WOOL GROWTH EFFICIENCY

A study of the effects of liveweight status and diet on wool growth

A Thesis
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by

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SUMMARY

The studies reported in this thesis investigated the influence of diet and liveweight change on the efficiency of wool production, defined as wool growth rate per unit of feed consumed. There is no general agreement among researchers as to the precise nature of the relationships and an examination of the literature revealed that this lack of unanimity may be largely due to omissions in design. Few wool growth experiments have taken into account a number of interacting variables that are likely to affect the relationships, namely seasonal wool growth rhythms, diet composition and intake level, the residual effects of previous diet, and the genotype of the experimental sheep. The first experiment reported in this thesis was initiated to test the hypothesis that the amount of wool produced at any level of dietary intake is influenced by the rate and direction of liveweight change. Sheep were fed different quantities of a standard barley/lucerne diet in a crossover design so that a range of weight changes was induced at each level of feed intake. In this way the impact of weight change *per se* on wool growth was estimated. Despite substantial individual bodyweight responses (-57 to +158 gd^{-1}), there was no evidence of any enhancement of wool growth during weight loss, nor of depressed wool growth as a consequence of weight gain. In this study wool growth rate was estimated at, or near, equilibrium with each new nutritional regime. The possibility remained that change in liveweight with its concomitant effects on nutrient availability, was responsible for a portion of the lag in wool growth response. A small experiment run concurrently

with Experiment 1 using autoradiographic and mitotic rate studies, revealed that the lag was related to the time required for changes in mitotic rate and follicle bulb dimensions to occur.

A feature of this study was the high variation in wool growth efficiency of sheep fed the standard barley/lucerne diet that was used throughout the experiment. The coefficient of variation increased from an estimated 12% at the beginning of the trial to as high as 40% at the end of the study. The responses of bodyweight to diet level, on the other hand, were within the range normally expected. Thus the statistical tests were not as sensitive as had been planned. Nevertheless, there was no suggestion of anything but a proportional relationship between intake level and wool growth rate, regardless of weight change.

In subsequent experiments the factors associated with the high variability in wool growth efficiency were examined in detail, since there is no evidence in the literature of a wool growth variability/diet interaction. Yet diets containing cereal grains are commonly used for drought feeding and fattening store animals. It was established in Experiment 3 that wool growth variance in efficiency was related to diet composition and not to any differences between sheep in genetic wool growth potential. The source of variability in efficiency was identified in Experiment 4 when the hypothesis was tested that the variations in wool growth were more a reflection of protein flow to the abomasum, then of events between absorption and synthesis. Postruminal protein flow, in turn was related to the pattern of ruminal fermentation induced in the sheep. This appears

to be the first time that variations in wool growth rate among sheep receiving the same diet in similar amounts has been related to the flow of digesta constituents from the rumen. The results indicate that selection of sheep for wool growth on diets containing a high proportion of cereal grain (and possibly starch), may be subject to substantial error in terms of potential wool growth ranking. Furthermore, the variations in protein flow may be substantial on this type of diet and studies designed to characterise rumen metabolism and duodenal protein availability on such diets would require a large number of sheep to obtain accurate estimates.

TABLE OF CONTENTS PAGE NO.

STATEMENT

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

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PREFACE

Ruminants, through their capacity to utilise cellulose and non-protein nitrogen as energy and protein substrates have assumed an important economic and ecological role in the production of meat and fibre from plant organic matter. However as competition from "non-animal" enterprises for land resources increases, so too does the necessity for maximising the efficiency of animal production. Furthermore, as the "terms-of-trade" facing the livestock producer inevitably decline, high output of produce per unit input becomes paramount.

In this regard, it has been asserted that "it is impossible for a sheep enterprise to achieve simultaneously the highest possible values for efficiency of meat production (Em) and efficiency of wool production (El) ..... due to the opposite effect of liveweight growth on Em and El" (Irazoqui, 1970). Evidence regarding this proposed interaction is equivocal, and it will be contended in this thesis that failure to account for the dynamic nature of wool fibre responses has led to the misconception that efficient body growth and efficient wool growth are incompatible objectives.

A further aspect studied in this thesis concerned the efficiency of wool production on diets of different composition to elucidate an apparent "diet x sheep" interaction.

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER 1: Factors influencing the efficiency of wool growth rate

REVIEW OF LITERATURE

Introduction

1.1 The measurement of wool growth rate.

- 1.1.1 Mid-side patch technique
- 1.1.2 Dyebanding technique
- 1.1.3 Autoradiography
- 1.1.4 The time responses of wool growth to nutritional change
  - 1.1.4.1 Possible causes of the time lag
  - 1.1.4.2 Implications of the time lag on experimental design and analysis

1.2 Diet intake, diet composition and wool growth rate.

- 1.2.1. The influence of protein and energy supply and utilisation on wool growth
- 1.2.2. The nature of the relationship between feed intake and wool growth rate
  - 1.2.2.1 Curvilinear relationships
  - 1.2.2.2 Linear relationships with declining efficiency as intake increases
  - 1.2.2.3 Simple proportional relationships
  - 1.2.2.4 Summary

Introduction

"Efficiency" is a term widely used in the context of animal production systems, and is based on the generality of output per unit input. Wool growth, like other production characters, is highly dependent on the nutritional status of the sheep. However, when correction is made for differences between sheep in dietary intake, there remain large variations in efficiency which have been attributed to the interaction of genotype, season, diet quality, method of measurement and bodyweight status. These factors have been the subject of several reviews (Hutchinson and Wodzicka 1961; Ryder and Stephenson 1968; Downes et al. 1976; Irazoqui 1978), and in the most recent publication, a detailed account has been provided of the physiological and environmental limitations to wool production (Black and Reis 1979). It is the object of the present review to examine the relationship between feed intake and wool growth rate (WGR) and the factors which modify the response curve, with special emphasis on the significance of each factor in the design and interpretation of wool growth experiments.

Section 1.1 The measurement of wool growth rate

The measurement of wool growth appears to be deceptively simple, yet the most common method, that of shearing the whole sheep, is not without error. When a sheep is shorn, the wool below the cutting piece is left behind to be included as part of next year's fleece. Provided the animals are shorn at the same time each year, and nutritional conditions are similar, the error is probably small.

The problem of measurement becomes more complex when an attempt is made to relate wool growth to nutritional changes especially when the feeding regimes are of short duration and shearing becomes impractical.

Wool follicle activity is very sensitive to variations in the intake of nutrients, and the rate at which wool is produced (WGR) fluctuates according to diet, endocrine status, photoperiod, and the time taken for WGR to come into equilibrium with a change in diet. All of these effects need to be quantified in studies of wool growth, and techniques have been developed which provide accurate estimates of WGR over relatively short periods. There are, however, problems of interpretation of these measurements which have led to a lack of unanimity in the literature on the relation of WGR and efficiency to diet.

1.1.1 The mid-side patch method

Repeated clipping of defined areas of skin has been used for some time to estimate WGR (Marston 1955 cites the use of the technique by Sir Charles Martin in 1932), and it remains a popular means of defining wool responses to nutrition (Ferguson 1956, 1962; Schinckel 1960; Arnold

et al. 1964, 1965), genotype (Dunlop et al. 1960), and season (Coop 1953; Bigham et al. 1978). While results are often expressed as patch wool production per unit time, differences in patch size can render such data insensitive to between-sheep (or treatment) differences since for each sheep the patch will represent a different proportion of the wool-bearing area. Nevertheless, in the adult sheep the number of follicles enclosed within a patch remains unaltered despite changes in patch size due to the growth of the animal, so that within-sheep differences are valid and provide a good estimate of the relative differences in WGR from occasion to occasion.

Yeates et al. (1975) suggest that for many purposes an area can be defined using the wool clippers. Subsequent wool harvests are then made taking care not to enlarge this area. More accurate estimates require the tattooing of the patch area, usually centred over the last rib midway along the dorso-ventral curvature. Wool is clipped within this area (usually 10 x 10cm) at intervals not less than 14 days, using an Oster small animal clipper fitted with a No. 40 blade. Wool weight thus obtained can be expressed per unit area (Ferguson et al. 1949), or per patch. The unit area method is open to serious criticism since it is influenced by changes in patch size that occur as a sheep grows. None of these methods estimate total wool production, a critical factor in many comparisons. An early method used to estimate total WGR from patch wool weight was based on the assumption that total skin surface area is related to bodyweight (Marston 1948; Ferguson 1972). Sheep are not geometrically uniform, neither is wool uniformly distributed over the body

and imprecision stems from poor choice of the factor relating total wool-bearing surface area to bodyweight. Moreover estimation of liveweight is itself imprecise (Hogg 1977). Thus, values based on production per unit area and estimates of total surface area are too crude for investigational work and may even be misleading.

A convenient refinement, of the patch technique, which estimates total wool grown by an animal, is based on the proportionality between patch production and total fleece production (Equation 1.1).

$$\underline{\text{Equation 1.1}} \ldots\ldots \text{WGR}(gd^{1}) = \frac{A.B}{C.D}$$

where A is the clean wool grown within the patch in any period; B is the clean dry fleece weight grown between shearings; C is the length of the clipping interval; and D is the total wool grown on the patch between shearings (after Langlands and Wheeler 1968)

Several assumptions are implicit in this technique;

1. that the midside region provides an unbiassed estimate of relative WGR in the whole fleece,
2. that the shearing pile is the same prior to, and at the conclusion of the trial, and
3. that clipping <u>per se</u> does not alter WGR.

Bigham (1974) and Wodzicka and Bigham (1968) present evidence that the patch/fleece wool weight ratio is not constant but varies with time after shearing and with season. These, and other reports of altered WGR on clipped patches, may be attributed to local cooling of the clipped

region. Depressed WGR at low temperatures has been widely reported (Bennett *et al.* 1962; Doney and Griffiths 1967; Slee and Ryder 1967; Downes and Hutchinson 1969; Lyne *et al.* 1970), the main determinant being reduced fibre length growth rate (Downes and Hutchinson 1969). That these effects are attributable to temperature and not clipping *per se* is supported by the rapid recovery of growth at warmer temperatures (Downes and Hutchinson 1969), and the absence of a WGR depression when patches are covered (Downes and Lyne 1961). At more moderate temperatures than those used in the experiment of Downes and Hutchinson (1969) ($2^{o}C$), repeated clipping and infrequent clipping produce similar total patch weights (Coop 1953: Downes and Lyne 1961; Bigham 1974). In contrast, there are two reports in the literature of an upwards biassing of fibre diameters estimated by clipping (Sharkey *et al.* 1962; Langlands and Wheeler 1968). No unequivocal statement regarding clipping effects can be made at present, although the weight of evidence indicates that at moderate temperatures or when the patch is covered, the method provides an accurate estimate of total fibre growth. Moreover, Henderson (1953) demonstrated the suitability of the midside region for the measurement of relative changes in WGR in the rest of the fleece.

The emergence time delay

A major problem with clipping techniques is the difficulty of harvesting wool at the same height above the skin each time, particularly in Merinos with wrinkly skin. Unclipped fibre between the site of fibre synthesis and the clipper level represents a source of error amounting to the equivalent of from 4-10 day's wool growth, depending on the

WGR (Downes and Sharry 1971). To account for this "emergence time" delay, the clipping can be delayed by an arbitrarily selected period, say 7 days after a change in diet, or, less frequently, the change in fibre output can be determined at the follicle bulb level by autoradiography (vide infra).

1.1.2 The dyebanding method

Apportioning of the fleece growth into short periods is also made by reference to wool grown between bands of dye applied to the base of the wool staples at intervals of not less than 3 weeks (Chapman and Wheeler 1963; Williams and Chapman 1966). The staple grown during a shearing interval is removed and cleaned, and the total wool growth in a dyeband interval is apportioned according to the proportion of wool grown in that period relative to total staple weight. The non-destructive nature of this method overcomes the major disadvantage of the clipping technique, namely the effect of clipping and exposure of the skin on WGR. Furthermore fibres are marked at the skin level, whereas a wool pile of approximately 1.5mm remains when the standard Oster clipper is used (Williams and Chapman 1966). A further advantage of dyebanding is the speed at which it can be carried out, thereby allowing a large number of sheep to be measured per unit labour input. The interval between successive dyebands must be greater than three weeks, because the dye tends to move up the fibre, particularly in genotypes with a high wool suint and grease content (Yeates et al. 1975). Consequently, when the interval is too short the previous dyeband may be obscured. To overcome this effect WGR over shorter periods WGR over shorter periods is

obtained by dyeing two adjacent sites at staggered intervals (Kenney 1978).

The precision of the dyebanding method is similar to that of the mid-side patch method (Langlands and Wheeler 1968; Wheeler et al. 1977).

1.1.3 Autoradiography of fibres

Downes and Lyne (1959) described a means of measuring fibre growth rate by labelling with tracer doses of intravenously-administered ^{35}S-cystine. Subsequent microscopic examination of the fibres and superimposed X-ray films enables both length growth rate and diameter to be accurately determined over periods as short as four days (Downes et al. 1967).

The technique has been used to determine the effects of temperature and nutrition on fibre growth (Downes and Hutchinson 1969; Downes and Sharry 1971; Reis and Tunks 1969; 1978). In contrast to the previously described techniques, autoradiography allows an instantaneous measure of fibre response to treatments without the "emergence time" delay described above. Precise length and diameter measurements are thus obtained. Cost limits this method to use over short periods and to small numbers of sheep.

1.1.4 The time responses of WGR to nutritional change

Following an alteration in diet quantity or quality, the output of wool fibre does not immediately reflect the new nutritional status of the animal (Marston 1948). While this has been known for some time, workers have varied in the emphasis placed on estimating equilibrium WGR, often because the measurement of wool growth has been a secondary

objective (Allden 1979).

Application of the autoradiographic technique to short term supplementation trials has revealed that fibre growth rates determined from changes in length and diameter are relatively stable after 8 days (Reis and Downes 1971; Reis & Tunks 1978). In these trials, supplements of individual amino acids or proteins were infused post ruminally and emergence time plus equilibrium time by this method would be 14-18 days. In contrast, when nutrition is improved by increasing dietary intake, the equilibration of WGR with diet is much slower, despite the rapid initial responses of the fibres (Downes and Lyne 1961). The time from nutritional change to WGR equilibrium has been termed the lag period, and Table 1.1 summarises this period as defined by each author in a number of wool growth experiments.

In each of these experiments, WGR was measured using the clipping technique, although Downes and Sharry (1971) also used autoradiography. The equilibrium times presented in the table varied between 2-20 weeks with no apparent significant trend related to the direction of the nutritional change.

Nagorcka (1977) analysed WGR data using a time-dependent description, rather than the normal static least squares method and determined a lag period of 25 days, a value consistent with that estimated by autoradiography. More recently the lag period has been defined in a more dynamic sense as the time taken for two-thirds of the WGR differential to occur (White et al. 1979), however failure to account for the remaining 33% of the wool growth response could lead to erroneous conclusions.

Table 1.1 Time required for WGR to equilibrate with a dietary change (based on each author's own definition of equilibrium)

Authors	Dietary change	Equilibrium time (weeks)
1. Marston (1948)	0.5 maintenance - 2.0 maintenance	12 weeks
2. Reis and Schinckel (1961)	Low nitrogen intake	8-10
	High nitrogen intake	2
3. Sharkey *et al.* (1962)	Grazing	4 (diameter 12)
4. Ferguson (1962)	$500gd^{-1}$-*ad lib.*-$500gd^{-1}$	4
5. Barry (1972)	($775gd^{-1}$ casein)-pasture	5
6. Barry (1973a)	methionine infusion	4-6
7. Hogg (1977)	restricted-*ad lib.*	4
8. Coop (1953)	n.a.	12
9. Langlands and Donald (1977)	pasture - (280, 403, 524, 644g DOM d^{-1})	20
10. Downes and Sharry (1971)	$400-1000gd^{-1}$; $1400-500gd^{-1}$	3

+ NB These estimates are based on the author's definition, and not necessarily supported by wool growth/time relationships.

It appears, then, that a lag period of from 14-25 days can be expected, allowing from 10-15 days for changes to become established in the follicle, and 4-10 days for emergence. The evidence also suggests that the response may be more rapid when simple nutrients are supplied postruminally (e.g. Reis 1969; Reis & Downes 1971) than when dietary intake is altered (Table 1.1).

1.1.4.1 Possible causes of the time lag of wool growth

Clearly then, factors other than the emergence time are responsible for the long equilibrium times apparent when the nutritional status of a sheep is altered.

Firstly there are physical considerations involving the wool follicle itself, there being a positive association between follicle bulb size and fibre synthesis (Schinckel 1962; Wilson and Short 1979a). The possibility that time is required for follicles to change dimensions in response to nutrition was suggested by Fraser (1965), although this appears to conflict with the rapid fibre responses observed by autoradiography. At the present time no satisfactory conclusion can be drawn on this point because there have been no serial estimates of changes in follicle bulb dimensions with nutrition.

There is also evidence that during a period of severe undernutrition, the active follicle population is reduced. After refeeding, the regeneration of mitotic activity in these quiescent follicles may take up to 12 weeks to be complete (Lyne 1961). Such an effect would be limited to only a small range of the nutritional changes commonly experienced.

Stabilisation of body protein stores with a new

nutritional regime may take up to 6 weeks in the sheep (Reis & Schinckel 1961), an effect which could delay wool growth responses. Thus Marston (1948) advanced the hypothesis that increased availability of amino acids from catabolised body tissues would maintain the WGR at its original level when sheep were offered a low level of nutrition. Indeed, short-term fibre growth responses to decreased intakes have been notably slower than to increased intakes (Downes and Sharry 1971). These authors postulated that keratin precursors (cystine) in the skin maintain the WGR for some time after a diet change. The changes in skin thickness and protein content of skin with nutrition, observed by Hutchinson (1957), lend support to this concept. Similarly, competition for nutrients between wool and "non wool" tissues at intakes above maintenance, may delay the wool growth response. This subject is considered in a later section.

If the wool growth response lag is a consequence of competition for nutrients, a longer lag would be anticipated when the rate of bodyweight gain is greatly enhanced. No such result was noted by Hogg (1977) when WGR responses during compensatory growth were examined. A lag period of more than 30 days recorded by this author is similar to that recorded commonly in the literature (Table 1.1). Moreover, the lag in response has been observed when changes in bodyweight were minimal (Moran 1970), although nitrogen retention was not estimated in this trial.

It is concluded that the prime causes of the wool growth lag have not been completely elucidated. Certainly the lag due to emergence time and that associated with the

establishment of changes in the follicle which influence fibre diameter and length growth rate would account for from 2-3 weeks, but it is not clear whether the delay at the follicle level is a consequence of slow physical changes in follicle bulb dimension or a result of reduced amino acid availability during rapid growth and enhanced availability during weight loss. These conclusions are based on fragmentary evidence since there has been no study that has measured WGR during short intervals following a change in diet, and related these to bodyweight status.

Section 1.2 Diet intake, diet composition and wool growth rate

In this section the relationship between feed intake and WGR is examined. The effects of season, genotype and weight change on that relationship are considered later in this review.

Firstly, the relative roles of protein and energy in altering WGR need to be examined because the impact of weight change on wool growth efficiency is dependent on the interaction between these nutrients.

1.2.1 The influence of protein and energy supply and utilisation on WGR

There is little doubt that wool production is largely dependent on the supply of amino acids to the follicle bulbs, a suggestion made as early as 1948 by Hedley Marston. The role of energy per se on the other hand, has only received attention more recently. The results of these investigations suggest that effects of energy on WGR are mediated via protein supply, either by altering microbial protein synthesis (Smith 1975) or by influencing

postabsorptive protein metabolism (Black et al. 1973).

Early work on the relationship between WGR and protein or energy intake was characterised by a failure to account for the modifying influence of the rumen on the supply of nutrients to the animal. An "oft-quoted" example of this is the experiment of Ferguson (1959) in which WGR was poorly related to protein concentration above 8% when a range of diets was fed. Subsequently work by Hogan and Weston (1967a, b) provided strong evidence that the postruminal amino acid supply was not increased on the higher protein rations. To overcome these effects of digestion in the rumen, nutrients have been supplied postruminally, in particular individual amino acids (Reis and Schinckel 1963, 1964; Reis 1967; Langlands 1970; Dove and Robards 1974; Reis and Tunks 1978) and whole proteins (Reis and Schinckel 1961; Reis 1969; Colebrook and Reis 1969; Egan 1970). Alternatively, proteins have been protected from ruminal catabolism by chemical treatment (Ferguson 1972; Barry 1972, 1973b, 1976), by using naturally protected proteins such as fishmeal (Kempton et al. 1978), or by maintaining the sucking reflex in lambs (Walker and Cook 1967; Walker and Norton 1971). It became clear from these studies that wool growth is closely associated with protein supply, and in particular with the supply of the sulphur amino acids (Reis and Schinckel 1963, 1964), a finding that is hardly surprising in the light of the rapid rates of wool protein turnover (Wilson and Short 1979a, b), and the high cystine content of wool keratin (Corfield and Robson 1955). It might thus be anticipated that enhanced ruminal microbial protein synthesis would increase wool production. Indeed, Ferguson (1972) found, on

examination of a range of diets, that the non-protein digestible organic matter fraction had a constant, positive effect on WGR, consistent with its effect on microbial protein synthesis.

Energy sources

There is also evidence that energy availability influences WGR apart from its effects on microbial protein synthesis. Bullough and Laurence (1958) studied hair follicles of mice "in vitro" and found that adequate supplies of carbohydrate substrate and oxygen were essential for active mitosis. Enzymes that inhibited the TCA cycle or glycolysis, depressed mitotic rate in these studies. Similarly, Ryder (1958) demonstrated that radioactive glucose was rapidly incorporated into mitotic cells and into the follicle outer root sheath, where it is stored as glycogen.

In a detailed examination of follicle bulb preparations Leng and Stephenson (1965) observed that both glucose and acetate were actively oxidised. They concluded that high turnover of DNA and RNA by bulb cells is a result of the production of ribose in the pentose phosphate pathway, and that non-essential amino acids for protein synthesis may be produced by transamination from TCA intermediates. Blood glucose or follicle glycogen (Ryder 1958) would provide suitable substrates for these reactions. Black and Reis (1979) have estimated the energy requirement for maximum WGR as 3.7 mmoles ATP/minute. Obviously the requirement for hexose to provide this energy will depend on the relative importance of anaerobic and aerobic pathways in the follicle metabolism. Assuming, in the absence of firm evidence, that

half of the available glucose is metabolised anaerobically, then 48g glucose would be required at the follicle level each day for maximum WGR. This would represent an appreciable drain on the animal's available glucose (Lindsay and Williams 1971). This value, in fact, may be an underestimate if additional glucose is required for ribose production as suggested earlier. That the glucose requirement will be high is supported by data of Adachi and Uno (1969). Only about one quarter of the glucose metabolised in the hair follicle entered the TCA cycle (Black and Reis 1979).

In an attempt to isolate the effect of energy supply per se on WGR, Ball et al. (1972) supplemented lucerne hay with oils from various oilseed crops. While liveweight gain was increased by supplementation, neither VFA pattern in the rumen, nor WGR was altered. The authors concluded that energy supply had little effect on WGR, although no direct measurement of microbial protein synthesis was made.

Infusion studies with protein and energy

In more recent studies the post-ruminal supply of protein and energy have been altered independently, by feeding lambs liquid diets, which enter the abomasum directly (Walker and Norton 1971), or by infusing liquid diets directly into the abomasum of mature sheep (Black et al. 1973). In both trials WGR was determined by the availability of both protein and energy. When protein supply was low, WGR was stimulated by additional protein but was reduced by additional energy. Conversely, when protein was not limiting, extra protein caused a slight decline in WGR, whereas added energy stimulated WGR. The results indicate

that there is an optimum ratio of absorbed protein relative to energy, the value of which has been calculated as approximately 12 to 1 although it may vary with digestible energy intake level (Kempton 1979).

Nutrients providing energy which are absorbed from the tract may influence WGR by altering the intermediary metabolism of amino acids. The provision of energy when protein supply is high may thus "spare" amino acids which would otherwise be deaminated during gluconeogenesis. Moreover the nutrients supplied to the sheep in the studies of Walker and Norton (1971) and Black et al. (1973) are not those normally absorbed by ruminants (Kempton 1979). Provision of high levels of glucose may thus have influenced WGR through changes in hormonal status or the efficiency of utilisation of other nutrients (Kempton 1979) and not as a consequence of energy level at all. The suggestion that the infusion of liquid diets into mature sheep may produce effects not normally present in functioning ruminants is supported by a recent trial in which a liquid diet similar to that used by Black et al. (1973) was infused intra-abomasally. Abnormal wool growth and shedding of fleeces occurred (Chapman and Black 1981).

The calculation of protein/energy ratios in the studies of Black et al. (1973) is based on the assumption that the nutrients supplied were absorbed with the same efficiency as normal nutrients. Alterations to the mucosae of the small and large intestines, described by Black et al. (1973), may suggest that this is not necessarily the case when sheep are maintained on liquid diets.

Clearly WGR is closely associated with the quantity and

composition of protein absorbed from the small intestine. Effects of absorbed energy are less well defined but appear to operate by altering the availability of amino acids to the wool follicle. Unless there is sufficient protein of the right quality there is unlikely to be any effect of energy intake on the WGR of normally fed sheep as has been noted by Egan (1970) and Dove and Robards (1974).

1.2.2 The nature of the relationship between feed intake and WGR

It has long been recognised that WGR, like other production characters is related both to the quality of a diet and to the level of intake of that diet (Weber 1931; Krishnan 1939; Marston 1948), or more correctly, to those components of the diet which influence duodenal protein supply. This section is not concerned with qualitative effects but rather with wool responses to quantitative intake of a particular ration. The nature of the association between WGR and intake of a diet has yet to be elucidated. There is no general agreement between different workers, and three relationships have been proposed. Two of these imply that the quantity of wool produced per unit of feed intake declines as the intake level increases, whereas the third represents a simple proportionality, so that WGR/Intake is constant. Clearly it is important to determine which of these is correct. As Langlands and Donald (1977) point out, a simple relationship means that wool production per unit area of land will be proportional to intake per unit area and independent of intake per animal. On the other hand, if WGR/Intake is inversely related to intake, then the highest wool production per area will be achieved when intake per

animal is lowest.

