THE EFFECT OF GENETIC SELECTION FOR FLEECE WEIGHT ON UREA METABOLISM AND DIGESTIVE FUNCTION IN ROMNEY SHEEP

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ABSTRACT

The study was undertaken to investigate the effect of 30 years selection for greasy fleece weight on rumen metabolism, apparent digestibility and nitrogen metabolism in the NZ Romney sheep. Previous studies had recorded a lower plasma urea concentration in the fleece weight selected (Fwt) animals as compared to the randomly selected control (C) animals, so most emphasis was placed on determining whether the lower plasma urea concentration in the Fwt sheep was accompanied by a lower irreversible loss (IRL).

Eight C and eight Fwt yearling rams (mean liveweight 42kg) were housed inside in individual metabolism crates. The study was divided into two almost identical experiments - the first one when the animals were fed on a chaffed meadow hay diet and the second on a lucerne chaff diet. Each experiment consisted of a two week adaptation period, a ten day digestibility period and a final period of six days during which ^{14}C-urea was infused intravenously for fourteen hours. At the end of the lucerne chaff experiment the animals were slaughtered and the rumen contents weighed and subsampled.

The Fwt animals had a one mM lower plasma urea concentration when fed on both diets. However there was no difference in the plasma urea IRL, urinary urea excretion or urea recycling to the digestive tract between the Fwt and C sheep when fed on either diet.

Relative to the C sheep, the Fwt animals had a higher creatinine clearance rate (63 vs 50 ml/min; $P<0.05$) when fed the meadow hay diet, and a higher urea clearance rate when fed both diets (meadow hay diet 42 vs 32 ml/min, $P<0.10$; lucerne chaff diet 72 vs 60 ml/min, $P<0.05$).

When fed the meadow hay diet there were no differences between the Fwt and C animals in voluntary feed intake (VFI) (Fwt animals 44 vs 42 gDM/kgW$^{0.75}$/d C animals) or apparent digestibility (DM digestibility 55% for both genotypes). However when fed on the lucerne chaff diet the Fwt animals had a higher VFI than the C animals (102 vs 94 gDM/kgW$^{0.75}$/d) and a lower apparent dry matter digestibility (56.1% vs 61.3%; $P<0.05$). These changes were associated with an increase in the rumen Fractional Outflow Rate (FOR) of lignin in the Fwt sheep (3.00 vs 2.58 %/h; $P<0.05$). There was a higher molar proportion of acetate present in the rumen fluid of the Fwt animals than in the C animals (68.6 vs 64.0%; $P<0.10$).

These results confirm that the Fwt animals have a consistently lower plasma urea concentration over a range of nutritional levels (0.6X maintenance and 1.5X maintenance) than the C animals, but there was no difference in urea metabolism as measured by plasma urea IRL and urea excretion. The urea and creatinine clearance rates suggest that selection for fleece production may have altered kidney function, but that the expression of these differences is related to the nutritional level. The greater FOR in Fwt sheep fed at the high level of nutrition (i.e. lucerne chaff) may mean that the amino acid

flow at the duodenum (undegraded dietary plus microbial protein) is greater than in the C animals and this could be a factor contributing to the superior wool production of the Fwt sheep.

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LIST OF ABBREVIATIONS

Acet	Acetic Acid
ADR	Acid Detergent Residue
But	Butyric Acid
C	Control Animals from the randomly selected line of sheep
°C	degrees Celsius
CCR	Creatinine Clearance Rate
Cl	Clearance
Conc	Concentration, []
Creat	Creatinine
d	Day
DDMI	Digestible Dry Matter Intake
Dig	Digestibility
DM	Dry Matter
DNI	Digestible Nitrogen Intake
FDPR	Fractional Disappearance Rate
FDR	Fractional Degradation Rate
FOR	Fractional Outflow Rate
F+	Australian Merino Fleece Weight Selection Line
F-	Australian Merino Selection Against Fleece Weight Line
Fwt	NZ Romney Fleece Weight Selection Line
G	Genotype
GE	Gross Energy
GFR	Glomerular Filtration Rate
GIT	Gastro Intestinal Tract

Hemi	Hemicellulose
I	Intake
IBut	Iso Butyric Acid
IVal	Iso Valeric Acid
IRL	Irreversible Loss Rate
IU	International Units
$L^{0.75}$	Metabolic Liveweight
Luc	Lucerne Chaff Diet
MH	Chaffed Meadow Hay Diet
mM	millimoles
MRT	Mean Retention Time
N	Nitrogen
ND	Not Determined
NDR	Neutral Detergent Residue
NH_3	Ammonia
OM	Organic Matter
PCC	Plasma Creatinine Concentration
Prop	Propionate
PUC	Plasma Urea Concentration
R	Room
Ret	Retention
RG/WC	Ryegrass White Clover Pasture
SA	Specific Activity
SE	Standard Error of the Mean
μ	Mean
UER	Urinary Excretion Rate
Val	Valeric Acid

VFA	Volatile Fatty Acids
VFI	Voluntary Feed Intake
Wt	Weight

CHAPTER 1

LITERATURE REVIEW

CHAPTER ONE: LITERATURE REVIEW

1.1 INTRODUCTION

Wool production is influenced by many factors including nutrition, the genetic potential of the animal, climatic factors and management techniques. This review will look at genetic selection, the effect of protein supply and nitrogen metabolism of the ruminant in relation to wool growth.

1.2 THE EFFECTS OF GENETIC SELECTION FOR FLEECE WEIGHT

1.2.1 INTRODUCTION

Improvements in wool production through genetic selection should be possible because there are large differences between individuals, strains and breeds of sheep.

There are three major trials selecting for fleece weight running in Australasia. There are two in Australia, involving the Australian Merino; one at Trangie Research Station and the other at Cunnamulla. The third is in New Zealand, involving the NZ Romney. In the Australian trials there are three lines of selection;

1) selection for fleece weight (F+),
2) no selection (C), and
3) selection against fleece weight (F-).

Both experiments have resulted in an increase in wool production in the F+ animals over the C and F- animals, in spite of utilising different selection pathways. In the Trangie flock selection was totally on wool production while in the Cunnamulla flock there was a limit placed on the fibre diameter and the amount of skin wrinkle.

At Massey University there are two selection lines a randomly selected control line (C) and a line selected for fleece weight (Fwt). Selection has been totally on hogget greasy fleece weight (Blair et al, 1984,1985). In the Massey Romney selection flock a one kilogram increase in greasy fleeceweight (or two standard deviations) over the control was achieved over 25 years of selection (Table 1.1) (Blair et al, 1984,1985; and McClelland et al, 1987a). The two kilogram increase in bodyweight is too small to account for the difference between the lines.

An important area of research that requires study is how this change has occurred and whether or not it has resulted in an improvement in the overall situation by increasing the output per unit input; - for example nutritional, labour and animal health inputs. For instance if the voluntary intake is increased as well as wool production then the farmer maybe no better off economically. For these reasons it is necessary to know what factors have actually been altered through the selection process. These factors include the effects on the voluntary intake, digestion, rumen function and the efficiency of food conversion to wool, as well as other physiological changes.

Table 1.1 The Effect of 25 Years of Selection on Wool Production and Liveweight at Hogget Shearing in the Massey Romney Flock

	GENOTYPE		
	Fwt	C	Sign
Wool Production(kg)	4.7	3.6	0.001
Liveweight(kg)	43.1	40.9	0.01

McClelland et al, 1987a

1.2.2 THE EFFECT ON THE VOLUNTARY FEED INTAKE

Wool production is dependent to a large extent on intake (Allden, 1979). In the Australian selection lines Ahmed et al (1963) found that the F+ flock consumed 8% more feed and produced 18% more wool than the randomly selected flock, however as the F+ animals were significantly heavier there was no difference in intake per unit of bodyweight. Williams and Millar (1965) found no difference in the voluntary feed intake between the F+ and F- Merinos when feeding three roughage diets and McClelland et al (1987a) found no

differences between the Romney Fwt and C lines on ad-libitum intakes of lucerne chaff under a pen feeding regime or in the faecal dry matter output on a range of pasture allowances (4500 to 1900 kgDM/ha) in ram hoggets.

Ahmed et al (1963) found no evidence that the F+ animals selected a higher protein diet when offered a range of protein levels. It is possible that dietary selection is more important in the field where the choice of diet is wider and the protein level is more likely to be limiting, however the importance of these selection differences, if present, is likely to be only small. The differences in diet selection that Weston (1959) found between the strong wool and fine wool Merinos were inconsistent.

The evidence suggests therefore that intake is not a major factor influencing the production differences between the F+, C and F- animals and some other factor (or factors) appears to be responsible.

1.2.3 THE EFFECTS ON RUMEN DIGESTION AND FUNCTION

Selection for or against wool production in the Australian Merino does not appear to have altered the digestibility of dry matter, organic matter, energy, nitrogen or sulphur; or the proportion of nitrogen reaching the small intestine (Weston, 1959; Piper and Dolling, 1969b; Williams, 1979).

Hamilton and Langlands (1969) found no difference in the content or digestibility of nitrogen using the faecal nitrogen technique between the F+ and F- lines in the Merino. In the Fwt and C Romney lines there was no difference in the dry matter or organic matter digestion or in the urinary or faecal nitrogen excretion on a lucerne chaff diet (McClelland et al, 1987b), although McClelland et al (1986) found that the Fwt animals had a lower dry matter digestibility when offered freshly cut mature ryegrass /white clover pasture ad libitum.

Williams (cited by Williams, 1979) found that the concentration and the proportion of the volatile fatty acids and the ammonia concentrations in the rumen fluid were similar in the Australian F+ and C animals fed on lucerne hay. Hough (1987) found no difference in the Trangie selection lines in the plasma non esterified fatty acid concentrations or in the blood concentration of three hydroxyl-butyrate. From these results it would appear that there has been no consistent change in the rumen function between the genotypes studied to date.

1.2.4 MINERALS AND VITAMINS

There is no information to indicate whether or not the availability and the utilization of minerals is associated with the genetic variation in wool production. Copper is important in the keratinisation of the wool fibre and therefore it may be involved in

the genetic determination of wool production. Zinc may also be important, in rats it influences the partitioning of the sulphur amino acids among tissues, especially where the skin is deficient in sulphur or zinc (Hsu and Anthony, 1970). Some vitamins may have links with wool production through the effects on the keratinisation process (for example vitamin A) or as co-factors in the metabolism of methionine (for example vitamins B_6 and B_{12}). However there is no information on the importance of these in determining genetic variation.

Therefore the reasons for the differences in fleece weight between the F+ and F- lines would appear to be in the efficiency of the post absorptive utilisation of nutrients.

1.2.5 THE EFFECT OF SELECTION ON THE EFFICIENCY OF FEED CONVERSION INTO WOOL PRODUCTION

McGuirk (1980) suggested that the two major components of fleece weight are the total surface area growing wool and the amount of wool produced per unit area of skin. It was found that in the Australian Merino, selection for an increase in the wool production per unit area of skin was effective in increasing the wool production per animal, whilst the changes due to altering the total area producing wool (i.e. bodyweight and skin wrinkle) were small and inconsistent. Some workers have found that more than 50% of the variation between animals can be accounted for by the differences in the efficiency of

the conversion of feed to wool rather than to changes in the level of the voluntary intake (Butler and Maxwell, 1984). For example in a grazing study Hamilton and Langlands (1969) found that 70% of the variation between their F+ and F- animals was due to the differences in efficiency while less than 30% of the variation could be explained by the differences in intake. However these differences in efficiency are only consistent when the total surface area producing wool remains constant (Williams, 1979).

The relationship between the genotype and the environment, especially nutrition, is important as it can reduce the phenotypic superiority of the genetically superior sheep. A number of workers have looked at this in the Cunnamulla flock (Turner et al (1968), Dolling and Moore (1960), and Dolling and Piper (1968)) and in the Trangie flock (Ahmed et al (1963), Williams and Winston (1965), and Williams (1966)).

Although the F+ animals were more efficient at all levels of intake, in both flocks there were some differences in the interaction between the efficiency and the level of nutrition. Piper and Dolling (1969a) found no significant interaction, while in the Trangie flocks the superiority of the F+ animals increased with the increasing nutrition level (Table 1.2). However the range of intakes was wider in the Trangie Trials.

Table 1.2 The Effect of the Feeding Level on the Relative Efficiencies of the Different Selection Lines

Reference	Brd[a]	Flock	Feeding Level	Ratio Wool Produced/ Feed Intake (control =100)
Williams and Winston (1965)	M	F+	High(Bwt gain)	126
	M	C		100
	M	F-		96
	M	F+	Mod(maint)	117
	M	C		100
	M	F-		87
	M	F+	Low (Bwt Loss)	106
	M	C		100
	M	F-		90
Williams (1966)	M	F+	Unrestricted	138
	M	C		100
	M	F-		75
	M	F+	Restricted	121
	M	C		100
	M	F-		72
Piper and Dolling (1969a)	M	F+	High	132
	M	C		100
	M	F+	Moderate	129
	M	C		100
	M	F+	Low	91
	M	C		100
McClelland et al(1986)	R	Fwt	High	150
	R	C		100
	R	Fwt	Moderate	116
	R	C		100
	R	Fwt	Low	86
	R	C		100

[a] M=Merino, R=Romney
(adapted from Butler and Maxwell, 1984)

Williams and Winston (1965) and Williams (1966) showed the efficiency of feed utilisation for wool growth of the F+ animals increased as the level of feed intake increased, and the differences in wool production and efficiency were greatest at the highest feeding level. McClelland et al (1986) found a similar effect in the New Zealand Romney Fwt and C lines. The capacity of the genetically superior animals to respond more to the increased level of nutrition was demonstrated in a trial by Williams et al (1972b) when the sulphur amino acids cystine and methionine were infused into the abomasum, the F+ animals increased wool production by 55% while the F- animals only increased wool production by 15%. Hamilton and Langlands (1969) found that the difference in both wool production and efficiency was greater when the sheep were grazing a phalaris pasture (3800kgDM/ha) than when grazing a similar pasture with 2000kgDM/ha - i.e. under a low nutritional regime the phenotypic superiority of the genetically superior sheep may be reduced. Under Australian conditions the stocking rates of 4.5 and 3.5 sheep per hectare have been compared. There was a large difference in the wool production per head although the F+ animals still maintained a 30 to 40% advantage even at the high stocking rate. These effects however need to be studied under a more temperate system.

Dolling and Moore (1960) found that the size of the difference varied between the F+ and the F- animals between the pen and the pasture diets (see Table 1.3).

Table 1.3 The Effect of Diet on the Differences Between the Two Selection Lines

	Pen	Pasture
F+	110	124
F-	100	100

Dolling and Moore (1960)

They suggested that this result could have been due to the F+ animals increasing their level of intake more on the pasture or that they were able to select a higher quality pasture diet than the F- animals.

It appears from the above, that the F+ animals convert feed to wool more efficiently than the F- animals and the difference in efficiency increases with intake.

1.2.6 PHYSIOLOGICAL CHANGES

Genotypes that differ in wool production have been shown to exhibit differences in several important physiological determinants of wool growth. These can be grouped into the following categories:

- changes to the follicle structure and formation
- changes in the sulphur and cystine metabolism
- changes in the plasma urea concentration
- changes in the blood flow
- hormonal changes

1.2.6.1 Changes To The Follicle Structure And Formation

The difference in the fibre output between genetically different sheep could result from

1. a reduced cell turnover time,
2. a larger basal cell population,
3. larger basal cells,
4. a larger proportion of the basal cells flowing from the bulb entering the fibre relative to those entering the inner root sheath (Williams, 1976a).

Williams and Winston (1987) found that the average diameter of the follicle bulbs were similar between lines but that the F+ bulbs had a ten percent larger area of mitotically active tissue. The follicle bulb cells of the F+ animals appeared to have a 15% larger volume than those cells in the F- animals in the preliminary trials,

whilst the volume of the cortical cells of the high producing animals tended to be more sensitive to the nutritional level. No difference was found in the rate of entry of the cells into mitosis between flocks, although the rate was greater in both flocks on the higher feeding level. In the Trangie flock the F+ animals had longer fibres regardless of the dietary intake (Williams, 1976a). However the mean fibre diameter of the F+ animals increased proportionately more with increasing dietary intake than did the mean diameter in the lower producing animals. This disproportionate change resulted in an interaction in the wool production per unit area between the environment and the genotype (Williams, 1966). Williams and Winston (1987) recorded a higher density of wool follicles in the F+ animals.

Selection at Trangie altered wool production at different sites on the body disproportionately (Table 1.4) leading to the possibility of an increase in the proportion of the wool remaining after skirting. Williams (1976a) suggested that this would increase the value of the fleece as this piece is the most valuable.

In the F+ animals the wool follicles were usually straighter and deeper in the skin (Nay and Hayman, 1969). Williams (1973) suggested that in spite of the increased growth rate of the fibres of these animals the mean emergence times were likely to be similar between the two lines. Nay and Johnson (1967) and Nay and Hayman (1969) observed that the arrangement of follicle groups in the skin differed between the genotypes and that this was often associated with differences in follicle morphology. The F+ animals whose primary

follicles were arranged in parallel rows, had well vasculated follicles, whereas the F- animals with their curved primary follicles in crescent shaped fields had a poor vascular supply. The vascularization of the epidermal layer of the F+ animals was also more extensive. Williams's (unpub cited by Williams, 1987) work indicated that the skins of the genetically higher producing animals had a lower content of collagen and a higher content of non-collagen soluble protein.

Table 1.4 The Efficiency of Selection for Fleece Weight on Wool Production ($mg/cm^2/d$) at Different Body Sites

	Sampling Site		
	Thigh	Midside	Shoulder
High	312	458	529
Low	288	392	415
Difference (%)	8	17	28

(McGuirk, 1980)

1.2.6.2 Sulphur and Cystine Metabolism

Several workers have recorded that the high fleece weight Merinos had a lower wool sulphur content as well as a lower plasma concentration of cystine relative to the F- animals (Piper and Dolling, 1966; Reis, 1979; Hough, 1987). Williams and Winston (1987) found that the sulphur content of the wool was reduced (12%) in the F+ ewes and that this difference was consistent across nutrition levels.

Despite this negative genetic relationship between the wool production and the wool sulphur content, the daily output of sulphur in the F+ animals wool was higher than the C animals at the higher intake levels. However there was no difference in the percentage of sulphur retained in the wool free body as the genetically superior sheep secreted less sulphur in the urine (Table 1.5) (Williams, 1976a).

There is an indication that the least efficient sheep in terms of wool output per unit of food input have wool with a higher sulphur content than the more efficient sheep under the same conditions. The sulphur content of the wool in these animals appears to be less sensitive to changes in nutrition (Williams, 1979). When L-^{35}S cystine was infused intravenously the skin was found to have a very significant role in the utilisation and apparent sensitivity to the quantity of cystine absorbed, as the skin makes up only 5% of the liveweight but retained 30% of the radioactivity of the ^{35}S.

Williams (cited by Williams, 1979) suggested that the skin composition and the uptake of ^{35}S into the soluble protein of the skin differs between genotypes.

Table 1.5 The Daily Output of Sulphur in the F+ and C Merino Lines

	F+	C
Urine (mgS/d)	388	460
Faeces (mgS/d)	593	583
	981	1043
WOOL		
g wool/d	9	7
% sulphur	3.1	3.2
mgS/d	288	231
Total Excreted	1269	1274

Williams (1976a)

This and the fact that more than 95% of the sulphur in the wool fibre is present as cystine suggests that the F+ follicles functioned differently during the synthesis of wool keratin. The metabolism of cystine could vary in several ways

1. different quantities of sulphur amino acids may be absorbed from the digestive system
2. the utilization of cystine for the synthesis of protein, in tissues other than the skin, within the wool-free body
3. the transport of cystine to the skin and wool follicles
4. the utilization of cystine by the follicle for the synthesis of the fibre.

