 0.1 | NS |
| Casein | 2 | 1.2 | NS |
| Genotype x Casein | 2 | 15.7 | NS |
| Error | 12 | 21.5 | |

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[a] Sample period where P1 = basal diet
P2 = basal diet plus casein supplements

[b] Means ± S.E., n = 9

[c] Analysis of variance based on mean ALT concentrations over sampling times.

TABLE 3.6 : Aspartate-amino transferase (AST) activity in plasma of Fleeceweight-selected and Control rams.

| Sample period[a] | Mean AST concentration ( $U.l^{-1}$ )<br>Control (C) | <br>Fleeceweight (FW) |
|---|---|---|
| Stabilisation period (P1) | 117.0 ± 4.73[b] | 123.9 ± 5.82 |
| Casein Supplementation (P2) | | |
| 0 g | 132.6 ± 17.88 | 141.6 ± 9.68 |
| 30 g | 139.6 ± 21.04 | 119.2 ± 5.82 |
| 60 g | 129.7 ± 10.94 | 121.2 ± 6.65 |

Analysis of Variance[c]

| Source of variation | df | Mean Square and Significance | |
|---|---|---|---|
| Stabilisation period | | | |
| Genotype | 1 | 154.9 | NS |
| Error | 16 | 237.0 | |
| Casein Supplementation | | | |
| Genotype | 1 | 698.1 | NS |
| Casein | 2 | 22.6 | NS |
| Genotype x Casein | 2 | 64.1 | NS |
| Error | 12 | 522.1 | |

[a] Sample period where P1 = basal diet
P2 = basal diet plus casein supplements

[b] Means ± S.E., n = 9

[c] Analysis of variance based on mean ALT concentrations over sampling times

# CHAPTER IV

# EFFECT OF AN INTRAVENOUS INFUSION OF METHIONINE ON PLASMA UREA CONCENTRATION IN CONTROL AND FLEECEWEIGHT RAMS

# CHAPTER IV

# EFFECT OF AN INTRAVENOUS INFUSION OF METHIONINE ON PLASMA UREA CONCENTRATION IN CONTROL AND FLEECEWEIGHT RAMS

## Introduction

A lower concentration of urea in the plasma of FW rams compared to that of C rams was observed consistently during the trials reported in Chapters II and III. The difference was evident both when rams were fed *ad libitum* and when intake was fixed irrespective of liveweight. It is possible that FW sheep conserve amino acids for wool production while C sheep "waste" a proportion of available amino acids therefore excreting a greater quantity of urea. A limited number of Australian studies have shown that Merino sheep selected for increased fleeceweight incorporate sulphur amino acids at a lower rate (by % wool weight) than those selected for low wool production (Piper and Dolling, 1966; Reis *et al.* , 1967). It is known that a shortage of sulphur amino acids limits the synthesis of wool protein. Given the lower cystine content in the wool of high fleeceweight sheep, this could allow incorporation of a greater proportion of non-sulphur amino acids into wool and consequently decrease the deamination rate. Based upon this information the hypothesis was advanced that supplementation with sulphur amino acids, such as methionine, would reduce the "wastage" rate in C animals (allowing the utilisation of a greater proportion of non-sulphur amino acids) and so decrease the difference between the lines in plasma urea concentration. Thus a switchback experiment was designed with a continuous intravenous infusion of L-methionine during the treatment period and saline infusion both pre- and post-treatment. The plasma urea concentrations of FW and C rams were then measured in all periods.

Another possibility is that differences between the lines in plasma urea concentration could reflect differences in kidney function. If variation existed between the lines in glomerular filtration rate (GFR), the selection line exhibiting a high GFR would have low plasma concentrations of those substances not actively secreted or reabsorbed in the nephron. Creatinine is an example of such a substance. Urea concentration might be similarly affected by a higher GFR although the magnitude of the difference may be altered due to the passive movement of urea across the more distal parts of the nephron. The possibility that variation in GFR contributes to variation in plasma urea concentration has not been examined in those selection lines known to differ in plasma urea (Mersmann *et al.*, 1984; Sejrsen *et al.*, 1984; Carter *et al.*, 1986). Creatinine concentrations in plasma were therefore measured to provide an indicator of possible between-line differences in kidney function.

Limited attempts have been made to relate the concentration of reduced glutathione (GSH) in the erythrocytes to wool production in both selected and unselected lines. Saltykov (1956) first suggested the possible value of blood GSH measurements in estimating wool production. A positive phenotypic association between fleeceweight and GSH level was found in unselected Merino sheep (Agar *et al.*, 1972). However, later studies showed that selection for low fleeceweight significantly increased the erythrocyte GSH level in Merino sheep (Hopkins *et al.*, 1975) and Indian sheep (Kalla and Ghosh, 1975). This implies a negative genetic correlation between these traits.

The opportunity was therefore taken during this trial to measure the erythrocyte GSH concentration in the two lines of sheep and to investigate the effect of the methionine infusion on GSH level.

Further investigation of plasma T4 concentration was prompted by the difference between the FW and C lines shown in Chapter III. The possibility that FW and C lines vary in their plasma concentrations of glucose was also examined.