1.2.2.1 Curvilinear relationships

Ferguson et al. (1949) proposed "that the relation of wool growth rate to nutrient intake would follow the familiar law of diminishing returns", as defined in Equation 1.2.

Equation 1.2.....$WGR=A-Ae^{-k(I-Io)}$

where WGR is wool growth rate, I is intake, I_o is intake when WGR is zero, A is the asymptotic WGR and k is a constant dependent on diet and sheep genotype. It was considered that the asymptote (A) represents the maximum wool growth potential of the animal, a value genetically determined (Ferguson 1956). At this asymptote the rate of follicle bulb cell division is maximal, coinciding with a minimal cell turnover time of about 15 hours (Black and Reis 1979). To attain this maximum WGR, Reis (1969) has estimated that about 150g of protein would need to be digested in the intestines each day. To supply this amount of protein large quantities of a protein-rich feed would be required. Even so the attainment of such high levels of WGR on herbage diets would be doubtful because of the substantial loss of nitrogen across the rumen wall during digestion (Egan et al. 1975). Furthermore, only 75% of the nitrogen leaving the abomasum is truly digested in the intestines (Hogan and Weston 1968). The required intake of most diets is probably beyond the intake capacity of the animal, or can only be maintained for a short period of time (Daly and Carter 1955; Ferguson 1959; Schinckel 1960). This may explain why there

are few reports of curvilinear wool growth responses to increasing levels of intake.

The concept of a ceiling WGR for any genotype is nonetheless valid and has been demonstrated in trials in which the obligatory high protein requirement has been met by postruminal protein supplementation (Reis 1969; Reis and Downes 1971; Black et al. 1973). Hogan et al. (1979) have assessed the maximum rates of wool growth of Australian merino genotypes, albeit on limited available data. It is noteworthy that few experiments with Merinos have approached these maxima.

1.2.2.2 Linear relationship with declining efficiency as intake increases

While there are few reports of a curvilinear relationship, there are many which indicate that as intake increases the WGR per unit intake decreases (Ferguson et al. 1949; Ahmed et al. 1963; Williams 1966; Pattie and Williams 1967; Moran 1970; Saville and Robards 1972; Robards et al. 1974; 1976b).

Equation 1.3.....$WGR = a + bI$

where 'a' is WGR when intake is zero, 'I' is intake and b is a constant dependent on both diet and genotype. A positive WGR value when intake is zero lends support to the concept that wool is growing at the expense of body tissues. However there is some suggestion that experimental design has confounded the effects of time (see Section 1.4), season (Section 3) and diet digestibility (Allden 1979).

As dry matter intake increases, the digestibility of

some feedstuffs decreases (Blaxter et al. 1956; Armstrong 1964). For this reason the wool growth response to high intakes might be diminished because less nutrients are available per unit of intake, although changes in digestibility with intake are unlikely to be solely responsible for the form of the relationship. (Allden 1979) This author also demonstrates clearly from data of Langlands and Donald (1977) that failure to account for the time lag of wool response can lead to erroneous conclusions regarding the relationship. As previously mentioned in section 1.1.4, the measurement of WGR responses before equilibrium has been attained will produce a result in which the calculated WGR/Intake response is of the form in Equation 1.3.

1.2.2.3 Simple proportional relationship

In other studies, WGR has been directly proportional to the intake of a given diet (Equation 1.4).

Equation 1.4.....WGR = bI (Pattie and Williams 1967; Allden 1968a; Ferguson 1972; Langlands and Donald 1977).

1.2.2.4 Summary

The evidence suggests that WGR approaches a genetically determined maximum as intake increases but this ceiling level appears to be more hypothetical than real. The reason is that for diets digested predominantly in the rumen, the required intake for maximum WGR is unlikely to be maintained, so that most reports are of a linear response. There is a lack of agreement as to the form of this linear regression, although those trials in which time has been allowed for wool growth to equilibrate with diet suggest one of simple proportionality. Other reasons for conflicting

results in the literature are that animals have commonly been fed different amounts sequentially, without account being made of seasonal growth rhythms or of any possible interaction between intake and growth rhythm. Failure to correct for these factors would alter the response curve.

Section 1.3 Seasonal wool growth rhythms

1.3.1. Historical

As early as the mid 19th Century it was recognised that WGR was not constant throughout the year (cited by Hutchinson and Woodzicka 1961), although the effects of nutrition and reproduction were commonly confounded with any inherent rhythm that may have existed (Fraser 1931; Coop 1953). Since these early observations a recurring annual cycle of WGR has been clearly demonstrated in non reproducing sheep on a constant nutritional level, the WGR being maximal in summer and declining during the autumn/winter months. Thus Ferguson et al. (1949) reported seasonal variations in the WGR of Camden Park Merino and Corriedale ewes fed a uniform diet throughout the year, and noted a high correlation between WGR and ambient temperature. However temperature is also correlated with day length, and the known effects of photoperiod on breeding cycles in ewes (Yeates 1949), led to studies in which daylength patterns were reversed (Morris 1961; Hart et al. 1963), kept constant (Coop and Hart 1953), or completely removed by hooding the sheep (Hart 1961). Similarly, the effects of temperature were examined by reversing and exaggerating the temperature cycle (Morris 1961; Bennett et al. 1962). From these studies it is apparent that the seasonal wool growth rhythm is photoperiod-dependent, with

the intensity of light source a further modifying factor (Symington 1959; Slee 1965).

The rhythm is related to "an archaic pattern of shedding, regrowth and quiescence, involving a loss of the shedding phase" (Hutchinson and Wodzicka 1961). Selection for wool production has greatly diminished the magnitude of the cycle in the domesticated sheep which grows wool continuously, although shedding still occurs on the legs and face of modern sheep (Jefferies 1964). Long-wool breeds and their crosses exhibit a wide seasonal WGR difference (Hart et al. 1963), whereas fine-wool merinos grow wool at a uniform rate throughout the year (Slee and Carter 1961; Williams 1964; Doney 1966).

1.3.2. Mathematical descriptions of the cycle

The shape of the seasonal wool growth variation follows a trigonometric function of the form shown in Equation 1.5.

Equation 1.5..... $W = A_o + b_o \cos(wt - \phi)$

where A_o is the mean WGR, b_o is the half amplitude of the variation, t is time in days, w is $\frac{2\pi}{365}$ and ϕ is the phase (Jan.1 = day o) (Nagorcka 1979). This author, on examination of data from Ferguson et al. (1949) and Hart et al. (1963), concluded that an additional term could be added to this equation to account for a slight tendency for the pattern to be bimodal.

More commonly used descriptions of the rhythm are firstly, the summer/winter WGR ratio (Hill 1970) and secondly, the amplitude of the rhythm (Equation 1.6) viz;

Equation 1.6.....$A = \frac{H-L}{(H+L)/2}$

where A is the amplitude, H is the maximum WGR and L the minimum WGR (Hutchinson and Wodzicka 1961). The extent of the genetic effect on seasonal variance is reflected in the wide range of A values presented in tabular form by Nagorcka (1979).

1.3.3. Implications of seasonal wool growth rhythms on the design and analysis of experiments

Seasonal variation in WGR may be accounted for in experimental designs by the inclusion of a reference group of sheep maintained at a constant level of nutrition throughout the trial. Data for other treatment groups are subsequently corrected on the basis of WGR changes in these sheep. Implicit is the assumption that the amplitude of the cycle would be similar irrespective of the level of nutrition selected for the reference group. There are few data available to determine the impact of intake level on the non-nutritional variation, despite its importance in defining the means of adjustment.

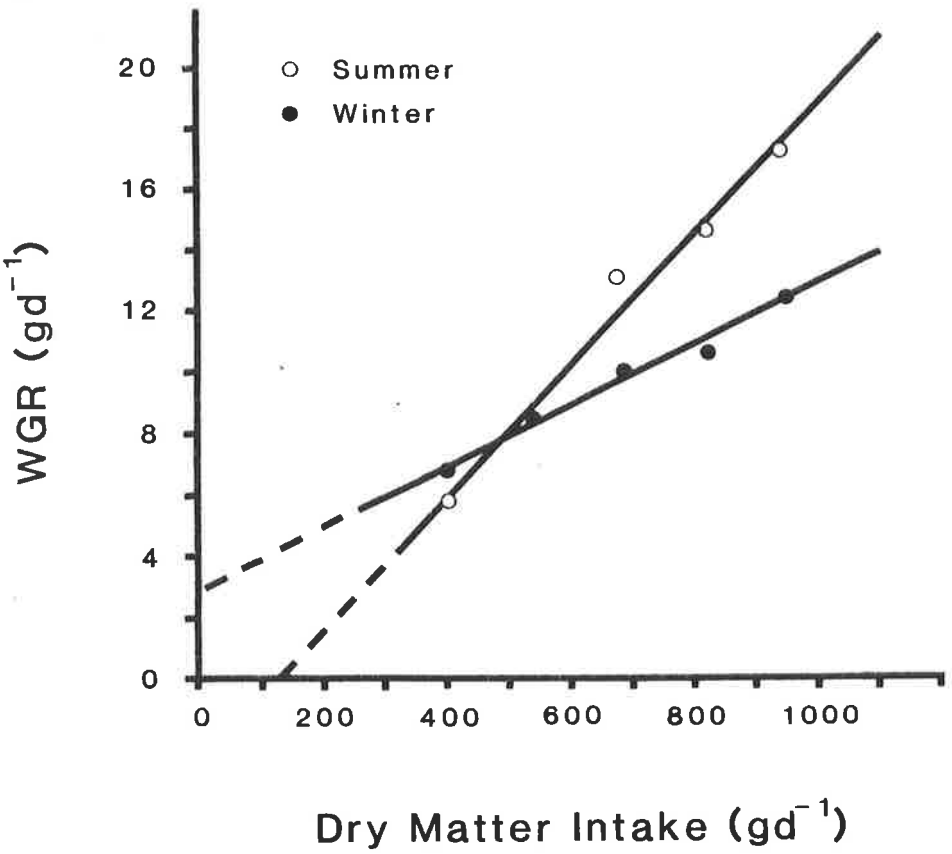
Sumner (1979) fed groups of Romney Marsh, Coopworth, Perendale and Corriedale wethers concurrently at 5 intake levels during late winter, spring and early summer. The relationship between feed intake and WGR changed with season (Fig 1.1a). That is, there was a disproportionate reduction in wool growth responses to high intakes in winter in comparison to the summer feeding period. At low intakes there was little effect of season on WGR, while at high intakes a large seasonal effect was apparent. Similar responses are evident for British breed sheep. In winter,

Figure 1.1 The relationship between WGR (gd^{-1}) and dry matter intake (gd^{-1}) in Summer and Winter:

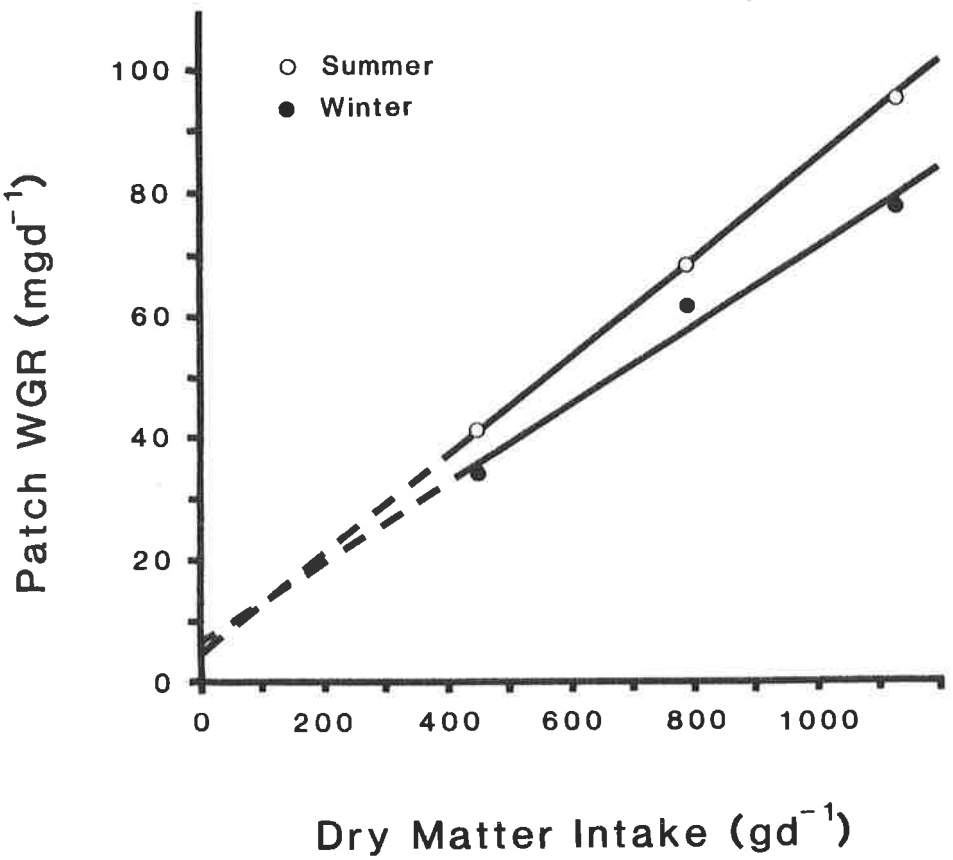
a) for Romney Marsh, Coopworth, Perendale and Corriedale wethers at 5 intake levels (adapted from data of Sumner 1979)).

b) for Merino wethers at 3 intake levels (adapted from data of Hill (1970).

a)


Summer
Winter
WGR (gd^{-1})
0
4
8
12
16
20
0
200
400
600
800
1000
Dry Matter Intake (gd^{-1})

b)


Summer
Winter
Patch WGR (mgd^{-1})
0
20
40
60
80
100
0
200
400
600
800
1000
Dry Matter Intake (gd^{-1})

the follicles of Blackface (Doney 1964) and Cheviot sheep (Doney 1966), are insensitive to nutrition.

For sheep with a less defined seasonal rhythm there is evidence that the amplitude of the cycle is unaltered by nutritional level. For example, Hill (1970) noted WGR differences between winter and summer when medium-wool merinos were fed at three intake levels for 2½ years, but, the relative WGR differences at each intake were similar (Fig 1.1b).

These results indicate that little error is likely when data are adjusted on the basis of the relative WGR changes of a uniform intake group, at least for sheep with an inherently low seasonal wool growth rhythm, such as the Merino. For British breeds and their crosses, on the other hand, no simple correction can be made because the correction factor would depend on the degree of follicle refractoriness in winter.

Removing photoperiod effects by partial regression analysis of correlations between WGR, temperature and daylength (Ferguson 1962), or by adjustment on the basis of known amplitudes of rhythm for each genotype (Hutchinson 1962) would not be sufficiently precise for most experimental designs.

Section 1.4 Genotype and wool growth rate

In comparison to some other production characters in animals, the heritability of clean fleece weight of 0.30 (Schinckel 1958) represents a high genetic component of the variance in WGR. The following review examines the extent and probable causes of variation in WGR between sheep.

1.4.1 WGR of sheep of different breed or strain

Variations in WGR are apparent between breeds and strains of sheep as a result of differences in both feed

intake and the efficiency of utilisation of the diet (Dolling and Moore 1961; Williams and Winston 1965; Williams 1966; Dolling and Piper 1968). Table 1.2 summarises the few studies conducted to compare several breeds and strains. In this table the intake differences have been removed by expressing WGR per unit feed intake, and all efficiencies thus determined are expressed relative to that of the fine wool Merino. The similarity of efficiency rankings between trials conducted on a wide array of diets and intake levels supports the contention that relative efficiencies of wool growth are not altered by nutrition (Dolling and Moore 1961; McManus et al. 1966; Dunlop et al. 1966) unless seasonal growth rhythms are manifestly different. There is little doubt that the Long wool breeds grow significantly more wool than the Down breeds when fed the same amount of feed. (Yeates et al. 1975). Similarly, the strong wool merino appears to be more efficient than the fine wool strains by approximately 16-20%. Other differences are small and variable.

1.4.2 Variability in WGR between sheep within flocks

It is not uncommon within a flock for some sheep to produce twice as much wool as others, partly as a result of differences in intake and diet selection but also due to variation in the efficiency of nutrient utilisation. The relative importance of these factors has varied between trials in which high and low wool producers have been compared, but in general about 50% of the WGR differences are associated with efficiency of utilisation (Ahmed et al. 1963). Table 1.3 indicates typical variances of efficiency observed under experimental conditions.

Table 1.2 Mean WGR and wool growth efficiencies (WGR/dry matter intake) for different breeds and strains of sheep. (Efficiency is exxpressed relative to efficiency of finewool merinos in each trial).

Breed or Strain	Mean WGR (gd^{-1})	Relative Efficiency (% of fine merino)	Conditions	Author
Lincoln	16.1	111-123		
Corriedale	14.3	102-109	Pen fed	Daly and Carter (1955)
Polwarth	10.7	106-110		
Finewool merino	8.2	100		
Border Leicester	7.3	73		
Dorset Horn	5.8	55		
Southdown	3.0	47	Grazing	Langlands and Hamilton (1969)
Strong merino	(10.4)	154		
Fine merino	7.9	100		
Medium merino	10.0-12.6	115-124		
Strong merino	11.3-13.9	124	Pen fed	Dunlop et al. (1966)
Fine merino	8.2-9.8	100		
Strong merino	10.0	118	Pen fed	Weston (1959)
Fine merino	6.6	100		
Strong merino	10.0	119	Grazing	
Fine merino	7.2	100		
Strong merino	11.1	116		
Medium peppin A	10.3	112		
Medium peppin B	9.9	109	Group fed	Dunlop et al. (1960)
Medium non-peppin	10.3	111		
Fine merino	9.1	100		
Strong merino	-	122	Grazing	Weston (1956)
Medium merino	-	111		
Fine merino	-	100		

+Estimated in another trial.

Table 1.3 Coefficients of variation (CV) in WGR per unit intake for sheep in the same flock, under experimental conditions.

Author	Diet	Sheep	CV(%)
Weston (1959)	Lucerne chaff/ Wheaten chaff (50/50)	Finewool Strongwool	10 15
Schinckel (1960)	Lucerne/Maize (50/50)	Peppin merinos	15-22
Dolling and Moore (1961)	Lucerne/Oaten chaff	Peppin merinos	10-20
Pattie and Williams (1967)	500gd^{-1}) 700gd^{-1}) Lucerne 900gd^{-1}) Hay ad lib.)	 Peppin merinos	5 10 6 19
Piper and Dolling (1969a)	Hi protein) Sorghum straw, Med protein) Wheaten starch, Low protein) Linseed meal, sorghum grain	Peppin merinos (unselected)	17 15 26
Saville and Robards (1972)	Lucerne pellets	Bungaree, Collinsville, Random, fleece plus, Nucleus	2-5

On herbage diets the coefficients of variation (c.v) of wool growth efficiency fall within the range of 2-20%, whereas on diets that include cereal grains and starch products, the c.v fall in the range 15-26%. Whether these represent real differences associated with diet has not been determined experimentally. Despite substantial efficiency differences between individual sheep of the same strain, the relative rankings remain unaltered when the nutritional regime is changed (Weston 1959; Dolling and Moore 1961).

1.4.3. Potential sources of variability "between-sheep" in efficiency of wool growth

Sheep on a constant intake of a uniform diet may differ in the quantity of wool produced as a result of differences in one or more of the following: (a) the digestion of nutrients and supply of amino acid nitrogen to the small intestine, (b) the proportion of amino acid nitrogen absorbed from the tract, (c) the post absorptive metabolism of nutrients and (d) the efficiency with which the follicle population converts available nutrients into fibre (Schinckel 1960; Piper and Dolling 1969a).

Examination of the literature reveals that the main determinants of genotypic wool growth differences are dietary intake (Ahmed et al. 1963) and the efficiency of utilisation of nutrients for fibre production (Williams 1979). Digestive efficiency appears to be of minor importance (Weston 1959; Hutchinson 1961; Dunlop et al. 1966; Piper and Dolling 1966). Of the postabsorptive factors, the efficiency of intermediary amino acid metabolism (Williams et al. 1972; Williams 1976; 1979) and the arrangement and morphology of the follicles (Nay and

Hayman 1969; Jackson et al. 1975) are clearly of importance in generating genotypic differences. Poor producers have a lower proportion of the active germinal cells entering the fibre cortex (Vseboldov and Prusova 1966; Butler and Wilkinson 1979; Wilson and Short 1979a), and therefore lower fibre growth despite follicle mitotic activity similar to that of high producers.

These inherent wool growth differences between experimental sheep are best accounted for by use of covariance statistics provided there is no interaction between level of nutrition or treatment, and genotypic wool growth. The previously mentioned results of Weston (1959) and Dolling and Moore (1961) suggest that this is the case. Estimation of WGR at one nutritional level applies to any other, so that this statistical technique is valid.

Section 1.5 The interaction of body tissues changes with wool growth

1.5.1 Concept

Marston (1948) noted a significant time lapse before WGR equilibrated with level of feed intake (Section 1.1.4) and proposed that this was a result of changes in the status of non-wool tissues. At sub maintenance intakes, "steady depletion of the fat reserves was the major factor which determined the quantity of amino acids drawn up for fuel, and so the quota that became available for wool production". Conversely, when sheep were fed above their maintenance requirement, Marston considered that "synthetic processes other than wool production were mainly responsible for depletion of of the substrate".

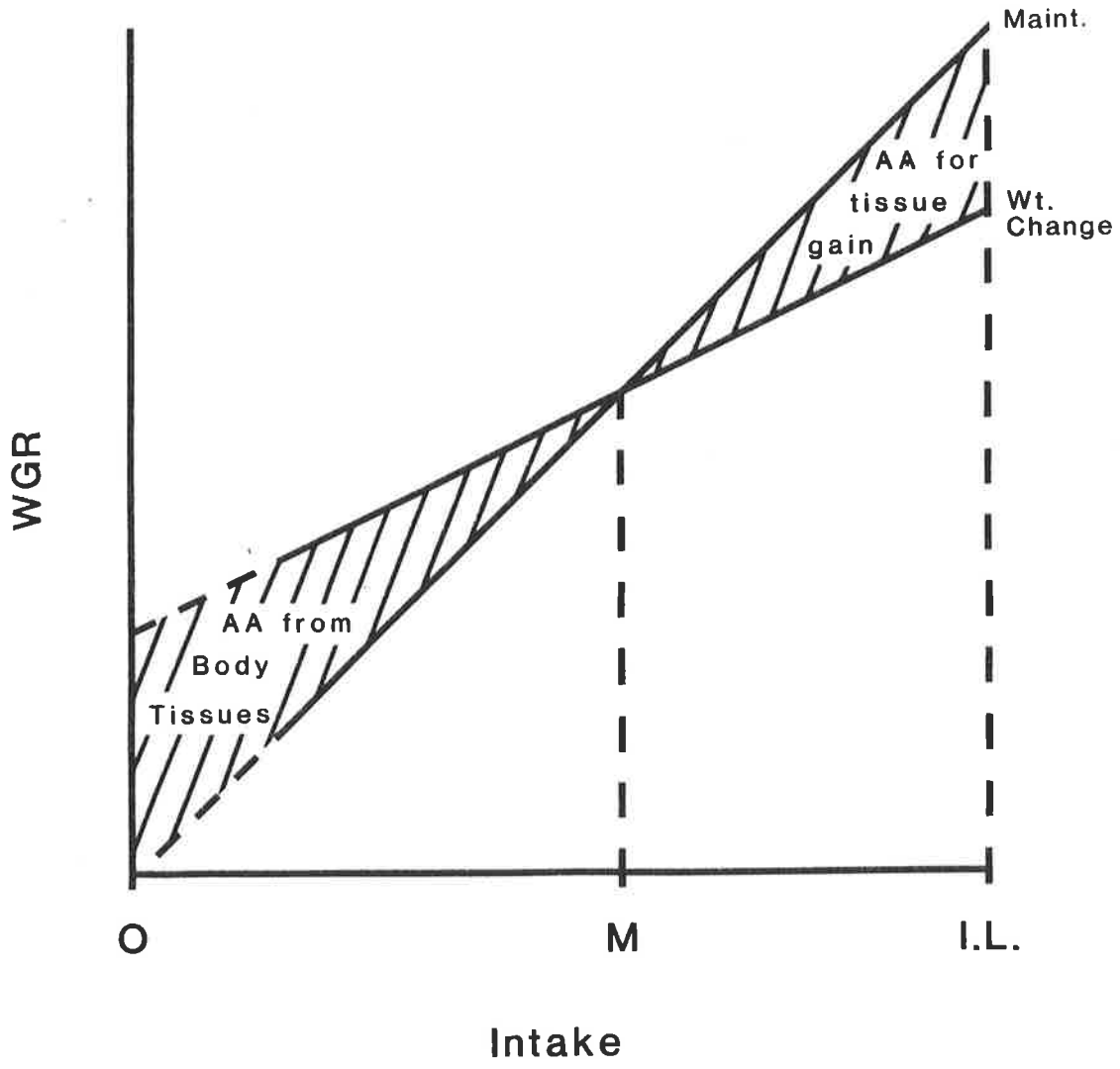
Black and Reis (1979) elaborated on the concept when

they attempted to quantify the synthetic processes using the Michaelis-Menten kinetic equation (Conn and Stumpf 1972 p173). Briefly the approach was as follows. The outcome of competing biochemical reactions in different tissues depends on the relative rates of reaction and the affinities and concentration of substrates for each reaction. Available substrate, in turn, is a function of its concentration in the blood, and the blood flow to the tissue in question. These concepts were incorporated into a simulation model in which the principal nutrients were the sulphur amino acids. As the maximum rate of reaction of methionine in tissues other than wool increased, the WGR decreased.