The lower concentration of cystine in the plasma of the F+ animals would however limit the synthesis of wool (Williams, 1976). Williams et al (1986)found that the plasma cystine concentration remained 17% lower in the F+ animals than the F- during fasting. When ACTH injections were given the cortisol levels increased in both groups but this increase was not associated with a change in the plasma cystine concentration.

Williams et al (1972a) found no difference in the cystine entry rate using a continuous infusion however when a single injection technique was used the F+ animals had a lower entry rate of cystine (Williams, 1976b). Both experiments found that the F+ animals had a lower plasma cystine concentration. Therefore there is no indication from either the concentration or the mean entry rates to suggest that the F+ animals have a greater availability of cystine at the follicle

level in the Australian Merino. It would appear that the greater efficiency of the superior sheep is derived from their ability to incorporate a unit of cystine into a greater amount of fibre compared to the F- animals. This suggests that cystine is utilized differently by the different genotypes during the passage from the plasma into the follicle and during its conversion to keratin.

Williams (1976a) argued that the genes responsible for the expression of wool growth determined the concentration of cystine in the plasma. It has been suggested that the high ionic concentration of cystine in the plasma and/or the cellular fluids surrounding the follicle and the presence of the curved follicles in the F- sheep actually enhances the initial keratinization of the inner root sheath and the synthesis of the high sulphur proteins in the cells destined for the cortex. Either or both of these factors could result in a depression in wool growth.

1.2.6.3 Plasma Urea Concentration

The plasma urea concentration (PUC) was consistently 1mM lower in the Romney Fwt line than the C animals and this difference has been maintained over a range of diets, intakes and feeding patterns although the differences have not always been significant (Clark, 1987; McClelland et al, 1987b; McCutcheon et al, 1987). Supplementation with protected casein increased the PUC equally in both lines while a methionine infusion lowered the PUC more in the control animals (McClelland et al , 1987b). Hough (1987) however

found no differences in the urea level between the F+ and the F- Merino selection lines. In these sheep however they did find a 15% higher level of the plasma α-amino-N in the selected animals. In a later trial no differences were found (Hough cited by Williams, 1987).

1.2.6.4 Blood flow

Black and Reis (1979) used a computer model to simulate the effects of blood flow and the distribution of blood amongst the tissues, upon wool growth. This model predicted that an increase in the blood flow to the skin would result in an increased wool production and an increase in the proportion of the sulphur present as ultra high sulphur proteins. However the high fleece weight animals have a lower wool sulphur content and Hales (unpub) has found no significant differences in blood flow to the skin between the genetically different Trangie lines. Differences in the blood flow have been measured when using radioactive microspheres in other organs. In the adrenal gland, the pineal gland ($P<0.005$), the kidney ($P<0.10$), the subcutaneous fat, the ometum fat ($P<0.01$), the perirenal fat there was a higher blood flow in the F+ animals (Hales unpub). Ahmed et al (1963) recorded a decrease in the seasonality of the wool growth pattern and McClelland et al (1987a) found a less marked depression in winter wool growth in high producing animals. However this has not been measured in the Australian Merino flocks where the increase in the blood flow to the pineal gland has been recorded.

McClelland et al (1987b) suggested that the kidney may play a role in the differences observed in the urea plasma level in the Romney selection lines. In one experiment the Fwt animals produced 30% more urine per day implying a higher daily water intake. In a later trial where the water intakes were recorded no differences were found however an association between the PUC and the creatinine clearance rate was found. This acts as a measure of the glomerular filtration rate in the kidney thereby providing an indication of the kidney function. The blood flow to the kidney has not been measured in the Romney where these differences have occurred and the differences that have been recorded in the Merino have only been small. This may be a breed difference or some other factor may have been altered, for example kidney size. Further information is required on the importance of the kidney in the differences between the lines.

In the blood flow work by Hales the blood flow to the thyroid gland, the reticulum and the rumen were all lower in the F+ animals.

1.2.6.5 Hormonal changes

In the Trangie flock Hough (1987) has observed a difference in the plasma thyroxine levels but this difference has been inconsistent. There was no difference in the plasma concentrations of insulin or growth hormone and the sensitivity level was not studied. Williams et al (1986) recorded similar levels of corticoid in the plasma for both lines and the responses to adreno-corticotrophic hormone were the same.

1.2.6.6 Summary

The physiological differences between the flocks appear to be related to the post absorptive protein metabolism, especially the availability of cystine at the follicle, therefore the effect of protein supply on wool production and protein metabolism needs to be studied.

1.3 THE EFFECT OF PROTEIN SUPPLY ON WOOL GROWTH

After the removal of the wax, suint, dirt, and vegetable matter from wool the remainder is basically protein. Therefore the protein absorbed from the intestine i.e. the protein supply to the follicle, is likely to be an important factor influencing wool growth. Cystine which appears to play an important role in the regulation of wool growth, makes up approximately thirteen per cent of the wool protein while methionine makes up about half a percent (Marshall and Gillespie, 1977). This is higher than the levels commonly found in forages and abomasal digesta (Table 1.6).

Table 1.6 The Levels of Sulphur Amino Acids Commonly Found in Lucerne, Abomasal Digesta and Wool (%AA /%Total Nitrogen)

	Cystine	Methionine	Total
Lucerne			
Hogan et al, (1970) (hay)	0.6	1.2	1.8
Chibnall et al, (1963) (leaf)	1.2	1.3	2.5
Abomasal Digesta (Phalaris/clover diet)			
Hogan et al, (1970)	1.2	1.3	2.5
Wool			
Marshall and Gillespie (1977)	13.0	0.5	13.5

1.3.1 THE SULPHUR CONTENT OF WOOL

Considerable differences have been observed both within and between breeds of sheep in the amino acid composition of the wool (Reis, 1979). Under an adequate nutritional regime the sulphur content of the wool ranges from 3.5 to 3.7% for most animals, however

when a wider range of nutritional levels is applied the values fall between 2.7 and 4.7%. It has been suggested that the lower value may represent a fundamental limiting structure for wool production. Between these two extremes however the wool sulphur content appears to be related to the level of nutrition (Reis, 1979).

The proteins in wool can be fractionated into three major groups - the low sulphur proteins, the high sulphur proteins and the high tyrosine proteins. The major proportion of the variation in the sulphur content of the wool is due to alterations in the biosynthesis of the ultra-high sulphur proteins (Broad et al, 1970).

The sulphur content of the wool may be a function of the length of time spent in the region of the follicle where the synthesis of the high sulphur proteins is occurring i.e. a fast growth rate is associated with a lower sulphur concentration, as well as on the availability of the substrate. The availability of the sulphur amino acids is a function of the nutritional status of the animal, whilst the follicle activity may be related to nutritional factors it is also affected by non-nutritional and genetic factors. Doney and Evans (1968) suggested that sheep produce wool with a characteristic sulphur content unless this is altered by changing the relationship between the follicular activity and the available sulphur substrate.

1.3.2 THE EFFECT OF PROTEIN SUPPLEMENTATION

The response in wool production to protein supplementation in the mature animal is a function of the following criteria :

- diet and digestible energy intake
- site of supplementation
- type of protein used especially the solubility, degradability and the amino acid composition of the protein
- the genetic potential of the animal which may limit the ability of the animal to respond.

Dietary protein supplementation increases the overall nitrogen supply. As dietary supplements of sulphur amino acids are normally degraded in the rumen, they do not stimulate wool growth unless the diet is deficient in sulphur relative to the energy available for microbial growth (Downes et al, 1975 cited by Reis, 1979). However when the protein is infused into the abomasum of sheep thereby by-passing the rumen degradation, the rate of growth and the cystine content of the wool can be increased with (Reis and Schinckel, 1961, 1962; Reis, 1969). This appears to be variable over a relatively wide range of diets. Wright (1971) found a response on a concentrate diet based on ground maize when abomasally infusing methionine, and Dove and Robards (1974) recorded a response on both lucerne hay and wheaten hay diets, although the response was larger on the lucerne. However there was no apparent effect found on a wheat diet (Reis and Tunks, 1974) or on an oat hay diet (Lindsay et al, 1978). Therefore it appears that protein synthesis of the follicle is regulated by the

quantity of cystine (or methionine) that enters the follicle (Williams, 1973). Williams (1976a) found that the response to DL-methionine (41%) was greater than the response (32%) to L-cystine when compared on an equimolar basis. The interaction between the flocks and the treatments suggested that methionine could have a specific effect on the rate of wool growth as well as its effect through transulphuration to cystine. However cystine appears to be the primary amino acid limiting wool growth.

On roughage diets abomasal supplementation with any individual amino acids other than the sulphur amino acids, does not affect wool production, although the amino acid composition is important. For example, gelatin is markedly inferior to casein even when supplemented with the sulphur amino acids (Reis and Schinckel, 1963) and if lysine is administered with zein then wool production is stimulated to a greater extent as the same extent as supplementation with an equimolar amount of casein (Reis, 1979).

The sulphur content of the wool of the high producing animals is lower than the low producing animals (Piper and Dolling, 1966), therefore it would appear that the supply of sulphur amino acids is less limiting in these low producing animals as they are nearer to their genetic limit.

As protein absorption, especially of the sulphur amino acids at the small intestine is important in wool production the factors affecting rumen protein output need to be studied.

1.4 NITROGEN METABOLISM IN THE RUMINANT

1.4.1 PROTEIN DIGESTION IN THE RUMEN

The nitrogen in the rumen can come from many sources including:

- the diet
- recycled urea, via the saliva or across the rumen wall from the blood
- the breakdown of dead bacterial cells
- the breakdown of epithelial cells that have been sloughed off the walls.

(Leng and Nolan, 1984).

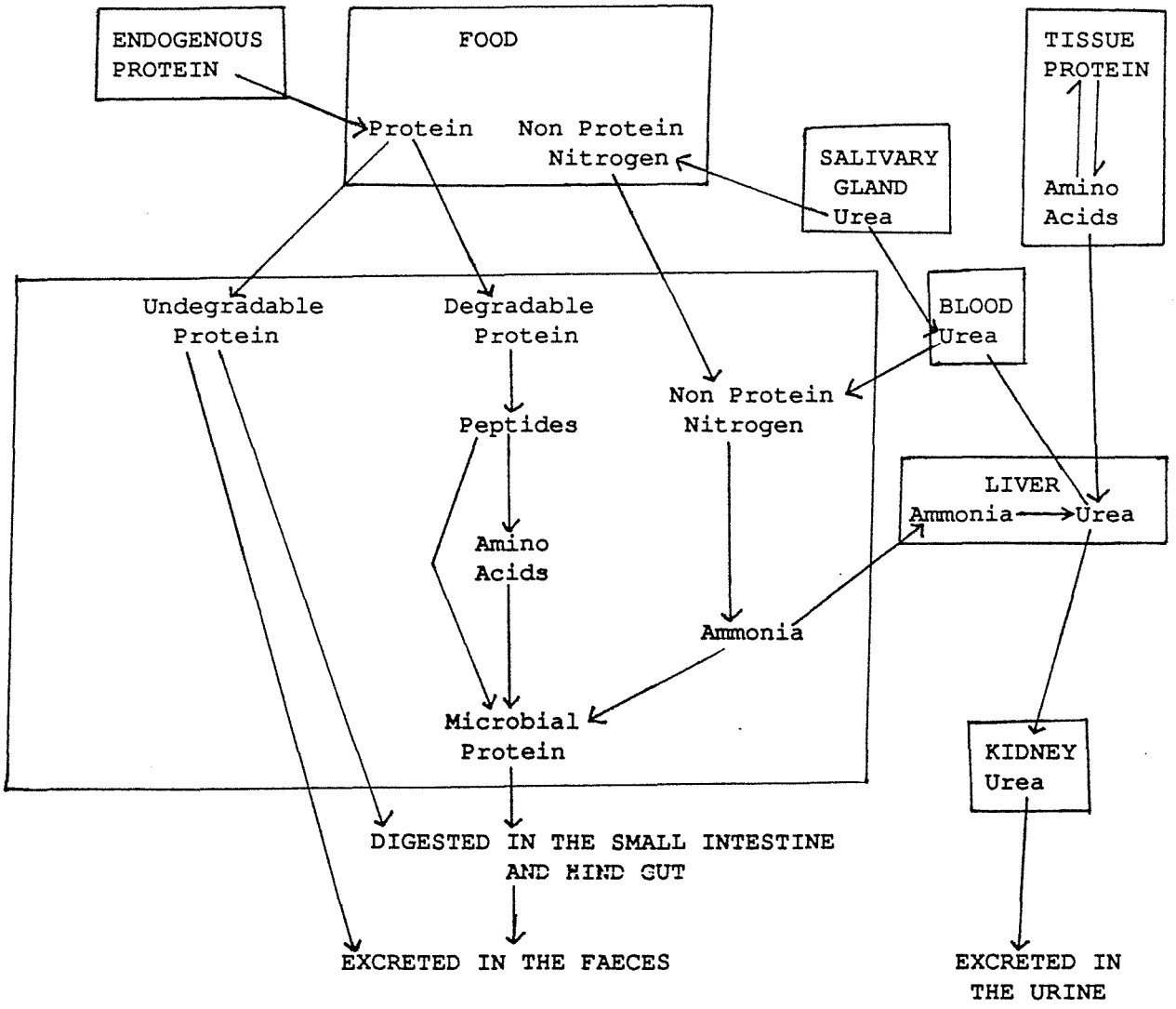
In sheep where the dietary nitrogen intake is low, the endogenous nitrogen may provide up to 25% of the available nitrogen in the rumen (Nolan and Stachiw, 1979; Kennedy and Milligan, 1980).

Degradable protein is broken down by proteases and peptidases into amino acids and ammonia in the rumen (Figure 1.1) (Nolan and Leng, 1972). The ammonia can then be incorporated into microbial cells, absorbed across the rumen wall or pass into the abomasum.

If the ammonia is incorporated into the microbial cells it will become reavailable on the death of the cell. Ammonia nitrogen contributes to between 50 and 80% of the nitrogen incorporated into bacterial cells (Nolan and Leng, 1972; MacRae and Reeds, 1980), therefore a readily degradable nitrogen source is required to supply

this ammonia. Kempton et al (1979) found that the microbial requirement for dietary fermentable nitrogen over a range of diets was approximately 1.2 gN/MJME. If the degradable nitrogen or the organic matter content of the diet is too low, or if the protein and energy breakdown are out of sequence then the number and activity of the rumen microorganisms will be restricted resulting in a decrease in the efficiency of the ammonia capture by these organisms.

Figure 1.1 Digestion And Metabolism Of Nitrogenous Compounds In The Ruminant



Any large excesses of ammonia flowing out of the rumen ammonia pool are likely to be absorbed in the abomasum (Mathison and Milligan, 1971). Any undegraded protein in the rumen is available for digestion in the small intestine. Mathison and Milligan (1971) found that for every 100g of dietary organic matter fermented in the rumen 2g of dietary nitrogen was digested in the rumen and 2g of nitrogen passed into the abomasum incorporated in the cell material.

The nitrogen flow to the duodenum is a mixture of ammonia, endogenous secretions, undegraded dietary and bacterial protein, and some microbial nucleic acids. The nitrogen flow to the duodenum is related to the nitrogen intake. Ulyatt and Egan (1979) derived two regression equations : one for fresh feeds and one for dried diets (Equation 1, 2), using a range of the values in the literature for sheep on intakes between two and fifty eight grams of nitrogen per day.

Dried diets $Y = 0.87X + 3.36$ (Equation 1)

Fresh diets $Y = 1.188X - 0.11X^2 - 0.018$ (Equation 2)

where X is the nitrogen intake in g/d

Y is the total nitrogen entering the duodenum in (g/d)

Ulyatt and Egan (1979)

Beever et al (1986) looked at the relationship between the duodenal NAN flow and the nitrogen intake in yearling cattle on fresh ryegrass and white clover swards (Equation 3).

Fresh Diets $Y = -0.01854X + 1.5074$ (Equation 3)

Where X is the nitrogen intake per unit of organic matter (g/kg)

Y is the duodenal NAN flow per unit of total N intake (g/g)

Beever et al (1986)

This result was similar to Hogan and Westons(1970) and Ulyatt (unpub cited by Beever et al, 1986) (Equation 4).

$Y = -0.01691X + 1.4304$ (Equation 4)

Where X is the nitrogen intake per unit of organic matter(g/kg)

Y is the duodenal NAN flow per unit of total N intake (g/g).

Ulyatt (cited by Beever et al, 1986)

With both fresh and dried diets there were gains in the nitrogen across the rumen at low levels of nitrogen intakes, presumably these were due to the endogenous nitrogen secretions, recycled urea and/or microbial protein synthesis. On fresh diets there was a net loss in nitrogen across the rumen when the nitrogen intake reached 18g/d, whilst the corresponding figure for the dried diets was approximately 25g/d. Once these nitrogen intakes were reached the percentage loss of additional dietary nitrogen appeared to be around 45% for the fresh diets and 13% for dried diets (Ulyatt and Egan, 1979). The higher proportion of dietary nitrogen reaching the duodenum in sheep fed dried diets is possibly due to the reduced solubility of the nitrogenous compounds as a result of the drying process (Beever et al, 1976), resulting in a larger proportion of the dietary protein escaping rumen degradation. Therefore the NAN flow can be increased by increasing the efficiency of the microbial growth by the provision of readily available carbohydrates or by the reduction of dietary protein degradation in the rumen.

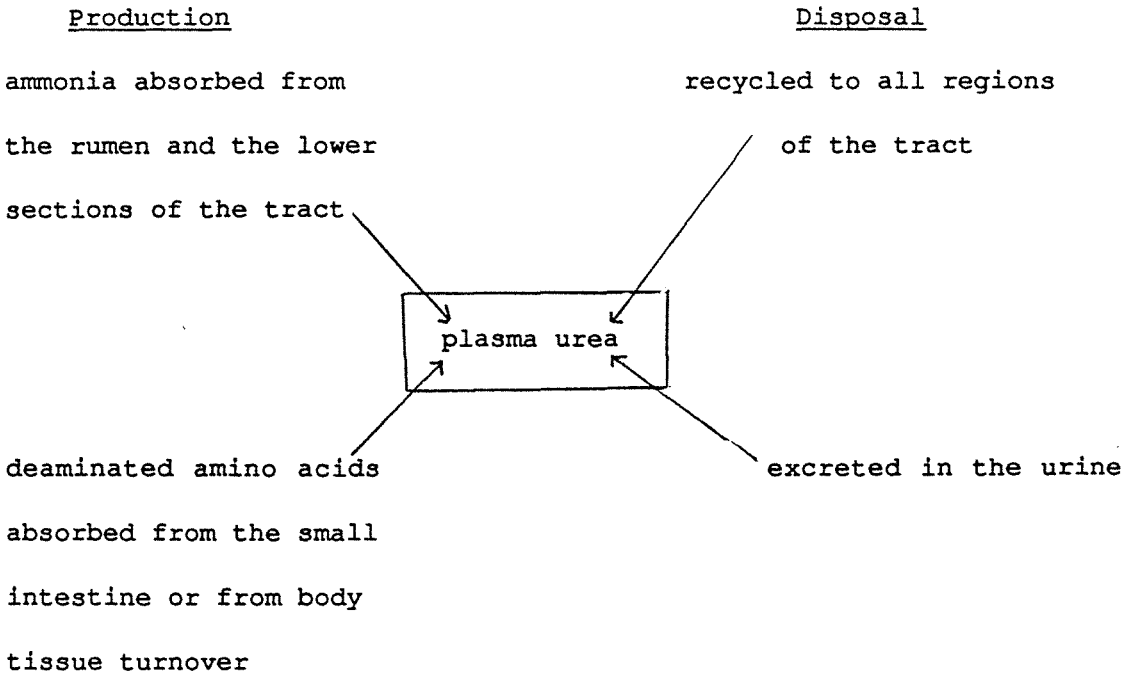
The later can be achieved by protecting the protein from fermentation in the rumen, for example by treating it with formaldehyde, or by using a naturally occurring compound that will also protect the protein, for example condensed tannins. Barry et al (1986) recorded an increased nitrogen retention in the sheep fed cultivars of Lotus pedunculatus with high levels of condensed tannins. This was due to an increase in the NAN flow to the small intestine and an increase in the level of nitrogen recycling to the rumen. Kempton et al (1979) found that the NAN flow to the duodenum

in growing lambs supplemented with formaldehyde treated casein was twice that of the animals supplemented with urea or untreated casein.