## Materials and Methods

The study involved 5 C and 5 FW 2 year-old rams and was conducted over 3 experimental periods (P1-P3; mid July-August). In P1 and P3 a continuous intravenous saline infusion was administered (for 4 and 5 days respectively) while P2 involved a 7-day methionine infusion. The rams were housed indoors in metabolism crates and throughout the experiment were fed chaffed lucerne hay (crude protein content 16.1% on a dry matter basis). All animals were fed to provide 110% maintenance (0.61 MJ ME/kg$^{.75}$ assuming the feed contained 9.5 MJ ME/kg DM and 85% DM; Rattray, 1986). On alternate days a mineral supplement (2g per head of 95% sodium chloride and 5% sodium molybdate) was given to counteract possible copper toxicity. Feed was offered four times daily. Three equal portions (comprising half the ration) were fed at 0800h, 1100h and 1400h and the balance bulk fed at 1700h.

Liveweight was measured at the commencement and conclusion of the experiment. Fleeceweight was recorded at shearing during the previous October.

Jugular cannulae (Deseret Pharmaceutical Co. Inc.) were inserted bilaterally under local anaesthetic (10% xylocaine), one acting as an infusion cannula and the other as the sampling cannula. Patency of the sampling cannulae was maintained with heparinised saline (100 IU heparin/ml saline). A daily 6 ml antibiotic injection (Streptopen; Glaxo N.Z., Ltd.) was given to each animal.

A peristaltic pump was used to deliver the continuous infusion. The pump, a Desage PLG Multipurpose Peristaltic Pump, Model 132100

(Desage GmBH, Germany), using silicon tubing of internal diameter 0.55 mm (supplied by the same manufacturers), was calibrated over ten channels to deliver approximately 20 (range 19-24) ml/hour/channel. The pump was connected to infusion bags and cannulae via polyethylene tubing (internal diameter 1.00 mm, external diameter 1.50 mm; Dural Plastics and Engineering, NSW, Australia). Throughout the experiment 1000 ml single-dose infusion bags containing sterile, non-pyrogenic saline (0.9% sodium chloride, pH5; Travenol Laboratories N.Z. Ltd. Auckland) were used. Antibiotic (Terramycin; 1 ml/500ml saline) was added to each infusion bag.

During P2 methionine was infused daily at a rate of 25.7 mg methionine/kg bodyweight (approximately 1.5 g/ram/day). Stock methionine solution (6g/100 ml in sterile saline) was added to the infusion bags through an autoclaved millipore filter for a final infusate concentration of 3.0 mg/ml.

Blood samples (5 ml) were withdrawn 20 minutes prior to feeding at 1400h and then at 40 minute intervals on four consecutive occasions until 1640h. Haematocrits were measured and a 0.2 ml sample immediately deproteinised for GSH assay. Plasma was separated by centrifugation (using 35% sodium citrate as the anticoagulant) and stored frozen at -12°C for the determination of urea, creatinine and glucose concentrations.

Plasma urea and glucose concentrations were determined as described in Chapter II. For analysis of creatinine, the samples were volumetrically bulked across sampling times within days and assayed by the autoanalyser method of Chasson *et al.* (1961). For determination of T4, samples were volumetrically bulked across sample times and days (within periods) and analysed as in Chapter III. GSH concentration was determined by the method of Beutler *et al.* (1963).

Statistical analyses of plasma hormone and metabolite concentrations were carried out on data from the last three days of each experimental period. For urea, glucose and GSH the concentrations determined by assaying the five samples collected on each day were used to generate mean daily concentrations. The daily means were then subjected to repeated measures analysis to test the interactions between main effects and sampling times (days). Since these interactions were generally nonsignificant the three daily means were averaged to give a period mean concentration. These means were then subjected to analysis of (co) variance for a switchback design to test the effects of selection line, treatment and their interaction. The same procedure was used for the bulked daily mean creatinine concentrations and the period mean thyroxine concentrations. Bodyweight, weight gain and fleeceweight data were analysed by analysis of variance as previously described and all statistical analyses were performed using the statistical package 'REG' (Gilmour, 1985).

## Results and Discussion

The mean values and standard error for initial and final liveweight, liveweight gain and fleeceweight of the C and FW rams are given in Table 4.1. Both genotypes had similar initial liveweights and liveweight gain over the experiment. FW rams grew significantly more wool than C rams ($P < .01$).

Plasma urea concentrations for C and FW rams during each period are shown in Figure 4.1. Analysis was conducted on data from the last three days of each period and can be found in Table 4.2. The results of the present study are consistent with those reported in Chapters II and III in that throughout the experiment C rams maintained consistently higher plasma urea concentrations than FW rams ($P <.01$).

During the second period, when methionine was infused, the plasma urea concentration of FW rams remained stable (Figure 4.1) but that of C rams declined so that the difference between the lines was reduced by approximately 50%. Following the conclusion of the methionine infusion, the levels in both lines increased and the difference which had existed in the first period (i.e. approximately 1 mM) was re-established. Although the difference between FW and C rams during the methionine infusion period was substantially less than that in the saline periods the interaction was non-significant. Nevertheless, the decline in plasma urea concentration which was observed lends support to the original hypothesis that infusion of methionine might reduce the "wastage" rate of amino acids in C animals, thereby decreasing the plasma urea concentration. The significant regression of urea on creatinine after correction for genotype and

TABLE 4.1 : Liveweight and fleeceweight of Fleeceweight-selected and Control rams.

| Variable | n | Control (C) | Fleeceweight (FW) |
|---|---|---|---|
| Initial liveweight (kg) | 5 | 57.9 ± 2.1 [a] | 59.2 ± 3.3 |
| Final liveweight (kg) | 5 | 59.7 ± 2.3 | 62.1 ± 2.8 |
| Liveweight gain ($g.d^{-1}$) | 5 | 65.4 ±30.4 | 112.3 ± 24.3 |
| Fleeceweight (kg) | 5 | 4.1 ± 0.3 | 5.5 ± 0.1 |