The processes proposed by Marston (1948) when sheep lose weight are similar in that nutrient availability is the operative factor. The catabolism of body tissues is presumed to supply endogenous substrate which will have the same effect on WGR as nutrients absorbed from the gastrointestinal tract. Consequently the WGR at any intake level is greater for a sheep losing weight than for one at maintenance with that intake.

This concept is demonstrated in Fig. 1.2. The line passing through the origin represents the relation of WGR to intake when sheep are maintaining bodyweight at each intake level (Maint). The line intercepting the Y axis (Wt. change) represents the relation of WGR to intake according to whether a sheep is at maintenance (M), gaining weight (I.L) or losing weight (O). During weight gain wool growth is depressed, whereas during weight loss it is enhanced (Ferguson 1972). WGR per unit intake when weight change is zero has been termed net efficiency by Ferguson (1962),

Figure 1.2 The relationships proposed by Ferguson (1962), between WGR and dietary intake when sheep are maintaining weight at each level of intake, or changing weight at each intake level. M represents the point of energy balance for the weight-change group, below which loss of weight is incurred and above which the sheep gain weight. The shaded areas represent the WGR increments associated with weight change. IL is the maximum intake capacity of the sheep.


Maint.
AA for
tissue
gain
Wt.
Change
WGR
AA from
Body
Tissues
O
M
I.L.
Intake

while gross efficiency refers to wool production under conditions of changing liveweight. The evidence to confirm or refute this postulate is now examined.

1.5.2. Liveweight status and efficiency of wool growth

1.5.2.1. Catabolised body tissues as a source of nutrients for wool growth

The concept of enhanced wool growth efficiency when sheep are losing weight (Marston 1948) is based on the assumption that endogenously-derived nutrients are available for wool growth processes. There is some evidence that this is not the case. During periods of undernutrition there is a net catabolism (i.e. catabolism less synthesis) of body proteins, particularly in skeletal muscle (Waterlow and Stephen 1968). Amino acids derived from these labile protein stores would enhance the plasma free amino acid pool but may not necessarily be available for fibre synthesis if they are metabolised to provide energy in glucogenic pathways (Judson and Leng 1973b). Wool growth will probably not be increased by this supply of additional energy unless there is a concomitant supply of extra amino acids (see section 2.1).

Some confirmation for this is found in the data of Black et al. (1973). When energy was limiting, additional postruminal protein had little effect on wool growth. Apparently this protein was serving energy needs because addition of energy nutrients when protein was in excess, enhanced WGR. Similarly, Barry (1973b) demonstrated that there was no wool response to additional amino acids in sheep fed at submaintenance levels, while significant increases in WGR occurred when sheep were supplemented at maintenance levels. Liveweight loss was reduced by protein

supplementation on the submaintenance ration, suggesting that the extra amino acids were being utilised for the energy demands of basal metabolism.

In contrast to these studies there is some evidence that catabolism of skin proteins may temporarily enhance WGR by increasing the cystine pool in the fluid spaces surrounding the follicles (Downes 1961; Downes and Sharry 1971). Because skin is a relatively large organ (Downes 1965) its catabolism would be of some significance during periods of poor nutrition (Downes et al. 1976). Indeed, Hutchinson (1957) noted a loss of some 40g of protein from the skin of sheep subjected to poor nutrition for eight weeks. Additional amino acids from the non follicular extravascular pool (Downes 1961) are probably incorporated into follicles before they enter general circulation and the catabolic pathways of the liver. While this may account for a short-term enhancement of WGR when feed intake is reduced (Downes and Sharry 1971), there are other reports of much longer equilibrium times to low intakes (Section 1.4).

1.5.2.2 Competition for nutrients between wool and non-wool tissues during weight gain

Because methionine (cystine) is the first limiting amino acid for both WGR (Reis et al. 1973) and liveweight gain (Fennessy 1976), it seems reasonable to assume that rapid weight gain would increase competition between tissues for this substrate and possible reduce WGR. Only indirect evidence on this point is available. Corbett (1979) considers that the gross efficiency of wool production may increase in the first few years of life "because nutrient demands for body growth will presumably diminish,

progessively leaving greater proportions of the nutrient intake available for wool growth". Evidence to support this proposition can be drawn from the studies of Atkins & Robards (1976) who showed that sheep selected for high weight gain grew faster as lambs and produced less wool per unit digestible organic matter intake than a randomly selected group. Both groups were more efficient wool producers as adults than as lambs, a result Oddy and Annison (1979) suggest is not surprising "in view of the nutrient requirements for tissue growth". In contrast, Langlands and Hamilton (1969) observed no consistent effect of age, and by association weight change, on efficiency. Interpretation of these studies is difficult because postnatal follicle maturation and fibre production by each follicle may take up to 6 to 12 months to be complete (Schinckel and Short 1961). Similarly, the competition for nutrients during pregnancy and lactation (Barry 1969; Williams et al. 1978) are confounded by the hormonal status of the reproducing ewe (Corbett 1966). Hormonal aspects of the growth of wool will not be discussed.

1.5.2.3 The WGR of sheep fed to gain, maintain or lose bodyweight

An early experiment, designed to investigate the influence of thyroxine on wool growth, revealed an inverse relationship between wool and bodyweight responses (Ferguson 1958). These were further examined in a trial in which 36, two year old, medium-wool merinos were pen-fed diets of varying protein content but similar energy concentration (Ferguson 1959). The diets were fed at 500 gd^{-1} for 8 weeks, ad libitum for 12 weeks and again at 500 gd^{-1} for 32 weeks

(Ferguson 1962). Before the results were analysed, WGR was adjusted by partial regression analysis for the effects of temperature and time (see section 1.3.3.). While the bodyweight response to intake was rapid, the wool growth response was delayed, so that the responses of the two tissues were in opposite directions when expressed per unit of feed intake. (Fig 1.3)

The relationship, thus derived, was of the form:

Equation 1.7..... $W/_I = E - k.c/_I$

where W is wool growth rate, I is dry matter intake, c is bodyweight change, and E is the efficiency of wool growth at maintenance. The 'k' term represents the value of nutrients required for weight gain or derived from weight loss, its value in this experiment being 0.03329. "E" was 0.01188 so that the effect on WGR of a 1g change in bodyweight was equivalent to 2.8g of feed. Because an earlier experiment indicated no wool growth responses to crude protein above 8% of the diet, Ferguson expressed all values in equation 1.7 on an energy basis. He concluded that wool growth could be expressed as the sum of metabolisable energy in the diet and the energy content of bodyweight change, although an alternative explanation for the responses in terms of protein supply was also considered (Ferguson 1962). Nagorcka (1977) re-examined these data using a statistical technique which accounts for the time lag (25 days in this instance) in wool growth reponse (section 1.1.4). When this period was removed, there was no dependence of wool growth efficiency on bodyweight change.

Figure 1.3 Relationships between WGR per unit intake (gg^{-1}) and bodyweight change per unit intake (gg^{-1}). Mean data for all sheep. (Source: Ferguson 1962).

$$Y = 0.01188 - 0.03329X$$

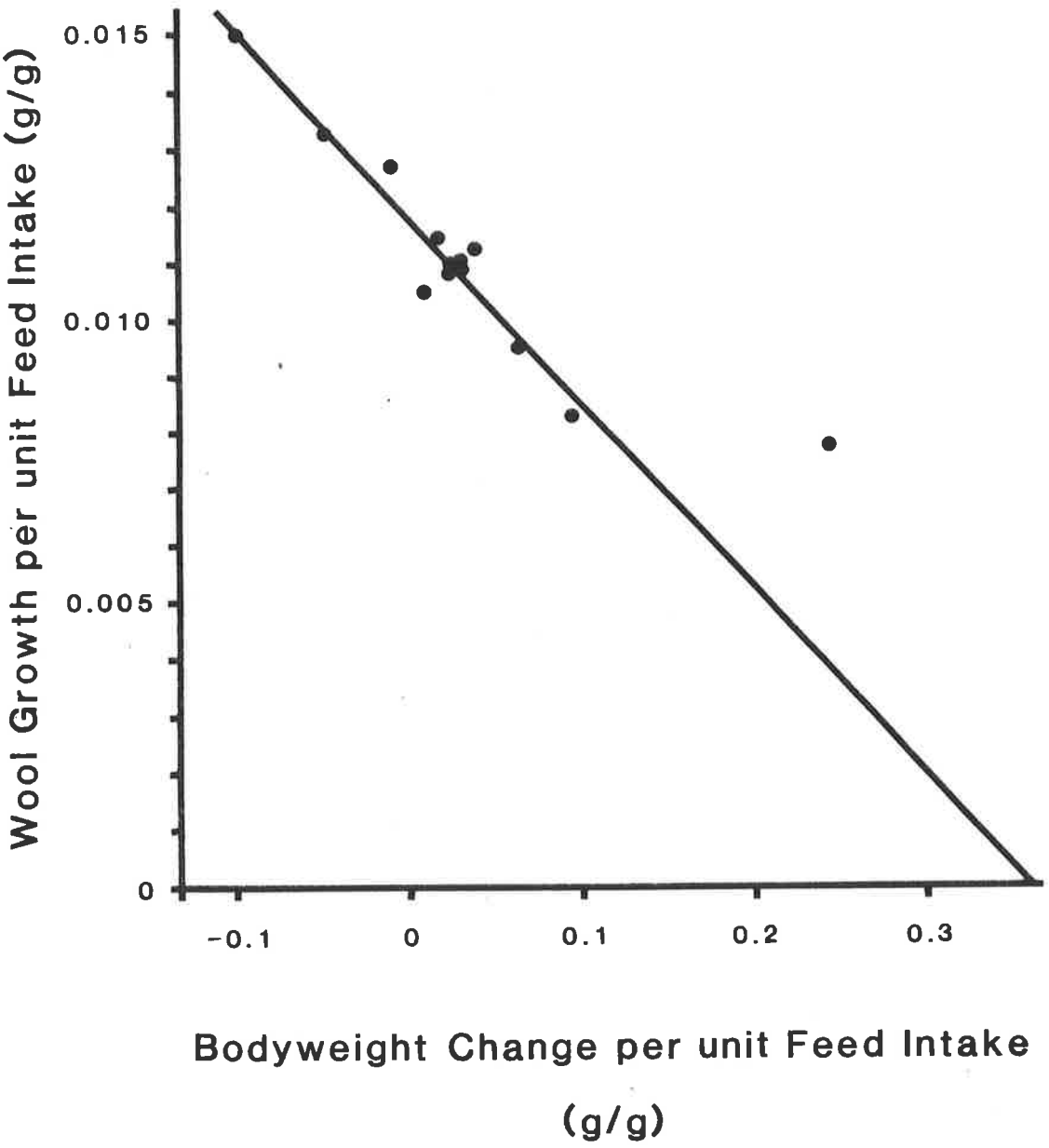
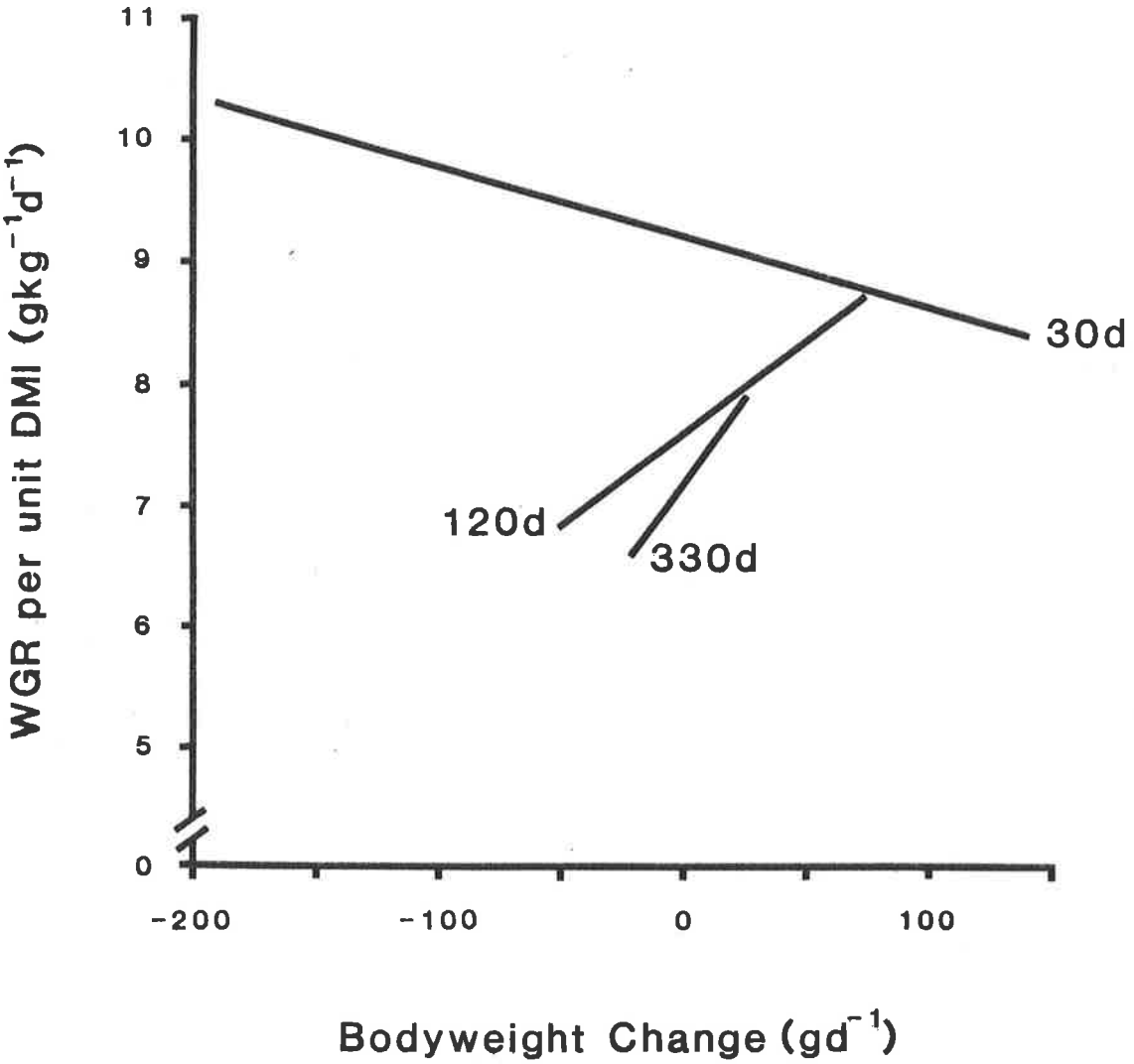

Wool Growth per unit Feed Intake (g/g)
0.015
0.010
0.005
0
-0.1
0
0.1
0.2
0.3
Bodyweight Change per unit Feed Intake
(g/g)

Figure 1.4 The changing relationship between wool growth efficiency (g wool per kg dry matter per day) and bodyweight change (gd^{-1}), with time (days) after nutritional change (adapted from Irazoqui 1970).


11
10
9
8
7
6
5
0
-200
-100
0
100
WGR per unit DMI (gkg^{-1}d^{-1})
Bodyweight Change (gd^{-1})
30d
120d
330d

There are few experiments in which wool and body responses to different intakes of the same diet have been measured serially thereby allowing an estimation of equilibrium time for WGR. Table 1.4 presents for these experiments the influence of the time lag (Section 1.1.4) on the "interaction" of these variables.

WGR per unit intake of dry matter was plotted against bodyweight change so that the regression is of the form $W/_I = a - bc$ where 'a' represents maintenance wool growth efficiency, and 'b' the impact of weight change on efficiency.

With the exception of the data of McInnes (1970), there is no significant effect of bodyweight change on the amount of wool produced at a given intake, if more than 4 weeks is allowed for WGR to stabilise with intake. The diets used by Robards et al. (1976a) and McInnes (1970) varied in composition throughout the trial and results should be accepted with some reservation.

Irazoqui (1978) believed that data from his 1970 experiment support Ferguson's (1962) proposition, but a closer examination of his results reveals that the effect of weight change on WGR per unit intake depended on the time of measurement (Table 1.4 and Fig 1.4).

For the first 30 days, when the greater weight change occured there was a negative relationship between wool growth efficiency and weight change, but by 120 days, when the range of weight changes was still considerable (-54 to +78gd) the efficiency of wool growth was unaltered by gain or loss of weight and remained so until 330 days.

1.6 Conclusion

There is little doubt that WGR is dependent on the

Table 1.4: The relationship between weight change and efficiency of wool growth with time after nutritional change

Author	"a"[1]	"b"[2]	Sig of 'b'	Max Weight loss (gd^{-1})	Max Weight gain (gd^{-1})	Time of wool measurement after Intake change (weeks)
Robards et al. (1976[a])	17.48	0.0187	n.s	-82	70	10
McInnes (1970)	15.37	0.0379	n.s	-124	19	3
	18.30	0.0452	***	-106	77	5
	17.10	0.0855	*	-51	-4	10
Irazoqui (1970)	9.21	0.0059	*	-190	143	4
	7.62	-0.0146	n.s	-54	78	17
	7.25	-0.0268	n.s	-24	35	47
Marston (1948)*	5.11	-0.0772	n.s	-2.83	4.48	12

n.s. = non significant

* = P<0.05

*** = P<0.01

*Marston (1948) data based on nitrogen intake and nitrogen balance (gd^{-1})

1 Wool growth efficiency at maintenance

2 Effect of weight change on efficiency

supply and composition of amino acids in the capillaries and extracellular fluid spaces surrounding the follicles. Energy availability, however, modifies this supply by influencing both microbial protein production and the metabolism of proteins absorbed from the tract, so that the relative availability of both protein and energy is important. The subsequent response of wool fibres to these nutrients is then reliant on the interaction between season, genotype and nutritional level, which determine the sensitivity of the follicles.

At present there is only indirect evidence concerning the proposal that rapid body growth reduces WGR by competition for common substrate. On close analysis the proposition that more wool is produced per unit of feed eaten when sheep are losing weight, also receives little support from the literature. However no definitive trial has been conducted to examine either of these hypotheses.

CHAPTER 2: An investigation of the influence of liveweight change on wool growth efficiency

2.1 Introduction

Grazing animals subsist on pastures which vary substantially in both quantity and quality in the short term (seasonal effects) and over longer periods (drought and flood effects). Consequently, the animal's liveweight fluctuates from periods of rapid gain to periods when considerable liveweight losses are incurred. While the impact of such patterns of growth on body composition and life time productivity has been examined (Allden 1970; Hogg 1977), there has been little investigation into the effects of liveweight fluctuation on current wool growth. In the preceding literature review, the concept of depressed wool growth efficiency during weight gain and enhanced efficiency during weight loss, was outlined (1.5.1). The work which supports this contention (eg. Ferguson 1962; Irazoqui 1978) is based on the proposition that the lag in wool growth response to nutritional change (1.1.4), is a consequence of the change in nutrient availability coincident with liveweight responses. A major problem here is that it is difficult to distinguish between cause and effect. Indeed, by the time WGR has equilibrated with a new level of intake, the weight change has diminished substantially, because the maintenance requirements of the animal have been altered due to weight gain or loss during this period. Thus comparisons are not possible when weight gains and losses are maximal. Another possibility is that the lag period is independent of weight change and nutrient availability changes, being a function of the time required for structural alteration to

follicle bulbs to take place (Yeates et al. 1975). This aspect of the lag period is examined in Chapter 3.

A factor favouring this view is that once equilibrium WGR has been established, the direction or rate of bodyweight change appears to have little impact on efficiency (see Nagorcka 1977 and Section 1.5.2.3). Moreover, when sheep were in negative energy balance (Barry 1973a) there was little wool growth response to postruminal methionine supplementation possibly because the additional methionine was catabolised to provide energy. Endogenous substrates may suffer a similar fate, so that they don't enter general circulation before being catabolised in the liver.

It is important to assess the extent of the interaction between wool and non-wool tissues for several reasons. Firstly, this knowledge may aid in the development of management strategies, for instance, if weight loss does enhance efficiency, "efficiency will be maximal when the farmer buys heavy weight animals and sells them after a given liveweight has been lost (the enterprise will be producing wool at the expense of feed consumed in another enterprise)" (Irazoqui 1978). Secondly, and more importantly, it would allow the prediction of wool growth responses to feed supplements such as protected proteins or individual amino acids, under the conditions of changing bodyweight status observed in the field. Finally, it is necessary to know the extent of the relationship between wool and non-wool tissues to define the level of energy balance at which wool growth experiments are carried out. No experiment has been reported in which the

interrelation of liveweight change per se and the amount of wool produced per unit of feed eaten (wool growth efficiency) has been examined. Neither has there been any study in which the factors responsible for the time taken for wool growth to equilibrate with diet have been investigated. The first two studies reported in this thesis (Chapters 2 and 3) examined these aspects to test the proposition that when WGR has reached equilibrium after a change in intake level, it is independent of liveweight change.

To undertake such a study the following criteria had to be met:

1. Time should be allowed for WGR to equilibrate with intake level (Section 1.1.4).
2. The effects of seasonal variations in WGR should be taken into account (Section 1.3).
3. The diet source should remain unchanged throughout the experiment (Section 1.2.1).
4. The inherent differences between sheep in WGR under the conditions of the experiment should be minimised (Section 1.4).

   and finally,
5. Any interaction between intake level and efficiency of wool growth, which is not associated with weight change must be taken into account (see Sections 1.2.2.1, 1.2.2.).

An experimental design was employed which accounted for each of those factors so that weight change, and the dietary circumstances which produced it, were the major variables.

2.2 Materials and Methods

2.2.1 Animals

48 South Australian, strongwool merino wethers (Bungaree strain,) aged 12 months, were selected from a commercial flock grazing at the Mortlock Experiment Station, Mintaro S.A. The sheep were selected on the basis of uniformity of fleece weight and skin characteristics after 5 months of common grazing. Wool production of the group, expressed as WGR per unit bodyweight, had a coefficient of variation of 12%. Twins and sheep of low birth weight or with wrinkled skin were excluded. After footparing and treatment for internal parasites, the sheep were allocated at random to individual pens.

2.2.2 Design

A total experiment period of 50 weeks was divided into 3 subperiods (I, II and III) of 12, 18 and 20 weeks respectively. Period I was a uniformity, or covariance period, in which 44 sheep received a common level of feed at about maintenance, so that inherent WGR differences under the experimental conditions could be ascertained and used as a covariate in the analysis of the production results of subsequent periods. During this period and Period II an additional 4 sheep were fed ad libitum to provide an estimate of WGR for sheep whose growth path was not interrupted by the period of maintenance feeding.

During the second experimental period (II), groups of sheep were fed different quantities of the same ration to induce liveweight gains and final bodyweights at the end of the period. Consequently, when sheep in the different groups were fed similar amounts of the experimental feed in Period

III, they gained, lost or maintained weight depending on the weights attained at the end of Period II. This allowed contemporaneous comparisons to be made between sheep whose intake level was identical, but whose liveweight status differed, thereby fulfilling requirement 5 in the Introduction to this Chapter.

A group receiving a constant level of feed throughout the 50 week period was included to allow WGR in periods II and III to be adjusted for seasonal growth rhythms, thereby enabling WGR and efficiency of wool growth to be compared both within and between periods.

The possibility of an interaction between intake level and non-nutritional wool growth variance was accounted for by maintaining groups of sheep at each intake level in period III.

The planned growth paths of groups, for Periods I, II and III were estimated from metabolisable energy intakes and requirements described in the MAFF Technical Bulletin 33 (1975), and are illustrated in Fig 2.1.

In period III the following comparisons were made at the same intake level:

Groups	Liveweight status
1. AA_1 v DA_1	1. Maintenance v gain
2. BB_1 v Ad lib.B_1 v AB_1 v DB_1	2. Maintenance v loss v loss v gain
3. CC_1 v AC_1	3. Maintenance v loss
4. DD v AD	4. Maintenance v loss

To achieve this array of weight changes the following intake levels (gd^{-1}) were used in Periods II and III: (Table 2.1)

Figure 2.1 Planned growth paths for groups of sheep in Periods I, II and III, Experiment 1.