1.4.2 UREA METABOLISM

The production and disposal of urea is important in the ruminant, as nitrogen is frequently one of the major nutrients limiting wool production. Under steady state conditions the production of urea via ammonia absorption and the deamination of amino acids equals the disposal by the recycling and excretion of urea in the urine (Figure 1.2).

Figure 1.2 UREA METABOLISM



1.4.2.1 Production

When the ammonia is absorbed across the rumen wall it is transported in the portal blood to the liver where it is converted to urea. Lewis (1957) showed that the rate of absorption of ammonia into the portal system was related to the rumen ammonia concentration and it increased markedly at 20-30mg/100ml when the corresponding plasma urea levels were 24mg/100ml. This appeared to indicate that there was a constant relationship between the rumen ammonia concentration and the plasma urea nitrogen level, except when the diet contained large quantities of soluble protein. However McIntyre and Williams (1970) found that the plasma urea levels rose during an intravenous infusion of urea while the ammonia concentration did not. They suggested that the rumen ammonia concentration and the plasma urea levels are regulated by the nitrogen intake.

Neutze et al (1986) recorded an increase in the transfer of nitrogen from the rumen ammonia pool to the plasma urea pool and vice versa with an increase in the nitrogen intake in sheep fed on alkali treated wheat straw(Table 1.7), although the proportions of blood urea derived from ruminal ammonia remained relatively constant.

Table 1.7 The Effect of Increasing the Nitrogen Intake on the Transfer of Nitrogen Across the Rumen Wall

	The Amount of Urea Added to the Alkali Treated Wheat Straw Diet (g/kg DM)		
	3.5	5.9	11.5
N Intake (g/d)	7.1	11.5	18.6
Prop of Blood Urea N Derived From NH_3	0.56	0.65	0.56
Prop of Rumen NH_3 Derived From Urea N	0.22	0.23	0.11
Transfer Blood Urea N to Rumen (g/d)	3.8	4.7	2.6
Transfer Ruminal NH_3 to Blood Urea (g/d)	3.5	6.7	8.9
Net Transfer (est) (g/d)[a]	+0.3	-2.0	-6.3

[a] Blood Urea Nitrogen into the Rumen Ammonia-Nitrogen Pool (estimated as the difference between the transfers between the two pools) (Neutze et al, 1986)

Nolan and Leng (1972) calculated that 40-50% of the plasma urea was derived from rumen ammonia and that the rest apparently came from the deamination of amino acids and ammonia absorbed from the lower sections of the digestive tract in sheep fed on lucerne with a nitrogen intake of around 23g per day. However Kempton et al, (1979) found that in growing lambs more ammonia was absorbed across the rumen wall in high nitrogen diets. Nolan et al (1976) calculated that 6.1 g/d urea was formed from the degradation of amino acids in the body excluding the microbial amino acids derived from the rumen ammonia.

1.4.2.2 Disposal

1.4.2.2.1 Urea Recycling Via The Digestive Tract

The urea that is recycled via the gastrointestinal tract (Figure 1.1) can provide an important source of nitrogen, thereby influencing the production level and in extreme situations the survival of the animal itself. The amount of nitrogen recycled reduces the nitrogen deficit by that same amount. Recycling is only of nutritional significance when the level of nitrogen is limiting microbial growth and fermentation in the rumen.

The quantity of urea recycled from the blood to the digestive tract of the sheep, is primarily dependent upon the plasma urea concentration which is affected by the nitrogen content of the diet (Ford and Milligan, 1970). Cocimano and Leng (1967) found that in

sheep on a low nitrogen diet 73% of the plasma urea was recycled to the tract as compared to 25% in animals on a high nitrogen diet. This was the result of a lower degradation and excretion rate(Table 1.8).

Table 1.8 The Effect Of Diet On The Irreversible Loss Rate (IRL) Of Urea, Urea Degradation And The Percentage Of Urea Entering The Tract (mg/min)

%Crude Protein In Diet	Urea IRL	Urea Degraded In Gut	Urea Excreted In Urine	% Urea IRL Re-entering The Tract
3.5	2.6	1.9	0.7	73
4.5	2.6	1.6	1.0	62
13.5	22.9	12.9	10.0	56
17.0	23.2	5.8	17.4	25
27.3	41.8	10.0	31.8	23

(Cocimano and Leng, 1967)

When the dietary level of nitrogen is low then the transfer of endogenous nitrogen to the rumen makes up a relatively larger

component of the rumen nitrogen supply. For example in the trial by Neutze et al (1986) the recycled nitrogen (from plasma urea) provided 53%, 29% and 12% additional nitrogen on the low, medium and high nitrogen intakes (Table 1.7).

In the ruminant, urea can be returned to the digestive tract via:

1. the saliva
2. by diffusion across the rumen wall
3. across the wall of the hind gut.

The relative importance of these factors varies with the diet and feeding level.

The quantity of salivary urea nitrogen entering the rumen is limited by the total salivary output and the blood urea concentration, both of which depend on the diet. On the brome grass Kennedy and Milligan (1978) found that the salivary urea supplied less than 10% of the recycled urea (or about 0.4 to 0.6 g day) while the transfer of urea across the rumen epithelium accounted for up to 9 g (or 90%). However in sheep on a lucerne diet most of the daily nitrogen transfer of approximately 1.3g, appeared to be maintained by the salivary secretion of urea (Nolan and Leng, 1972)

Table 1.9 The Effect of Diet on the Amount of Urea That is Transferred to the Rumen

Reference	Diet	Transfer of Urea[a] mgN/h/kgBwt
Nolan and Leng (1972)	lucerne	1.44
Nolan et al (1976)	lucerne	1.39
Kennedy and Milligan (1978)	lucerne	1.08
Kennedy and Milligan (1978)	bromegrass	5.80
		5.65
		4.10
		4.17
		1.85
Nolan et al (1979)	low quality hay	0.67
		0.60
		0.48
Nolan and Stachiw (1979)	low quality hay plus molasses	2.65

[a] transfer of urea to the rumen

(Kennedy and Milligan, 1980)

On low quality and lucerne diets the amount of blood urea entering the rumen is small compared to the amount on the brome grass diets (Table 1.9). Kennedy and Milligan (1978) suggested that the low rate of blood urea transferred on the lucerne and native pastures was due to the transfer being limited by the high rumen ammonia concentration on the lucerne diet and the low dietary organic matter digestibility on the low quality feeds.

Obara and Shimbayashi (1980) suggested that in ruminants on a diet containing sufficient or excess nitrogen the rumen is replaced as the principal site of the appearance of the recycled urea by the lower sections of the digestive tract. Nolan and Leng (1972) found that of the 5.1 gN/d of urea apparently degraded in the digestive tract only 1.2gN/d appeared in the ruminal ammonia pool. They suggested that the remainder may have been degraded in the lower digestive tract.

It is possible that there are differences in the nitrogen metabolism and urea recycling between the different genotypes and flocks. A method of measuring this is required.

1.4.2.2.2 Measurement Techniques

Isotope tracer technology is currently the most favoured technique available for measuring the movement of urea in the conscious, relatively undisturbed animal feeding normally (Nolan and Stachiw, 1979).

There are basically two application methods -single injection and continuous infusion. With the single shot method a single injection of tracer is given and the decay curve is plotted against time. By ignoring the initial mixing period the extrapolation of the rectilinear portion of the curve to time zero can be used to provide an estimate of the level of activity at the time of the injection (Figure 1.3). With continuous infusion the tracer is administered over a period of time and the analysis is preformed on samples taken after the activity level has reached plateau (Figure 1.4). Cocimano and Leng (1967) used a regression analysis and found a similar relationship between the irreversible loss rate and the plasma concentration for the single shot and continuous infusion techniques, however they did find that with the single shot technique there were small experimental errors in the specific radioactivity turnover after six hours. These errors were probably due to the low concentrations of the tracer present after this point and could result in relatively large errors in the estimate of the extrapolation value for time zero. Therefore Cocimano and Leng (1967) concluded that the continuous infusion technique would give a better approximation of the true values for the irreversible loss rate.

In order for these techniques to be used successfully the animals should be in a metabolically steady state situation, i.e. fed at least once an hour (Conrad, 1972), in which case the pool sizes will remain constant therefore the inflow rate will equal the outflow rate (Nolan and Leng, 1974). These techniques also assume that the

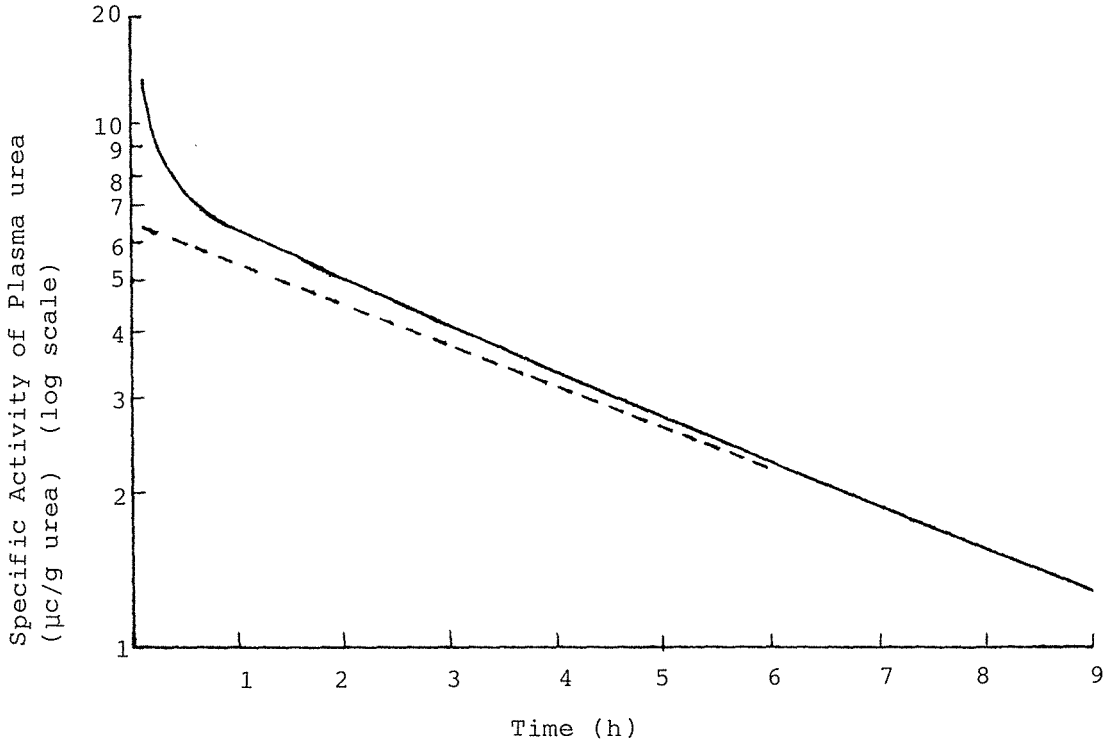


Figure 1.3 Specific Activity of Plasma Urea Over Time After a Single Injection of [^{14}C] urea (-- linear extrapolation back to zero).

(Cocimano and Leng, 1967)

Equation 5

(a) Pool size (g) = $\frac{\text{Injected dose } (\mu c)}{SR_0 \quad (\mu \text{ c/g urea})}$

(b) IRL = $\frac{\text{Pool size}}{1.44t_{\frac{1}{2}}}$

where SR_0 is the specific activity at time zero

$t_{\frac{1}{2}}$ is the time for half the urea to be lost from the urea pool

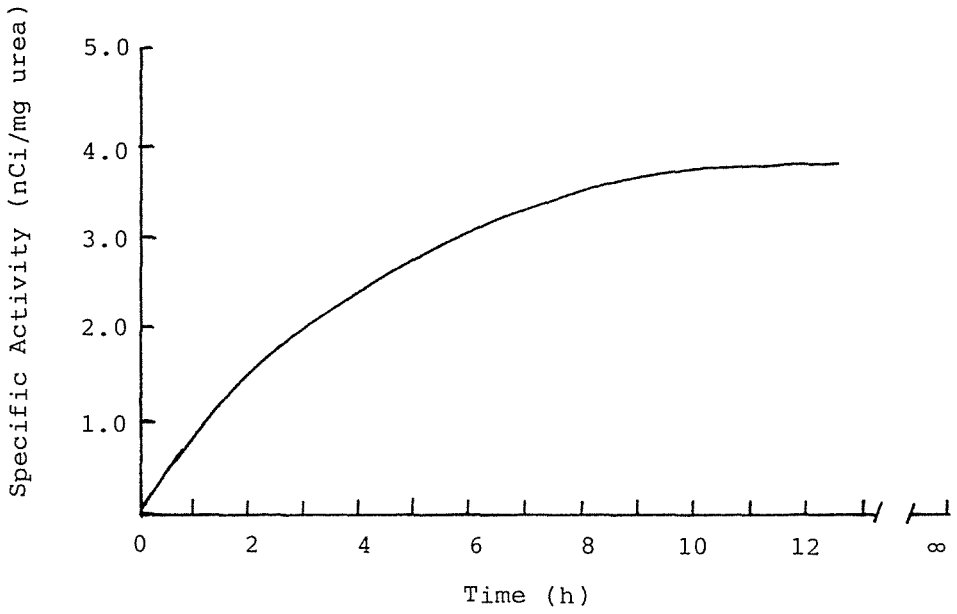


Figure 1.4 Specific Activity of Plasma Urea Over Time for a COntinuous Intravenous Infusion of [^{14}C] urea In Sheep

(Nolan and Leng, 1972)

Equation 6

$$IRL = \frac{\text{Infusion Rate (DPM/min}}{\text{SA Plateau (DPM/mg)}}$$

metabolic breakdown of the compound is at random (Conrad, 1972). The other assumption which is that the tracer is rapidly mixed throughout the pool, is usually valid when the plasma urea pool is used as the primary pool, but it is a potential source of error when the rumen or the caecum pools are being studied (Nolan and Leng, 1974).

If the measurements are taken over a relatively short time period (for example 0-1600 minutes as in the experiment carried out by Nolan and Stachiw, 1979) the results only apply to compartments with relatively high turnover rates. Other compartments, like muscle protein, act as short term sinks over short periods of time and will result in small over estimates of the irreversible losses of any tracer from the primary pool. The isotopes commonly used include the radioactive isotope ^{14}C and the stable isotope ^{15}N. The ^{14}C is involved in marking urea-C which is an end product of metabolism, while ^{15}N is used to mark the nitrogen which may be recycled through the various nitrogen pools in the body (example see Table 1.10).

Tracer techniques are often used in conjunction with compartmental analysis techniques to interpret the nitrogen kinetics. The isotope is placed into one of the primary pools - usually the blood urea pool or the rumen ammonia pool or the caecal fluid ammonia pool, and the level of activity is monitored in the primary pool and any of the associated secondary pools. The results of several of these experiments are grouped together to build up a model of inter-related metabolic events.

Table 1.10 The IRL, Flux Rate and Recycling Rate Measured Using Radioisotope Techniques

Pool Sampled	Total Flux Rate (g/d)	IRL (g/d)	Recycling Rate(g/d)	Size of Pool (g)
Rumen NH_3-N	15.0	10.7	4.3	1.46
Plasma Urea-N	15.1	13.5[a]	1.6	3.64
Caecal NH_3-N	4.8	4.2	0.6	0.14
Plasma Urea-C	16.1	15.7[b]	0.4[c]	3.50

[a] The rate of IRL of urea-N is a measure of the rate of urea synthesis from N which has not already been recycled during the process of urea degradation

[b] The rate of IRL of urea-C is a measure of the total urea synthesis in the body

[c] Only small quantities of urea-C are recycled

(Nolan et al, 1976)

The other area of urea disposal by the animal is urea excretion by the kidney.

1.4.2.2.3 Urea Excretion By The Kidney

Urea which is the major end product of vertebrate protein metabolism, is a toxic waste product that is removed by the kidneys in most vertebrate animals. An isotonic solution containing low molecular weight solutes and water from the blood is filtered through the capillary and the Bowmans capsule walls into the capsule lumen. As this filtrate moves slowly through the tubule physiologically important substances are reabsorbed into the blood, while the hydrogen and potassium ions, and any foreign compounds are secreted in the tubular urine (Hoar, 1982). The various nitrogenous end-products react differently in the tubule. Creatinine is not reabsorbed in the proximal convulated tubule and therefore its concentration increases with the reabsorption of water (Gans and Mercer, 1977) and thus can be used as index of kidney function.

Urea diffuses across most biological membranes and therefore the reabsorption of urea is probably by passive diffusion. Over the normal range of urine flow rates 40-60% of the filtered urea is excreted and the rate increases with flow (Sullivan, 1974), while the plasma urea concentration has little effect.

The level of nutrition appears to influence the movements of urea in the kidney, although most of the work done so far has only looked at the effects of protein intake (Roch-Ramel and Peters, 1981). The amount of urea produced and therefore the amount of urea excreted in the adult animal is approximately proportional to the

nitrogen intake (Schmidt-Nielsen, 1958). In many species, including sheep, it has been observed that a drop in the nitrogen intake results in a decrease in the faecal endogenous urea excretion if the urine flow remains constant. The size of the change ranges from a slight increase in the beaver to a dramatic increase in the ruminant (Roch-Ramel and Peters, 1981).

All of the changes that appear to have occurred in the renal handling of urea in animals on a low dietary protein diet could be due to a change in the urea permeability of the terminal nephron. These could be brought about by changes in the adrenocortical secretions, as in rats it has been shown that dexamethasone will standardise urea excretion in protein deficient animals. Some workers have suggested that some of the changes indicate the presence of urea and methyl urea carriers. In some tissues experiments have demonstrated that urea and water cross the cell membranes by slightly different pathways(Roch-Ramel and Peters, 1981). However more evidence is needed especially with respect as to whether this occurs in the kidney and its importance to the animal.

1.4.3 SUMMARY

In the rumen degradable nitrogen is fermented to ammonia by the rumen microorganisms. Once the microbial requirements for nitrogen have been met there is a reduction in the efficiency of nitrogen utilisation by the animal due to the loss of ammonia from the rumen.

In a low quality diet there is less nitrogen available in the feed so these losses are usually small, however on a high quality diet these losses are larger and can result in a significant reduction in the efficiency of nitrogen utilisation, especially on fresh forages.

One of the ways excess ammonia can be lost from the rumen is by being absorbed across the rumen wall into the plasma. In the liver this ammonia is converted to urea, and then it may be recycled back into the digestive tract or excreted in the urine. This recycled urea can provide an important source of nitrogen in an animal on a low quality diet, while in animals on a diet where nitrogen is not limiting, more urea is excreted and therefore lost from the system. The section of the digestive tract to which the recycled urea is returned in an animal on a low level of nitrogen intake influences the usefulness of this recycling, i.e. if the urea is recycled in the saliva or to the rumen it is more likely to be utilised than if it is recycled to the hind gut.

The amount of ammonia produced in the rumen by microbial breakdown is affected by the rate of passage of digesta down the tract. In a high quality diet an increase in the level of intake is likely to increase the rate of passage and therefore reduce the time available for protein degradation.

The development of isotope tracer technology has allowed scientists to study these changes in detail.

1.5 CONCLUSION

Physiological differences have been found between the different selection lines in sheep selected for fleece weight and the control line both in the Australian Merino and the New Zealand Romney. Little work has been done on the changes in nitrogen metabolism especially with regard to the irreversible loss of urea from the plasma. This is particularly important in the New Zealand Romney where in several trials it has been found that the fleece weight line has a lower plasma urea concentration than the control line.