Analysis of Variance

| Source of Variation | | df | Mean Square | Significance |
|---|---|---|---|---|
| Initial liveweight | Genotype | 1 | 3.5 | NS |
| | Error | 8 | 37.6 | |
| Final liveweight | Genotype | 1 | 14.4 | NS |
| | Error | 8 | 33.6 | |
| Liveweight gain | Genotype | 1 | 5499.0 | NS |
| | Error | 8 | 3795.0 | |
| Fleeceweight | Genotype | 1 | 4.6 | ** |
| | Error | 8 | 0.3 | |

[a] Mean ± S.E.

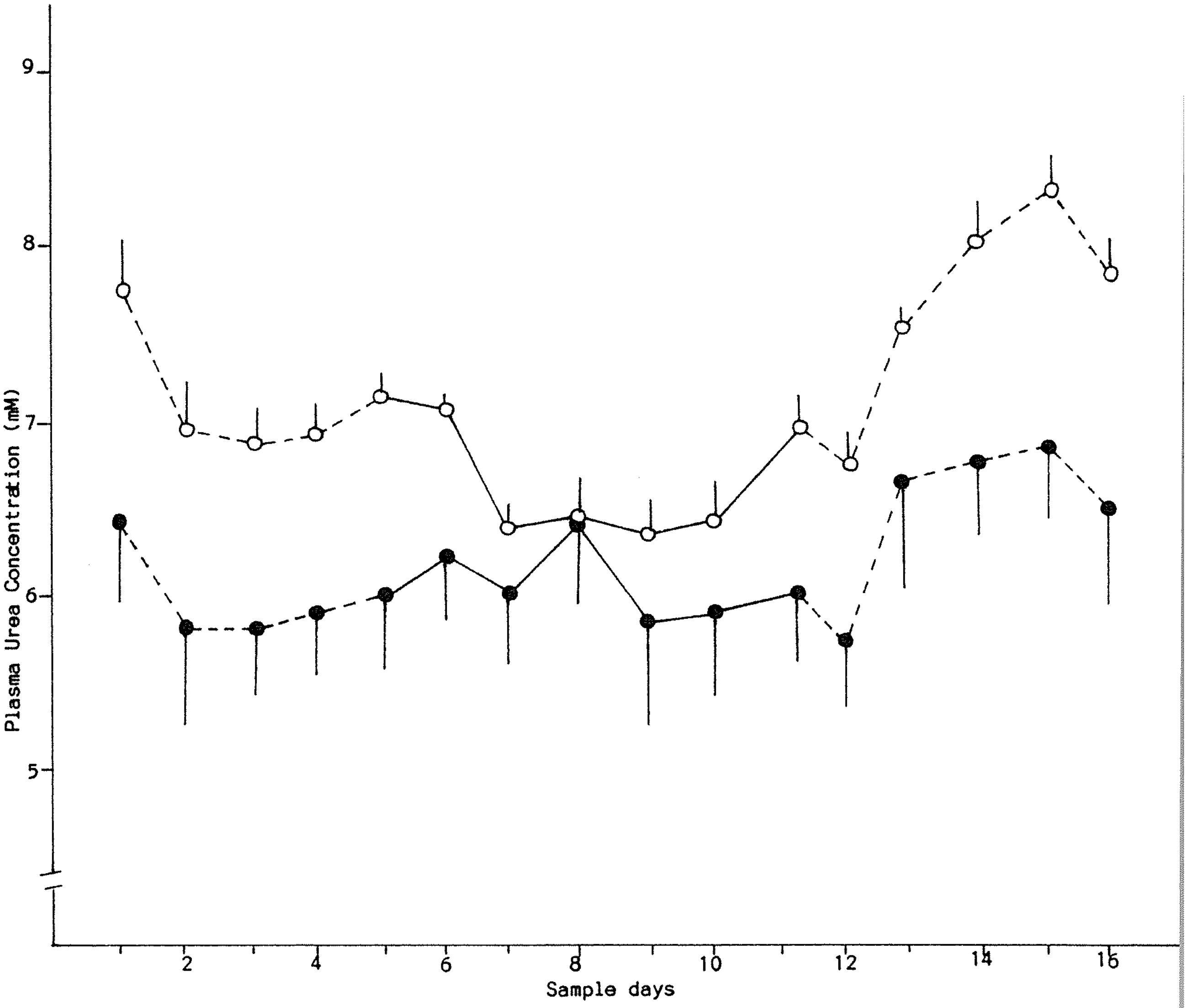

FIGURE 4.1 : Plasma urea concentration during the saline (---------) and methionine (————) treatment periods in Control (o) and Fleeceweight-selected (•) rams. Bars indicate standard errors about the mean.

TABLE 4.2 : Plasma urea concentration in Fleeceweight-selected and Control rams during saline and methionine infusion periods.

| | | Plasma urea concentration (mM) | |
|---|---|---|---|
| Treatment | n | Control (C) | Fleeceweight (FW) |
| Saline | 5 | 7.5 ± 0.2 | 6.5 ± 0.3 |
| Methionine | 5 | 6.6 ± 0.1 | 5.9 ± 0.5 |

Analysis of Variance[b]

| Source of Variation | df | Mean Square and Significance | |
|---|---|---|---|
| Genotype | 1 | 7.8 | ** |
| Treatment | 1 | 2.6 | † |
| Genotype x Treatment | 1 | 0.6 | NS |
| Creatinine[c] | 1 | 3.3 | * |
| Error | 29 | 0.7 | |

[a] Mean ± S.E.