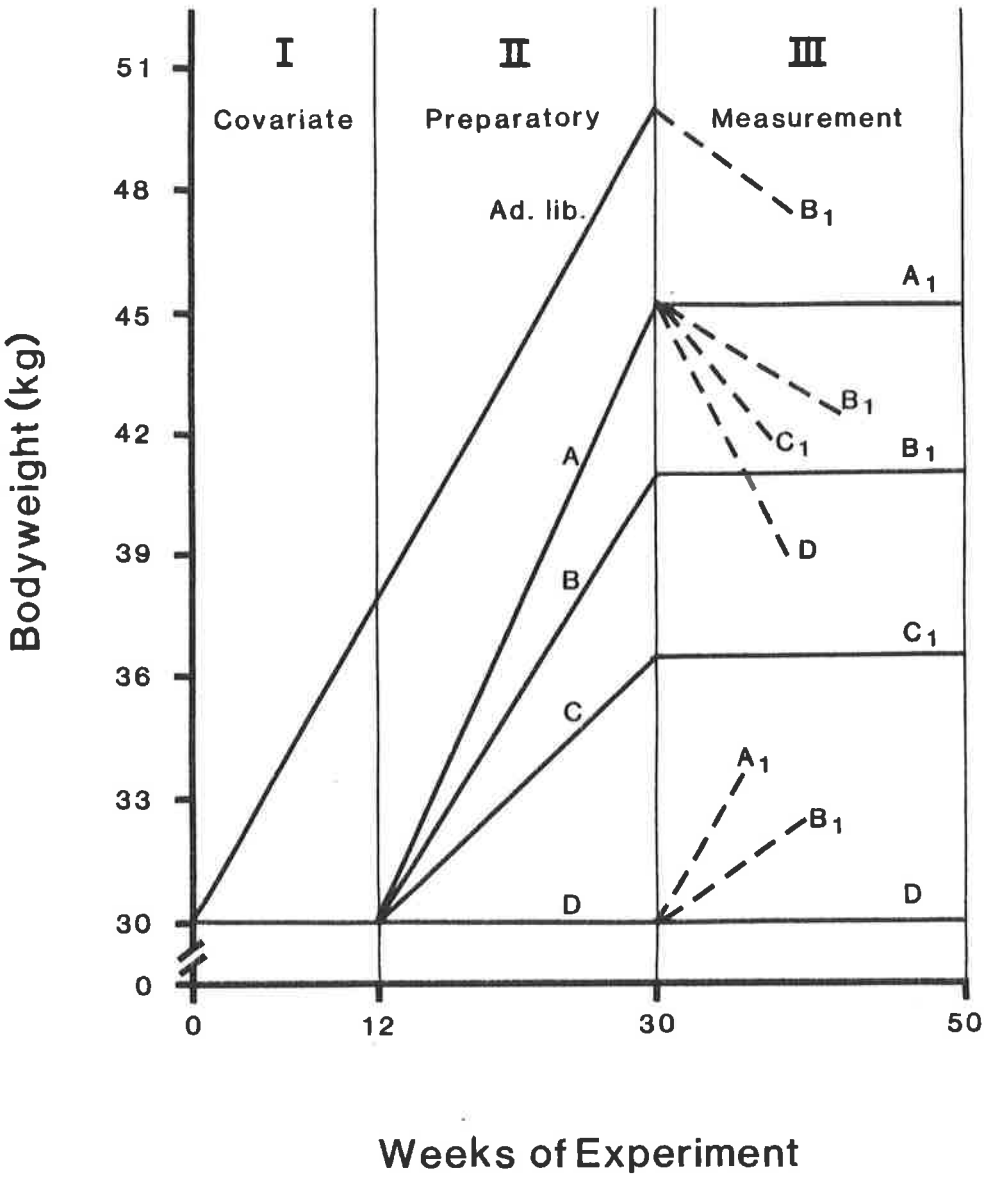

I
Covariate
II
Preparatory
III
Measurement
Ad. lib.
A
B
C
D
A1
B1
C1
B1
A1
B1
C1
D
D
51
48
45
42
39
36
33
30
0
0
12
30
50
Bodyweight (kg)
Weeks of Experiment

Table 2.1 Air-dry and dry matter intakes (DMI) of experimental groups during Periods II and III

Period II	Air-dry(gd^{-1})	DMI(gd^{-1})	Period III	Air-dry(gd^{-1})	DMI(gd^{-1})
A	1000	895	A_1	700	627
B	850	760	B_1	650	582
C	700	627	C_1	600	537
D	500	448	D	500	448

Groups of 4 sheep were considered to be a satisfactory minimum group size because the variability in wool production at pasture was low and the covariance period would account for inherent differences in wool growth capacity between individual sheep. Table 2.2 outlines the allocation of the 48 sheep to each of the experimental groups in Periods II and III.

2.2.3 Feeding

The diet comprised 60% barley grain (cv Clipper) and 40% lucerne chaff and was selected for its high energy concentration. Components were finely hammermilled, mixed and pelleted. The chemical composition of the ration is shown in Table 2.3.

To minimise variation in diet composition throughout the trial, the total feed requirement was prepared at one time and each week's ration was chosen at random from the supply available, thereby eliminating feed source as a factor contributing to variation.

The diet was offered once daily at 0.800 h after removal of the previous days residue. Refusals were bulked over 7 days and dried at 103°C for 24 h to enable mean daily dry matter intake to be estimated.

A mineral mix (Moir and Harris 1962) was also provided at $5gd^{-1}$ per sheep.

2.2.4 Methods

The experimental protocol, which involved the clipping of midside patches on 26 occasions to estimate wool production of the 48 sheep, and the determination of

Table 2.2 Allocation of sheep to groups in experiment 1

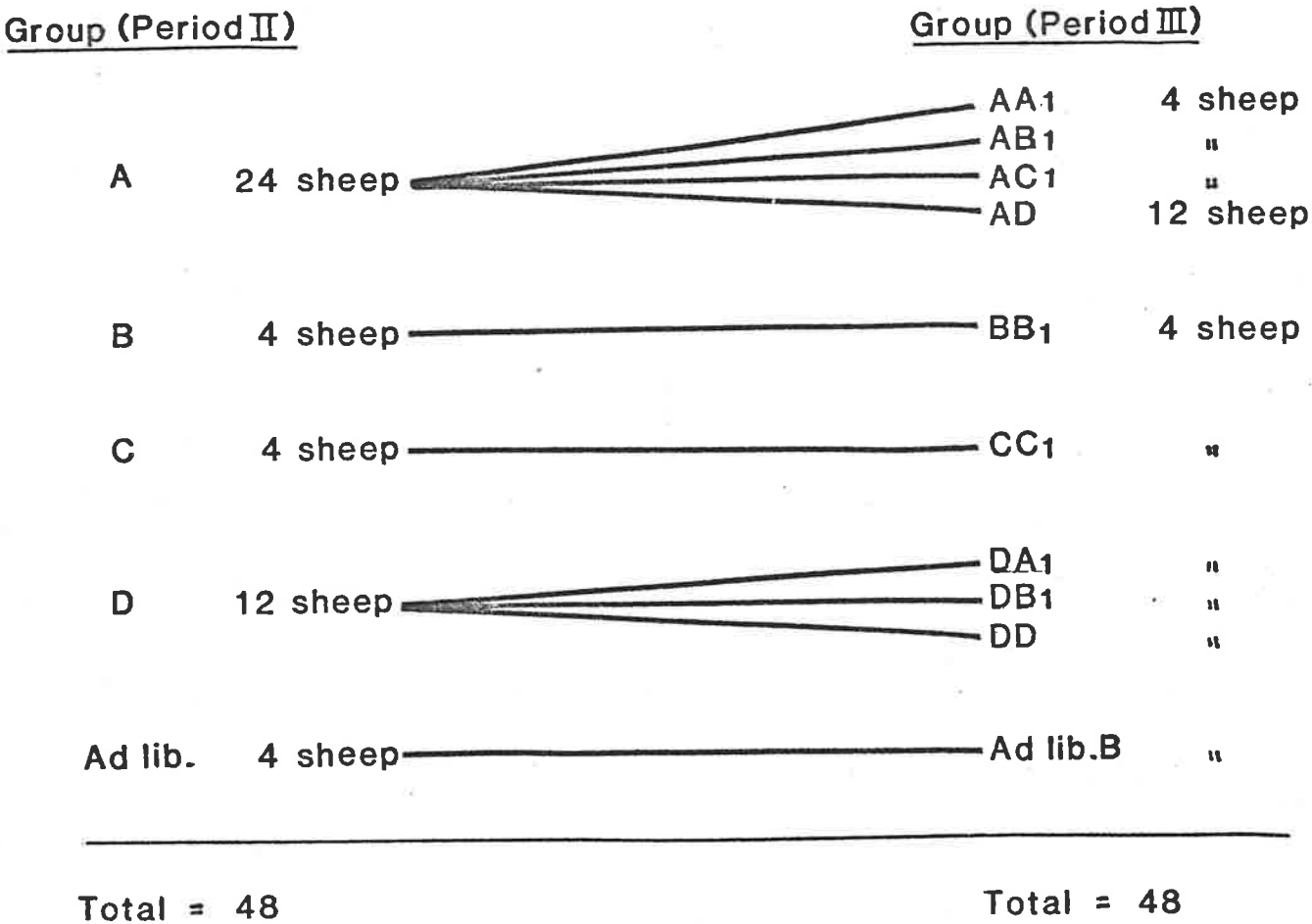


Table 2.3 Chemical composition of the pelleted ration

Component		Mean ± S.E.M. over experiment
Dry matter	(%)	89.5 ± 1.3
Nitrogen	)	2.60 ± 0.23
Organic matter	) (% DM)	95.7 ± 0.2
Ether Extract	)	3.76
Metabolisable energy	(MJ/kg DM)+	11.50
Phosphorus	(% DM)	0.231
Sulphur	(% DM)	0.250

+ME = 0.15 DOMD% (MAFF Tech. Bull. 33)

bodyweight by regular weighings and body composition by tritium dilution on 6 occasions, is presented in Table 2.4.

Wool Growth Rate

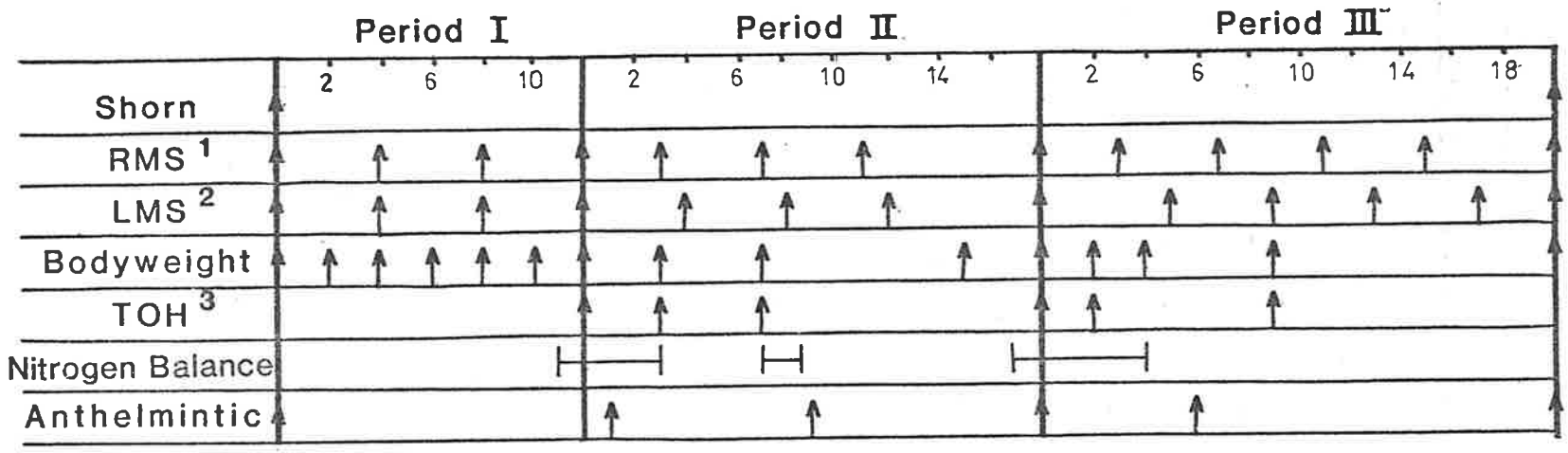
Immediately after shearing, at the beginning of Period I, sheep were tattooed on closely clipped areas of skin on both the right and left midside regions. The areas thus delineated were 10cm x 12cm and were clipped at intervals of not less than 3 weeks (Table 2.4) using Oster small animal clippers fitted with No. 40 blades. The patch wool samples were weighed, placed into sealed muslin bags and immersed in 400ml. of commercial petroleum ether (Shell X_4). The wool was agitated and squeezed repeatedly for 10 minutes. This process was repeated in a second beaker of clean X_4, followed by a final rinse in water at 50°C. Samples were then dried at 70°C for 24 h. and weighed. More than 95% of the total grease present, as estimated by Soxhlet extraction, was removed by this scouring technique.

At shearing, fleece subsamples of approximately 200g. were taken from the midside region, weighed, and then scoured in tubs using the method described by Yeates et al. (1975 p. 331). Daily WGR was then estimated by apportioning the clean dry fleece weight according to the proportion of total patch wool grown in each clipping period (Langlands and Wheeler 1968).

Estimates of WGR were made each 2 weeks by clipping right and left midside patches at alternate 4 week periods. Both patches on the same sheep provided similar WGR estimates when they were simultaneously clipped during Period I:

(r^2 = 0.985 (Right side WGR = 0.058+0.966 Left side WGR)

Table 24 The outline of Experiment 1.



Key : 1. Right midside patch harvest.

2. Left midside patch harvest.

3. Tritiated water space estimation.

The intercept did not differ significantly from zero and the regression coefficient was not significantly different from unity.

Bodyweight

Bodyweight was measured at intervals as outlined in Table 2.4. To reduce errors of "gut-fill", sheep were weighed prior to feeding at 0.800h. Fleece-free liveweight was estimated by subtracting greasy fleece weight, calculated from wool growth data at the time of weighing, from total liveweight. Fleece-free liveweight change was then determined for each period by regression of fleece-free bodyweight at each measurement, against time.

Body composition

Body composition was estimated on 3 occasions in each of Periods II and III, by reference to tritiated water space of each sheep (Searle 1970a, b). After a 16h waterless fast, an intramuscular injection of 200 µci of tritiated water (TOH) was made. Five ml of venous blood was taken by jugular puncture 6h after the injection and 1h after bodyweight was measured. The plasma water was removed by sublimation in Thunberg tubes and 0.5ml was added to 6.0ml scintillation fluid (1000ml toluene, 500ml Triton X 100; 4.0g. PPO, 0.1g POPOP). Samples were counted 3 times (10 min. each) in a Tri Carb liquid scintillation counter. A 25 µci/litre standard was counted with each batch, and the TOH space estimated.

Total body water, fat, protein and lean (kg) were then determined using the general regression equations derived by Searle (1970a) for sheep of all ages.

This technique was considered suitable for the current experiment because it allowed estimates to be made of body

composition changes with time, in the same animal.

Nitrogen balance and diet digestibility

Nitrogen retention, defined as nitrogen intake less nitrogen in faeces, urine and wool, was determined by the total collection method from sheep in metabolism crates. Faeces were weighed and a 10% subsample freeze-dried. The remainder was dried at $103^{o}C$ for 48h. for dry matter determination. Urine was collected into 1.0M H_2SO_4 so that the pH remained below 2. A 10% subsample was taken after the total daily output was recorded. Samples of feed, faeces and urine were assayed for nitrogen on a Technicon Autoanalyser after a micro Kjeldahl digestion.

Nitrogen balance was not determined for all sheep, but only for representatives from some groups. Three sheep from each of groups A($1000gd^{-1}$) and D($500gd^{-1}$) were placed in metabolism crates and collection made for the first 3 weeks and for the seventh week of Period II.

In Period III, nitrogen balance was measured in 3 sheep from each of groups DA_1($500-700gd^{-1}$), AA_1($1000-700gd^{-1}$) and DD ($500-500gd^{-1}$) for the first 21 days of this period.

An alternative means of estimating nitrogen retention (NR) over longer periods of time and for all sheep was by reference to the changes in body protein (P) as measured by TOH space viz:

$$NR(gd^{-1}) = \frac{P(kg)t_n - P(kg)t_o}{t_n - t_o \text{ (days)}} \times \frac{1000}{6.25}$$

NR, thus determined, does not include nitrogen retained in wool.

Dry matter and organic matter digestibilities were measured during all collection periods. Organic matter of feed and faecal samples was determined by ashing at 550°C for 12 h.

Statistical analysis

Group means were tested in an analysis of covariance as described by Finney (1971). Individual wool growth efficiencies at the end of Period I were used as the covariate, on the assumption that the relative performance of individual sheep is unaltered by level of nutrition. Indeed, this has been previously noted for sheep on different rations (Dolling and Moore 1961).

An alternative method of analysis was by regression techniques. This option had the advantage of allowing the data from Periods II and III to be combined, so that a wider range of weight changes and WGR's could be examined. Seasonal wool growth rhythms, which would render such analysis invalid, were taken into account by correction of all data on the basis of the relative WGR changes of the uniform intake group (DD), a procedure which appears to be justified for merinos (see Section 1.3.3).

All other comparisons were made in an analysis of variance with differences in group means examined by a simple t-test.

2.3 Results

2.3.1 Bodyweight change

A considerable range of weight changes was generated by the different dry matter intakes of sheep in Periods II and III (Table 2.6, Appendix 2.1), thereby achieving a primary objective of the design. Group mean weight changes are

presented in Table 2.5 together with the planned liveweight responses. These data are also presented graphically for each group throughout the experiment (Fig 2.2). In general the planned direction of weight changes was adhered to, although the extent of the changes was lower than predicted.

The group of sheep maintained on a constant intake of the diet for the whole trial, gained weight slowly at an average of 6.9 gd^{-1}. This small net gain comprised an initial loss in Period I, with subsequent gain in the remainder. Weight loss in Period I was in fact a characteristic of all groups.

Liveweight change was closely related to the intake level relative to the maintenance requirement, the latter estimated as fleece-free bodyweight raised to the 0.75 (Fig 2.3). Significant curvilinearity of this relationship indicates more efficient use of the diet at submaintenance intakes. At maintenance (0 gd^{-1} bodyweight change), the corresponding metabolisable energy requirement was 0.391 MJ per kg $^{.75}$, a value which closely approximates the standard maintenance energy requirements as specified by the MAFF Technical Bull. 33 (eg. a 20kg sheep required 3.7 MJd^{-1}, MAFF value = 3.8 MJd^{-1}, 30kg = 5.0MJd^{-1}, MAFF value = 5.1 MJd^{-1}). Throughout the whole experiment individual weight changes ranged from -57 to +158 gd^{-1}, although short-term weight change responses were considerably greater than this. Sheep offered the diet ad libitum in periods I and II consumed, on average, 1100gd^{-1} dry matter and gained weight at 118gd^{-1} over periods I and II. Overall, intakes ranged from a maintenance level for a 30kg sheep to approximately 2.5 times maintenance. The mean intakes of all groups during

Figure 2.2 The growth pattern of groups of sheep in Experiment 1. Plotted values are group means.

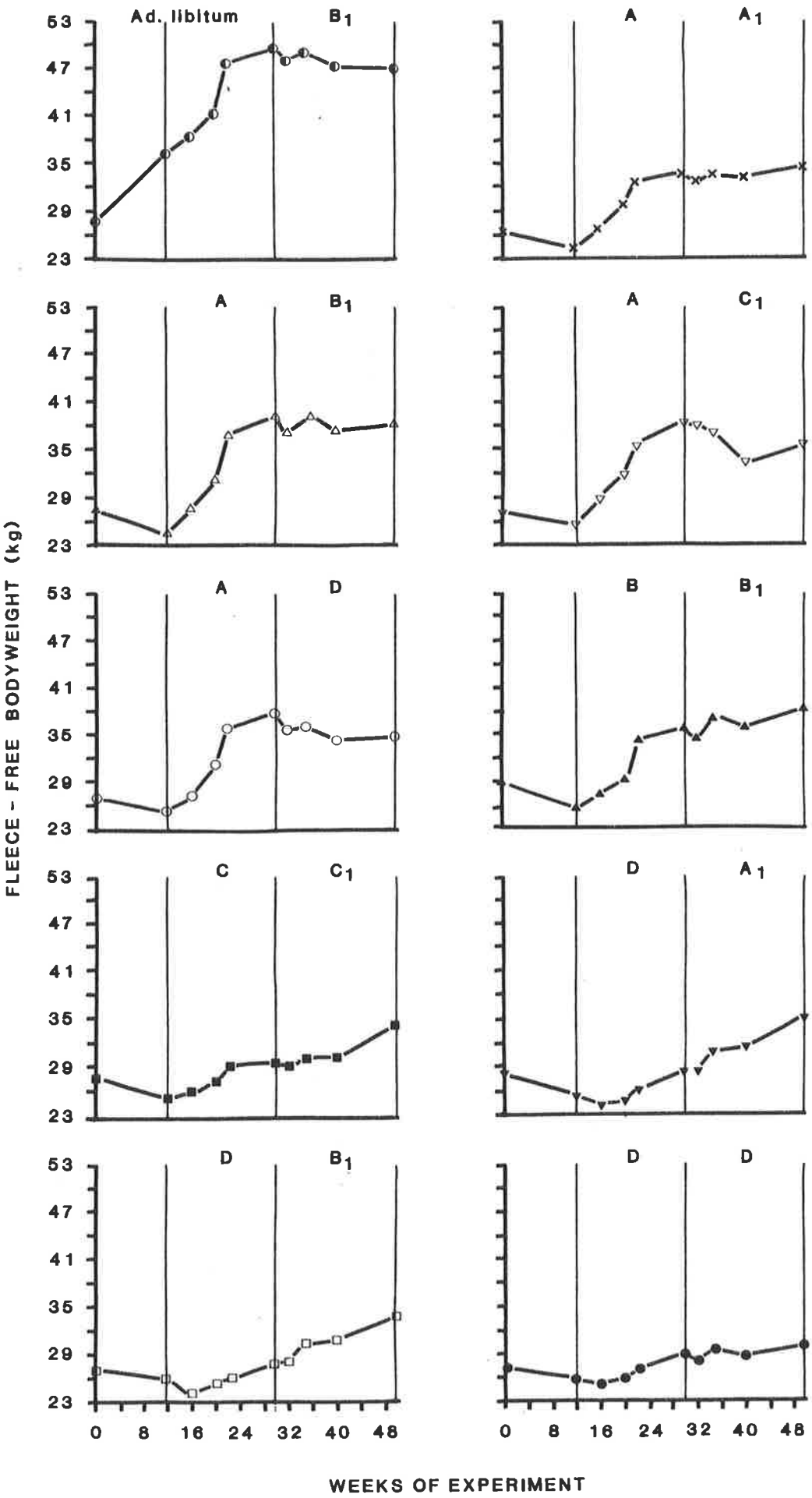

Ad. libitum
B1
A
A1
A
B1
A
C1
A
D
B
B1
C
C1
D
A1
D
B1
D
D
53
47
41
35
29
23
0
8
16
24
32
40
48
FLEECE - FREE BODYWEIGHT (kg)
WEEKS OF EXPERIMENT

Figure 2.3 The relationship between dry matter intake, as a proportion of maintenance requirement ($g/kg^{.75}/d$), and fleece-free liveweight gain (gd^{-1}) in Periods II and III of Experiment 1. The association is described as follows:

$$Y = -132.2 + 4.47X - 0.02.X^2 \quad (P\ 0.001)$$

The curvilinearity was significant.

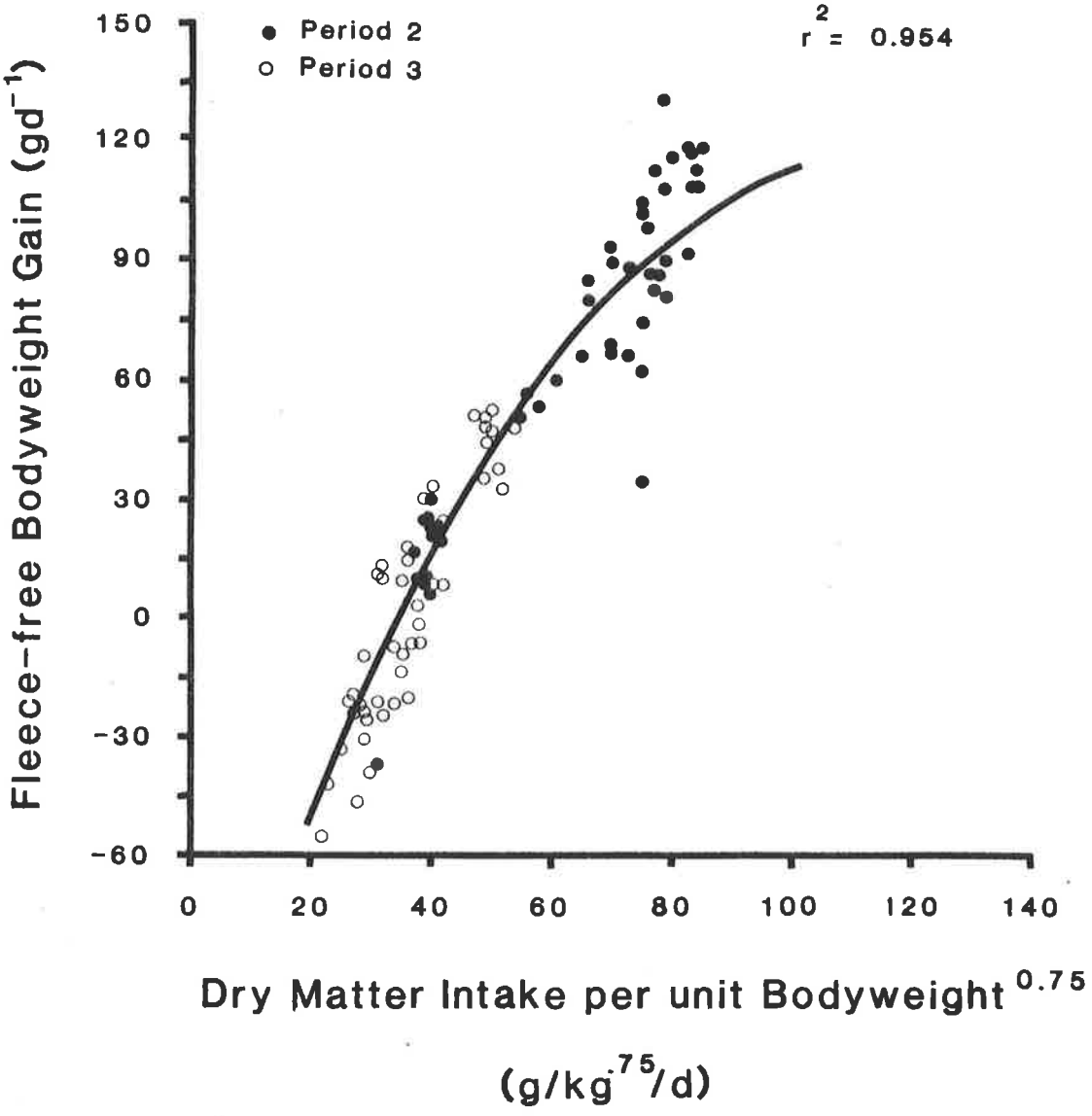

Period 2
Period 3
r^2 = 0.954
Fleece-free Bodyweight Gain (gd^{-1})
150
120
90
60
30
0
-30
-60
0
20
40
60
80
100
120
140
Dry Matter Intake per unit Bodyweight $^{0.75}$
($g/kg^{.75}/d$)

Table 2.5 Planned and actual liveweight changes (gd^{-1}) for all groups in experiment 1. (Means ± S.E) +

Group	PERIOD II		PERIOD III	
	Planned	Actual	Planned	Actual
Ad lib. B_1	-	118 (17)	gradual loss	-9 (23)
AA_1)	110	85 (27)	0	7 (28)
AB_1)	110	133 (12)	gradual loss	-8 (7)
AC_1)	110	118 (36)	moderate loss	-26 (13)
AD)	110	112 (21)	rapid loss	-22 (20)
BB_1	85	92 (9)	0	19 (13)
CC_1	53	40 (49)	0	15 (25)
DA_1)	0	26 (9)	62	50 (6)
DB_1)	0	15 (8)	50	47 (6)
DD)	0	27 (4)	0	9 (11)

+ (Individual liveweight changes are presented in Appendix 2.1)

Table 2.6 Mean daily dry matter intakes (gd^{-1}) (± SEM) for all groups in Periods I, II, III.