Previous work has found that the magnitude of the differences in the gross efficiency of the conversion of feed to wool increases with increasing nutrition level. As well as this the loss of ammonia across the rumen wall is lower on a low nitrogen intake so that majority of the urea produced in the liver should originate from tissue turnover, whilst in animals on a high nitrogen intake more ammonia is absorbed across the rumen wall and converted to urea.

For these three reasons a medium quality hay and a high quality hay were used in this study carried out on the NZ Romney selection lines.

The aims of this study were

a. to look at the differences in the nitrogen digestibility and balance between the Fwt animals and the C animals fed on a

medium quality meadow hay and a higher quality lucerne chaff diet

b. to confirm that the plasma urea concentration is lower in the Fwt selected Romney line than the C line when fed dried forage feeds under steady state conditions (once hourly feeding)

c. to determine if the lower plasma urea concentration in the Fwt line is due to a lower rate of urea production in the body, here defined as the irreversible loss rate(IRL) from the plasma and measured using ^{14}C urea isotope

d. to measure urea disposal in quantitative terms in the two selected lines, here defined as the urinary urea excretion and recycling from the plasma to the gut

e. to determine whether selection has altered rumen metabolism by studying the rumen mean retention time, weight of rumen contents, ammonia concentration and the volatile fatty acid composition. It is normally assumed that selection for increased fleece weight has not affected the digestion process (Williams, 1979; McClelland et al, 1987b) and only affects the partitioning of absorbed nutrients but further study is required in the NZ Romney selection lines to confirm this in this situation.

CHAPTER 2

INTRODUCTION

CHAPTER TWO INTRODUCTION

Maximising the amount and efficiency of wool production is of major concern to the NZ farmer, and genetic selection for greasy fleece weight has produced major increases in wool production in some flocks. In order to gain an understanding of the mechanisms through which genetic selection has improved the efficiency of wool growth, it is useful to compare the digestion and utilisation of nutrients in sheep selected for high wool production and randomly selected sheep.

The present study was carried out in the fleece weight selected (Fwt) and control (C) lines of Romney sheep at Massey University, NZ. The management and selection processes have been described in detail by Blair et al, (1984,1985). The flocks were closed in 1958 after three years of preliminary selection from the NZ national flock. Each flock contains 70-80 mixed aged breeding ewes which are culled at five and a half years of age. Each year these ewes are mated to four one and a half year old rams selected from within the same flocks. Lambs are born during August/September and shorn, as lambs, in late November/early December of that same year. Selection is carried out on the basis of the greasy fleece weight at hogget shearing in September the following year. Both flocks are grazed together on high quality ryegrass/white clover pasture and managed under conditions that are as close to commercial conditions in New Zealand as possible. This selection has resulted in a one kilogram increase in hogget greasy fleece weight by 1987.

In the physiological studies carried out to date comparing the Fwt and C animals, the most consistent effect observed has been the lower plasma urea concentration in the Fwt animals (Clark, 1987; McClelland et al, 1987b and McCutcheon et al, 1987). No differences have been observed between the Fwt and the C sheep in voluntary feed intake (VFI), and the results from studies on the apparent dry matter digestibility have been inconsistent (McClelland et al, 1986; McClelland et al, 1987b).

The objectives of this present study were to confirm that the plasma urea concentration is lower in the Fwt than the C animals when fed diets of chaffed meadow hay and lucerne under controlled and steady state conditions, and to determine whether the lower plasma urea concentration is due to a difference in urea production (here defined as the irreversible loss; IRL) or to a different pattern of urea disposal. The other main objective was to try to resolve the conflicting results for the differences in the DM digestibility by studying the VFI, DM and N digestibilities, and N retention in sheep fed both diets. Rumen function was also measured in the sheep fed on the lucerne chaff diet, with an emphasis on the mean retention time (MRT) of particulate matter, as a possible way of explaining any differences found between the Fwt and the C animals in the voluntary feed intake and apparent digestibility.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER THREE MATERIALS AND METHODS

3.1 GENERAL

The experiment commenced in mid-October and carried through to mid December 1986. During the trial the animals were housed in individual metabolism crates at the Biotechnology Division of DSIR, in Palmerston North.

There was a preliminary adaptation period of seven days during which lucerne chaff was fed, prior to the start of the experiment, to allow the animals to adjust to being inside in crates. The experiment itself consisted of two almost identical sections-the first section was carried out with meadow hay as the diet (Expt. A), and this was followed by an experiment in which lucerne chaff was the diet (Expt. B). At the end of Expt B the animals were slaughtered to measure the rumen mean retention time of particulate matter and the ruminal ammonia pool size. A twenty four hour fasted liveweight was recorded at the beginning and end of each experiment.

3.2 ANIMALS

Eight yearling rams from the Massey Romney Fleece weight selection line (Fwt) and eight from the Control line (C) were randomly selected after the 1986 September hogget shearing. Prior to

the start of the experiment the Fwt animals weighed 44.89 (±1.64(SE))kg as compared to the C animals mean liveweight of 42.05(±1.24)kg. The hogget fleece weights were 3.86(±0.09) and 3.09 (±0.14)kg for the Fwt and C animals respectively for a ten month fleece.

These animals were crutched, drenched with Ivomec (Ivermectin, Division Merck Sharp and Dohme (NZ) Ltd) and treated with Wipeout pour on dip (Deltamethrin, Coopers Animal Health NZ Ltd) before being placed in the metabolism crates. The trial was carried out with the animals being housed in two rooms, with four controls and four fleece weight animals in each room.

The animals were cannulated in both jugular veins two and a half days prior to the start of each ^{14}C-urea infusion and the cannulae were removed at the end of each infusion. Each animal was clipped around the cannulation area and a 14 gauge needle inserted into the vein. The cannulation tube (medical grade polyethylene tubing with an internal diameter of 1.00mm and an external diameter of 1.5mm; Dural Plastics, Sydney, Australia) was then threaded down the needle into the vein. The needle was removed and the tube was then glued and bandaged into position. The tubes were flushed with heparinised saline (100IU heparin/ml) twice daily between the cannulation and the start of the ^{14}C-urea infusion to reduce the risk of the tubes becoming sealed with fibrin.

At the end of Experiment A one of the C animals had to be removed from the trial due to ill health.

3.3 DIET

The meadow hay was a medium quality hay and the lucerne was a higher quality hay (Table 3.1). Both feeds were chopped into lengths less than 10cm long, prior to feeding and while the animals were on the lucerne chaff mineralised salt blocks were continuously available.

Table 3.1 The Composition of the Feed on Offer

	Meadow Hay	Lucerne
Organic Matter(OM) (g/kgDM)	900.0	920.5
Nitrogen (N) (g/kgDM)	21.07	32.04
Energy(MJ/kgDM)	17.77	19.19
Neutral Detergent Fibre (NDF) (g/kgDM)	704.7	524.4
Acid Detergent Fibre (ADF) (g/kgDM)	417.8	342.2
Hemicellulose (g/kgDM)	287.0	182.2
Lignin (g/kgDM)	66.9	88.6

3.4 EXPERIMENTAL DESIGN

3.4.1 Feed Adaptation Period

After the preliminary period the feed was changed from lucerne chaff to chaffed meadow hay for Experiment A (30 days) and then back to lucerne chaff for Expt B (30 days). During the first two weeks of each experiment the animals were fed ad-libitum to establish the feeding level for the digestibility trial. The voluntary intake data from the last four days of the adaptation period was used to compare the voluntary intakes of the two genotypes.

3.4.2 Digestibility Trial

The amount of feed on offer was reduced to the level of the animal with the lowest dry matter intake (DMI)/kgW$^{0.75}$/d at the end of the adaption period, to ensure that the results were not confounded by differences in voluntary intake. In Experiment A this feeding level was set at 37.5gDM/kgW$^{0.75}$/d on the meadow hay and in Experiment B the level was 89.8gDM/kgW$^{0.75}$/d on lucerne chaff.

Following the 14 day adaptation period, measurements were taken over the next ten days for the digestibility of dry matter, organic matter, nitrogen and energy on both diets, and in addition, for NDF, ADF and lignin on the lucerne chaff diet. The feed was placed on belt feeders which delivered one twenty-fourth of the day's ration every hour. Duplicate feed subsamples were taken each day for dry

matter and two 200 gram samples were taken daily, pooled and stored for chemical analysis.

All the feed residues were collected daily and stored for later analysis. The feed left in the feed bin and the feed that had fallen through the crate were kept separate and pooled for each animal over the digestibility period. As there appeared to be a physical difference in the bin refusals between individuals no refusals were bulked across animals.

Over the digestibility period the animals were fitted with harnesses and faecal collection bags. The faeces were collected daily, weighed and bulked over time for each animal and stored at -20°C. At the end of the digestibility period they were mixed and subsampled for analysis.

The urine was collected in buckets containing sufficient 50% sulphuric acid to keep the pH below 3 -this was 20mls on the meadow hay diet and 100mls on the lucerne chaff diet. The weight of urine produced each day was recorded and a 20% subsample stored at -20°C. During the digestibility trial period the amount of drinking water consumed each day was also recorded.

3.4.3 Infusion Trial

The infusate was made up by diluting one mCi of ^{14}C-urea (50-60mCi/mMol; Amersham, England) in four litres of sterile saline

(9gNaCl/l), and infused at the rate of 30.9(±0.39)mls per hour (7.75 μCi/h) for fourteen hours using a peristaltic pump with planetary gears (Desaga, Heidelberg West Germ.). A sample of the infusate was taken before and after each set of infusions.

A blood sample was taken prior to the start of the ^{14}C labelled urea intravenous infusion at 800 hours to provide a measure of the background DPM. The first experimental blood sample on the first animal was taken ten hours after the start of the infusion (1800 hours) and the infusion ceased at 2215 hours on the same day. The animals were grouped in pairs with five minutes between each pair and with each pair being blood sampled every 40 minutes, over the period 10-14 hours after the start of the infusion.

A ten ml sample was taken from the opposite vein to the infusion using a syringe. The sample was then placed into a heparinised vacutainer and separated by centrifugation (1500g for 15mins). The plasma was then stored at -20°C until required for urea, creatinine and ^{14}C-urea analysis. Prior to each blood sample one to two mls of blood was withdrawn and discarded. After each blood sample the cannulation tubes were flushed with heparinised saline.

The urine was collected for forty eight hours after the start of the infusion, with an 80ml subsample being taken after 24 hours and a subsample after the second 24 hours. These two subsamples were then stored at -20°C.

Two days after the first group of eight animals (four Fwt and four C animals) were infused, the same procedure was carried out on the remaining animals.

3.4.4 Slaughter Procedures

Three days after the final fasted liveweight in Experiment B the animals were killed with an injection of Euthetal (Sodium Pentabarbitone, May and Baker, NZ.). Prior to slaughter a blood sample was taken for urea analysis. The animals were fed manually on the hour to ensure that the animals were as close to steady state as possible and slaughtered on the half hour. The abdominal cavity was opened up and the rumen was tied off at the oesophagous and the omasum, and then removed. The weight of the total rumen contents was recorded and subsamples taken for

- dry matter
- freeze drying for organic matter and carbohydrate fibre analysis
- ammonia concentration
- volatile fatty acid (VFA) analysis

The subsamples for ammonia and the volatile fatty acid analysis were filtered through three layers of muslin cloth. The VFA subsample was deproteinised by precipitating with a metaphosphoric acid:formic acid mixture, separated by centrifugation (1500g for 15 mins)and stored at -20°C. The ammonia subsample was deproteinised using 2N sulphuric acid saturated with magnesium sulphate. This was

then left to stand for ten minutes, centrifuged (1500g for 15 mins) and the supernatant stored at -20°C until required.

3.5 LABORATORY METHODS

The dry matter samples were oven dried at 105°C until dry (as determined by a constant weight). The feed, faeces and digesta samples for organic matter, nitrogen, energy and carbohydrate analyses were freeze dried, ground through a 1mm sieve and stored until required. The organic matter was determined by ashing the samples at 500°C for sixteen hours.

The total nitrogen content for all samples was determined by the Kjeldhal procedure, using a Kjeltec Auto 1030 Analyzer (Tecator, Sweden). The samples were digested in concentrated sulphuric acid using a selenium catalyst, at 550°C to transform all the nitrogenous products into ammonium sulphate. The ammonia was then automatically distilled off and titrated against standardised hydrochloric acid. The ammonia content of the deproteinised rumen fluid was analysed on the same machine but without the digestion in concentrated sulphuric acid step. Gross energy content was calculated using bomb calorimetry (Gallenkamp Autobomb; UK.). The fibre determination was done using Van Soest's Neutral Detergent and Acid Detergent method (Robertson and Van Soest, 1981) with hemicellulose and lignin being determined by difference. Urea was analysed on a Technicon Autoanalyzer using the method of Marsh et al (1965). Creatinine was

analyzed using the method of Chasson et al's (1961) on the autoanalyzer. Volatile fatty acids in the rumen digesta were analyzed by gas chromatography (column length 1.85m, temp 195°C, packing 20% FFAP on 80-100 mesh Chromosorb W; model Shimadzu Gas Chromatoph Gz-8A PF (Japan)). The plasma and urine samples were deproteinised with barium hydroxide and zinc sulphate (see appendix A) prior to counting on a liquid scintillation counter (model LS 3801, Beckman Instruments Incorp.; U.S.A.). The quench curve was set up using chloroform to quench. This curve was then evaluated by varying the ratios of the different reagents used at each stage of the sample preparation (Appendix A).

3.6 CALCULATION OF THE DATA

3.6.1 Calculation of the FDPR, FDR, FOR and the True Mean Retention Time (MRT)

The Fractional Disappearance Rate (FDPR), Fractional Degradation Rate (FDR) and the Fractional Outflow Rate (FOR) of lignin were calculated as:

$$\text{FDPR } (h^{-1}) = \frac{\text{Lignin Intake (g/h)}}{\text{Rumen Lignin Pool Size (g)}}$$

$$\text{FOR } (h^{-1}) = \frac{\text{Faecal Output (g/h)}}{\text{Rumen Lignin Pool Size (g)}}$$

where faecal output of lignin is assumed to equal abomasal outflow

As FDPR = FDR + FOR ; FDR was calculated by the difference.

True Mean Retention Time (MRT) was defined as the mean time (h) that undegraded particulate matter spent in the rumen (Faichney, 1980) and is therefore exposed to microbial attack. It is the reciprocal of the FOR and was calculated using lignin as a marker.

3.6.2 Kidney Function

The plasma clearance rate expresses the degree to which substances are removed from the plasma by the kidney and are excreted in the urine, for example urea or creatinine clearance. To measure kidney function the creatinine clearance rate is commonly used (Frandson, 1979).

$$\text{Clearance Rate} = \frac{\text{Rate of Urine Prod (l/d) x Urine Conc (mg/l)}}{\text{Plasma Conc (mg/l)}}$$

3.6.3 The Irreversible Loss of Plasma Urea

The irreversible loss rate (IRL) of plasma urea provides a measure of the rate at which urea is being removed from the plasma and consists of the losses in the urine and the urea that is recycled back to the digestive tract. Under steady state conditions this is equal to the entry rate or production of urea

$$\text{IRL} = \frac{\text{Infusion Rate of } ^{14}\text{C-urea } (\mu\text{Ci/h})}{\text{Plasma Plateau Specific Activity } (\mu\text{Ci/mg urea})}$$

Recycling Of Urea To The Tract = IRL(gN/d) - Urinary Urea Prod (gN/d)

Cocimano and Leng, (1967)

All values were corrected for background before the activity levels were calculated.

3.7 STATISTICAL METHODS

A basic model was tested using (CO)ANOVA using the SAS (Primos Version, Ref 5.03 Model 9955, SAS Institute Inc., U.S.A) statistical package.

Model $Y_{ijk} = \mu + G_i + R_j + e_{ijk}$

where μ is the overall mean

G_i is the effect of the ith genotype

R_j is the effect of the jth room

e_{ijk} is the random residual effect unique to Y_{ijk}

The model was corrected for any room effects where there had a significant effect. All daily feed intakes, water intakes and urinary outputs were tested for a time x genotype interaction using a repeated measures analysis. This interaction was only significant in the lucerne voluntary feed intake data, although the time effect was significantly different in all the models.

CHAPTER 4

RESULTS

CHAPTER FOUR RESULTS

4.1 LIVEWEIGHT

The Fwt animals were always at least two kilograms heavier than the control animals (Table 4.1) in spite of losing more weight on the meadow hay diet (Table 4.2). However none of these differences were significant (P>0.05).

Table 4.1 The Mean Fasted Liveweights(kg) For Each Trial Period

	GENOTYPE					
Diet	Control (n=7)		Fleece Weight (n=8)			
	Mean	SE	Mean	SE	Diff	Sign
Meadow Hay	40.1	1.19	42.0	1.42	1.90	NS
Lucerne	39.6	1.25	41.9	1.52	2.10	NS

Table 4.2 The Liveweight Gain(g/d) Over the Four Week Experimental Periods for Both Genotypes Fed Each Diet

Diet	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Meadow Hay	-190	13	-221	17	0.17
Lucerne	193	11	211	15	NS

4.2 VOLUNTARY INTAKE

On the meadow hay diet there was no difference in the voluntary feed intakes between the two genotypes over the last four days of the adaptation period and the overall values were low. But on the lucerne chaff the voluntary feed intake overall was high and the VFI of the Fwt animals was higher than the C animals (Table 4.3). A repeated measures analysis showed that there was a significant time effect ($P<0.01$) and that there was an interaction between the time and the genotypic effects ($P<0.05$). The reason for this interaction appears to be because the mean voluntary feed intake of the C animals appeared to have stabilised, whilst the mean intake of the Fwt

animals appeared to be still increasing (as a limit was placed on three Fwt animals on day 3 and 4).

Table 4.3 The Mean Daily Voluntary Feed Intakes Of Dry Matter ($gDM/kgW^{0.75}$) Of Each Genotype Over The Last Four Days Of The Adaptation Period

	GENOTYPE				
Diet	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Meadow Hay[a]	44.4	1.57	41.9	2.46	NS
Lucerne[a]	94.1	3.83	101.8	2.46	
Day 1	95.5	3.45	102.9	2.07	0.04
Day 2	84.6	7.18	96.6	4.69	0.08
Day 3	101.9	5.25	102.2	3.55	NS
Day 4	95.5	5.21	105.4	3.21	0.01

[a] mean over the four days

4.3 DIGESTIBILITY TRIAL RESULTS

During the digestibility periods there were no differences in the metabolic intakes on either diet between the two genotypes, however on the lucerne chaff diet the Fwt animals had a significantly lower digestible dry matter intake (Table 4.4).

Table 4.4 The Dry Matter and Digestible Dry Matter Intakes on Both Diets During the Digestibility Periods ($g/kgW^{0.75}/d$).

	GENOTYPE				
Diet	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Meadow Hay					
DMI	36.2	0.79	36.2	1.28	NS
DDMI	20.1	1.15	19.9	0.73	NS
Lucerne					
DMI	87.1	2.39	86.0	0.66	NS
DDMI	57.3	3.19	48.3	1.72	0.05

4.3.1 Digestibilities

On the meadow hay diet there were no differences in the digestibility of any feed component between the genotypes (P>0.05) (Table 4.5). On the lucerne chaff the Fwt animals had lower digestibility values for dry matter (P<0.05), organic matter (P<0.05), nitrogen (P<0.10) (Table 4.5).