[b] Analysis of variance based on mean urea concentration

[c] Regression on plasma creatinine concentration

treatment effects (Table 4.2) indicates that variation in plasma creatinine accounted for a substantial proportion of the variation in plasma urea concentration not explained by these effects.

Plasma creatinine concentrations of C and FW rams and analysis of these data are shown in Figure 4.2 and Table 4.3 respectively. C rams maintained greater plasma creatinine concentrations than FW rams by approximately 0.01 mM. Despite the decrease in plasma urea concentration of the C rams during the methionine infusion, there was no effect of methionine treatment on the difference in plasma creatinine levels between the two genotypes. Thus the mechanism responsible for the decline in the plasma urea concentrations of C rams did not affect their plasma creatinine levels.

Variation between genotypes in plasma creatinine concentration may reflect differences in either the glomerular filtration rate (GFR) or the entry rate of creatinine into the plasma pool. That C rams had higher plasma concentrations of both urea and creatinine suggests a difference in the GFR and implies a lower GFR in C than FW rams. It also suggests that the methionine infusion decreases the plasma urea concentration by a mechanism other than a change in GFR. Such a mechanism would, in all likelihood, involve a change in the amino acid balance for wool growth. The fact that the plasma urea concentrations of the C and FW rams did not converge completely during the methionine treatment provides evidence that, even during the methionine infusion, differences existed between the lines in GFR.

Mean GSH concentrations during the three periods are shown in Figure 4.3 and analysis of the data is given in Table 4.4. Although

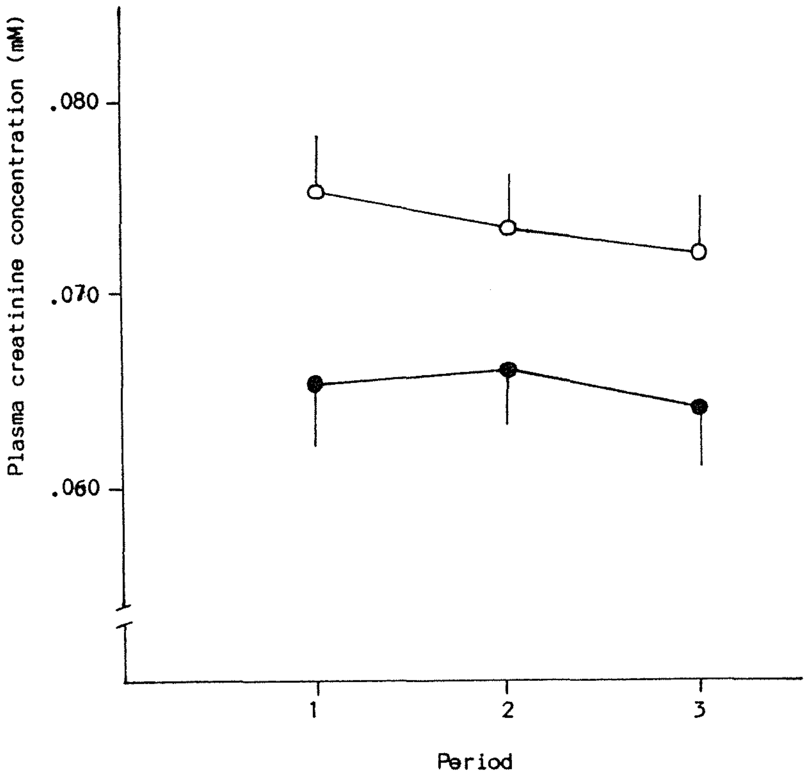

FIGURE 4.2 : Mean plasma creatinine concentrations during Period 1 (saline infusion), Period 2 (methionine infusion) and Period 3 (saline infusion) in Control (o) and Fleeceweight-selected (•) rams. Bars indicate standard errors about the mean.

TABLE 4.3 : Plasma creatinine concentration in Fleeceweight-selected and Control rams during saline and methionine infusion periods.

| | | Plasma creatinine concentration (mM) | |
|---|---|---|---|
| Treatment | n | Control (C) | Fleeceweight (FW) |
| Saline | 5 | 0.074 ± 0.002[a] | 0.064 ± 0.002 |
| Methionine | 5 | 0.073 ± 0.003 | 0.066 ± 0.003 |

Analysis of Variance[b]

| Source of variation | df | Mean Square and Significance | |
|---|---|---|---|
| Genotype | 1 | 0.0006 | ** |
| Treatment | 1 | 0.000002 | NS |
| Genotype x Treatment | 1 | 0.000008 | NS |
| Error | 26 | 0.00005 | |

[a] Mean ± S.E.