	PERIOD		
	I	II	III
Ad lib. B_1	985 (75)	1143 (64)	549 (56)
AA_1	425 (13)	845 (34)	543 (61)
AB_1	429 (8)	885 (41)	585 (0)
AC_1	431 (7)	846 (106)	482 (99)
AD_1	433 (8)	870 (35)	427 (39)
BB_1	425 (12)	766 (5)	585 (0)
CC_1	442 (5)	574 (111)	540 (0)
DA_1	419 (10)	456 (2)	630 (0)
DB_1	436 (3)	457 (0)	585 (0)
DD_1	433 (7)	457 (0)	450 (0)

Periods I, II and III are presented in Table 2.6. The digestibility of the diet was not influenced by the level of feeding and averaged 78.4%, although there was some evidence of depressed digestibility for a short period after the DMI was increased (Table 2.7).

2.3.2 Body composition and nitrogen balance

The changes in bodyweight outlined above, were accompanied by changes in the estimated quantity of protein and fat in the body tissues. The mean total body protein content (less wool protein) for each group is presented in Appendix 2.2 for the whole of period II and the first half of period III. These data were used to estimate the mean nitrogen retentions for the experiment (Table 2.8). Clearly, gain and loss of weight by sheep in period III was reflected in changes in the body nitrogen stores.

In the nitrogen balance studies, equilibration of nitrogen balance with diet was achieved within about 2 weeks (Table 2.9). There was considerable discrepancy between data estimated from "in vivo" body composition and that determined by the total collection technique, even when the contribution of wool nitrogen was taken into account. The latter method produced higher retention estimates, in accordance with the directional bias of the collection method (i.e. nitrogen retained is overestimated because all errors in collection tend to be losses and never gains of nitrogen). In further calculations nitrogen retention data by TOH estimation were used because a) the period assessed was longer and b) the bias mentioned above does not apply to this technique.

Table 2.7 Changes in dry matter digestibility (%) with time after intake change (± SEM)

Group	DMI change	Weeks after DMI change				
		0	1	2	3	7
A	448-895gd^{-1}	78.2 (2.3)	75.0 (2.8)	79.4 (0.9)	78.2 (0.6)	81.1 (1.8)
A_1	448-636gd^{-1}	80.0 (1.9)	76.9 (1.5)	79.4 (2.0)	79.3 (1.6)	-
D	448-448gd^{-1}	78.7 (1.3)	78.2 (3.0)	76.9 (1.7)	77.9 (2.3)	79.0 (0.6)

Table 2.8 Group mean nitrogen retention, excluding wool nitrogen (gd^{-1}), estimated from body protein content changes as determined from in vivo body composition data. (Means ± S.E).

Group	Proposed weight changes in Periods II and III	Nitrogen retention (gd^{-1}) Period II (18 weeks)	Period III (9 weeks)
Ad lib. B_1	Gain/loss	1.82 (.69)	-2.45 (.73)
AA_1	Gain/maintenance	0.74 (.36)	-1.05 (.30)
AB_1	Gain/loss	2.42 (.19)	-2.27 (.30)
AC_1	Gain/loss	1.62 (1.00)	-1.32 (.41)
AD	Gain/loss	1.57 (.78)	-1.77 (1.09)
BB_1	Gain/maintenance	1.29 (.51)	-0.61 (.71)
CC_1	Gain/maintenance	1.09 (.25)	-0.15 (.44)
DA_1	Maintenance/gain	-0.29 (.31)	0.43 (1.16)
DB_1	Maintenance/gain	0.14 (.33)	0.38 (.33)
DD	Maintenance/maintenance	0.28 (.33)	-0.64 (.73)

Table 2.9 Mean (N Balance + Wool N) (gd^{-1}) estimated by collection for sheep in groups A and D (Per II) and AA_1, DA_1 and DD (Per III) - 3 sheep/group (Mean ± S.E).

		WEEKS				
Group II	DMI Change	0	1	2	3	7
A	448-895	2.00 (1.40)	9.25 (0.12)	5.32 (1.84)	4.36 (.63)	5.34 (1.23)
D	448-448	3.34 (1.05)	3.63 (.55)	2.82 (1.04)	1.42 (.14)	2.51 (.09)
	Difference:	ns	P<.01	ns	P<.01	P<.02
			Days after DMI change			
Group III	DMI Change	0	5	10	15	20
AA_1	895-627	4.29 (.39)	4.00 (1.78)	2.73 (.65)	2.89 (1.53)	2.93 (1.15)
DA_1	448-627	3.55 (.86)	6.64 (1.77)	3.76 (.78)	5.33 (.46)	3.88 (.65)
DD	448-448	2.71 (.80)	2.66 (.33)	2.27 (.95)	2.26 (.11)	3.46 (.74)

2.3.3. Wool growth

The dietary regimes imposed, generated a wide range of wool growth responses in addition to the bodyweight changes previously described, the WGR of individual sheep varying from as low as 0.6g of clean, dry wool per day to 22.1gd^{-1}. The latter value approaches the genetic maximum for the strain of merino sheep under study (Hogan *et al.* 1979). Individual WGR throughout the experiment are presented in Appendix 2.3. The relationship between equilibrium WGR and DMI is shown in Fig 2.4 for all experimental groups during Periods II and III. All values have been adjusted by reference to the WGR of the uniform intake group. The unadjusted regression lines relating WGR and DMI in Periods II and III, are also plotted, indicating the significant displacement that can occur when a reference group is not included.

The regression equations which describe the relationships between WGR and DMI in Fig. 2.4, are presented in equations 2.1 and 2.2 for Periods II and III respectively and in Equation 2.3 for the "adjusted" WGR data from both periods.

Equation 2.1...Period II

$$WGR(gd^{-1}) = 0.78+0.0129DMI(gd^{-1}) \qquad r^2 = 0.36 \ (P<0.001)$$

Equation 2.2...Period III

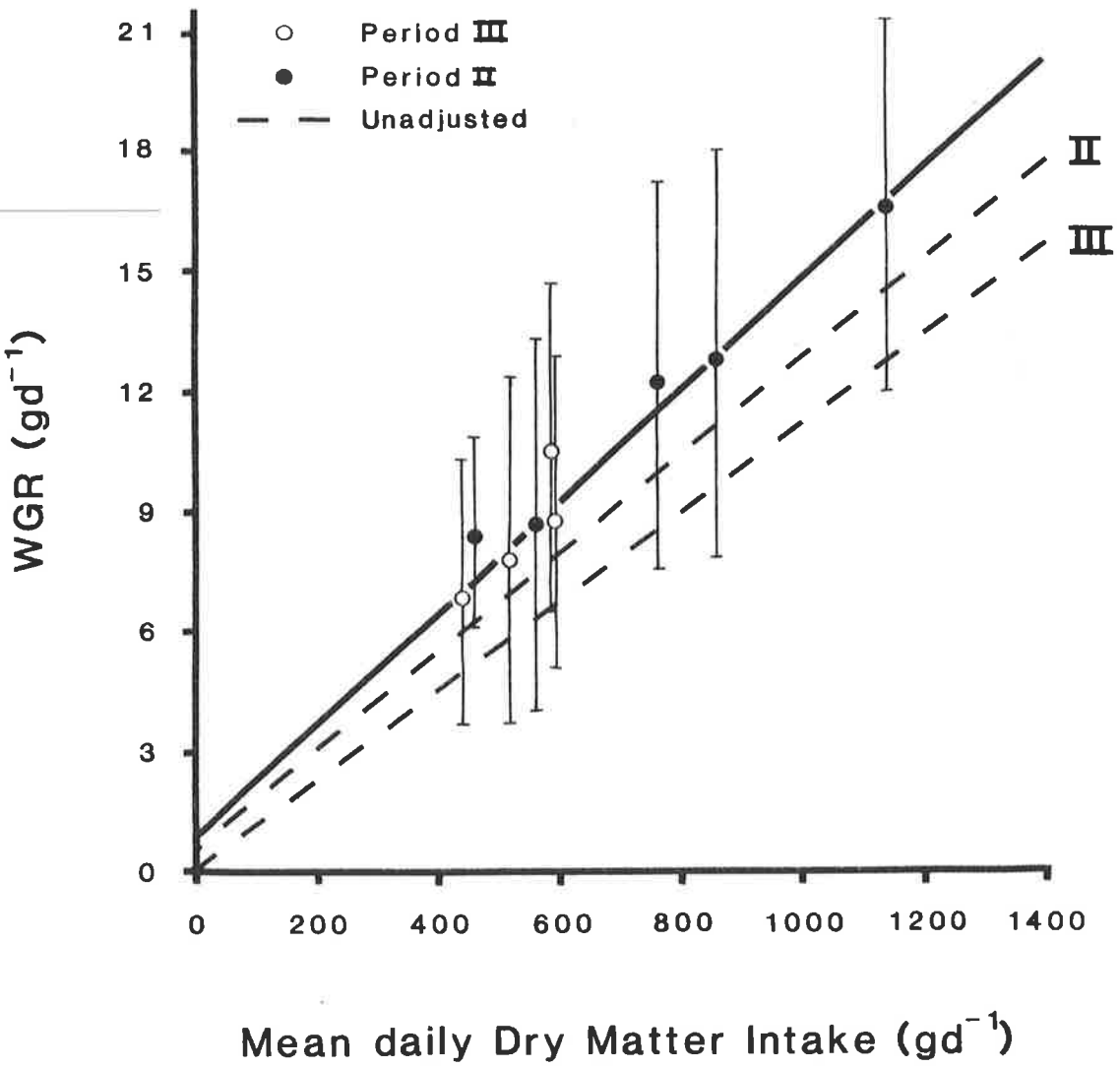
$$WGR(gd^{-1}) = 0.00 + 0.0112DMI(gd^{-1}) \qquad r^2 = 0.14(P<0.01)$$

Figure 2.4 WGR (gd^{-1}) as influenced by dry matter intake (gd^{-1}) in Periods II and III. The solid line represents the response when WGR data were corrected for relative changes in the WGR of a uniform-intake group. The dashed lines indicate the relationship in Periods II and III when WGR data were unadjusted. Regression points are mean values ± S.D. The corresponding regression equations are:

$Y = 0.78 + 0.0129X$ (Period II)

$Y = 0.00 + 0.0112X$ (Period III)

$Y = 1.24 + 0.0140X$ (Both periods adjusted)


Period III
Period II
Unadjusted
II
III
WGR (gd⁻¹)
21
18
15
12
9
6
3
0
0
200
400
600
800
1000
1200
1400
Mean daily Dry Matter Intake (gd⁻¹)

Equation 2.3...Both Periods

$WGR(gd^{-1}) = 1.24 + 0.0140DMI(gd^{-1})$ $\quad r^2 = 0.34$ $(P<0.001)$

At no time did the relationship between WGR and DMI differ significantly from one of simple proportionality, all lines passing through the origin, so that there was no suggestion of an "intake x wool growth efficiency" interaction.

Although a significant portion of the variability in wool growth was associated with intake differences between sheep, there remained a substantial residue that could not be accounted for by variations in intake. This variance is indicated by the standard errors depicted in Fig 2.4, and contrasts with the close relation of liveweight change to intake (as a proportion of maintenance requirement) (see Fig 2.3). An important feature of of the results for WGR which detracted from the sensitivity of the tests, was the increase in the coefficient of variation (%) in wool growth efficiency throughout the experiment. At the beginning of Period I, the variance in efficiency (estimated as WGR per unit bodyweight) was 12.0% and increased to 19.1%, 29.1% and 39.9% at the end of Periods I, II and III respectively (the latter efficiencies being estimated as WGR per unit intake). Consideration of the covariate period wool growth efficiencies accounted for only an additional 5% of the variance.

All WGR data were analysed without transformation as there was no evidence that variances were heteroscedastic, the Fmax test (Sokal and Rohlf 1973 p.210) being non significant.

Wool production of the uniform intake group

It was mentioned above that wool growth data were corrected for observed WGR changes of the uniform intake group. Figure 2.5 illustrates the quite considerable fluctuations in the mean WGR of these sheep throughout the experiment. In Period I, the WGR of all sheep on the maintenance intake declined, presumably indicating the residual effect of the nutritional status under grazing conditions. From weeks 16-42 only minor fluctuations in the WGR of the uniform intake occurred, and once again the 4 sheep on a constant intake for the entire experiment, were representative of other sheep in Period II on the same intake. The mean WGR of DD sheep from weeks 16-42 was 7.77 ± 0.27 gd^{-1} (n=4). Toward the end of Period III, there was a notable drop of $2.2gd^{-1}$ in the WGR of these sheep, possibly in response to the onset of winter. Similar decreases at this time were apparent in sheep on other intakes. Thereafter, there was little relationship between season and WGR.

The changes in WGR of the 4 uniform-intake sheep are summarised in Table 2.11 together with the bodyweight responses. The overall decline in WGR contrasted with a gradual increase in liveweight. Sheep 29 had the most pronounced decline in WGR, and there was some evidence that the fibre density of this animal was reduced during the latter part of Period III.

Time taken for WGR to equilibrate with diet

It is clear from Table 2.10 that wool growth continued to change for a long time after a change in feed intake. In Period II, about 90% of the total WGR change was complete

Figure 2.5 Changes in mean WGR (gd^{-1}) of 4 sheep on a constant, maintenance intake of the experimental ration for 76 weeks. Mean fleece-free bodyweights are also indicated, as are the seasons. The standard deviations about the mean at the end of each Period were $2.33gd^{-1}$, $2.42gd^{-1}$ and $2.37gd^{-1}$.

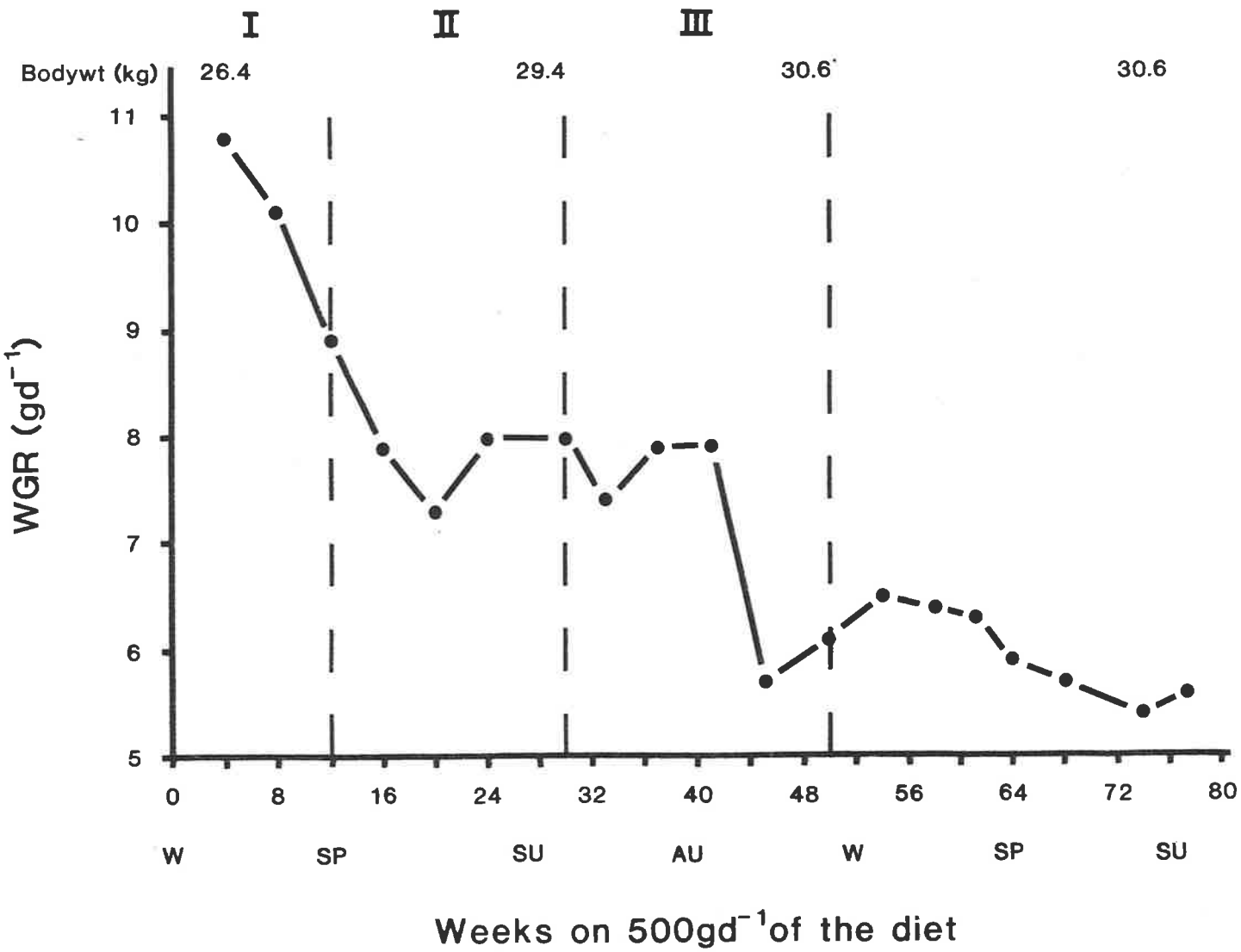

I
II
III
Bodywt (kg)
26.4
29.4
30.6
30.6
11
10
9
8
7
6
5
WGR (gd^{-1})
0
8
16
24
32
40
48
56
64
72
80
W
SP
SU
AU
W
SP
SU
Weeks on 500gd^{-1}of the diet

Table 2.10 Mean WGR changes with time after intake change. Values are expressed as a percentage of total WGR change final WGR - initial WGR)

Group (II)	DMI change (gd^{-1})	WEEKS 0	4	5	8	9	12	13	18	WGR change (gd^{-1})
A	500-1000	0	13	30	91	93	85	91	100	+ 5.4
B	500-860	0	8	18	77	72	67	62	100	+ 3.9
C	500-700	0	11	45	90	87	90	92	100	+ 3.8

Group (III)	DMI change (gd^{-1})	WEEKS 0	3	5	7	9	11	13	15	17	20	WGR change (gd^{-1})
Ad lib. B_1	Ad lib.-650	0	24	57	85	92	88	89	87	92	100	-8.4
AA_1	1000-700	0	77	108	135	154	162	162	127	112	100	-2.6
AB_1	1000-650	0	16	44	60	74	76	80	80	94	100	-8.8
AC_1	1000-600	0	11	43	69	80	94	91	80	83	100	-3.5
AD	1000-500	0	29	51	88	100	106	102	94	94	100	-5.1
BB_1	850-650	0	Very low WGR change in this group								100	-0.4
CC_1	700-600	0	"	"	"	"	"	"	"		100	-1.3
DA_1	500-700	0	33	33	50	54	63	71	121	125	100	+2.4
DB_1	500-650	0	61	55	71	76	103	118	142	134	100	+3.8

Table 2.11 WGR, as a percentage of final WGR in Period I for 4 sheep fed a constant intake of the experimental ration. (Bodyweight gains (gd^{-1}) are shown in parenthesis.

Sheep No	Period I	Period II	Period III
2	100 (-15)	99 (28)	85 (16)
16	100 (-20)	88 (30)	72 (9)
24	100 (-25)	93 (27)	71 (13)
29	100 (-12)	73 (21)	37 (-7)
$\bar{X}$	100 (-18)	88 (27)	66 (8)

after 8 weeks at the increased nutritional level, but only about 11% of the response was apparent after 4 weeks. The equilibration of WGR with intake is also illustrated in Fig. 2.6.

In Period III responses were more variable, so that after 3 weeks, from 11 to 77% of the total WGR change had occurred in different groups. It was not until week 13 that all groups had attained more than 70% of the response, in this case both positive and negative. There was, however, no indication that the direction of the dietary intake change had any impact on the stabilisation time. For instance, a comparison of groups DB_1 and AC_1 reveals that, at similar absolute WGR changes but in different directions, about 70% of the response was complete by 7 weeks. Moreover, the time lags appeared to be little affected by the extent of the wool growth change.

A more detailed investigation of the lag period is reported in Chapter 3.

2.3.1 The relation of liveweight change to wool growth efficiency

The WGR changes of the experimental groups in Periods I, II and III are shown in Fig. 2.6a, b, c and d, together with the group mean daily dry matter intakes. Analysis of covariance in Period III revealed that none of the WGR differences between groups were statistically significant. A comparison of the wool growth efficiencies of groups of sheep of similar intakes but with differing rates and directions of weight change is made in Table 2.12. While efficiency varied from 13.7 to 20.6g wool per kg feed, these differences were not related to the

Figure 2.6 WGR (gd^{-1}) changes with time for all groups in Experiment 1.

a) Groups AB_1, BB_1 and DB_1.

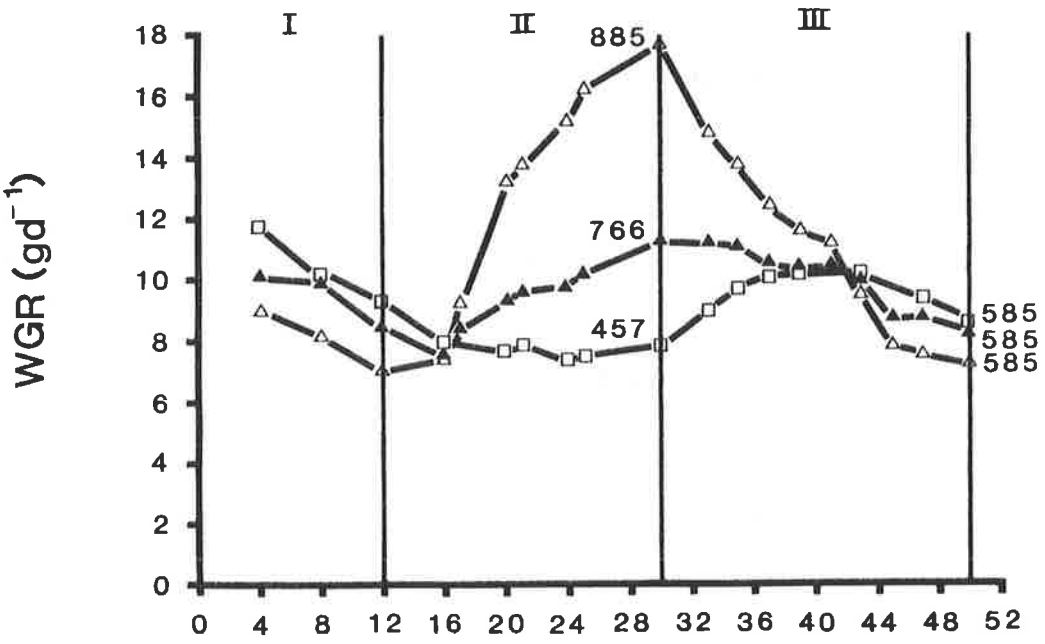
b) Groups AD and DD.

c) Groups AC_1 and CC_1.

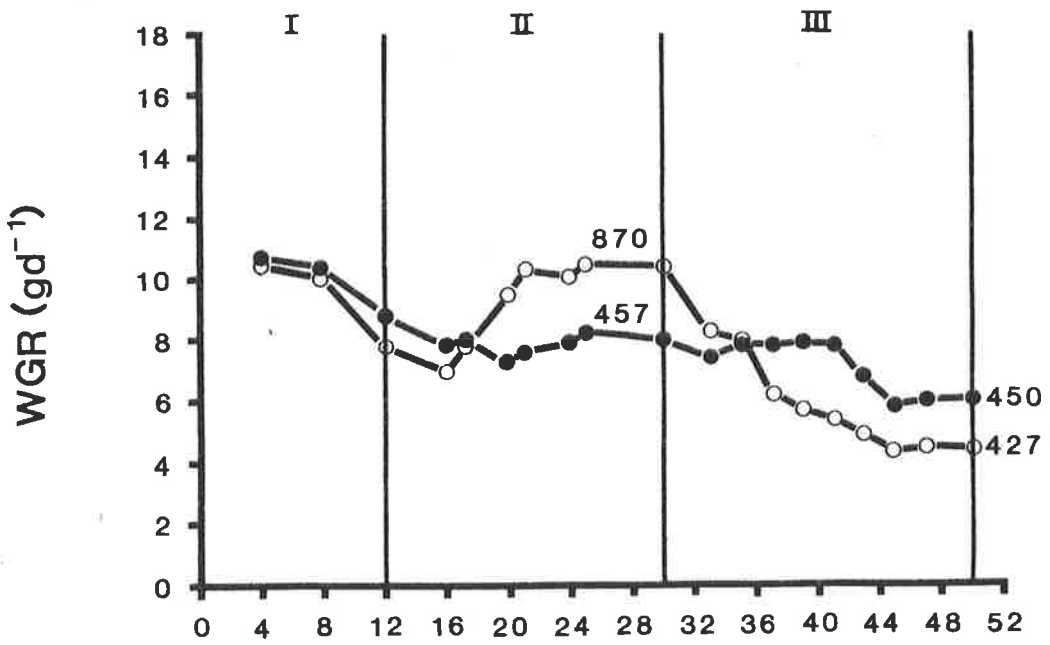
d) Groups AA_1 and DA_1.