Table 4.5 A Comparison Of the Apparent Digestibilities (%) of the Feed Components by the Different Genotypes During the Digestibility Periods

	GENOTYPE				
Diet	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Meadow Hay					
DM	55.3	2.41	54.9	1.12	NS
OM	57.4	2.30	56.9	0.91	NS
Total N	60.3	1.91	60.8	1.59	NS
Lucerne					
DM	61.3	1.22	56.1	1.82	0.02
OM	62.8	1.39	58.6	1.68	0.04
Total N	68.6	1.11	65.4	2.08	0.10
NDR	55.1	1.77	51.8	2.32	NS
ADR	47.2	1.55	44.4	2.79	NS
Hemi	68.7	3.24	64.7	1.96	NS

4.3.2 Nitrogen Balance Data

There was no difference in the nitrogen retention between the two genotypes when fed either diet, but the values in the Fwt animals covered a wider range (Table 4.6). However the nitrogen retentions on the two diets were very different, with the animals losing nitrogen on the meadow hay diet and gaining it on the lucerne diet.

Table 4.6 A Comparison of the Nitrogen Balances of the Two Genotypes On the Two Diets During the Digestibility Periods

Diet	GENOTYPE Control (n=7) Mean	SE	Fleece Weight (n=8) Mean	SE	Sign
Meadow Hay					
NI (g/d)	11.44	0.44	12.04	0.48	NS
NI (g/kgW$^{0.75}$/d)	0.72	0.02	0.73	0.02	NS
Faecal N (g/kgW$^{0.75}$/d)	0.28	0.01	0.29	0.01	NS
Urinary N (g/kgW$^{0.75}$/d)	0.52	0.03	0.56	0.04	NS
Apparent N dig %	60.34	1.91	60.80	1.59	NS
Dig NI (g/kgW$^{0.75}$/d)	0.44	0.02	0.44	0.02	NS
N Ret (g/kgW$^{0.75}$/d)	-0.08	0.02	-0.12	0.03	NS
Lucerne					
NI (g/d)	43.04	0.94	44.53	1.76	NS
NI (g/kgW$^{0.75}$/d)	2.73	0.03	2.70	0.04	NS
Faecal N (g/kgW$^{0.75}$/d)	0.86	0.03	0.93	0.05	NS
Urinary N (g/kgW$^{0.75}$/d)	1.56	0.04	1.47	0.09	NS
Apparent Dig N %	68.59	1.11	65.39	2.08	0.10
Dig NI (g/kgW$^{0.75}$/d)	0.69	0.01	0.65	0.02	0.10
N Ret (g/kgW$^{0.75}$/d)	0.31	0.03	0.30	0.12	NS

4.3.3 Fluid Balance

When fed either diet, voluntary intake of supplementary water and total water were higher for the Fwt animals than for the control sheep ($P<0.05$; Table 4.7 and 4.8). However, when expressed as $g/kgW^{0.75}/d$, although the values still tended to be higher for the Fwt animals than the controls, the difference did not reach significance ($P>0.05$). The Fwt animals also tended to excrete more urine per day but the difference between the genotypes only attained significance when uncorrected for liveweight, when meadow hay was fed ($P<0.06$). However there was a strong correlation between water intake and urinary output on both diets ($R^2=0.95$, $P<0.0001$ on the meadow hay; and $R^2=0.92$, $P<0.0001$ on the lucerne).

There were no differences between genotypes in the total excretion of nitrogen, urea or creatinine on either diet.

Table 4.7 A Comparison Between the Two Genotypes For Differences In The Daily Water Intake And Urinary Output On The Meadow Hay Diet During The Digestibility Periods

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Supplementary Water Intake (g/day)	1296.9	73.85	1699.9	96.04	0.02
Total Water Intake (g/d)	1428.8	85.95	1786.9	94.10	0.06
Total Water Intake ($g/kgW^{0.75}/d$)	90.9	6.20	109.6	7.80	0.07
Urine Weight (g/day)	840.4	73.00	1102.4	113.40	0.06
Urine Weight ($g/kgW^{0.75}/d$)	55.2	5.55	68.1	8.45	NS
Urinary N Output ($g/kgW^{0.75}/d$)	0.52	0.03	0.56	0.04	NS
Urinary Urea ($g/kgW^{0.75}/d$)	0.48	0.04	0.48	0.05	NS
Urinary Creatinine ($g/kgW^{0.75}/d$)	0.05	0.01	0.06	0.01	NS

Table 4.8 A Comparison Between the Two Genotypes For Differences In The Daily Water Intake And Urinary Output On The Lucerne Chaff Diet During The Digestibility Periods

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Supplementary Water Intake (g/day)	4369.7	181.29	5189.7	274.21	0.04
Total Water Intake (g/d)	4597.5	181.34	5429.3	274.24	0.05
Total Intake ($g/kgW^{0.75}/d$)	315.2	23.35	332.7	20.98	NS
Urine Weight (g/day)	2013.2	182.00	2418.0	98.10	NS
Urine Weight ($g/kgW^{0.75}/d$)	139.4	19.04	149.6	20.53	NS
Urinary N Output ($g/kgW^{0.75}/d$)	1.56	0.04	1.47	0.87	NS
Urinary Urea ($g/kgW^{0.75}/d$)	1.52	0.83	1.39	0.88	NS
Urinary Creatinine ($g/kgW^{0.75}/d$)	0.09	0.01	0.10	0.01	NS

4.3.4 Lignin Digestion and Kinetics

There was no difference ($P>0.05$) in the lignin intake on the lucerne diet between the two genotypes. However the Fwt animals had a lower apparent lignin digestibility than the C animals ($P<0.05$). There was no difference in the ruminal pool size of lignin or in the Fractional Disappearance Rate (FDPR) between the Fwt and the control animals ($P>0.05$). However in the Fwt animals the Fractional Degradation Rate (FDR) was lower than the controls and the Fractional Outflow Rate (FOR) was higher (Table 4.9). The Fwt animals had a significantly lower ($P<0.05$) rumen mean retention time (MRT) of lignin than the control animals.

Table 4.9 A Comparison of the MRT, FDPR, FOR and FDR of Lignin Between the Two Genotypes, in Animals Fed the Lucerne Chaff Diet

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Intake (g/kgW$^{0.75}$/d)	7.5	0.14	7.6	0.62	NS
Faecal (g/kgW$^{0.75}$/d)	5.7	0.15	6.7	0.37	0.01
App Digestibility %	23.1	2.42	11.3	5.24	0.05+
Ruminal Pool (g)	140.8	6.61	154.5	5.33	NS
FDPR (%/h)	3.35	0.108	3.38	0.380	NS
FOR (%/h)	2.58	0.132	3.00	0.195	0.03
FDR (%/h)	0.77	0.081	0.38	0.172	0.03
FDR/FOR	0.31	0.041	0.15	0.066	0.03
MRT (h)	39.4	2.06	34.3	2.32	0.03

+ significance when water intake included in the model 0.02

4.4 RUMEN POOL SIZES IN SHEEP FED ON LUCERNE CHAFF

When the effect of the time of slaughter was removed the Fwt animals had significantly more total material and fluid present in the rumen ($P<0.05$), but these effects were removed by correcting for the metabolic liveweight of the animal. None of the other components of rumen fill were significantly different ($P>0.05$) between the two genotypes (Table 4.10).

There was no difference in the total VFA pool size or in the individual VFA pool sizes ($P>0.05$) but there was a difference in the relative molar proportions of acetate and propionate, with the fleece weight line producing a higher proportion of acetate and a lower proportion of propionate (Table 4.11).

Table 4.10 A Comparison Of The Ruminal Pool Sizes Between The Two Genotypes On A Lucerne Chaff Diet

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Wet Weight (kg)	7.74	0.30	8.44	0.20	0.02
Wet Weight ($kg/kgW^{0.75}$)	0.49	0.022	0.52	0.018	NS
Fluid Pool (kg)	6.67	0.25	7.32	0.19	0.03
Fluid Pool ($g/kgW^{0.75}$)	42.48	1.929	44.78	1.682	NS
Dry Matter Pool(kg)	1.06	0.15	1.12	0.41	NS
Organic Matter Pool (kg)	0.96	0.048	1.01	0.036	NS
Total N Pool(g)	38.4	2.27	39.9	1.54	NS
NH_3 Conc (mgN/l)	230	10	200	20	0.13
NH_3 Pool (gN)	1.54	0.107	1.44	0.131	NS
NAN Pool (gN)	36.9	1.85	38.6	1.29	NS

Table 4.11 Molar Proportions Of The Individual VFAs In Ruminal Fluid Obtained At Slaughter In Sheep Fed Lucerne Chaff

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
VFA	Mean	SE	Mean	SE	Sign
Total VFA (me/100ml)	27.4	2.58	35.8	12.57	NS
Acetate	64.0	1.12	68.6	1.72	0.08
Propionate	27.7	1.35	22.9	1.60	0.08
Butrate	7.0	0.57	7.2	0.52	NS
Valeric	1.4	0.14	1.3	0.10	NS
Acet/Prop	2.4	0.19	3.1	0.27	0.07
But/Val	5.1	0.27	5.7	0.74	NS

4.5 UREA METABOLISM

There was no difference in the mean urine volumes between the genotypes on either diet over the twenty four hours of the infusion ($P>0.05$). The plasma urea concentration was 17% higher on the meadow hay diet ($P<0.05$) and 12% higher on the lucerne chaff diet ($P<0.01$) in the fleece weight animals (Table 4.12 and 4.13). There was no

difference in the Irreversible Loss Rate (IRL) of urea from the plasma or the urinary urea excretion or in the amount of urea recycled to the digestive tract, between the genotypes on either diet ($P>0.05$). The Fwt animals had a significantly higher urea clearance rate on both diets, but in the meadow hay this effect was removed when the values were corrected for liveweight differences (Table 4.12 and 4.13).

There was no difference in the plasma creatinine concentration or urinary excretions of creatinine between the Fwt and C sheep when fed on the lucerne chaff diet. However the plasma creatinine concentration was significantly higher in the control animals. The creatinine clearance rates were not significantly different on the lucerne chaff diet, but on the meadow hay diet creatinine clearance rates were greater in the Fwt animals than the C animals, both before ($P<0.05$) and after ($P<0.10$) correction for metabolic bodyweight (Table 4.14).

Table 4.12 Plasma Concentrations, IRL and Clearance Rates Of Urea Between The Two Genotypes, Measured During The ^{14}C-urea Infusion Period On The Meadow Hay Diet

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Plasma Urea Conc (mM)	5.6	0.24	4.7	0.36	0.04
Urine Urea Conc(g/l)	17.3	1.14	16.5	1.58	NS
Urea Clearance (ml/min)	32.0	3.38	42.2	3.88	0.07
Urea Clearance ($ml/kgW^{0.75}/min$)	2.3	0.22	2.8	0.28	NS
Urea IRL (gN/d)	14.2	0.41	13.9	0.92	NS
Urinary Urea Loss (gN/d)	8.0	0.66	8.4	0.71	NS
Urea Recycled to Gut (gN/d)	6.2	0.81	5.6	1.09	NS
% IRL Recycled	43.4	4.99	38.3	6.79	NS

Table 4.13 Plasma Concentrations, IRL and Clearance Rates Of Urea Between The Two Genotypes, Measured During The ^{14}C-urea Infusion Period On The Lucerne Chaff Diet

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
	Mean	SE	Mean	SE	Sign
Plasma Urea Conc (mM)	9.4	0.27	8.2	0.25	0.01
Urine Urea Conc (g/l)	23.6	1.97	22.3	2.02	NS
Urea Clearance (ml/min)	59.7	2.94	71.8	3.03	0.02
Urea Clearance ($ml/kgW^{0.75}/min$)	3.8	0.15	4.4	0.09	0.03
Urea IRL (gN/d)	36.1	1.11	35.3	1.28	NS
Urinary Urea Loss (gN/d)	23.6	1.97	22.3	2.02	NS
Urea Recycled To Gut (gN/d)	13.5	1.21	11.5	1.02	NS
% IRL Recycled To The Gut	37.9	2.38	32.4	2.26	NS

Table 4.14 Plasma Creatinine Concentrations and Clearance Rates in Sheep Fed Both Diets

	GENOTYPE				
	Control (n=7)		Fleece Weight (n=8)		
Diet	Mean	SE	Mean	SE	Sign
Meadow Hay					
Plasma Conc (mM)	0.083	0.003	0.073	0.003	0.03
Urine Conc (g/l)	0.73	0.027	0.75	0.056	NS
Urine Excretion (g/d)	0.76	0.050	0.85	0.063	NS
Creat Clearance (ml/min)	49.6	3.38	63.1	5.21	0.05
Creat Clearance ($ml/kgW^{0.75}/min$)	3.15	0.21	3.77	0.30	0.10
Creat Cl:Urea Cl	1.45	0.13	1.33	0.15	NS
Lucerne					
Plasma Conc (mM)	0.06	0.003	0.056	0.002	NS
Urine Conc (g/l)	0.52	0.042	0.53	0.041	NS
Urine Excretion (g/d)	1.18	0.060	1.23	0.091	NS
Clearance (ml/min)	109.6	12.08	115.1	6.04	NS
Clearance ($ml/kgW^{0.75}/min$)	6.96	0.620	6.99	0.26	NS
Creat Cl:Urea Cl	1.7	0.13	1.6	0.07	NS

CHAPTER 5

DISCUSSION

CHAPTER FIVE DISCUSSION

5.1 THE EFFECT OF SELECTION ON DIGESTIVE FUNCTION

5.1.1 Voluntary Feed Intake and Apparent Digestion

Any changes in digestive function need to result in an increase in the net amino acid-N absorption from the small intestine, to have a significant effect on wool production. Three possible ways that this could occur are through a change in the voluntary feed intake (VFI), digestibility, and nutrient partitioning. A number of experiments have looked at the first two factors in the Massey Romney selection lines in non-pregnant, non-lactating animals (Table 5.1).

On the lucerne chaff diet, in Experiment B, the Fwt animals had a higher voluntary feed intake and a lower digestibility than the C animals. In the trial by McClelland et al (1986) no difference in VFI was found but the dry matter digestibilities were significantly lower in the Fwt animals, on a fresh ryegrass white clover diet (Table 5.1). However these differences were not observed on the meadow hay diet, or in any of the other trials on a lucerne chaff diet in the NZ Romney selection lines (Table 5.1), or in any of the Australian selection line trials.

Table 5.1 The Effect of Genetic Selection For Wool Production on VFI (g/kgW$^{0.75}$/d) and the Apparent Dry Matter Digestibility (%) in Young Rams

		Digestibility			VFI	
Author	Diet	Level[b]	C	Fwt	C	Fwt
Expt A (present study)	MH	36	55	55	44	42
Expt B (present study)	lucerne	83	61	56[a]	94	102[a]
McClelland et al (1986)	RG/WC	71	63	60[a]	73	69
McClelland et al (1986)	lucerne	68	61	61	ND	
McCutcheon et al (1987)	lucerne	58	64	64	ND	

ND Not Determined

[a] $P<0.05$

[b] Feeding Level (gDM/kgW$^{0.75}$/d) at which the DM digestibility was measured

There are two likely reasons for the differences between Expt B and the other trials. These are the differences between diets (i.e. meadow hay vs lucerne chaff) and the differences in the feeding level which is the most likely option, as a very high level of VFI was achieved. It is possible that the high intakes achieved on the lucerne chaff diet in the present study could be due to carryover effects from feeding the lower quality meadow hay diet. McManus et al (1972) found that sheep fed below maintenance increased their intake in the compensatory growth period that followed, above those intakes of animals that had not undergone a restriction. It is unlikely that this would have differentially affected the two genotypes or biased the comparison.

During the digestibility period of Expt B the intakes were restricted below ad lib and it is possible that if the feeding level was higher or if the differences in VFI between the genotypes was allowed to be fully expressed, that the digestibility differences would be even larger.

5.1.2 Rumen Digestion and Disappearance rate

It is logical to suggest that the changes in apparent digestibility and VFI could be associated with a change in the rumen volume, degradation rate (FDR), passage rate (FOR), or a combination of these effects. The passage rate is defined as the rate that material passes out of the rumen into the duodenum, and the degradation rate as the rate at which material is digested and absorbed from the rumen.

5.1.2.1 Rumen Pool Size Of The Various Rumen Constituents

Rumen pool sizes were larger in the Fwt animals but only the wet weight and the fluid pool sizes were significantly different between the two lines of sheep, although this significance was removed by correcting for the metabolic liveweight. Therefore it would appear that the rumen pool sizes are not a major factor in the differences in the VFI and apparent digestibility observed between the Fwt and C animals in Experiment B.

5.1.2.2 The Ruminal Fractional Outflow Rates (FOR) and Degradation Rates (FDR) of Lignin

No differences ($P>0.05$) were recorded in the rumen pool size or FDPR of lignin. However using lignin as a particulate marker, there appeared to be a difference between the genotypes in how the material disappeared from the rumen on the lucerne chaff diet. In the Fwt animals there was a higher FOR and a correspondingly lower FDR of particulate matter, indicating that more material left the rumen undegraded in the Fwt animals than in the C animals. This appears to be the principle mechanism through which the VFI and the apparent digestibilities are likely to have been altered in Experiment B.

As there appears to be an increase in the FOR of particulate material from the rumen in the Fwt animals, then there is a possibility that the fluid FOR also increased. In this trial there was no direct measure of this, but Harrison et al (1976) showed that

on a semi-purified diet the molar proportion of propionate was inversely proportional to the rumen dilution rate (or FOR). On the lucerne chaff diet, the Fwt animals had a lower proportion of propionate and a higher proportion of acetate present in the rumen, indicating that there is a possibility that the fluid FOR could have been higher in the Fwt animals than for the C animals. The level of significance of the genotypic effect in the lignin digestibility model increased when the water intake was included in the model. It is possible that the increase in water intake observed in the Fwt animals could be a contributing to a greater rumen liquid FOR in these animals. This suggests that the higher the water intake, the higher the FOR and the lower the digestibility. A number of authors have observed that an increase in the rumen fluid FOR is associated with an increase in the amount of microbial protein leaving the rumen, both for in vivo studies (Harrison et al, 1975, 1976) and in vitro continuous culture studies (Isaacson et al, 1975)

5.1.2.3 The Possible Effect Of Altering Ruminal FOR And FDR On Protein Flow To The Duodenum

The increase in particulate matter FOR and the decrease in the FDR in the Fwt sheep could result in more dietary protein reaching the duodenum undegraded, assuming that it was associated with the same particles as lignin. This could result in less ammonia being produced in the rumen from dietary protein, so less would be absorbed across the rumen wall. This is supported by the fact that there is a positive correlation between the rumen ammonia concentration and the

plasma urea concentration (R^2 =0.5, P<0.06) suggesting that the higher the rumen ammonia concentration the higher the plasma urea concentration.

This would mean that more amino acids are available for absorption in the small intestine (undegraded dietary and microbial protein), and therefore it is possible that the amino acid uptake from the digestive tract maybe higher in the Fwt sheep. This would result in an increased supply of amino acids to the follicle and may partially explain the differences in wool production between the two genotypes. The possibility that this is occurring in the Fwt animals needs to be tested by direct experimentation, using animals cannulated at the rumen and duodenum, with digesta flow estimations using indigestible markers.

As none of these results have been found in the Australian Merino selection lines it is possible that there is a difference in the effect of selection on the two groups of animals. This maybe due to a difference in the initial genetic base, from which the selection was made, or to differences in the conditions under which the different lines were selected, especially with regards to diet quality. As the Australian selection was made on a poorer quality diet than the Romney selection, and as Table 4.1 shows the differences in dry matter digestibility and VFI only occurred in the NZ Romney at the relatively higher levels of nutrition.

When fed the lucerne chaff diet in the present study it appears that selection for increased wool production may have altered

digestive function in such a way that amino acid flow to the duodenum could have been increased.