[b] Analysis of variance based on mean creatinine concentration

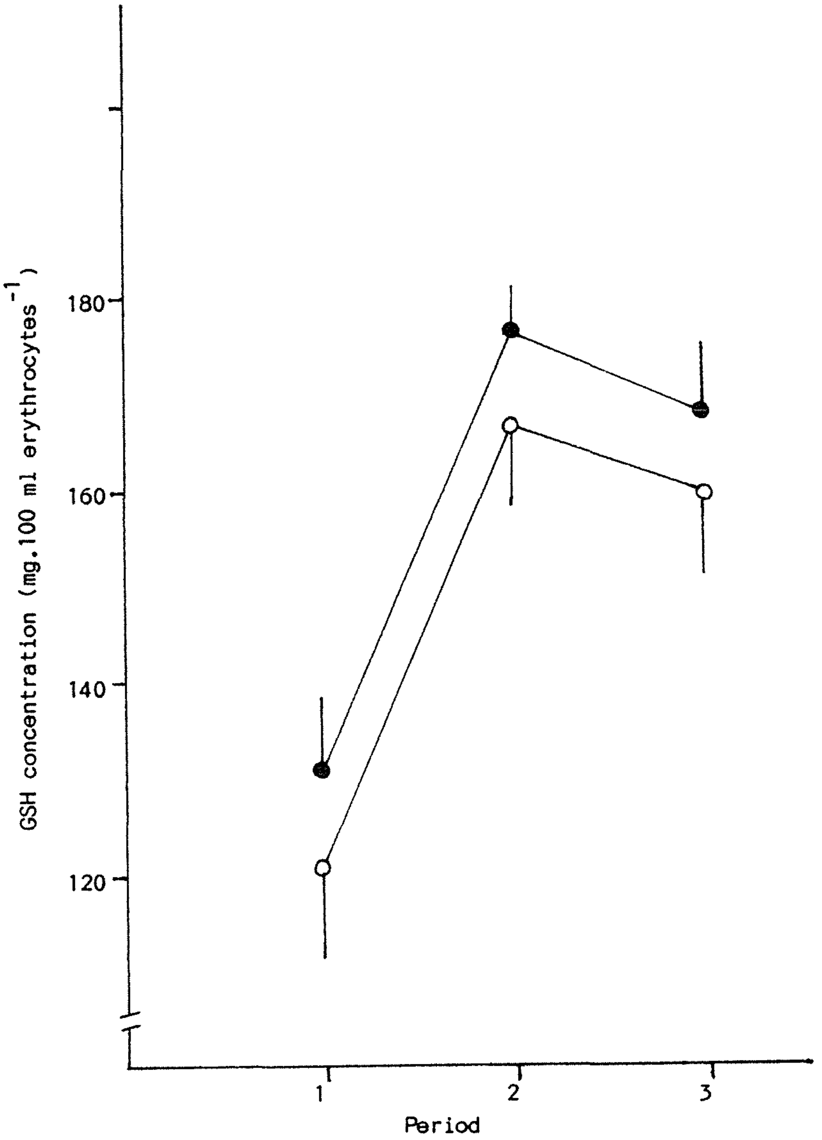

FIGURE 4.3 : Mean erythrocyte GSH concentrations during Period 1 (saline infusion), Period 2 (methionine infusion) and Period 3 (saline infusion) in Control (o) and Fleeceweight-selected (●) rams. Bars indicate standard errors about the mean.

TABLE 4.4 : Erythrocyte concentration of reduced glutathione (GSH) in Fleeceweight-selected and Control rams during saline and methionine infusion periods.

| | | GSH concentration (mg.100ml erythrocytes$^{-1}$) | |
|---|---|---|---|
| Treatment | n | Control (C) | Fleeceweight (FW) |
| Saline | 5 | 140.1 ± 8.8[a] | 149.3 ± 7.5 |
| Methionine | 5 | 166.7 ± 8.6 | 176.1 ± 4.3 |

Analysis of Variance[b]

| Source of Variation | df | Mean Square and Significance | |
|---|---|---|---|
| Genotype | 1 | 644.9 | NS |
| Treatment | 1 | 4743.7 | ** |
| Genotype x Treatment | 1 | 0.03 | NS |
| Error | 26 | 536.2 | |

[a] Mean ± S.E.

[b] Analysis of variance based on mean GSH concentration

FW rams maintained greater erythrocyte GSH concentrations than C rams during all periods, the difference between the lines was not significant. The results suggest that methionine infusion elevates GSH concentration in both lines and that the return to pre-methionine levels occurs only slowly. The significant increase in GSH concentration following methionine infusion is consistent with the suggested role of GSH as a cystine carrier.

Consistent with the results reported in Chapter II, there was no difference between genotypes in the plasma concentration of glucose (Figure 4.4). Analysis of the data showed neither an effect of treatment on plasma glucose nor an interaction with selection line (Table 4.5).

Plasma thyroxine concentration for each genotype is shown in Figure 4.5. As shown previously in Chapter III there was a significant difference between the lines in the plasma concentration of T4 ($P < .01$; Table 4.6). C rams maintained higher concentrations of T4 in the plasma by approximately 1 $\mu g.dl^{-1}$ throughout the experiment. There was no effect of treatment and neither was there an interaction. These results are consistent with those of Hough *et al.* (1986) and clearly require further investigation to determine the origin of the difference.