Mean daily dry matter intakes (gd^{-1}) for each group are indicated.

a)

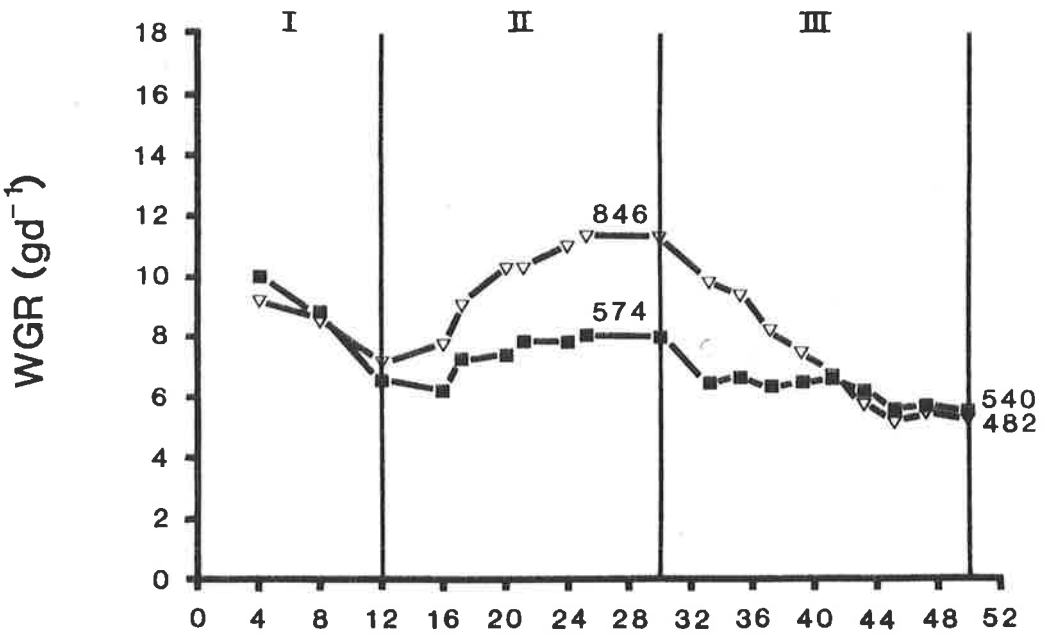


b)

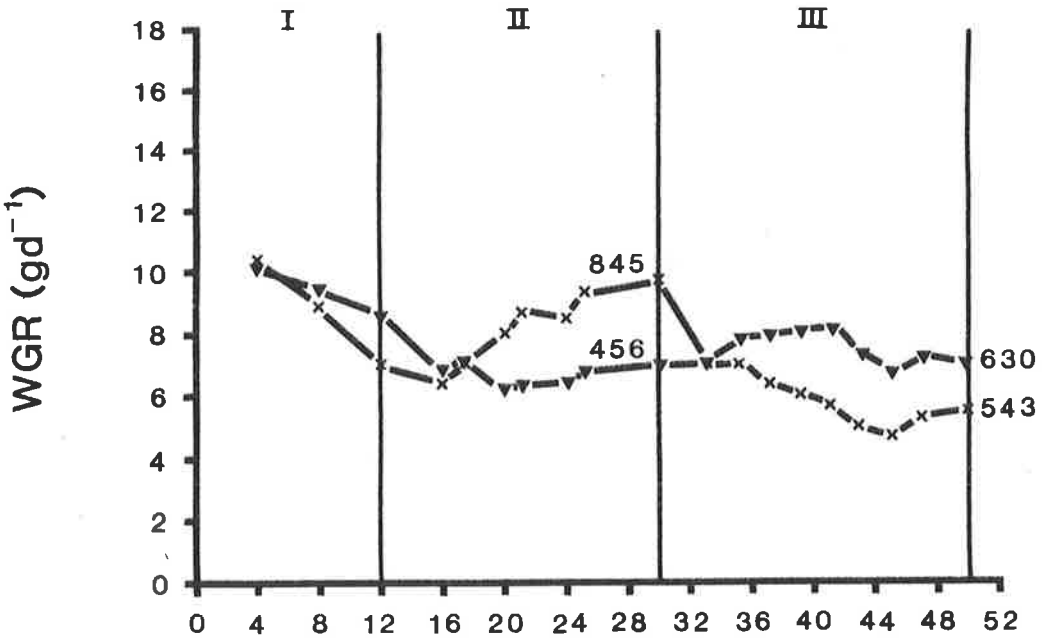


WEEKS OF EXPERIMENT

c)



d)



WEEKS OF EXPERIMENT

effects of weight change, nor were they a reflection of the inherent differences in efficiency determined during the covariate period.

Because the liveweight responses generated in Period III were relatively minor (group means:-26 to +50 gd^{-1}), a more appropriate analysis was to combine data from Periods II and III, so that a wider array of weight responses could be included. Such an analysis was possible because WGR data could be corrected for seasonality as previously described, and there was no "efficiency x intake" interaction. Equation 2.4 describes the relationship between efficiency of wool growth (g kg^{-1}) and weight change (gd^{-1}) for all sheep in Periods II and III. Individual bodyweight changes ranged from -57 to + 158gd^{-1}.

Equation 2.4....

$$WGR/DMI = 16.39-0.0034 \text{ weight change}(gd^{-1}) (r^2=0.004)$$

Nevertheless, the above results were obtained after WGR had equilibrated with dietary intake. To investigate the possibility that diminished weight changes by this time were responsible for the non-significant relationship in Equation 2.4, a similar regression analysis was used to derive the relation of wool growth efficiency at 5-7 weeks after the dietary change, to weight change from weeks 0-5. The appropriate comparisons are presented in Table 2.13 for all groups in Period III. Clearly, sheep losing weight were not more efficient wool producers than those gaining weight. At the extremes of weight change, group AD(-51gd^{-1}) and group DB_1 (+87gd^{-1}) produced 18.6 and 19.1 g wool per kg feed respectively.

Table 2.12 Mean DMI (gd^{-1}), efficiency of wool growth (WGR gd^{-1} per DMI kgd^{-1}) and fleece-free liveweight change (gd^{-1}) for all groups in Period III. Efficiency was estimated from final WGR in the period corrected for seasonality. (Means ± S.E).

Group		DMI (gd^{-1})	Efficiency (gd^{-1}/kgd^{-1})	Weight change (gd^{-1})
	AA_1	543	14.5 (7.4)	7 (28)
	DA_1	630	15.9 (6.4)	50 (6)
Ad lib.	B_1	549	13.7 (4.1)	-9 (23)
	AB_1	585	17.8 (3.9)	-8 (7)
	BB_1	585	20.3 (7.8)	19 (13)
	DB_1	585	20.6 (7.7)	47 (6)
	AC_1	482	15.1 (6.9)	-26 (13)
	CC_1	540	15.0 (7.8)	15 (25)
	AD	427	14.4 (6.3)	-22 (20)
	DD	450	19.8 (7.7)	9 (11)

Table 2.13 Mean DMI (gd^{-1}), efficiency of wool growth at 5-7 weeks (gd^{-1}/kgd^{-1}) and liveweight change to 5 weeks (gd^{-1}). All WGR data were corrected for seasonality. (Mean ± S.E). (Period III)

Group		DMI (gd^{-1})	Efficiency (gd^{-1}/kgd^{-1})	Weight change (gd^{-1})
	AA_1	543	13.8 (5.7)	-4 (63)
	DA_1	630	14.0 (3.2)	79 (28)
Ad lib.	B_1	549	18.5 (3.9)	-14 (79)
	AB_1	585	25.4 (1.9)	-4 (13)
	BB_1	585	20.7 (6.7)	45 (28)
	DB_1	585	19.1 (2.9)	87 (9)
	AC_1	482	20.5 (3.0)	-25 (13)
	CC_1	540	13.4 (3.5)	5 (10)
	AD	427	18.6 (4.9)	-51 (57)
	DD	450	19.8 (6.2)	14 (8)

2.3.5 The influence of body protein status on wool growth efficiency

Because wool growth is highly dependent on the protein nutrition of the host, the changes in body protein status were measured to determine if wool growth efficiency was more dependent on nitrogen balance than on weight change per se. To test this concept, the body nitrogen retention, estimated from TOH data over the first 9 weeks of Period III, was plotted against the wool growth efficiency determined at about the corresponding time i.e. after 11 weeks of Period III. Regression analysis of the results indicated that efficiency (E) was independent of the rate of body nitrogen gain or loss (N.B) within the observed range of -2.97 to +1.19 gd^{-1} (Equation 2.5).

Equation 2.5...

$$E = 17.34+1.10NB \qquad (r^2 = 0.04\ P>0.20)$$

2.4 Discussion

The experiment reported in this chapter is one of the most detailed and long term wool growth studies yet reported. The experimental design employed in this investigation differed from most nutritional studies in that responses to diet were invoked by feeding similar quantities of the diet to sheep of different weight rather than feeding different amounts to sheep of similar bodyweight. The technique was successful in that a range of liveweight, body protein content and WGR changes was generated at each level of dietary intake (Tables 2.5 and 2.8).

At no time throughout this trial did the relationship between WGR and intake differ significantly from one of simple proportionality (Fig 2.4), provided the former had stabilised at each nutritional level. In other words, the efficiency of wool production (WGR/Intake) was unaltered by absolute intake, a result which has been noted by other workers (Allden 1968b; Ferguson 1972; Langlands and Donald 1977). As suggested by Langlands and Donald (1977) this implies "that the quantity of wool produced per unit area of land will be proportional to intake per unit area and independent of intake per animal". Other authors have reported depressed efficiency at high intakes even though the genetic maximum WGR for the sheep in question (Hogan et al. 1979) has not been approached (Ferguson et al. 1949; Ahmed et al. 1963; Pattie and Williams 1967; Saville and Robards 1972). Failure to account for the lag period, and the interaction between feed intake and digestibility of some rations (Allden 1979) may account for this discrepancy. In the current study digestibility was not affected by intake for more than a short period (Table 2.7), and for the estimation of efficiency the lag period of 7-11 weeks (Table 2.10) was taken into account. A similar stabilisation period has been noted by other workers (Marston 1948; Coop 1953; Reis and Schinckel 1961). Contrary to the suggestion by the latter workers that the lag may be influenced by the direction or extent of the nutritional change, no such effect was apparent in the present trial (Table 2.10), although variability of response was high and may have overridden the effect. Similarly while the regression of WGR on intake was one of simple linearity, no unequivocal

statement can be made regarding this relationship because the error variance about the regression line was high. The fact that this regression never produced a significant intercept despite a decreasing slope throughout the experiment (Fig. 2.4), is in accord with the data of Hill (1970) in which season altered the regression line. However, the present study indicates that correction of wool growth data on the basis of the relative WGR changes of a uniform intake group would be imprecise, because individual variation was high in this study (see Section 1.3.3).

There was no evidence that WGR at any intake level was influenced by the rate or extent of liveweight changes within the range -26 to $+50gd^{-1}$ for groups (Table 2.12), or -57 to $+158gd^{-1}$ for individuals. Neither did the group mean efficiency differences approach statistical significance when allowance was made for inherent variation in WGR using covariance analysis.

Nevertheless, while mean weight changes for any period were substantial, the majority of the liveweight response occurred in the first five weeks after an intake change. Examination of the relationship between weight change and wool growth efficiency over this period (in Period III) again revealed no significant interaction, despite quite appreciable weight gains and losses (-51 to $+87gd^{-1}$ for groups and -157 to $+117gd^{-1}$ for individuals) (Table 2.13). Moreover, the gain or loss of body protein *per se* was not associated with variations in efficiency (Equation 2.5). In a statistical analysis of the data of Ferguson (1962), Nagorcka (1977) failed to detect any significant interaction between liveweight change and WGR, provided a time delay of

25 days was allowed. The current data, obtained in a more definitive examination of the hypothesis, lend support to his mathematical analysis. It appears that other than a short period immediately after a nutritional change there is no significant negative correlation between bodyweight change and efficiency, even when rates of weight change are substantial (Downes and Sharry 1971; Schoeman & De Wet 1973) (see Section 1.5.2.3 for re-analysis of data in the literature). The present result is also in keeping with work in which postruminal protein supply has been increased in sheep differing in energy balance (Black et al. 1973; Barry 1973b). Additional protein supplied to sheep that are losing weight does not increase wool production, presumably because the protein is catabolised for energy production (Judson and Leng 1973a) and wool growth has a low energy requirement (Section 1.2.1).

The results provide no evidence, therefore, that a more efficient wool-producing enterprise can be obtained by growing wool at the expense of body tissues accrued elsewhere (Irazoqui 1978). Maximum wool production per area is best obtained by optimising stocking rate to ensure maximum pasture utilisation, regardless of intake per animal. Neither does there appear to be any need to ensure static liveweight for the estimation of WGR (so-called "net efficiency" (Ferguson 1962)) in wool growth experiments. Of much greater importance is the necessity to ensure that WGR has equilibrated with diet i.e. that the carryover effect of previous nutrition is negligible, (see Fig 1.4, from Irazoqui 1970), that seasonal growth rhythms can be corrected for, and that the dietary source is unchanged.

The above conclusions cannot be made unequivocally because even though a significant proportion of the variance in WGR was accounted for by dietary intake, at no time was more than 50% of the between-sheep differences removed (Fig 2.4). This was reflected in the high coefficient of variation in wool growth efficiency, a value of 40% being recorded at the end of the trial. This is in contrast to the value of 12% noted for the same sheep during grazing prior to the experiment (when efficiency was estimated as WGR per unit bodyweight), and to estimates of variance presented by other workers (see Table 1.3). Bodyweight responses, on the other hand, were in line with those proposed when the experiment was planned (Table 2.6) and 95% of the variance in weight gain was attributed to differences in intake relative to maintenance requirements (Fig 2.3). Higher variability of wool growth efficiency during the experiment than was recorded at pasture, and the poor correlation between individual efficiency on pasture versus pen feeding (r^2=0.004) raises the possibility that the efficiency ranking of sheep on one diet may not always be related to the ranking on another diet, either because of some inherent character in the individual sheep, or because of variations in rumen metabolism that are independent of the individual. Such a concept, although not in keeping with the limited evidence of high repeatability of efficiency of sheep in a flock on different diets (Weston 1959; Dolling and Moore 1961), clearly needs further study in relation to the grain-herbage diet used in the current experiment.

Further studies, reported later in this thesis, were initiated to examine the nature and source of the

extraordinarily high variance in wool growth efficiency observed in this trial.

2.5 Conclusion

1. The experimental design described in this chapter allowed contemporaneous comparisons of the efficiency of wool growth of sheep differing only in the direction and rate of bodyweight change. Thus, interactions between diet composition, level of intake, seasonal growth rhythms and genotype were taken into account.
2. At no time was the linear relationship between WGR and intake of diet significantly influenced by liveweight change or gain and loss of body protein, even when these were extensive.
3. The efficiency of all sheep declined as the experiment progressed. However, the regression of WGR on intake over a given measurement period, always produced an intercept not significantly different from zero. Consequently the relative wool growth decrease with time, at any given intake level, was similar.
4. The efficiency of wool growth of individual sheep varied widely and independently of intake level or weight change. In contrast, liveweight responses were generally in line with those anticipated from dietary intake and the maintenance requirements. It is proposed that the wool and body tissue responses were at variance in some sheep because of some characteristic of the diet used which affected sheep differentially in terms of their wool producing capacity. Such effects could be due to some inherent character in the sheep that affected diet utilisation, or alternatively to

some character of the diet that induced instability in metabolism. The elucidation of this postulate is reported in subsequent studies (Chapters 4 and 5).

5. The time taken for WGR to equilibrate following a change in diet took up to 7-11 weeks, a period that is in accord with wool growth measurement studies reported in the literature using clipping techniques, but significantly greater than the period adduced from autoradiography studies. Autoradiography and follicle bulb studies were undertaken on a few sheep in the current experiment and the findings are reported in Chapter 3.

CHAPTER 3: A study of factors influencing the time lag in wool growth response

3.1 Introduction

It was concluded from experiment 1 (Chapter 2) that neither the direction nor the rate of bodyweight change had any significant effect on equilibrium WGR. Nevertheless, for sheep on a constant intake, the rate of liveweight gain will always diminish with time, as more and more of the ration is used to provide for the greater maintenance needs of the growing animal. Thus wool growth reaches the maximum at a time when the rate of weight gain is diminishing, a situation that makes it more difficult to determine the consequence of competition between the two tissues (Marston 1948; Schoeman and de Wet 1973). An alternative explanation is that the lag is induced by gradual changes in the structural dimensions of the follicle bulbs, as it has been clearly demonstrated that fibre output is closely related to the follicle bulb size (Fraser 1965).

Autoradiographic studies of wool fibre responses to nutrition have demonstrated that changes in fibre output are rapid, and seem to be complete within 10 days (Reis and Downes 1971). WGR equilibrium times estimated by clipping, on the other hand, are substantially longer, even when allowance is made for the emergence time delay described by Downes and Sharry (1971) (see Table 1.1). It has been considered that gradual changes in follicle bulb dimensions, with subsequent effects on fibre dimensions, are responsible for the long lag periods (Section 1.1.4) (Yeates et al. 1975). Were gradual bulb diameter changes (with accompanying fibre diameter changes) to occur after the initial fibre

response to nutrition, the impact on total WGR would be substantial because of the great influence of diameter on total fibre volume, and hence weight.

During the course of Experiment 1, supporting studies, of necessity on a limited scale, were undertaken to examine (a) the time sequence of changes in both follicle bulb cell mitotic rate and follicle bulb dimensions, in response to nutrition and (b) the responses of fibre length and diameter to intake change, using autoradiography.

Previous studies of mitotic activity in follicle bulbs have involved the blocking of metaphase nuclei with colchicine or colcemid administered intravenously (Schinckel 1961, 1962; Fraser 1965; Wilson and Short 1979a). The large number of fatalities recorded by these authors testifies to the detrimental impact of this procedure on general metabolism. Serial estimates of mitotic activity in the same animal are thus precluded. Recently a technique has been devised to overcome these "whole-body" effects of intravenous colchicine administration. Colchicine, at a very low dose rate, is injected intradermally at a depth of about 1mm; skin biopsy samples are then taken and processed to allow differentiation of mitotically active nuclei. (Phillips, unpubl). This method has been used successfully to monitor the re-establishment of activity in follicle bulb cells following cyclophosphamide administration (Schlink 1977). In the present experiment it was employed to monitor the pattern of mitotic changes in individual sheep whose nutritional level was increased or decreased. Follicle bulb dimensions were measured on the same preparations as mitotic activity.

3.2 Design

3.2.1 Fibre responses to nutritional change using autoradiography techniques

Fibre response times and total WGR changes were measured for each of the 4 sheep listed in Table 3.1. The sheep were selected on the basis of the extent and direction of dietary changes, the criterion being that contrasting differences in the degree of weight change and WGR would be established. Resources and time constraints limited the number of animals used, and statistical tests were based on within-sheep changes. Comparisons between sheep were not possible.

The results obtained using autoradiography were compared with the actual lag period determined by sequential clipping of tattooed areas.

3.2.2 Changes in follicle bulb cell mitotic activity and bulb dimensions with time after a nutritional change

Mitotic responses were estimated in two sheep only; sheep 1, whose intake was changed from 1166 to 582 g DMd^{-1}, and sheep 48, whose DMI was increased from 448 to 627g dmd^{-1}. Estimates were made on days 0, 8, 17, 23 and 100 after the intake change. These times were chosen because it was anticipated that the majority of the responses would occur in the first 3 weeks, while long-term effects would be complete after 14 weeks. Bulb dimensions were measured on the biopsy samples.

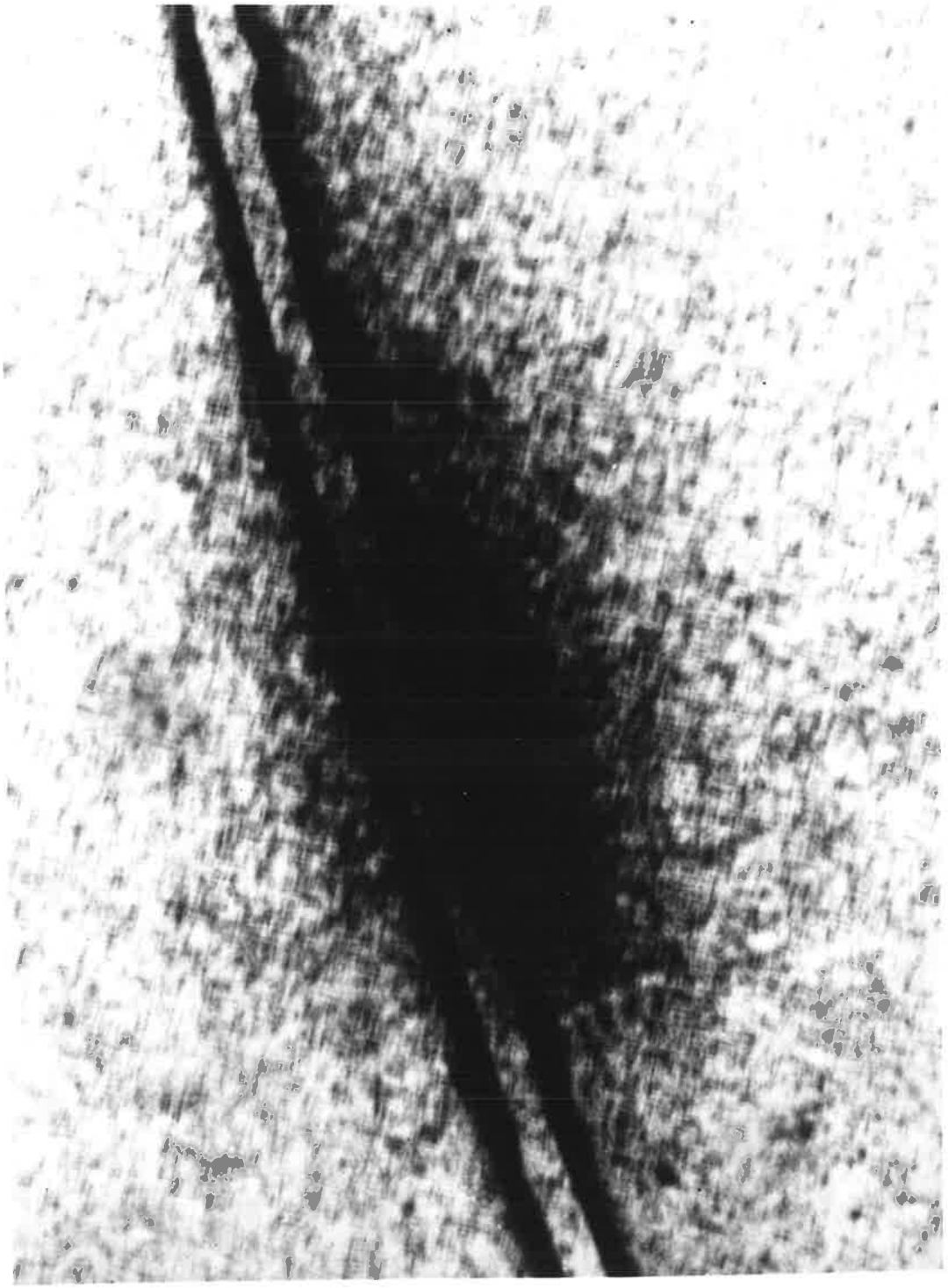
Prerequisite of the method

An absolute requirement of techniques by which the follicle bulb cell mitotic activity is estimated by

Table 3.1 The DMI change, previous weight gains (gd^{-1}) and new weight changes (gd^{-1}) of the 4 sheep in experiment 2

Sheep	Group	DMI change (gd^{-1})	Previous weight change (gd^{-1})	New weight change gd^{-1})
1	Ad lib. B_1	1166-582	+118	+9
43	AD	895-448	+133	-53
48	DA_1	448-627	+35	+60
29	DD	448-448	+28	-1

Plate 3.1 An autoradiographed fibre after an intravenous injection of 40μCi L^{35}S-cystine hydrochloride. The superimposed X-ray film is slightly off-centre but the point on the fibre at which the injection was made can be accurately identified. (Mag. 63x).


40 µm

colchicine blocking at metaphase, is that the accumulation of cells entering mitosis is linear from the time of administration to the time of sampling (Schinckel 1961). It might be anticipated that a local intradermal dose is less susceptible to factors such as nutritional status which alter general metabolic processes and hence the clearance of colchicine from the follicle bulbs (Schinckel 1961). To verify this, a sheep consuming $1000gd^{-1}$ of a high protein ration (see Table 4.2 for composition) was given an intradermal dose of colchicine at 4 sites on the midside region at zero hours. Subsequently, skin biopsies were taken at 0, 2, 4 and 6 hours post injection and the number of mitotic nuclei were estimated as described in Section 3.3. The injection sites were separated by more than 5cm, as Phillips (unpubl. data) established that activity was not influenced at this distance.

3.3 Methods

3.3.1 Fibre volume responses and WGR

Individual fibre response times were estimated for sheep 29, 43 and 48 by autoradiography after an intravenous injection of 40µCi ^{35}S-cystine hydrochloride (Radiochemical Centre, Amersham England) administered on days -5, 0, 5, 10, 15, 20 and 40 days after the DMI was changed (see Downes and Lyne 1959). Unfortunately the autoradiogram for sheep 1 was poor and no estimate was made for this sheep. A photomicrograph of an autoradiographed fibre taken at high magnification is presented in Plate 3.1. The lower edge of the "hot-spot" represents the injection time.

WGR was determined by the midside patch method described in section 2.2.4.

3.3.2 Follicle bulb cell mitotic activity, and bulb dimensions

1. An injection of 50mg colchicine in 0.5ml physiological saline was made at a depth of 1.2mm using a 1.0ml syringe and 25 gauge hypodermic needle. The site, on the right midside behind the tattooed patch, was marked with "Texta Color".
2. Exactly 3h later a 1cm diameter circle of skin was cut with a trephine and removed using curved scissors. The sampling site was then dusted with *Cicatrin (Wellcome, Aust) antibiotic powder.
3. The biopsy sample was immediately "fixed" in Zenker's fluid for 5h., then washed in running water for 12-14h. Excess wool was clipped from the sample which was then placed in iodised alcohol (1% KI in 50% ethanol) 12-24h., followed by 70% iodised ethanol for a further 12-24h. Samples were then stored in 70% alcohol.
4. Blocking of tissues was carried out in an Automatic Tissue Processor as follows:

   Skin biopsies were dehydrated in alcohol baths of 70%, 80%, 80%, 95%, 95% and 100% ethanol. 3 chloroform baths and 2 histological wax baths completed the process.