5.2 THE EFFECTS OF SELECTION ON THE POST ABSORPTIVE METABOLISM

5.2.1 The Effects of Selection On Plasma Urea Concentration

There was a lower plasma urea concentration in the Fwt animals when fed both diets, with the difference being relatively constant over the two diets (meadow hay 1.0mM;lucerne chaff diet 1.1mM), in spite of different basal levels in the C animals (meadow hay 5.6 mM; lucerne chaff 9.4 mM). This effect is consistent with other experimental work involving the Massey Romney fleece weight selection flocks, although it has not been studied before in animals fed under steady state conditions on a low feeding level (Table 5.2).

Table 5.2 The Differences Found in Plasma Urea Concentration Between the Fwt and C Animals in Various Trials Involving the NZ Romney Selection Lines (mM; C-Fwt)

Author	Diet	Diff	Sign
Thomson (present study)	MH	1.0	0.04
Thomson (present study)	Luc	1.1	0.01
Clark (1987)	RG/WC	0.7	0.10
	PELM[a]	1.1	0.05
	Luc	0.9	0.01
	Luc[b]	0.5	NS
McCutcheon et al (1987)	Luc	0.8	0.01

[a] Diet was Protein Extracted Lucerne Meal supplemented with varying levels of casein

[b] Plus an intravenous methionine infusion (25.7mg/kgbodyweight/d)

5.2.2 The Effect of Selection on the Irreversible Loss and Disposal of Urea From the Plasma

Originally the lower plasma urea concentrations in the Fwt sheep suggested that the plasma urea IRL rate could have been lower in this

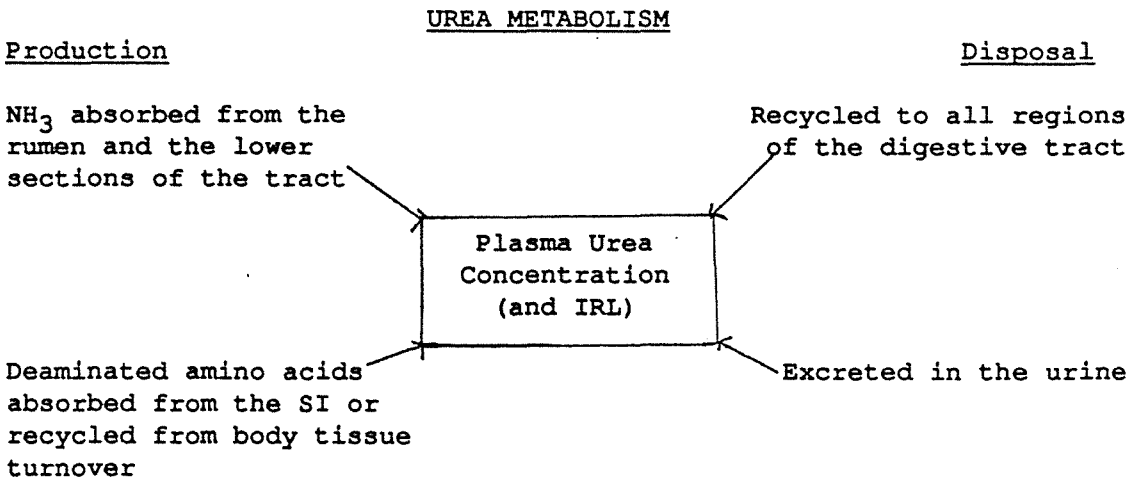
genotype and this could have conferred an advantage on the fleece weight animals, thereby accounting for their greater wool production. However in the present trial there were no differences in plasma urea IRL rate, urinary urea loss rate or the rate of urea recycling to the digestive tract between the Fwt and C animals, fed at the same dry matter intake per unit of metabolic weight, on either diet.

In sheep fed on the meadow hay diet there was a lower plasma urea IRL rate (14gN/d) as compared to sheep fed the lucerne chaff diet (36gN/d) and a higher proportion of this was recycled to the entire digestive tract (meadow hay 41%; lucerne chaff 35%). Cocimano and Leng (1967) found that 30% of urea was recycled on a similar nitrogen intake to the animals fed on the lucerne chaff diet. However the sheep fed a lower nitrogen intake ($0.9gN/kgW^{0.75}/d$) had a higher rate of recycling (56% IRL) than was observed in the sheep fed meadow hay in the present trial.

Under steady state conditions the urea disposal rate is equal to the production rate. The production rate is a combination of the production of urea from the deamination of amino acids released by the degradation component of protein turnover, deamination of absorbed amino acids, and from absorbed ammonia (Figure 5.1). There may have been a difference in the relative importance of these two components between the two sheep selection lines, however this is unlikely as the ammonia pool sizes were not significantly different. On the meadow hay diet urea production from tissue turnover is likely to constitute a larger proportion of the plasma urea production, as

ruminal ammonia absorption is likely to be lower than on the lucerne chaff diet. This is because in sheep fed on the lucerne chaff diet there is likely to be a greater production of ammonia resulting in a net loss of nitrogen across the rumen, while on the meadow hay diet there is likely to be a net gain. According to Ulyatt and Egan's (1979) equation (Equation 1) the predicted nitrogen flow to the duodenum on the meadow hay diet was 13.7gN/d, which represents a net gain of 1.8gN/d. However on the lucerne chaff diet where the nitrogen intake was 43.0g/d the predicted nitrogen flow to the duodenum was 40.8gN/d, resulting in a predicted net loss of 2.2gN/d. Ulyatt and Egan (1979) suggested that in animals on a nitrogen intake of less than 25gN/d that there would be a net gain of nitrogen across the rumen in sheep fed on dried diets, and that 13% of any additional dietary nitrogen, consumed above 25gN/d, would be absorbed as ammonia across the rumen wall.

Figure 5.1 Urea Metabolism



The lower plasma urea concentration in the Fwt sheep does not appear to be related to a lower urea production rate or a different pattern of urea disposal. This suggests that some other factor must be responsible for the consistently lower plasma urea concentrations in the Fwt sheep.

It is possible that the same amount of urea is distributed in a larger body space in the Fwt animals than in the C animals. As correction for metabolic liveweight did not remove the between line differences in plasma urea concentration, it may be that the Fwt animals have a larger capillary blood space exposed to the skin. If so it suggests that the follicles in the Fwt animals may have a better blood supply than the follicles in the C animals. Neither the urea pool size or blood flow have been studied in the Romney selection lines, and these should be studied in further experiments.

Another possible factor that could be involved in the lower plasma urea concentration in the Fwt animals is in the area of kidney function.

5.2.3 The Possible Effects on Kidney Function

Plasma clearance rate expresses the degree to which any substance is removed from the plasma by the kidney and excreted in the urine. As creatinine is not reabsorbed in the kidney it can be used to provide an estimate of the glomerular filtration rate (GFR), as well as the rate of creatinine removal (Frandson, 1979).

There was no difference between the genotypes for creatinine clearance rate when fed the lucerne chaff diet, but when fed on the meadow hay diet the Fwt animals had a higher clearance rate ($P<0.05$), although this effect was removed by correcting for liveweight. The Fwt animals had a higher urea clearance rate than the C animals when fed both the meadow hay diet ($P<0.10$) and the lucerne chaff diet ($P<0.05$). The difference when fed on the lucerne chaff diet was not removed by correcting for the metabolic liveweight.

The volume of urine was consistently higher in the Fwt animals although this difference did not attain significance. This difference however did not appear to be related to the creatinine clearance rate on either diet or to the plasma urea concentration.

Factors accounting for the variation in plasma urea concentration (PUC; mM) between individual sheep were assessed using the following linear model (Equation 7), where R and G represent the effects due to the different rooms and to the two genotypes respectively. When this model was fitted there was a significant effect of genotype on both diets (MH $P<0.05$; Luc $P<0.01$).

Base Model $PUC = mean + R + G + residual$ (Equation 7)

In an attempt to identify those components responsible for the genotypic effect various covariates were fitted into the model until the genotypic effect became non significant ($P<0.05$). The covariates used included urine volume (UV; $g/kgW^{0.75}/d$), creatinine clearance

rate (CCR; ml/kgW$^{0.75}$/min), urea excretion rate (UER; g/kgW$^{0.75}$/d), and plasma creatinine concentration (PCC; mM). Fitting urine volume had very little effect and the influence of the other factors varied with diet.

In the animals fed on the meadow hay diet the genotype effect was rendered non-significant ($P<0.05$) by the following model:

PUC =mean + R + G + CCR + residual (Equation 8)

(R^2= 0.48)

(n=14)

This indicates that in animals fed on the meadow hay diet that a large proportion of the genotypic difference is accounted for by including the creatinine clearance rate in the model, suggesting that selection has altered the glomerular filtration rate.

In the animals on the lucerne chaff diet however the situation was different. On the lucerne chaff diet the creatinine clearance rate had only a small effect, and the effect of including the plasma creatinine concentration and urinary urea excretion together had more effect in rendering the genotypic effect non-significant (Equation 9).

PUC =mean + R + G + PCC + UER + residual (Equation 9)

(R^2 = 0.59)

(n=14)

In an attempt to quantify the above relationships, regression analyses were performed on the data from both diets.

In the sheep fed on the meadow hay diet the result for the relationship between the plasma urea concentration and creatinine clearance rate was Equation 10.

$$PUC = 7.967 - 0.808\ CCR \qquad \text{(Equation 10)}$$

$(R^2 = 0.33;\ P<0.05)$

(n=14)

However the most variation was explained by Equation 11

$$PUC = 4.07 + 36.39\ PCC + 1.65\ UER - 0.76\ CCR \qquad \text{(Equation 11)}$$

$(R^2 = 0.48;\ P<0.10)$

In the animals fed on the lucerne chaff diet the prediction equation that resulted from the ANOVA associated with Equation 9 was Equation 12

$$PUC = 4.831 + 0.109\ UER + 61.740\ PCC \qquad \text{(Equation 12)}$$

$(R^2 = 0.30)$

(n=14)

However the most variation was explained by Equation 13

$$PUC = 1.471 + 16.46\ PCC + 3.04\ UER - 0.13\ CCR \quad \text{(Equation 13)}$$

$$(R^2 = 0.48)$$

$$(n=14)$$

Although both equations 12 and 13 failed to attain significance ($P>0.05$), they do show that a substantial proportion of the variation in plasma urea concentration in sheep fed on the lucerne chaff diet can be accounted for by variation in the urinary urea excretion rate and the plasma creatinine concentration.

From these equations and the analysis of variances (Appendix B) it was concluded that in the animals fed on the meadow hay diet that the creatinine clearance had been altered by selection for fleece weight. However on the lucerne chaff diet a large proportion of the genotypic difference was accounted for by the plasma creatinine concentration and the amount of urea excreted, with the creatinine clearance having only a small effect. This suggests that on the lucerne chaff diet that any differences present in the GFR are masked by some other factor, for example solute load. McCutcheon et al (1987) found that in Romney sheep fed 110% maintenance on a lucerne chaff diet that the plasma urea concentration was positively correlated with the plasma creatinine concentration and the urea excretion rate and negatively correlated with creatinine clearance rate, after correction for bodyweight (Table 5.3).

Table 5.3 Factors Related To Plasma Urea Concentration In The Fwt And C Romney Sheep Fed Roughage Diets At Different Levels Of Energy Intake

Author	Level[a]	Factors Affecting[b]
Thomson (present study)	0.6	. Creatinine clearance rate(-)
McCutcheon et al (1987)	1.1	. Creatinine clearance rate(-)
		. Urea excretion(+)
		. Plasma creatinine concentration(+)
Thomson (present study)	1.5	. Urea excretion(+)
		. Plasma creatinine concentration(+)

[a] the feeding level as a proportion of the maintenance requirement

[b] The factors were identified by Thomson as described in this study and by McCutcheon et al (1987) by an analysis of variance technique

This suggests that there is an interaction between the nutritional level and the mechanisms apparently controlling plasma urea concentration.

5.3 THE EFFECT OF DIET ON THE PHENOTYPIC DIFFERENCES BETWEEN THE FWT AND C ANIMALS

During the digestibility periods the differences in VFI and apparent digestibility between the two genotypes, were only found on the high plane of nutrition (i.e. the lucerne chaff diet). Therefore the conflicting results observed between other studies comparing the C and Fwt Romney sheep, may be due to the nutritional level at which the measurements were recorded. As the animals have been selected for greasy fleece weight production when grazing a high quality ryegrass/white clover pasture, it seems logical for the differences between the two lines to be most marked under high planes of nutrition.

When looking at the postabsorptive utilisation of nutrients in sheep fed the two diets, the Fwt animals had a lower plasma urea concentration on both diets. However the factors related to and perhaps explaining, this difference varied with diet and hence level of nutrition. For example on the meadow hay diet the difference between the genotypes disappeared when creatinine clearance rate was included in the model, whilst on the lucerne chaff diet a combination of urea excretion and plasma creatinine concentration was necessary to account for the genotypic effect. On the high level there will be a heavier load of solutes, including urea, which maybe of more importance to the animal in regulating plasma urea concentration than the change in the GFR (as indicated by the creatinine clearance rate).

Therefore it would appear that the level of nutrition at which the measurements are made has a major influence on the differences expressed between the two lines in terms of VFI, apparent digestibility and postabsorptive utilisation of nutrients. This may account for a large number of the conflicting results in the earlier trials in the NZ Romney selection lines.

CONCLUSIONS

1. From the results of the present investigation it appears that differences in voluntary feed intake (VFI) and apparent digestibility between the Fwt and C sheep are more marked at the high nutritional level. This may explain why there is an increase in the size of the differences between the two genotypes, in overall wool production and in the efficiency of feed conversion to wool with increasing feeding level. However more work is required in these areas to determine when these differences in intake and digestion occur, especially with regard to whether they occur in the selection flocks when fed on a typical NZ ryegrass/white clover pasture diet and whether any compensatory effects (if present) are important. It would be worthwhile studying these factors in an animal with a high nutrient demand, for example the young growing animal or a lactating ewe, when fed on a high quality pasture diet.

2. In the present work, selection appears to have altered rumen function as measured by - the digestibility coefficients, the rumen MRT of particulate matter and its pathway of disappearance from the rumen when the sheep were fed the high quality lucerne chaff diet. The Fwt animals appeared to have a higher FOR and a lower FDR of particulate matter than the control animals. Further studies looking at VFI, apparent digestibility, rumen particulate matter and fluid FOR need to be carried out over a range of nutritional levels and diets.

3. These changes in digestive function could possibly result in an increased supply of amino acids to the duodenum, possibly resulting in more amino acids being absorbed in the fleece weight animals, or a change in the amounts of some individual amino acids absorbed. This could result in an improved supply of amino acids to the wool follicle and therefore account for at least some of the differences in wool production between the two genotypes. This needs to be further investigated at both the duodenum, using cannulated animals, and at the wool follicle level. Experimental work is required on the amino acid flows to the duodenum, and absorption in the small intestine in the two lines of sheep.

4. Plasma urea concentration appears to be consistently lower in the Fwt sheep than in the C sheep. These differences do not appear to be explained by differences in the plasma urea IRL or in the pathways of urea disposal. It is possible that a difference in the size of the body urea space or a change in the kidney function may be involved. When fed on the meadow hay diet the difference in creatinine clearance rate suggests that selection has altered the GFR. However when fed on the lucerne chaff diet it would appear that other factors are responsible and that any differences in the GFR are of lesser importance to the animal in regulating the plasma urea concentration. Egan et al (1986) suggested that a high blood urea concentration could be sustained by a reduced kidney clearance, thereby the potential for the entry rate of urea into the GIT will be increased. For these reasons it would appear that the difference in plasma urea concentration between the Fwt and C sheep, is not

directly related to the increased wool production of the Fwt animals. It is possible that the differences in urea concentration may be correlated with some other factor that is directly related to the increased wool production, for example plasma amino acid concentrations. None of the studies so far show precisely how or why the Fwt animals have a consistently lower plasma urea concentration. As a mechanism for investigating the control of wool growth the plasma urea concentration would appear to be of limited value, although it may have a use as a selection tool when the factors influencing urea concentration are better understood.

5. From the results of the present work it would appear that the differences observed between the Fwt and C animals in VFI, apparent digestibility and post absorptive utilisation of nutrients vary with the nutritional level applied. This may explain the conflicting results from the earlier digestibility and voluntary feed intake trials in the NZ Romney selection lines.

6. No differences have been found in VFI, apparent digestibility, rumen function or plasma urea concentrations in the Australian Merino lines selected for and against fleece weight. This suggests that it may not be possible to extrapolate results obtained with the NZ Romney fleece weight selection lines to the Australian Merino fleece weight selection lines and vice versa.

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APPENDICES

APPENDICES INDEX

APPENDIX A: DEPROTEINISATION OF PLASMA

1. Barium Hydroxide Method

Boil the water for fifteen minutes in a conical flask, cool rapidly and blow N_2 gas over the surface to prevent the uptake of CO_2 by the water. Add $Ba(OH)_2$, cover and stir for 2-3hr;, cool and filter through No1 filter paper. Then add an extra 5% CO_2 free water, gas with N_2 and store in a liquid dispenser or burette with CO_2 exclusion. The concentration of the final solution should be 0.1 - 0.12 molar. Titrate this solution against 5ml of 0.3M $ZnSO_4$ until pH 7 is reached.

2. Scintillation Cocktail

Reagents: 4g PPO (2,5-Diphenyloxazole)

0.1g di Methyl POPOP

(1,4-Di-2(4-methyl-5-Phenyloxaolyl)Benzene

670 ml toluene

330 ml Triton X-100

In a fume cupboard the PPO and the di Methyl POPOP was dissolved in approximately one third of the toluene. The remainder of the toluene was then added and thoroughly mixed. Then the Triton X-100 was added and the final solution well mixed.

3. Deproteinisation

3.1 Deproteinisation of Plasma

Reagents: $Ba(OH)_2$

$ZnSO_4$

Scintillation Cocktail

To 1ml of plasma add 3mls of distilled water, then 1ml $Ba(OH)_2$ solution (avoiding exposure to air), mixing well after both additions. Then add the $ZnSO_4$ solution gradually until the pH is 7.0+/-0.2. In this trial with the plasma the pH was found to be stable over a range of volumes so a constant mean value was added. Centrifuge at 1500g for 10mins and add 1ml of the supernatant to 10mls of scintillation cocktail. The supernatant can be stored frozen until required.

3.2 Deproteinisation of Urine

The urine was neutralised with 1M NaOH prior to deproteinisation and then the same techniques as were used with the plasma were carried out.

4. Quench Curve

The quench curve was set up by diluting 1mC of standardised C^{14} labelled toluene (Amersham, British Calibration Service) to 1 x 10^3 DPM/ml in toluene. Fifteen ten ml aliquots of this were then counted and the eleven closest vials were used to set up the quench curve.One ml of the pooled blank plasma was added to each tube and then these were recounted. To these vials 0,1,2,3,4,5,9,11,13 and 15 drops of chloroform were added with a pasteur pipette and the vials were then recounted. These values were used to set up the curve.

5. Method Checks

Prior to the pooling of the blank plasma samples 1ml of deproteinised plasma from two control and two fleeceweight animals on each feed were counted and then spiked. As no difference was found as to the amount of quenching occurring between animals, genotypes or feeds all the blank deproteinised samples were pooled.

The effect of an imbalance of any reagent was looked at by changing the deprotreinising ratios:

a) 0.8 ml of plasma

b) 1.2ml of plasma

c) 2.5ml of water

d) 3.5ml of water

e) 0.8ml of $Ba(OH)_2$

f) 1.2ml of $Ba(OH)_2$

g) 2.0ml of $ZnSO_4$

h) 2.4ml of $ZnSO_4$

These samples were counted, spiked and recounted and the efficiency of counting calculated. These efficiencies all fell on the quench curve and from this it was felt that the chloroform was quenching in a similar way to the actual quench that was occurring in the samples.