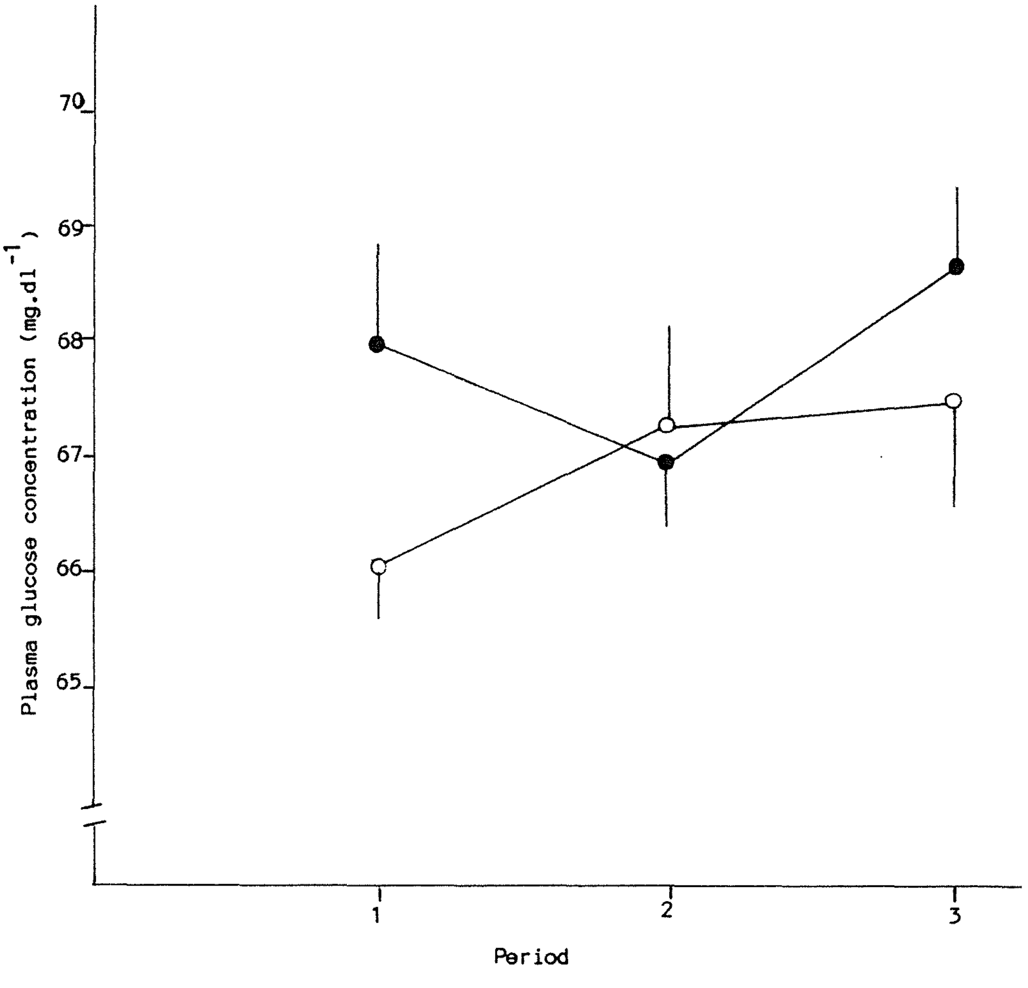

FIGURE 4.4 : Mean plasma glucose concentrations during Period 1 (saline infusion), Period 2 (methionine infusion) and Period 3 (saline infusion) in Control (o) and Fleeceweight-selected (●) rams. Bars indicate standard errors about the mean.

TABLE 4.5 : Plasma glucose concentration in Fleeceweight-selected and Control rams during saline and methionine infusion periods.

| | | Plasma glucose concentration (mg.dl$^{-1}$) | |
|---|---|---|---|
| Treatment | n | Control (C) | Fleeceweight (FW) |
| Saline | 5 | 66.8 ± 0.6[a] | 68.2 ± 0.6 |
| Methionine | 5 | 67.2 ± 0.9 | 67.0 ± 1.2 |

Analysis of Variance[b]

| Source of Variation | df | Mean Square and Significance | |
|---|---|---|---|
| Genotype | 1 | 5.6 | NS |
| Treatment | 1 | 0.9 | NS |
| Genotype x Treatment | 1 | 4.8 | NS |
| Error | 26 | 4.1 | |

[a] Mean ± S.E.