   The tissues were gently agitated for 30 minutes in each solution bath.
5. Sections of 10µm thickness were made on the embedded tissue using a rotary microtome (Erma optical). This thickness avoids split nuclei (Schinckel 1961). Sections thus obtained were attached to slides with dilute Mayer's albumen and dried at 45°C for 24h.

Staining

A counterstaining technique was used to enable clear distinction of metaphase nuclei.

Following wax removal in Xylol, and rehydration through decreasing alcohol solutions, the sections were hydrolysed in 1.0 N HCl at 45°C for 15-20 minutes and rinsed in H_2O (1-2 dips only). Excessive rinsing or inadequate hydrolysis causes the stain to be eluted from mitotic nuclei at the differentiation stage.

The staining technique is a modification of that described by Clarke and Maddocks (1963) in that longer periods of acid hydrolysis and staining in Crystal Violet and Lugol's Iodine were employed.

a 1.5 min in aqueous solution of Crystal Violet (1%).

b Rinse in H_2O (1 dip).

c 1.5 min in Lugol's Iodine.

d Rinse in H_2O.

e 5 secs. in 3% solution of the colour acid of Eosin in 70% alcohol.

f Rinse.

g Decolourise in 70% alcohol for 3 min.

h Continue differentiation in 90% alcohol (3 changes) for approx 15 mins.

i 10 min. 100% alcohol.

j 20-25 min Xylene.

k Coverslip mounted using DPX.

Counting

The frequency of follicles having 0, 1, 2, 3....n mitotic cells per section was recorded for every 4th serial section to minimise the possibility of counting the same bulb more than once. Moreover, only those bulbs sectioned along their central axis, and with clear distinction between mitotic and non mitotic nuclei, were counted. Mitotic cells in the papilla and outer root sheath were excluded.

More than 200 counts were required to characterise the mitotic index for each sample.

Concurrent estimates of bulb diameter were made, and mean bulb areas were determined on a projection microscope by tracing the bulb area, segmenting the tracing paper and using the weight/area index for that paper. Assuming symmetrical bulbs, the mean volume was calculated as mean bulb diameter x mean bulb area (Short et al. 1965, Wilson and Short 1979a).

Statistical analysis of mitotic rate differences

A non-parametric test was chosen, as there was evidence that the frequency distribution of mitotic rate was not "normal" (Schlinck, unpubl.). The Kolmogorov-Smirnovov test was used and is summarised below:

2 samples of n_1 and n_2 observations. A cumulative frequency table is made for each sample, and a difference column then made by subtracting the cumulative frequencies at each observation.

$Sn_1(x) = K/n_1$ where K = the number of scores x in sample 1,

$Sn_2(x) = K/n_2$ etc

The test is 2-tailed and the statistic of the test (K_D) is the maximum value of $|Sn_1-Sn_2(x)|$ when $n_1 + n_2$ 40. If K_D is greater than the confidence limit then the samples differ significantly at that level.

$$0.1\% \text{ level} = 1.93 \times \sqrt{\frac{n_1+n_2}{n_1 n_2}}$$

3.4 Results and Discussion

3.4.1 Comparison of the autoradiography and clipping techniques

Wool fibre output responded rapidly to both increased and decreased feed intake, and autoradiography showed that the majority of length growth rate changes were essentially complete 10-15d after the nutritional change (Fig. 3.1). It is also apparent from this figure that both fibre diameter (D) and length (L) responded together, so that the ratio of L/D remained constant, a result previously noted by Downes (1971). Moreover, rapid output responses have been recorded when nutrition has been improved by either postruminal amino acid supplementation (Reis et al. 1973) or increased feed intake (Downes & Sharry 1971). Good agreement between wool growth results determined by autoradiography and those obtained by the clipping technique corrected for the emergence time delay (Downes & Sharry 1971), led Reis et al. (1973) to conclude that satisfactory data can be obtained using autoradiography results from days 8-12 after an intake change. The data from the current trial are not in accord with this conclusion. While the fibre output response was rapid, the total WGR change estimated by clipping was

Figure 3.1 Responses of length growth rate (μmd^{-1}) and fibre diameter (μm), as estimated by autoradiography, to increased (sheep 48), decreased (sheep 43), and unaltered (sheep 29) dry matter intakes.

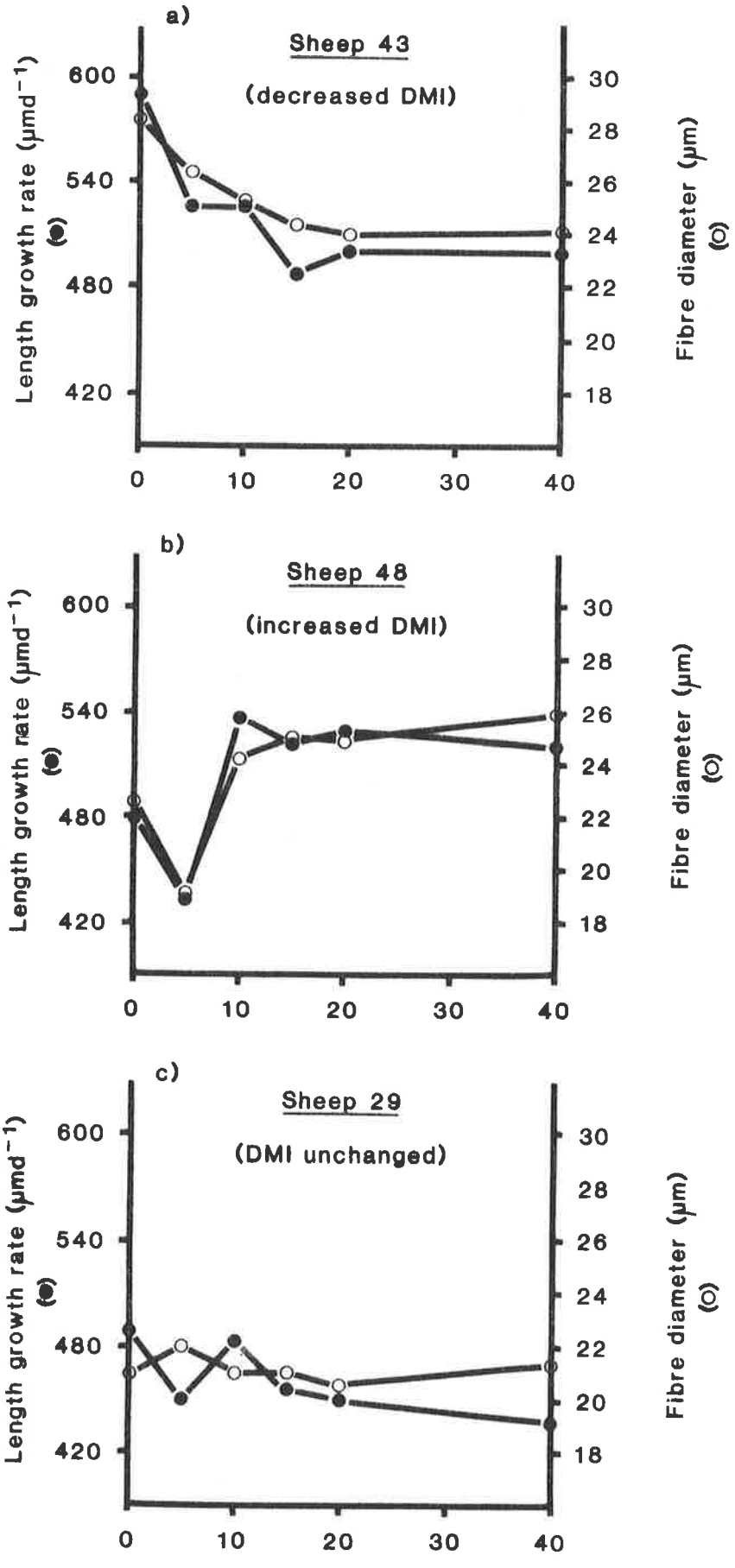
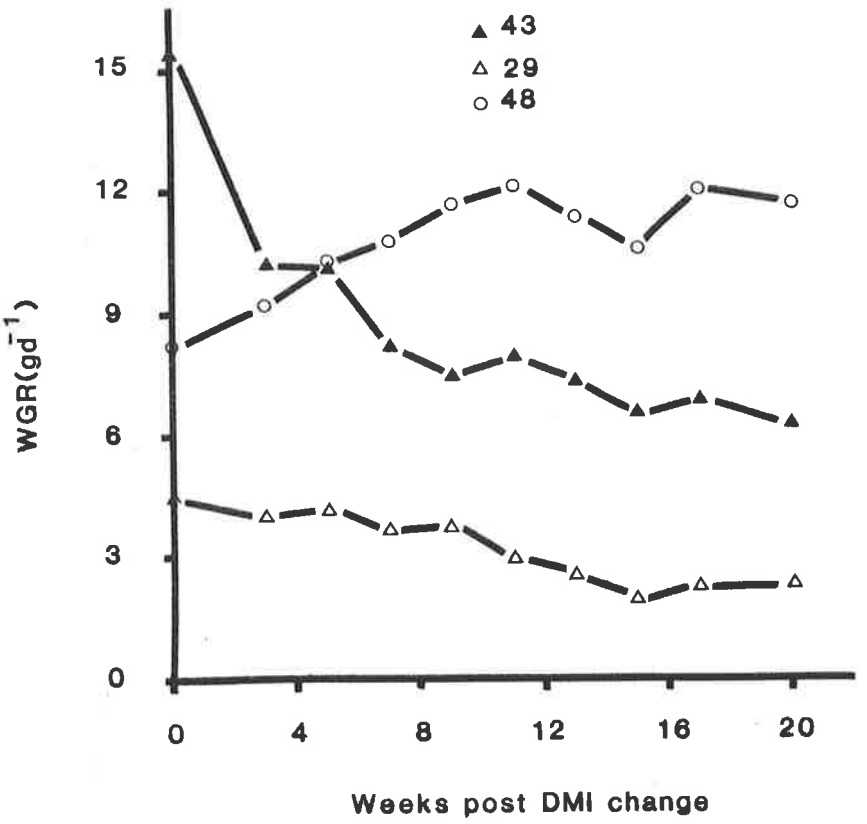
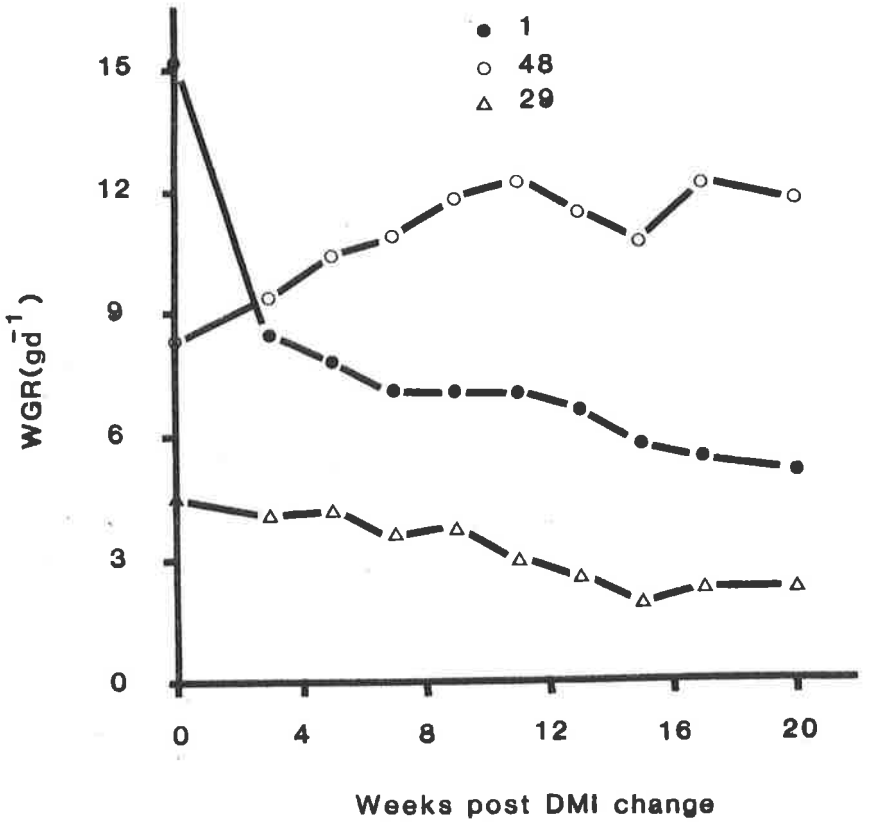

a)
Sheep 43
(decreased DMI)
Length growth rate (μmd⁻¹)
(●)
Fibre diameter (μm)
(○)
600
540
480
420
30
28
26
24
22
20
18
0
10
20
30
40
b)
Sheep 48
(increased DMI)
Length growth rate (μmd⁻¹)
(●)
Fibre diameter (μm)
(○)
600
540
480
420
30
28
26
24
22
20
18
0
10
20
30
40
c)
Sheep 29
(DMI unchanged)
Length growth rate (μmd⁻¹)
(●)
Fibre diameter (μm)
(○)
600
540
480
420
30
28
26
24
22
20
18
0
10
20
30
40

Figure 3.2 Changes in WGR (gd^{-1}) with time after nutritional change for sheep 1 (decreased intake), 43 (decreased intake), 48 (increased intake) and 29 (unaltered intake).


▲ 43
△ 29
○ 48
15
12
9
6
3
0
WGR(gd⁻¹)
0
4
8
12
16
20
Weeks post DMI change


● 1
○ 48
△ 29
15
12
9
6
3
0
WGR(gd⁻¹)
0
4
8
12
16
20
Weeks post DMI change

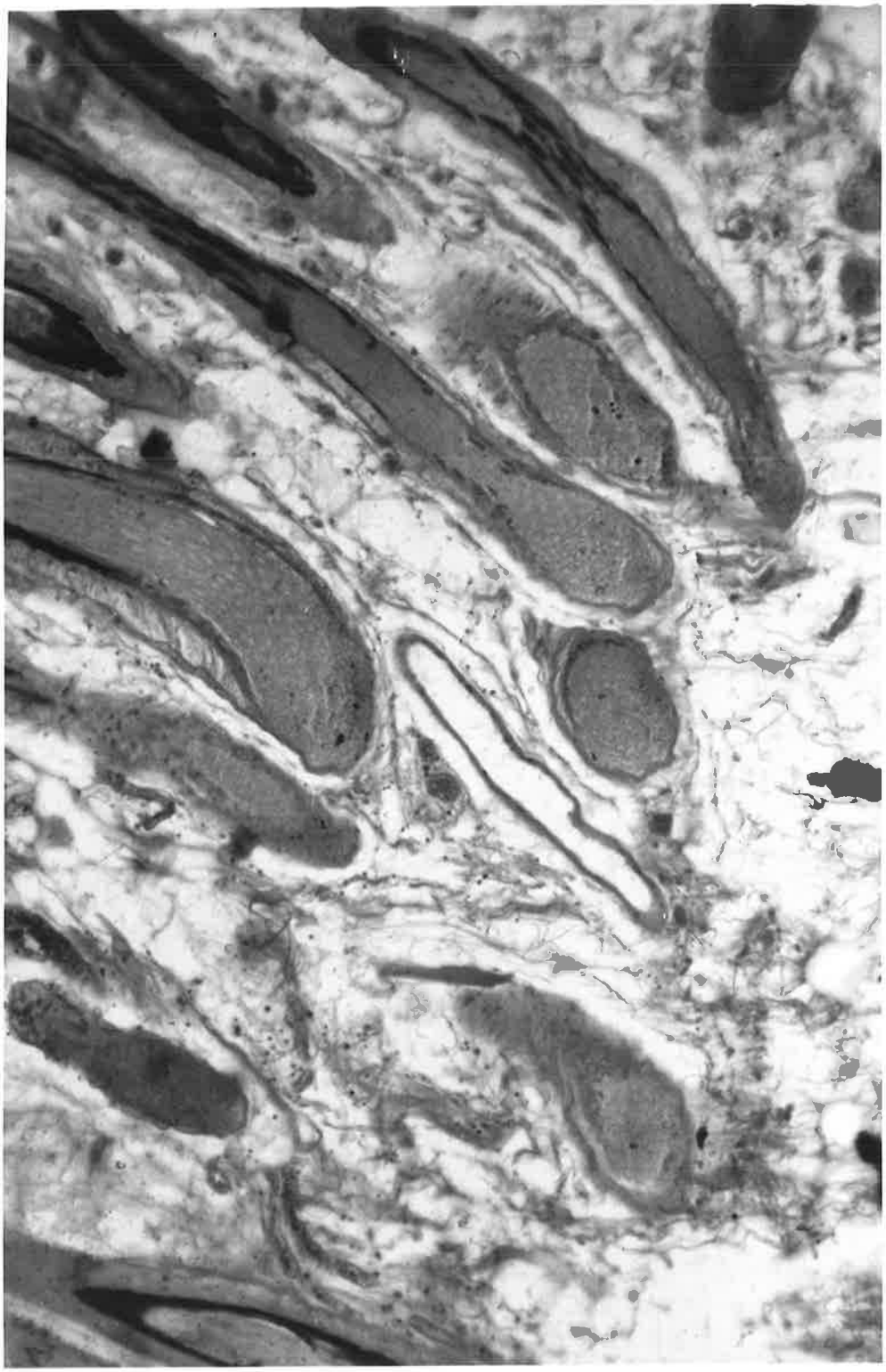
appreciably slower (Fig. 3.2). Equilibrium WGR, although complicated by some "non-nutritional" variance (see Sheep 29 Fig. 3.2), was not attained in response to either increased or decreased intake till 6 weeks, which leaves a difference of 3 weeks unaccounted for between the two methods after allowance of 7d for emergence time. (Downes and Sharry 1971). From the work of these authors, a lower emergence time than this might be anticipated for sheep 48 whose intake was increased, and this would exaggerate the discrepancy. Similarly, a slower fibre output would lengthen the emergence time of sheep 43 whose intake was decreased, and would account for some fraction of the 3 week anomaly. Nevertheless, in both cases the effect would only be of the order of 3-4 days.

Analysis of the data relating to follicle bulb mitotic activity and dimension changes in sheep numbers 1 and 48 support the contention that the clipping results are a more accurate reflection of the true wool growth responses.

3.4.2. Follicle bulb mitotic activity and dimension responses to nutritonal change

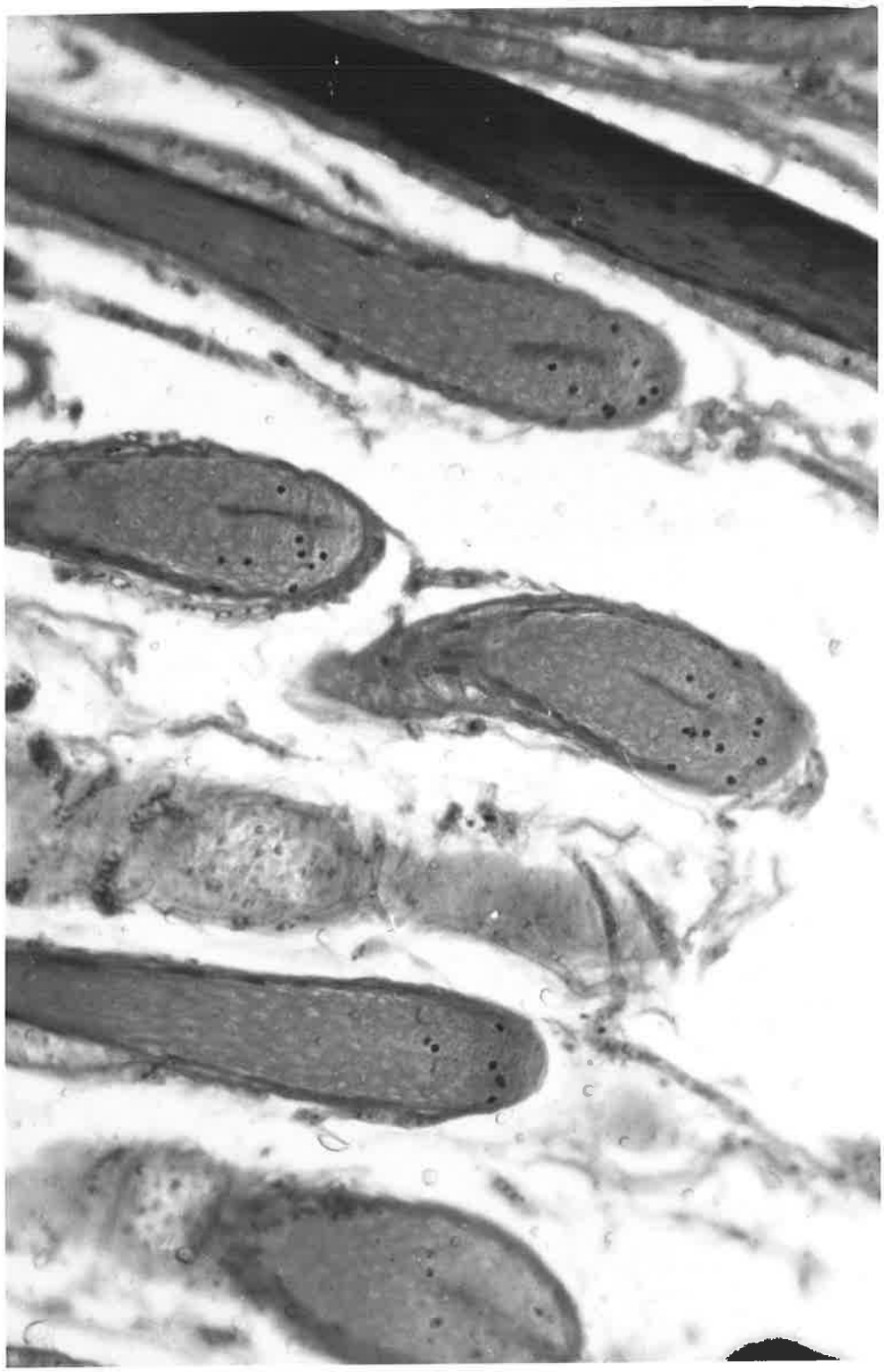
The technique described for estimating follicle bulb cell mitotic activity provided clear distinction of mitotic from non-mitotic nuclei. Typical sections at 0, 3 and 6h after colchicine administration in sheep 39 were photographed, and the resulting photomicrographs are presented in Plates 3.2, 3.3 and 3.4. Few mitotically active nuclei are apparent in non-follicular tissue even 6h after colchicine dose (Plate 3.4). Data obtained from sections such as these are presented in Fig. 3.3. Clearly, the mitotic nuclei were successfully arrested at metaphase with

Plate 3.2 Wool follicle bulb preparation made zero hours after an intradermal colchicine injection (50 mg). Very few mitotically active nuclei, indicated by dark-staining dots, can be noted in the germinal region of the follicle bulbs. Few mitoses are apparent in non-follicular tissue also. (Mag. 63x).



(63×)

Plate 3.3 Wool follicle bulb preparation made three hours after an intradermal colchicine injection (50 mg). From 6 to 12 mitotic nuclei are apparent in the bulb tissue. Mitotically inactive cells can also be seen in each follicle bulb. (Mag. 100x).



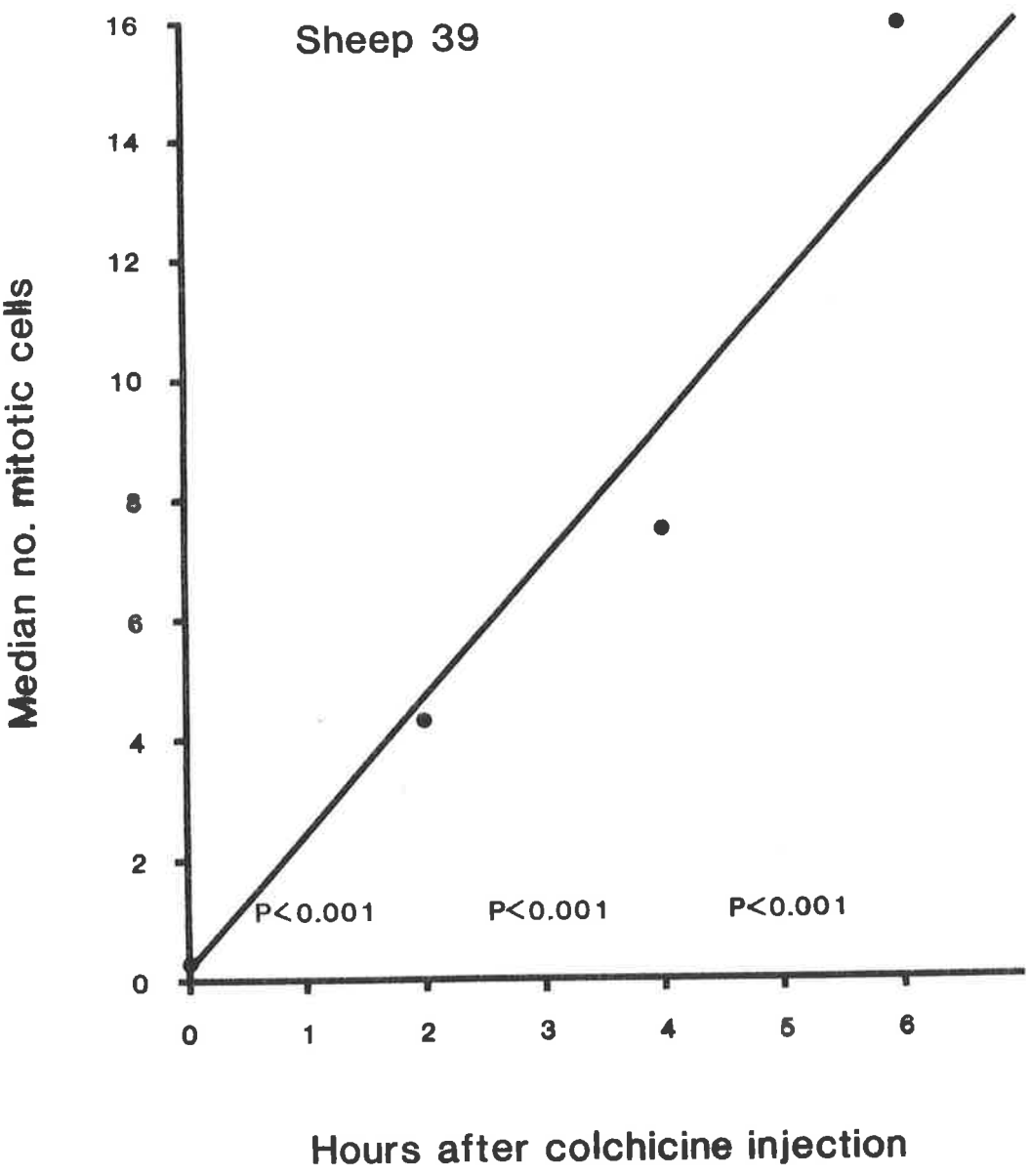
(100×)

Plate 3.4 Wool follicle bulb preparation made six hours after an intradermal colchicine injection (50 mg). Note the large number of active nuclei in each bulb. (Mag. 63x).