APPENDIX B: THE STATISTICAL ANALYSES OF THE KIDNEY FUNCTION DATA

MEADOW HAY DIET

Model 1

Plasma Urea Concentration (PUC)
= u + R + G + R*G + Error (R^2 0.30; P<0.21)

ANOVA TABLE

Source	DF	MS	Sign
Room	1	0.246	NS
Gene	1	3.670	0.05
G*R	1	0.385	NS
Error	12	0.825	

Model 2

PUC = u + R + G + CCR + Error (R^2 0.48; P<0.10)

ANOVA TABLE

Source	DF	MS	Sign
CCR	1	4.596	0.05
Room	1	0.272	NS
Gene	1	1.819	NS
Error	10	0.719	

Model 3

PUC= u + R + G + CCR + UER + PCC + Error (R^2 0.53; P<0.20)

ANOVA TABLE

Source	DF	MS	Sign
CCR	1	4.596	0.05
PCC	1	1.207	NS
UER	1	0.830	NS
Room	1	0.283	NS
Gene	1	0.507	NS
Error	8	0.806	

LUCERNE CHAFF DIET

Model 4

PUC = u + R + G + R*G + Error (R^2 0.43; P<0.10)

ANOVA TABLE

Source	DF	MS	Sign
Room	1	0.000	NS
Gene	1	4.785	0.05
R*G	1	0.128	NS
Error	11	0.588	

Model 5

PUC = u + R + G + UER + PCC + Error (R^2 0.58; P<0.10)

ANOVA TABLE

Source	DF	MS	Sign
Room	1	0.300	NS
UER	1	0.704	NS
PCC	1	1.840	0.10
Gene	1	1.855	0.10
Error	9	0.379	

Model 6

PUC =u + R + G + UER + PCC + CCR + Error (R^2 0.73; P<0.10)

ANOVA TABLE

Source	DF	MS	Sign
Room	1	0.006	NS
CCR	1	0.021	NS
UER	1	2.049	0.05
PCC	1	0.319	NS
Gene	1	1.213	0.10
Error	6	0.218	

APPENDIX C: RAW DATA

Table C1 History Of The Animals Prior To The Trial

a) CONTROL ANIMALS

Tag	Line	Sire	Dam	DOB	BR	RR	Wwt	Spwt	Fwt
80	1	166	143/83	23/8	1	1	20.3	44.5	2.95
115	1	19	68/80	28/8	1	1	22.5	41.3	2.75
161	1	208	117/81	31/8	1	1	19.2	40.5	3.05
195	1	209	168/80	4/9	1	1	21.9	42.8	3.50
199	1	19	166/82	4/9	1	1	20.1	-	-
203	1	166	197/83	9/9	2	2	15.9	36.9	2.60
208	1	209	125/83	16/9	1	1	22.4	46.3	3.20
212	1	208	164/80	16/9	1	1	21.3	45.7	3.60
Mean							40.5	42.6	3.09
SE							0.77	1.25	0.139

b) FLEECE WEIGHT ANIMALS

Tag	Line	Sire	Dam	DOB	BR	RR	Wwt	Spwt	Fwt
4	2	11	93/81	13/8	1	1	19.5	46.2	3.55
33	2	177	177/82	17/8	1	1	25.1	46.7	4.30
47	2	60	164/81	19/8	1	1	25.8	45.3	3.75
88	2	60	169/82	24/8	2	2	17.8	47.2	3.95
98	2	8	131/80	26/8	1	1	26.7	46.0	4.15
156	2	79	3/80	31/8	1	1	29.9	51.4	3.70
194	2	79	118/81	4/9	2	2	17.0	36.4	3.60
232	2	79	104/80	1/10	1	1	17.9	39.9	3.90
Mean							22.5	44.9	3.86
SE							1.76	1.64	0.093

DOB Date Of Birth
RR Rearing Rank
Spwt Spring Weight(kg)
BR Birth Rank
Wwt Weaning Weight(kg)
Fwt Fleece Weight (kg)

Table C2 Twenty Four Hour Fasted Liveweights (kg) Before and After Experiment A and B

a) CONTROL ANIMALS

Tag	Room	First Wt	Midwt	Final Wt	M-F	Fi-M
80	1	45.2	40.5	46.6	-4.7	6.1
115	1	42.4	37.7	43.2	-4.7	5.5
199	1	49.9	-	-	-	-
203	1	37.3	31.9	37.1	-5.4	5.2
161	2	40.2	33.3	39.3	-6.9	6.0
195	2	41.7	36.3	42.3	-5.4	6.0
208	2	45.4	41.4	44.9	-4.0	3.5
212	2	43.3	37.1	42.6	-6.2	5.5
Mean		43.2	36.9	42.3	-5.3	5.4
SE		1.34	1.31	1.21	0.37	0.34

b) FLEECE WEIGHT ANIMALS

Tag	Room	First Wt	Midwt	Final Wt	M-F	Fi-M
4	1	46.9	39.5	46.5	-7.4	7.0
33	1	48.4	42.3	48.9	-6.1	6.6
98	1	47.2	40.7	45.8	-6.5	5.1
156	1	49.3	43.3	50.7	-6.0	7.4
47	2	45.3	40.5	45.9	-4.8	5.4
88	2	45.5	37.6	43.7	-7.9	6.1
194	2	36.1	31.6	35.3	-4.5	3.7
232	2	41.8	35.6	41.4	-6.2	5.8
Mean		45.1	38.9	44.8	-6.2	5.9
SE		1.51	1.36	1.69	0.41	0.42

First wt = weight at beginning of meadow hay trial
Midwt = weight at the end of meadow hay trial and Beginining of lucerne trial
Final wt = weight at the end of the lucerne trial M-F =weight gain on the meadow hay diet
Fi-M =Weight gain on the lucerne chaff

Table C3 Daily Voluntary Feed Intakes (gDM/kgL$^{0.75}$) Over The Last Four Days Of The Meadow Hay Adaptation Period

a) CONTROL ANIMALS

Tag	Day 1	Day 2	Day 3	Day 4	Mean
80	39.27	43.14	40.00	32.99	38.85
115	53.14	47.69	41.97	41.69	46.12
203	40.57	58.56	55.49	38.06	48.17
161	40.78	46.06	47.93	34.62	42.35
195	41.63	46.63	42.53	30.64	40.36
208	46.09	-	41.28	43.50	43.62
212	37.24	43.33	41.19	48.43	42.55
Mean	42.68	47.57	44.34	38.56	43.15
SE	2.019	2.321	2.094	2.397	1.210

b) FLEECE WEIGHT ANIMALS

Tag	Day 1	Day 2	Day 3	Day 4	Mean
4	34.30	39.13	44.38	35.86	38.42
33	43.19	37.13	42.34	46.80	42.37
98	34.83	39.25	41.76	47.20	40.76
156	34.98	33.94	41.54	33.06	35.88
47	41.17	41.27	44.98	44.88	43.08
88	31.73	-	28.04	22.69	27.49
194	41.75	37.32	51.54	44.43	43.76
232	57.96	48.71	49.33	45.71	50.43
Mean	39.99	39.54	42.99	40.08	40.27
SE	2.958	1.755	2.488	3.103	2.368

Table C4 Daily Voluntary Feed Intakes (gDM/kgL$^{0.75}$) Over The Last Four Days Of The Lucerne Chaff Adaptation Period

a) CONTROL ANIMALS

Tag	Day 1	Day 2	Day 3	Day 4	Mean
80	100.64	72.37	117.65	96.09	96.69
115	78.47	64.33	89.14	80.76	78.18
203	106.57	102.96	-	120.39	109.97
161	100.38	116.58	115.83	101.02	108.45
195	98.72	70.26	104.71	95.77	92.37
208	92.38	78.82	88.78	79.03	84.75
212	91.38	86.53	95.48	94.51	91.98
Mean	95.48	84.55	101.93	95.37	94.63
SE	3.454	7.176	5.247	5.209	4.394

b) FLEECE WEIGHT ANIMALS

Tag	Day 1	Day 2	Day 3	Day 4	Mean
4	105.01	104.352	108.28	110.82	107.12
33	96.59	80.21	97.57	108.24	95.65
98	110.04	98.37	114.60	119.02	110.51
156	98.00	105.29	112.35	96.28	102.98
47	104.40	87.44	91.80	94.39	94.51
88	104.38	94.60	86.90	96.09	95.49
194	110.11	119.95	107.96	105.37	110.85
232	94.88	82.87	98.48	113.37	97.40
Mean	102.88	96.64	102.24	105.45	101.81
SE	2.066	4.685	3.547	3.211	2.455

Table C5 The Mean Intakes (g/d) Over the Meadow Hay Digestibility Perioc

a) CONTROL ANIMALS

Tag	Room	Fresh	DMI	OMI	NI	GEI
80	1	681.10	597.79	537.82	12.080	10.617
115	1	701.51	614.39	553.72	12.284	10.932
199	1	713.60	623.69	565.41	12.434	11.195
203	1	610.84	535.83	482.64	10.780	9.552
161	2	576.84	504.12	454.82	9.829	8.954
195	2	679.10	594.69	535.22	12.527	10.569
208	2	723.43	633.23	570.11	13.676	11.255
212	2	627.26	549.37	496.10	11.014	9.773
Mean		664.20	581.64	524.48	11.828	10.356
SE		18.75	16.41	14.83	0.429	0.296

b) FLEECE WEIGHT ANIMALS

Tag	Room	Fresh	DMI	OMI	NI	GEI
4	1	783.48	686.10	617.49	13.722	12.193
33	1	761.13	666.46	602.43	13.317	11.885
98	1	710.50	621.02	560.32	12.646	11.085
156	1	723.31	634.83	572.95	12.901	11.336
47	2	686.95	584.54	548.73	12.056	10.837
88	2	544.30	474.50	433.61	9.776	8.484
194	2	634.21	555.53	500.40	11.111	9.881
232	2	619.93	542.83	496.01	10.815	9.901
Mean		682.98	595.73	541.49	12.043	10.700
SE		28.09	24.85	21.66	0.483	0.432

Table C6 Mean Daily Faecal Outputs (g/d) on the Meadow Hay Diet During the Digestibility Period

a) CONTROL ANIMALS

Tag	Room	Wet Wt	DM Wt	OM Wt	N Wt	GE Wt
80	1	655.3	259.8	223.0	46.25	47.52
115	1	692.7	285.0	247.7	48.85	51.69
199	1	689.3	263.3	225.4	45.81	47.69
203	1	484.7	204.8	174.9	35.45	36.92
161	2	663.1	246.2	216.3	43.09	45.53
195	2	634.8	260.5	228.9	43.33	48.54
208	2	626.0	247.2	213.2	44.99	44.21
212	2	696.6	289.1	255.4	49.47	53.58
Mean		642.8	257.0	223.1	44.65	46.96
SE		24.44	9.25	8.62	1.545	1.792

b) FLEECE WEIGHT ANIMALS

Tag	Room	Wet Wt	DM Wt	OM Wt	N Wt	GE Wt
4	1	854.0	293.1	260.7	46.92	56.17
33	1	711.2	305.4	257.3	52.16	55.14
98	1	634.9	272.1	229.7	46.39	44.47
156	1	636.6	264.3	225.6	47.89	47.63
47	2	841.7	292.5	251.2	58.23	54.69
88	2	566.4	198.9	174.2	36.33	36.70
194	2	757.0	272.5	233.1	48.07	49.64
232	2	642.0	247.2	216.5	44.75	45.97
Mean		705.5	268.3	231.0	47.59	48.80
SE		36.93	11.86	9.87	2.201	2.331

Table C7 The Water Intakes And Urine Outputs On Meadow Hay During The Digestibility Period

a) CONTROL ANIMALS

Tag	Water (g/d)	Urine Vol(g/d)	N (gN/d)	Urea (gUrea/d)	Creat (gCreat/d)
80	1237.3	703.3	9.719	19.072	0.809
115	1340.8	834.3	9.327	17.403	0.918
199	1166.8	667.3	9.088	17.335	0.807
203	1581.3	1158.5	8.735	20.587	1.054
161	1157.9	784.5	7.374	12.944	0.541
195	1121.5	766.9	6.825	12.492	0.614
208	1619.0	1161.1	8.643	14.803	0.835
212	1150.4	648.0	6.914	16.226	0.447
Mean	1296.9	840.5	8.328	16.358	0.753
SE	70.53	73.00	0.3996	1.0020	0.0718

b) FLEECE WEIGHT ANIMALS

Tag	Water (g/d)	Urine Vol(g/d)	N (gN/d)	Urea (gUrea/d)	Creat (gCreat/d)
4	1838.0	1039.0	10.442	19.253	1.070
33	1560.5	946.4	11.686	22.118	1.079
98	1434.5	821.87	8.038	14.161	0.896
156	1403.8	953.3	8.799	14.413	0.944
47	1542.8	838.8	9.050	16.012	0.637
88	1855.9	1140.9	7.530	13.793	0.844
194	1745.14	1277.3	6.961	15.046	0.754
232	2218.39	1801.9	11.478	19.298	1.315
Mean	1699.9	1102.4	9.248	16.762	0.942
SE	96.04	113.40	0.6300	1.0850	0.0749

Table C8 The Daily Water Intakes (g) On The Meadow Hay Diet During The Digestibility Period

a) CONTROL ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Mean
80	907	725	1534	1893	584	1518	928	1809	1237
115	1873	1747	606	1949	30	1966	1282	1273	1341
199	1266	787	1208	1245	610	1337	1829	1052	1167
203	1876	1629	1299	-	927	2389	1060	1889	1581
161	80	1455	940	1348	156	2036	1845	1403	1160
195	539	887	1433	1875	295	513	2229	2001	1222
208	1473	1851	1250	1885	532	1823	2075	2063	1619
212	142	1530	733	1250	868	1579	1425	1676	1150
Mean	1020	1326	1125	1593	500	1645	1584	1646	1310
SE	254.4	160.8	117.4	122.0	113.3	199.9	169.4	129.4	67.0

b) FLEECE WEIGHT ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Mean
4	1374	2122	1715	2407	840	2360	1881	2005	1838
33	1373	1498	1400	1553	478	2552	2006	1624	1561
98	964	1303	1272	2181	564	1756	1252	2184	1435
156	1190	1548	1653	1686	671	1583	1837	1062	1404
47	871	930	1087	1703	1654	2063	1577	2457	1543
88	221	2208	480	1790	29	2951	4021	3147	1856
194	1131	2447	1713	2185	728	1950	2062	-	1745
232	1130	2173	1295	3925	1333	2577	2586	2732	2219
Mean	1032	1779	1327	2179	787	2224	2153	2173	1700
SE	131.3	188.2	145.8	270.0	178.8	164.2	299.5	246.1	96.0

Table C9 The Daily Urine Production(g) On The Meadow Hay Diet During The Digestibility Period

a) CONTROL ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Mean
80	857	752	549	673	509	713	800	777	704
115	1163	1251	691	743	509	775	768	774	834
199	809	937	422	639	645	668	626	592	667
203	2071	1618	878	1044	748	1052	834	1023	1159
161	717	723	949	786	675	648	822	956	785
195	941	697	830	749	557	583	1032	746	767
208	1805	1369	1226	1015	654	748	1293	1171	1160
212	762	614	901	666	398	714	581	608	656
Mean	1141	995	806	789	587	738	845	831	841
SE	182.3	131.2	88.5	55.2	40.2	49.7	80.4	71.8	72.6

b) FLEECE WEIGHT ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Mean
4	970	1227	-	1047	844	1184	940	1061	1039
33	1384	1309	603	701	-	733	881	1041	995
98	941	1572	479	734	681	777	669	722	822
156	994	1315	715	918	548	818	1062	1256	953
47	1124	665	610	656	443	727	832	1659	840
88	611	699	1192	736	429	1663	1968	1829	1141
194	1470	1551	1431	1039	851	1452	1403	1021	1277
232	2209	1669	1460	2273	1702	1888	1478	1736	1802
Mean	1213	1251	927	1013	785	1155	1154	1291	1109
SE	171.1	135.2	148.6	187.9	155.4	163.8	152.2	142.4	112.3

Table C10 The Mean Intakes On The Lucerne Chaff Diet During The Digestibility Period

a) CONTROL ANIMALS

Tag	Fresh	DMI	OMI	NI	GEI	NDFI	ADFI	HemiI	Lignin
80	1719	1470	1348	47.09	28.20	776.7	503.0	267.8	130.2
115	1544	1320	1214	41.61	25.34	702.5	454.7	242.1	115.6
203	1443	1234	1131	39.55	23.68	651.8	422.1	224.8	109.3
161	1499	1279	1178	41.13	24.63	678.3	439.3	233.9	113.7
195	1587	1357	1244	43.42	26.04	717.3	464.5	247.3	120.1
208	1576	1348	1235	44.16	25.90	696.9	450.7	240.2	114.0
212	1619	1385	1271	44.31	26.59	732.0	473.9	252.6	122.8
Mean	1570	1342	1232	43.04	25.77	707.9	458.3	244.1	118.0
SE	33.4	28.7	26.0	0.942	0.546	15.10	9.79	5.21	2.64

b) FLEECE WEIGHT ANIMALS

Tag	Fresh	DMI	OMI	NI	GEI	NDFI	ADFI	HemiI	Lignin
4	1691	1445	1325	46.22	27.73	764.2	495.1	263.3	128.3
33	1773	1517	1392	48.49	29.11	802.2	519.5	276.5	134.4
98	1686	1442	1322	46.19	27.66	761.7	493.3	262.7	127.7
156	1794	1534	1406	49.29	29.44	809.3	523.4	279.7	135.2
47	1739	1487	1365	47.60	28.55	786.1	508.9	271.2	131.9
88	1580	1351	1239	43.39	25.94	712.9	459.9	247.4	119.1
194	1444	1235	1132	34.27	23.70	652.7	422.7	225.1	109.4
232	1510	1290	1188	40.76	24.82	684.6	442.6	236.6	114.5
Mean	1652	1413	1296	44.53	27.12	746.7	483.2	257.8	125.1
SE	45.08	31.42	35.18	1.763	0.737	20.23	13.16	6.94	

Table C11 Mean Faecal Output (g/d) On The Lucerne Chaff Diet During The Digestibility Period

a) CONTROL ANIMALS

Tag	Wetwt	DM wt	OM wt	N wt	GE wt	NDRwt	ADRwt	Hemiwt	Lignin
80	1815	570.9	490.9	14.34	11.01	341.2	257.8	83.4	99.1
115	1570	538.5	463.9	14.13	10.36	621.9	243.8	78.1	97.6
203	1346	397.6	340.9	10.43	7.74	247.1	201.4	45.6	79.3
161	1781	564.7	493.1	14.03	10.77	352.5	242.6	110.0	80.5
195	1630	575.95	496.01	14.97	11.02	354.9	273.0	81.3	101.7
208	1624	539.9	467.9	13.32	10.29	302.5	247.2	55.3	92.0
212	1689	532.1	461.6	13.53	10.09	308.0	226.5	81.5	85.0
Mean	1636	531.4	459.17	13.536	10.18	361.2	241.8	76.5	90.7
SE	58.8	23.24	20.48	0.5562	0.4295	45.72	8.63	7.91	3.48

b) FLEECE WEIGHT ANIMALS

Tag	Wetwt	DM wt	OM wt	N wt	GE wt	NDRwt	ADRwt	Hemiwt	Lignin
4	1923	596.9	522.6	14.17	11.35	346.2	247.8	98.6	107.7
33	1668	553.7	477.7	13.73	10.52	302.9	224.7	78.1	94.1
98	1956	664.9	575.8	17.09	12.26	384.5	292.2	91.8	110.7
156	1852	589.0	513.0	12.79	11.27	342.5	258.2	84.3	101.4
47	2089	715.5	617.0	15.93	13.82	391.3	282.3	108.8	126.9
88	1839	677.6	580.1	17.54	12.85	420.1	313.7	106.4	124.4
194	1624	511.5	442.0	14.08	9.95	310.5	245.0	65.4	87.4
232	1755	631.1	550.7	16.29	11.63	364.8	272.3	92.5	129.4
Mean	1838	617.5	534.8	15.20	11.71	357.9	267.0	90.7	110.3
SE	54.4	23.98	20.37	0.354	0.441	14.26	10.18	5.14	5.53