[b] Analysis of variance based on mean glucose concentrations

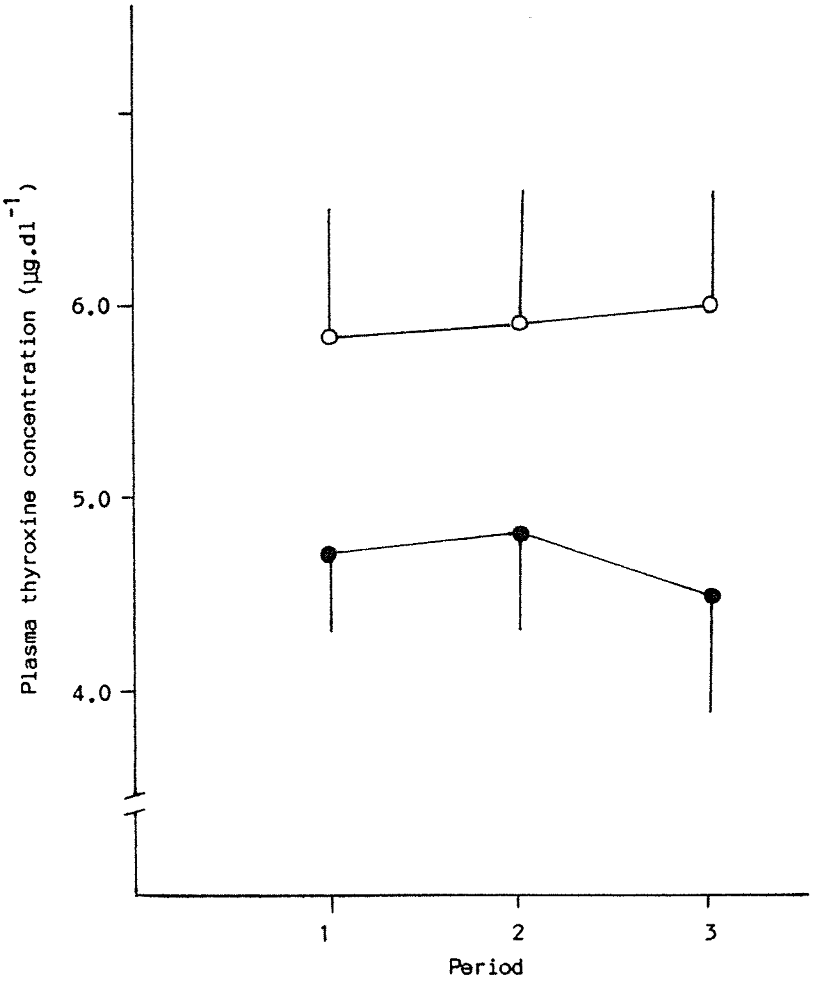

FIGURE 4.5 : Mean plasma thyroxine concentrations during Period 1 (saline infusion), Period 2 (methione infusion) and Period 3 (saline infusion) in Control (o) and Fleeceweight (●) rams. Bars indicate standard errors about the mean.

TABLE 4.6 : Plasma thyroxine concentration in Fleeceweight-selected and Control rams during saline and methionine infusion periods.

| | | Plasma thyroxine concentration ($\mu g.dl^{-1}$) | |
|---|---|---|---|
| Treatment | n | Control (C) | Fleeceweight (FW) |
| Saline | 5 | 5.90 ± 0.44[a] | 4.60 ± 0.34 |
| Methionine | 5 | 5.86 ± 0.68 | 4.82 ± 0.47 |

Analysis of Variance

| Source of Variation | df | Mean Square and Significance | |
|---|---|---|---|
| Genotype | 1 | 11.04 | ** |
| Treatment | 1 | 0.06 | NS |
| Genotype x Treatment | 1 | 0.11 | NS |
| Error | 26 | 1.58 | |

[a] Mean ± S.E.

# CHAPTER V

# DISCUSSION AND CONCLUSIONS

# CHAPTER V

## DISCUSSION AND CONCLUSIONS

The purpose of this study was to examine the physiological basis of the responses to selection for fleeceweight in Romney sheep and thereby to identify potential markers of genetic merit. The search for possible genetic markers was conducted with a view to increasing the rate of genetic gain in existing breeding programmes by decreasing the generation interval and/or improving the accuracy of selection. This is of particular relevance to the future use of artificial insemination in sheep since young rams in their first autumn may be used more extensively by artificial insemination than by natural mating.

The usefulness of a genetic marker depends upon the fulfilment of several criteria. First, a marker must be easily measured, its assay being both inexpensive and manageable with respect to ease and time involved. Results should, ideally, be obtained without the need for special sampling conditions (i.e. they should be obtained through infrequent sampling in the field). Second, a marker must be repeatable under a variety of conditions and, most importantly, it must be correlated with genetic merit for the production trait in question. Finally, use of the marker must not have detrimental effects on selection progress in other traits.

This study attempted to identify markers which could potentially be used to accurately discriminate between animals of high and low genetic merit, specifically those of the fleeceweight-selected and control lines of Romney sheep (Blair *et al.* , 1984, 1985). A variety of potential markers, generally levels of metabolites and hormones in the blood, were considered. An attempt was then made to determine the extent and repeatability of the differences between the selection lines and to provide some indication of the possible physiological basis for these differences.

The use of one such marker in sheep has already been reported in Soviet literature (Perchikhin, 1980). In this case selection for the level of activity of the enzymes aspartate aminotransferase or alanine aminotransferase in Lincoln ewes was considered to be a more effective means of increasing fleeceweight than direct selection. This selection criterion also has the advantage of being easily measured, there being a ready supply of inexpensive kits which adequately assay the enzyme activity. The current study using Romney selection lines found no significant difference in level of enzyme activity between genotypes, however, and neither was there a suggestion of a correlation with fleeceweight. Similarly no distinction could be made between the lines on the basis of plasma glucose concentration.

The study provided an indication of possible variation between the lines in erythrocyte GSH concentration. Throughout the methionine infusion study (Chapter IV) FW rams maintained greater concentrations of GSH than those of C rams. Although this difference between lines was not significant, there was a significant effect of treatment (i.e. methionine infusion) on GSH level. This finding is consistent with the suggested function of GSH as a carrier of cysteine. Further study is therefore warranted in this area both from the point of view of possible line differences in GSH level and also the effect of methionine on GSH concentration. Although there appears to be minimal diurnal variation in GSH, there is known to be marked seasonal variation, GSH concentration being at a minimum in winter and maximal in summer (Board *et al.* , 1976). Its concentration is also known to vary widely among sheep within a breed (Agar, 1975) and is affected by nutritional status, which alters the availability of substrates, thereby limiting the synthesis of GSH (Sumner *et al.* , 1979). Thus, it would seem that these factors combine to limit the potential usefulness of GSH as a genetic marker.

Of the metabolites studied the main difference between the selection lines was in plasma urea concentration. In all three experiments conducted, C rams had plasma urea concentrations consistently 1 mM higher than those of FW rams. This difference was evident over a variety of feeding conditions whether the animals were fed *ad libitum*, 110% maintenance or a diet fixed irrespective of liveweight. The results suggest that variation in plasma urea concentration may partly reflect differences between the lines in their efficiency of utilisation of amino acids for wool growth. Australian studies (Piper and Dolling, 1966; Reis *et al.* , 1967) have shown that Merino sheep selected for increased fleeceweight incorporate sulphur amino acids at a lower rate, by percentage wool weight, than animals selected for decreased wool production. This may indicate that the higher plasma urea concentration of C rams is the product of greater deamination of non-sulphur amino acids. Mersmann *et al.*, (1984) also suggested that differences in plasma urea concentration between fat and lean selection lines of pigs reflected differences in the efficiency with which amino acids were used for production vs deaminated.

This is consistent with the hypothesis that differences in wool sulphur content exist between the lines. Such differences may occur when calculated on a percentage wool weight basis but may not necessarily hold when calculated on the basis of total output. Wool sulphur content of the Romney selection lines has not been measured. Any future study should, however, consider this aspect.

Differences between the lines in plasma urea concentration may also reflect differences in kidney function. The lines were found to differ in plasma creatinine concentration in much the same way that they differed in plasma urea concentration. C rams maintained greater plasma creatinine concentrations by approximately 0.01 mM. These results imply variation between the lines in glomerular filtration rate (GFR), the line with the lower GFR (C) having a higher concentration of those substances not actively secreted or reabsorbed in the nephron (e.g. creatinine). GFR would also influence the plasma urea concentration despite the fact that urea concentration may be altered through passive movement across the more distal parts of the nephron.

The methionine study (Chapter IV) provided evidence that supplementation with a sulphur-containing amino acid allows C animals to utilise a greater proportion of the available non-sulphur amino acids thereby decreasing the deamination rate and plasma urea concentration. During the initial saline infusion C rams maintained higher concentrations of both plasma creatinine and plasma urea. When methionine was infused plasma urea concentration was decreased to a greater extent in C than FW rams, halving the difference that had previously existed between the lines. This difference (1mM) was again re-established on conclusion of the methionine treatment. Plasma creatinine concentration of both FW and C rams remained stable throughout all periods of the study. These results indicate that the decrease in plasma urea of C rams observed during the infusion of methionine can be attributed to an alteration in the utilisation of amino acids rather than to a change in kidney function. It should be noted that McCutcheon *et al.*,(1987) have recently shown that, in the same lines, much of the between-line variation in plasma urea can be accounted for by differences in creatinine clearance (a measure of GFR) and urinary urea excretion (which, it is presumed, reflects rates of deamination and hence entry of urea into the plasma pool).

The consistent difference in plasma urea concentration between the fleeceweight-selected lines and between other lines, (Tilakaratne *et al.*, 1980, observed 1mM difference in plasma urea between Friesian calves sired by bulls of low vs high genetic merit for milk production; Mersmann *et al.* , 1984, fat vs lean lines of pigs; Sejrsen *et al.*, 1984, dairy bulls of low vs high genetic merit; Carter *et al.*, 1986, fat vs meaty lines of Southdown sheep) indicates its possible use as a marker to predict genetic merit for a number of productive traits. The results further suggest that consideration of plasma creatinine, which is significantly associated with the variation in plasma urea, could enhance the accuracy of prediction. Both plasma urea and plasma creatinine are easily assayed and the differences between C and FW lines have been maintained under a variety of conditions in a number of separate trials conducted at different times of the year. There is also a strong indication that plasma urea is physiologically related to genetic merit for fleeceweight. In this way plasma concentrations of urea and creatinine meet several of the criteria necessary in a genetic marker. It appears, however, that in order to distinguish between genotypes on the basis of plasma urea/creatinine concentrations measurements must be made under controlled conditions. It has been observed that a low association exists between plasma urea measured in the field and genotype for fleeceweight (C.M. Clark, unpublished study; McCutcheon *et al.*, 1987). Although this fact would seem to limit the usefulness of plasma urea/creatinine concentrations as an effective marker, further study aimed at elucidating the physiological basis for line differences might, in turn, lead to more reliable markers.

Consistent with the results of Hough *et al.*, (1986) this study demonstrated variation between lines in plasma T4 concentration in two experiments (Chapters III and IV). FW rams maintained lower concentration of T4 (approximately 1 $\mu g.dl^{-1}$) than C rams. The differences between the lines, which were maintained throughout the course of each experiment, suggest the possible use of T4 as an indicator of genetic merit for fleece production. The criteria of a genetic marker are met by virtue of T4 being easily assayed and not subject to marked diurnal variation. However, the physiology of this T4 difference is unknown and at this point can only be speculated upon. There is little evidence in the literature of an increase in fleeceweight being associated, either phenotypically or genetically, with a lowered plasma concentration of T4. A change in the level of T4 may reflect a correlated response arising through linkage but not directly contributing to the between-line variation in wool production. If so this would imply that the same linkage relationship exists in both Merino and Romney sheep despite the fact that the breeds have been genetically distinct for many years.

In conclusion it may be stated that plasma concentrations of urea, creatinine and T4 hold considerable promise as potential markers for the identification of superior genotypes for wool production. There is an obvious need for further research pertaining to the origins of these differences as an improved knowledge of their physiology may lead to the development of more reliable markers.

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