Table C12 The Mean Water Intakes And Urinary Outputs (g/d) On The During The Digestibility Trial Period On The Lucerne Chaff Diet

a) CONTROL ANIMALS

Tag	Supp Water	Feed Water	Total Water	Urine Vol	N	Urea	Creat
80	4645	249	4894	2258	26.33	50.91	1.784
115	3816	224	4040	1586	24.82	51.49	1.348
203	4367	209	4576	2536	25.48	47.80	1.750
161	4867	220	5088	1670	20.89	40.60	0.960
195	4311	230	4541	1943	24.17	47.10	1.535
208	4897	228	5125	2635	25.35	47.33	1.792
212	3685	235	3919	1464	25.05	48.57	1.128
Mean	4370	228	4598	2013	24.58	47.69	1.471
SE	181.3	4.8	181.4	182.0	0.664	1.348	0.1269

b) FLEECE WEIGHT ANIMALS

Tag	Supp Water	Feed Water	Total Water	Urine Vol	N	Urea	Creat
4	4639	245	4884	1686	24.62	48.03	1.366
33	4938	256	5194	2180	25.55	50.40	1.788
98	4547	244	4791	1828	28.51	55.49	1.755
156	6614	260	6874	3452	24.03	46.09	2.106
47	5109	252	5362	1832	24.05	47.49	1.374
88	4400	229	4629	2159	30.03	57.38	1.705
194	6069	209	6278	3792	21.20	52.52	1.616
232	5202	220	5422	2389	15.86	33.08	1.338
Mean	5190	240	5429	2418	24.23	48.81	1.631
SE	274.2	6.5	274.3	98.1	1.542	17.257	0.0939

Table C13 The Daily Supplementary Water Intakes (g) During The Digestibility Period On The Lucerne Chaff Diet

a) CONTROL ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Mean
80	5245	2715	3301	5277	6259	5304	4412	4645
115	4726	2979	3062	3593	4985	4014	3353	3816
203	4837	3412	4074	4484	4956	5024	3784	4367
161	5618	2564	3705	4601	5076	4064	-	4863
195	4610	3623	3507	6018	4963	4137	3319	4311
208	5531	3742	4322	5545	5706	5342	4089	4897
212	4141	1893	3329	4779	4658	3052	3940	3685
Mean	4958	2990	3612	4900	5229	4420	3816	4370
SE	201.4	249.0	175.2	300.5	209.5	317.7	174.0	181.3

b) FLEECE WEIGHT ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Mean
4	5756	3680	2495	4907	5644	5530	4461	4639
33	5600	2333	4244	5999	6317	4410	5666	4938
98	5771	2960	3744	4269	5344	5258	4482	4547
156	7005	4646	5627	7256	7650	6708	7404	6614
47	5558	3921	5276	4994	5534	4767	5716	5109
88	5151	4142	3420	3861	4834	5535	3857	4400
194	7527	4856	6724	5310	7569	4964	5536	6069
232	6115	3892	5424	4924	5478	5415	5169	5202
Mean	6060	3804	4619	5190	6046	5323	5286	5190
SE	283.9	294.1	488.8	371.8	390.2	242.4	384.1	274.2

Table C14 The Daily Urine Volumes (g) On The Lucerne Diet During The Digestibility Period

a) CONTROL ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Mean
80	2513	2572	1340	1896	2666	2820	2092	2163	2258
115	2446	1502	1287	1512	1693	1694	1447	1105	1586
203	2318	2969	1654	2841	2348	3449	2136	2570	2536
161	1627	1365	1754	1491	1888	2012	1798	1424	1670
195	2463	1840	1472	2259	2303	1987	1798	1424	1943
208	3330	3154	1770	2734	2611	2527	2216	2740	2635
212	1951	1560	1298	1537	1482	1511	1244	1131	1464
Mean	2378	2137	1511	2039	2142	2286	1805	1728	2013
SE	530.1	745.7	213.3	581.0	459.1	684.5	368.3	746.8	182.0

b) FLEECE WEIGHT ANIMALS

Tag	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Mean
4	1400	1750	1430	1416	2050	1845	1847	1750	1686
33	2667	2084	2079	-	1999	2185	2164	2084	2180
98	2485	1848	1415	1519	1932	2006	1569	1848	1828
156	2432	3268	2938	4962	4221	3581	2945	3268	3452
47	1832	1850	1641	1786	1599	2021	2073	1850	1832
88	3887	2520	1210	1916	1472	2261	1701	2520	2186
194	5524	3103	4250	3567	4166	4093	2529	3103	3792
232	2684	2299	2140	2195	2500	2507	2484	2299	2389
Mean	2864	2340	2138	2480	2492	2562	2164	2340	2418
SE	1295	582.3	1017	1309	1094	822.4	466.4	582.3	98.1

Table C15 The Individual Rumen Pools(g) On The Lucerne Chaff Diet At Slaughter

a) CONTROL ANIMALS

Tag	Fresh	DMWt	OMWt	NWt	NDRWt	ADRWt	Hemi	Lignin	Time
80	8667	1298	1171	48.3	771	527	243	181	11.30
115	7622	1065	965	38.1	600	398	202	136	1.30
203	7947	1171	1057	43.1	675	455	221	157	10.30
161	7334	874	781	29.5	520	331	189	131	11.30
195	7126	1015	916	38.5	622	416	206	146	2.30
208	6680	962	863	34.7	564	369	195	133	1.30
212	8794	1054	949	36.4	627	393	234	151	10.30
Mean	7739	1063	957	38.4	626	413	213	148	
SE	296.5	52.4	48.2	2.27	30.5	23.9	7.7	6.6	

b) FLEECE WEIGHT ANIMALS

Tag	Fresh	DMWt	OMWt	NWt	NDRWt	ADRWt	Hemi	Lignin	Time
4	8848	1026	974	37.3	623	430	194	157	1.30
33	8851	1352	1225	48.2	817	551	266	181	10.30
98	7907	1128	1013	41.9	649	428	221	142	2.30
156	8902	1155	1042	40.7	712	476	236	163	11.30
47	8953	1142	1018	40.3	683	497	186	167	10.30
88	7582	1111	1000	40.7	634	408	225	139	2.30
194	8695	1043	928	36.7	605	363	242	140	11.30
232	7815	970	874	33.4	556	379	177	147	1.30
Mean	8444	1116	1009	39.9	660	442	218	155	
SE	202.1	40.7	36.3	1.54	28.0	22.3	10.81	5.35	

Table C16 The VFA (me/100ml) and Ammonia (ug/l) Concentrations of the Individual Animals Fed on Lucerne Chaff, At Slaughter

a) CONTROL ANIMALS

Tag	Acet	Prop	IBut	But	IVal	Val	Total	NH^3
80	15.08	4.58	1.11	0.68	0.14	0.22	21.81	260
115	14.81	6.82	1.10	0.61	0.11	0.19	23.65	250
203	24.46	11.27	1.77	1.03	0.25	0.42	39.20	250
195	17.78	8.19	0.9	0.72	0.10	0.17	27.85	220
208	14.56	6.71	1.32	0.70	0.22	0.17	23.69	180
212	18.34	8.45	0.70	0.68	0.10	0.19	28.45	210
Mean	17.51	9.67	1.15	0.74	0.15	0.23	27.44	228
SE	1.538	0.913	0.151	0.061	0.027	0.040	2.580	12.5

b) FLEECE WEIGHT ANIMALS

Tag	Acet	Prop	IBut	But	IVal	Val	Total	NH^3
4	77.62	23.89	6.10	2.38	0.33	0.57	110.88	190
33	19.66	6.31	1.54	0.78	0.12	0.27	28.69	300
98	13.78	4.20	1.11	0.70	0.08	0.19	20.06	150
156	13.71	6.31	0.95	0.54	0.11	0.18	21.80	150
47	14.68	3.66	0.81	0.58	0.15	0.18	20.05	190
88	18.43	4.99	0.61	0.52	0.12	0.21	24.88	220
194	17.20	7.94	-	0.52	0.04	0.16	-	190
232	14.97	6.89	1.24	0.52	0.15	0.21	23.97	180
Mean	23.76	8.02	1.77	0.82	0.14	0.25	35.76	196
SE	7.733	2.321	0.684	0.226	0.030	0.048	12.572	16.9

Table C17 Lignin Ruminal FDPR,FOR,FDR and Digestibilities For Individual Animals On The Lucerne Chaff Diet

a) CONTROL ANIMALS

Tag	MRT h	FDPR %/h	FDR %/h	FOR %/h	Dig %
80	43.80	3.00	0.72	2.28	0.24
115	33.32	3.55	0.55	3.00	0.16
161	47.50	2.90	0.79	2.11	0.27
195	39.08	3.61	1.05	2.56	0.29
203	34.57	3.42	0.52	2.89	0.15
208	34.75	3.57	0.69	2.88	0.19
212	42.67	3.39	1.04	2.34	0.31
Mean	39.38	3.35	0.77	2.58	0.23
SE	2.06	0.108	0.081	0.132	0.024

b) FLEECE WEIGHT ANIMALS

Tag	MRT h	FDPR %/h	FDR %/h	FOR %/h	Dig %
4	35.06	2.34	0.54	2.85	0.16
33	46.04	3.10	0.93	2.17	0.30
47	30.85	3.74	0.50	3.24	0.13
88	38.57	3.46	0.87	2.59	0.25
98	31.66	3.28	0.13	3.16	0.04
156	26.76	3.58	-0.16	3.74	-0.04
194	38.48	3.25	0.65	2.60	0.20
232	27.19	3.25	-0.43	3.68	-0.13
Mean	34.33	3.38	0.38	3.00	0.11
SE	2.32	0.072	0.172	0.195	0.052

Table C18 ^{14}C Urea Infusion Data And Urea and Creatinine Plasma Concentrations (mM) For The Animals On The Meadow Hay Diet

a) CONTROL ANIMALS

Tag	Group	Dose DPM	VolInf (ml)	Time (h)	DPM/ml Plasma	Plasma Urea	Plasma Creat
80	1	636400	423.1	13.22	930.29	5.73	0.087
115	1	636400	421.2	13.22	1006.88	5.04	0.073
199	1	636400	411.6	13.22	1039.13	4.59	0.090
203	1	636400	409.0	13.22	979.32	5.99	0.080
161	2	660115	435.6	13.42	945.81	5.47	0.097
195	2	660115	397.9	13.42	879.98	6.58	0.083
208	2	660115	374.0	13.42	961.59	5.16	0.077
212	2	660115	312.1	13.42	648.06	6.37	0.073
Mean						5.62	0.083
SE						0.24	0.003

b) FLEECE WEIGHT ANIMALS

Tag	Group	Dose DPM	VolInf (ml)	Time (h)	DPM/ml Plasma	Plasma Urea	Plasma Creat
4	1	636400	436.5	13.22	908.83	5.25	0.087
33	1	636400	406.3	13.22	839.12	5.46	0.070
98	1	636400	430.5	13.22	1346.29	3.97	0.070
156	1	636400	423.2	13.22	989.73	4.08	0.070
47	2	660115	224.7	7.90	680.89	4.93	0.067
88	2	660115	357.7	12.90	1081.50	4.23	0.077
194	2	660115	350.2	13.42	858.11	6.29	0.080
232	2	660115	384.0	13.42	1014.43	3.06	0.067
Mean						4.66	0.074
SE						0.360	0.0025

Table C19 Urinary Values For Individual Animals On The Meadow Hay Diet During The Infusion Period

a) CONTROL ANIMALS

	First 24 Hours				Second 24 Hours			
Tag	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM
80	1083	21.17	0.72	14161	1034	22.72	0.93	2948
115	1484	10.45	0.66	9547	984	20.59	0.64	1984
161	907	15.72	0.80	10469	1305	10.27	0.63	2005
195	939	15.42	0.61	4517	739	22.62	0.95	1898
199	844	18.13	1.03	13677	530	21.77	-	1363
203	1523	12.22	0.63	10417	1034	22.46	0.65	2433
212	483	17.09	0.42	9062	1011	19.26	0.94	5456
Mean	1038	15.34	0.70	11836	979	19.15	0.81	2568
SE	351	3.52	0.17	5350	245	4.71	0.14	1258

b) FLEECE WEIGHT ANIMALS

	First 24 Hours				Second 24 Hours			
Tag	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM
4	1030	18.38	0.92	14604	858	19.46	-	1946
33	1089	20.83	0.99	15215	1190	22.76	1.04	1983
47	1330	12.00	0.43	5721	713	22.87	0.96	2509
88	1085	11.79	0.70	10289	1295	12.97	0.78	1659
98	1126	16.74	0.45	13456	958	26.49	0.98	2339
156	1069	16.61	0.49	11863	1185	17.45	1.06	2081
194	1508	7.50	0.41	12708	1029	11.22	0.63	8043
232	1005	12.97	0.84	13294	1189	13.46	0.79	1153
Mean	1155	14.60	0.65	12144	1052	18.34	0.89	2714
SE	173.6	4.30	0.239	1065.9	70.3	1.945	0.061	775.2

Table C20 Urea Clearance,Creatinine Creatinine And N IRL Rate From The Plasma In Sheep Fed On Meadow Hay

a) CONTROL ANIMALS

Tag	NIRL (gN/d)	Recy (gN/d)	Excret (gN/d)	Prop Recy	UreaCl (ml/min)	CreatCl (ml/min)
80	14.72	3.88	10.84	26.39	48.88	53.34
115	13.54	4.61	8.93	34.02	43.97	57.95
161	15.23	8.53	6.70	55.98	34.53	-
195	14.95	7.51	7.44	50.22	43.63	55.22
199	12.82	6.42	6.39	50.18	30.31	43.31
203	13.52	2.97	10.54	22.00	28.06	41.66
208	12.86	6.22	6.64	4.36	31.91	59.29
212	15.93	9.59	6.33	60.24	24.63	36.70
Mean	14.19	6.22	7.98	37.92	35.74	49.63
SE	0.41	0.813	0.663	6.882	3.29	2.65

b) FLEECE WEIGHT ANIMALS

Tag	NIRL (gN/d)	Recy (gN/d)	Excret (gN/d)	Prop Recy	UreaCl (ml/min)	CreatCl (ml/min)
4	15.54	7.21	8.33	46.40	75.44	39.36
33	15.67	4.09	11.58	26.08	52.60	87.50
47	18.54	10.23	8.31	55.19	65.65	56.36
88	11.38	4.51	6.87	39.61	54.36	66.08
98	10.35	0.16	10.51	0.15	41.77	56.37
156	13.84	4.89	8.95	35.31	40.27	60.80
194	13.50	7.96	5.54	58.96	21.84	43.66
232	12.52	5.76	6.76	45.98	54.78	70.98
Mean	14.12	3.58	8.36	38.46	46.98	63.11
SE	0.98	3.14	0.708	6.617	5.06	5.21

Table C21 Infusion Information And Urea And Creatinine Plasma Concentrations (mM) In The Sheep Fed On The Lucerne Chaff Diet

a) CONTROL ANIMALS

Tag	Group	Dose DPM	VolInf (ml)	TDose (DPM)	DPM/ml Plasma	Plasma Urea	Plasma Creat
80	2	831686	454.8	450299	457.17	10.52	0.060
115	2	831686	458.7	454160	509.01	9.11	0.067
161	1	824321	446.0	437675	465.46	9.57	0.070
195	1	824321	419.6	411768	492.06	9.05	0.070
203	2	831686	429.3	425051	549.92	8.97	0.057
208	2	831686	419.7	411866	463.16	8.38	0.050
212	1	824321	443.0	434731	455.81	10.03	0.060
Mean						9.38	0.062
SE						0.272	.0028

b) FLEECE WEIGHT ANIMALS

Tag	Group	Dose DPM	VolInf (ml)	TDose (DPM)	DPM/ml Plasma	Plasma Urea	Plasma Creat
4	1	824321	457.2	452675	475.82	9.08	0.063
33	1	824321	429.6	425348	474.83	8.66	0.060
47	2	831686	437.3	429138	416.27	8.65	0.047
88	2	831686	449.3	441306	578.20	7.62	0.050
98	1	824321	460.4	455843	537.56	6.92	0.053
156	1	824321	453.0	448852	505.85	8.14	0.060
194	2	831686	421.9	414025	477.39	8.78	0.060
232	2	831686	447.2	438553	575.76	8.12	0.057
Mean						8.25	0.056
SE						0.250	.002

Table C22 The Individual Urinary Values For Animals On The Lucerne Chaff During The Infusion Period

a) CONTROL ANIMALS

	Day One				Day Two			
Tag	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM
80	2301	-	-	11944	3003	16.35	0.43	302.8
115	3034	12.72	0.34	7961	1775	28.23	0.60	500.0
161	1765	26.90	-	1279	1581	30.05	0.70	618.8
195	2201	23.72	0.67	11955	1564	27.85	0.56	627.3
203	2474	14.99	0.39	9179	2822	17.73	0.43	449.2
208	2229	24.42	0.65	1248	2459	19.27	0.51	228.7
212	1573	29.70	-	10807	1445	27.13	0.49	8259.5
Mean	2254	22.08	0.51	7768	2521	23.80	0.53	1569.5
SE	487.2	2.753		1765.1	612.0	2.177	0.014	1116.4

b) FLEECE WEIGHT ANIMALS

	Day One				Day Two			
Tag	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM	Vol(g)	[Urea] (g/l)	[Crt] (g/l)	DPM
4	2699	21.86	0.61	12526	1831	29.89	0.68	636.3
33	2594	21.22	0.58	10287	1955	28.62	0.75	489.3
47	1957	28.40	0.54	12089	1581	31.64	0.56	548.0
88	1971	21.91	0.31	11290	1606	30.64	0.61	1030
98	2847	15.82	0.44	9713	2143	22.66	0.58	380.4
156	2689	21.81	0.57	9856	2552	22.42	0.57	402.1
194	4405	8.80	0.22	5137	2819	15.25	0.37	263.4
232	3065	14.31	0.46	6832	1759	21.82	0.56	492.5
Mean	2778	19.27	0.47	9716	5874	25.37	0.59	530.3
SE	270.8	2.127		902.7	1536.5	2.023	0.039	81.78

Table C23 Urea Clearances, Creatinine Clearances And IRL Rates Of Animals on The Lucerne Chaff Diet During The Infusion Period

a) CONTROL ANIMALS

Tag	NIRL (gN/d)	Recy (gN/d)	Excret (gN/d)	Prop Recy	UreaCl (ml/min)	CreatCl (ml/min)
80	39.73	-	-	-	-	-
115	35.99	13.03	22.96	36.20	62.47	105.55
161	37.93	15.71	22.22	41.42	57.62	-
195	33.76	11.11	22.64	32.93	62.00	87.59
203	31.18	10.97	20.71	35.19	55.85	101.40
208	35.87	11.99	23.88	33.42	70.68	143.99
212	38.47	18.47	20.00	48.01	49.43	-
Mean	36.13	13.55	22.34	37.86	59.68	109.63
SE	1.11	1.214	0.589	2.378	2.934	12.080

b) FLEECE WEIGHT ANIMALS

Tag	NIRL (gN/d)	Recy (gN/d)	Excret (gN/d)	Prop Recy	UreaCl (ml/min)	CreatCl (ml/min)
4	38.38	11.04	27.34	28.77	74.61	122.22
33	36.14	9.70	26.44	26.83	75.71	133.50
47	41.59	16.81	24.77	40.43	70.99	110.33
88	30.79	8.87	21.92	28.80	71.31	87.14
98	34.21	11.82	22.39	34.54	80.25	126.43
156	35.77	8.73	27.03	24.42	82.33	131.84
194	34.99	14.72	20.26	42.09	57.18	94.05
232	30.75	10.44	20.31	33.94	62.03	114.89
Mean	35.33	11.52	23.81	32.48	71.80	115.05
SE	1.28	1.016	1.045	2.260	3.034	6.044