(×63)

(63×)

Figure 3.3 The accumulation of mitotically active nuclei with time after intradermal colchicine administration. This sheep was on a high nutritional level and had a WGR of 19.5 gd^{-1}. The significance levels refer to differences between sampling times as tested by the Kolmogorov-Smirnovov test.


Sheep 39
Median no. mitotic cells
16
14
12
10
8
6
4
2
0
P<0.001
P<0.001
P<0.001
0
1
2
3
4
5
6
Hours after colchicine injection

no decline in accumulation up to 6h after dosing, in contrast to some results obtained using the conventional colchicine technique (see Schinckel 1961). Biopsy sampling at 3h by the present technique is therefore justified, and the results obtained for the two sheep under study (Nos. 1 and 48) are presented in Table 3.2.

The mitotic rate in the follicle bulbs of sheep 48 increased slowly and linearly with time when intake was raised from 448 to 627 gd^{-1}, a result consistent with the steady fibre output responses as determined by clipping (Fig. 3.2). By day 17-23, the mitotic rate was about 10% greater, although not significantly so, and by day 100 was 40% greater than at day 0 ($P<.05$). While the initial increase in mitosis was statistically non-significant it corresponded precisely to the proportionate increase in both fibre length growth rate and fibre diameter over the same period (Fig. 3.1b).

Sheep no. 1 showed a rapid decline in WGR (by clipping) following a 50% reduction in diet intake (Fig 3.2b). Indeed, a 50% decrease in WGR was apparent in the first 3 weeks on the low nutritional level. While WGR continued to decline gradually for a further 4 weeks, thereafter the changes were in parallel to those of the uniform intake sheep no. 29 (Fig. 3.2). Depressed WGR was related to a decline in mitotic rate, the majority of which occurred by day 23 (at day 23 the mitotic activity was 74% of the original rate, and at day 100 the rate was 70% of the original).

A change in the number of mitotically active cells can result from either or both of the following:

1. a change in the total germinal cell number,

Table 3.2 Changes in mitotic activity, bulb dimensions and "turnover rate" for sheep numbers a) 48 and b) 1.

No. 48 (Increased intake)	Days post intake change 0	8	17	23	100
Mitotic No. (MN/3h)	4.2^{a}	n.a.	4.6^{a}	4.6^{a}	5.9^{b}
Bulb Diameter (μm)	66.5^{a}	69.8^{b}	73.2^{c}	75.6^{cd}	79.9^{e}
Bulb Volume ($\mu m^3 \times 10^4$)	4.05	n.a.	4.78	4.74	5.87
MN/Bulb Vol. ($no/\mu m^3 \times 10^4$)	1.04	n.a.	0.96	0.97	1.01
No. 1 (Reduced intake)	**0**	**8**	**17**	**23**	**100**
Mitotic No. (MN/3h)	6.6^{a}	6.4^{a}	n.a.	4.8^{b}	4.6^{b}
Bulb Diameter (μm)	74.7^{a}	78.4^{b}	72.3^{a}	75.8^{ab}	69.5^{c}
Bulb Volume ($\mu m^3 \times 10^4$)	6.47	n.a.	5.93	6.21	5.41
MN/Bulb Vol. ($no/\mu m^3 \times 10^4$)	1.02	n.a.	n.a.	0.77	0.86

Means in the same row not followed by the same letter differ significantly at $P<0.05$.

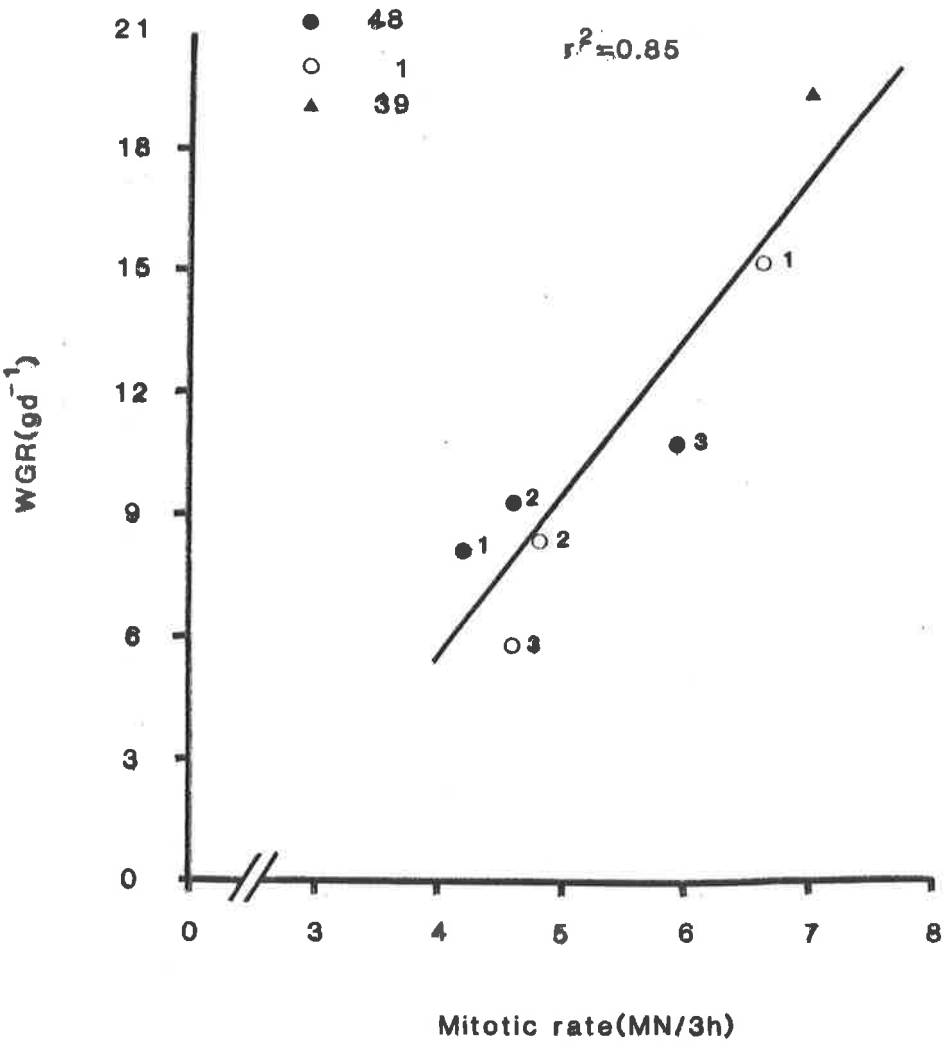
n.a. = not available, usually due to poor differentiation of mitotic nuclei.

2. a change in the rate of division of these cells (Fraser 1965). The latter, termed the turnover rate, can be estimated as the ratio of mitotic cells per unit time, to total bulb volume, an indicator of the total number of germinal cells (Wilson & Short 1979a). A large number of mitoses per unit bulb volume (MN/Bulb Vol. in Table 3.2) represents a rapid cell population turnover. Increased fibre output in sheep 48 was apparently achieved without any change in "turnover rate", while the lowered WGR of sheep 1 was in part a function of a slower cell turnover rate as well as less cells. Such differences may not be a reflection of differential responses to plane of nutrition, but rather of the mechanism of fibre production in different sheep. This speculation is supported by limited evidence of Fraser (1965) and warrants further investigation using the local colchicine technique described in this Chapter.

The mean maximum bulb diameter increased rapidly in sheep 48 and was still changing 23 days after intake was increased (Table 3.2). Furthermore, the bulb diameter at day 100 was significantly greater than at day 23 ($P<0.05$), a result in accord both with the gradual elevation of mitotic rate over this period and the wool growth response by clipping (Fig. 3.2). In this context it is of interest to note that there was an increase in mean fibre diameter (by autoradiography) from day 20 to day 40 in this sheep (Fig. 3.1b). These results may be interpretated as implying that the rate of bulb expansion (with concomitant changes in the number of germinal cells and hence, mitotic rate) is the limiting factor governing the rate of wool growth response.

When the intake of sheep 1 was halved, there was little

Figure 3.4 The relationship between wool growth rate (gd^{-1}) estimated by clipping, and mitotic rate (no./3h) for sheep no's 1 (decreased DMI) and 48 (increased DMI) with time. Numbers 1, 2 and 3 refer to times 0, 23 and 100 days after DMI change. The estimate for sheep 39 (high plane of nutrition) is included.


● 48
○ 1
▲ 39
r^2=0.85
21
18
15
12
9
6
3
0
WGR(gd^{-1})
0
3
4
5
6
7
8
Mitotic rate(MN/3h)
1
2
3
1
2
3

change in mean follicle bulb diameter up to 23 days after the intake change, a surprising result in the light of the rapid WGR decline over this period, and the responsiveness of follicle structure to improved nutrition demonstrated by sheep 48. Apparently the initial wool growth decrease was mainly induced by a slower cell turnover rate or a change in cell size, so that the bulb volume/cell number ratio was altered. It may be significant that enhanced fibre diameter can only occur if the follicle area is increased, whereas decreased diameter is not dependent on immediate changes in follicle area.

The close relationship observed between mitotic rate (MN/3h) and WGR (gd^{-1}) estimated by clipping, is depicted in Figure 3.4, for sheep 48 and sheep 1. The data for sheep 39 used to verify the technique prerequisite, is also included. As mitotic rate changed with time after a nutritional change, so the wool fibre output responded to the change in the number of germinal cells entering the fibre cortex.

It is clear from the results for both sheep that wool growth is intimately related to the rate of mitosis in the follicle bulb cells, and that the lag in wool output response to nutrition is a function of the time for mitotic rate to equilibrate with nutrient supply. The limited evidence from the autoradiography and mitotic studies indicates quite clearly that changes in wool follicle activity are immediate. The autoradiography data suggest that the major part of the change is complete within 2-3 weeks, whereas the mitotic studies indicate that mitotic activity, bulb diameter, bulb volume and the number of mitotically active cells per unit bulb volume may continue

beyond 23 days. As there was no sampling between days 23 and 100 the precise time of cessation of change cannot be estimated for each sheep. Such changes would be consistent with the longer times taken for wool growth to equilibrate with diet observed in the clipping studies.

3.5 General Conclusions

In this study it was recognised that the limited number of animals used would allow no more than general indications of changes in wool growth and follicle bulb parameters with diet. Nevertheless the following conclusions can be drawn.

Firstly, while not defining the cause(s) of the wool growth lag, the results indicate that the lag is related to the slow rate at which changes in follicle dimensions and mitotic activity occur. While wool response to decreased nutrition was more rapid than to increased nutritional level, in both cases equilibrium was not approached until some 6 weeks after dietary change. This period, as determined from clipping data, was longer by 3 weeks than equilibrium estimated by autoradiography, even when the emergence time delay was taken into account.

Secondly, the local intradermal colchicine technique which has been little used, appeared to be satisfactory and had none of the deleterious effects associated with the systemic administration of this chemical. Fibre output was related to mitotic rate measured using this technique and mitotic rate, in turn, was a function of the number of cells in the bulb, and the turnover rate of these cells.

The present data do not indicate whether the lag in wool growth response to nutrition is a consequence of changing nutrient supply or of obligatory structural changes in the follicle bulbs.

CHAPTER 4: Diet composition and the variability of wool growth rate between sheep

4.1 Introduction

Throughout Experiment 1 it was apparent that the discrepancies in the amount of wool produced per unit of feed intake between sheep, were greater than those normally recorded for sheep of similar genetic origin. At the conclusion of this trial the coefficient of variation in efficiency was 40%, in contrast to the value of 12% noted for the same animals under grazing conditions.

This high variation in WGR by sheep consuming the same ration prompted an important change in the proposed program of studies for this thesis. Originally it was planned to examine aspects of the partitioning of nutrients between wool fibre and body tissues in the growing, mutton-type sheep and the merino fed a cereal grain diet similar to that used in Experiment 1. Clearly it was important to elucidate the source of variation in animal performance on this type of diet, as rations containing a large proportion of cereal grain are used in wool growth and fattening studies (Schinckel 1960; Hutchinson 1961; Allden 1968a), and for drought feeding wool-producing sheep (Hemsley 1976). Furthermore, sheep at the extremes of production performance were ready at hand for closer examination.

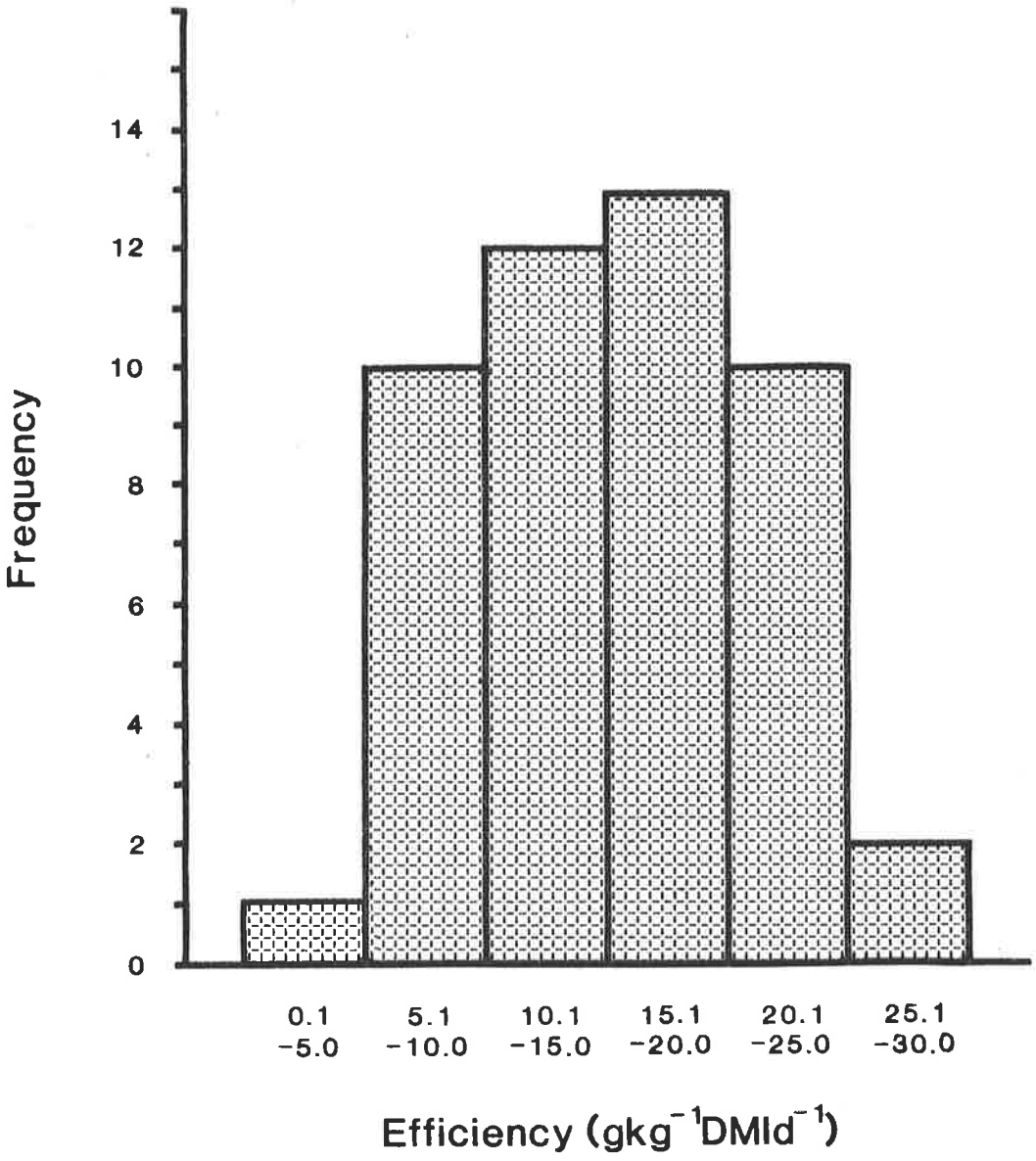
It was postulated that the variation was due to one of two major factors. Firstly, and less likely, was the possibility that some of the experimental animals were constrained by their genetic capacity to produce wool at the higher level of intake, and were tending to reach an asymptotic value for wool production, whereas others with a

high production potential continued to grow wool in proportion to intake (Ferguson 1956).

A second, more probable reason, was that some factor(s) associated either with the type of diet or of individual sheep consuming this type of diet, contributed to the wide variability. This raises a concept that hitherto has not been noted in the literature, namely that relative to its mates in the flock receiving a comparable amount of feed, a sheep may do well on one diet but indifferently on another.

To examine this second proposition, an experiment was planned using sheep chosen from Experiment 1. There was no evidence of anything but a normal distribution of efficiency of wool production when the 48 sheep were examined in the second period of Experiment 1. (Fig 4.1). To ensure the widest possible variation for analysis of the differences, sheep were selected from the extremes of this distribution curve. The objective was to define whether the variation in wool growth efficiency would be reduced when sheep were offered a diet that was formulated to provide a large amount of "good quality" amino acids postruminally. If protein supply on the diet used in Experiment 1 was marginal for some sheep and less so for others, then the WGR of the low efficiency sheep might be expected to increase substantially under an improved nutritional regime. Equally important, the experiment also sought to determine whether the wool growth efficiency of a sheep on the diet used in Experiment 1 was a repeatable or a random event. If repeatable, this would provide evidence of a genetic origin, whereas if the effect occurred randomly it would suggest some instability in digestion and metabolism associated with the diet. This

Figure 4.1 The frequency distribution curve for efficiency of wool growth (g/kg D.M.I.) of 48 sheep in Period 2 of Experiment 1.


Frequency
0
2
4
6
8
10
12
14
0.1
-5.0
5.1
-10.0
10.1
-15.0
15.1
-20.0
20.1
-25.0
25.1
-30.0
Efficiency (gkg⁻¹DMId⁻¹)

aspect was determined by returning sheep to the grain concentrate diet after a period on the high quality diet.

4.2 Materials and methods

4.2.1 Animals and feeding

Twenty of the sheep from Experiment 1 were allocated to two groups on the basis of their wool growth efficiency in that trial, sheep of low efficiency comprising the LE group and sheep of high efficiency the HE group. Table 4.1 details for each animal the wool growth efficiency recorded during the period of highest intake of the experimental ration (Diet A), and compares these values with the wool production per unit bodyweight at the beginning of Experiment 1, this being the only index of efficiency when sheep are grazed together on pasture (Weston 1959). On the basis of these data there was little difference in the efficiency of these groups at pasture, whereas more than a two-fold difference in mean efficiency was apparent in Experiment 1.
(10.3 cf. 22.1 g wool per kg. feed).

The sheep were shorn, footpared, and treated for parasites one month prior to commencement of the trial. During this time, one animal from group LE died, so that this group was reduced to 9 members.

Diets

Diet A, the feed remaining from Experiment 1, was used in the current trial. It comprised 60% barley (cv Clipper) and 40% lucerne hay, pelleted after hammermilling and mixing.

Diet B contained 65% lucerne hay, 15% extracted soybean meal, 15% extracted linseed meal and 5% fishmeal. Ingredients were pelleted after hammermilling and mixing.

The composition of the rations is shown in Table 4.2.

Table 4.1 The efficiency of wool growth of selected sheep on diet A (WGR(gd^{-1}) per DMI (kgd^{-1}) and and during grazing prior to experiment 1 (WGR gd^{-1}) per bodyweight (kg)).

Sheep No.		Grazing efficiency (gd^{-1} kg^{-1})	Diet A efficiency (gd^{-1}/kgd^{-1})
LE	3	0.44	11.43
	14	0.56	7.87
	41	0.55	8.03
	47	0.50	10.16
	5	0.60	9.43
	11	0.57	11.24
	12	0.65	14.58
	23	0.56	8.85
	36	0.71	10.78
Mean± S.D		0.571± 0.074	10.26± 1.96
C.V(%)		13.0	19.1
HE	13	0.51	23.19
	17	0.51	21.56
	19	0.55	21.32
	44	0.48	21.76
	4	0.46	19.09
	18	0.61	19.74
	25	0.48	21.73
	38	0.58	24.24
	39	0.56	22.22
	48	0.51	26.35
Mean± S.D		0.525± 0.046	22.12± 1.99
C.V(%)		8.8	9.0
Grand Mean S.D		0.547± 0.065	16.50± 6.24
C.V(%)		12.0	37.8

Table 4.2 The dry matter (DM), nitrogen (N), metabolisable energy (ME) and dry matter digestibility (DMD) of the experimental diets. (Means ± S.E.M.).

	Diet A	Diet B
DM (%)	88.30±0.01	86.39±1.70
N (% of DM)	2.71±0.10	5.15±0.05
DMD (%) +	77.3	73.7
ME ($MJkg^{-1}DM$) *	10.64	10.11

+ both determined at $1000gd^{-1}$ air dry feed intake.

* determined from ME = 0.15DOMD% (MAFF Bull.33 p65).

Initially, the 19 sheep (9LE, 10HE) were transferred to Diet B (1000 gd^{-1} air dry) for a period of 8 weeks with the object of determining whether the relative differences in wool growth efficiency observed in Experiment 1 were sustained or eliminated. At the end of this period, 8 sheep (4 LE and 4HE) were transferred back tc Diet A (1000 gd^{-1} air dry) for 14 weeks. The object of this comparison was to assess whether there was a return to the high wool growth variation observed in Experiment 1 and whether the efficiency performance of individual sheep in that experiment was repeatable. The number of sheep that could be transferred to Diet A was constrained because the quantity of feed remaining from Experiment 1 was limited. The remaining 11 sheep (5 LE, 6HE) stayed on Diet B for a further 7 weeks to ensure that any differences observed in the first 8 weeks of feeding were sustained.

To monitor non-nutritional variation in WGR the 4 sheep on a uniform intake in Experiment 1 were maintained on this regime for the entire 22 weeks of the trial.

The opportunity was also taken to examine the wool growth responses attained at high intakes of Diet B, to determine if efficiency was reduced when a large quantity of protein was provide *per os*. This was a subsidiary aim, but it was considered that knowledge of the relationship between WGR and nitrogen intake would be helpful in the interpretation of the dietary responses in this trial. Five sheep (3LE, 2HE) were offered 1500 gd^{-1} (air dry) of Diet B for 7 weeks after these sheep had previously been consuming 1000 gd^{-1} Diet B for 15 weeks. Likewise, it provided evidence regarding the wool producing capacity of HE and LE

sheep at high intakes of protein.

The feeding sequences described above are summarised as follows:

PERIOD	GROUP AND FEED
Weeks 1-8	All sheep on Diet B (1000gd^{-1})
Weeks 8-22	8 sheep (4LE, 4HE) returned to Diet A (1000 gd^{-1})
Weeks 8-15	11 sheep (5LE, 6HE) continued on Diet B (1000 gd^{-1})
Weeks 15-22	5 sheep (3LE, 2HE) previously receiving Diet B (1000 gd^{-1}) received Diet B (1500 gd^{-1})
Weeks 1-22	4 sheep (Group DD from Experiment 1) received Diet A (500 gd^{-1})

4.2.2 Wool growth

WGR was derived from right midside patch wool production over intervals of not less than 3 weeks (Section 2.2.4 describes the clipping and scouring procedures employed).

4.2.3 Statistical analysis

Differences in WGR and efficiency between groups and diets were examined in an analysis of variance, the means compared using a t-test. Repeatability of the wool growth response to diet A was estimated by simple linear regression of original efficiency in Experiment 1 with efficiency in the current trial for each individual, the magnitude of the correlation coefficient reflecting the extent of repeatability.

4.3 Results

4.3.1 Wool growth

4.3.1.1 Response to the high protein diet (Diet B)

When the experimental animals were transferred to the high protein Diet B there was a dramatic response in WGR by all sheep, but the LE group responded to a much greater extent than the HE group. Within 8 weeks the WGR and efficiency differences that existed between the two groups had been eliminated, and for those sheep that continued on Diet B for a further 7 weeks the differences remained non significant. More importantly, the coefficient of variation in wool growth efficiency of 42% on Diet A was reduced to 7.7% on Diet B for these 11 sheep. Figures 4.2 a) and 4.2 b) illustrate the time sequence of the wool growth responses of both the high and low efficiency sheep on Diet B. No correction was made to wool production data because the mean WGR of the constant intake group was stable (Fig. 4.2a). The increase in wool production of the LE sheep was 17.0 gd^{-1}, whereas the HE sheep increased by only 11.5 gd^{-1}. The factor of greater significance is that a highly significant difference in WGR and WGR per nitrogen intake between the groups on Diet A, was reduced to a non significant level when the sheep received Diet B.

Figure 4.2b shows that the efficiency of both groups declined initially when Diet B was offered, because the nitrogen intake was rapidly increased while WGR responses were delayed. The small advantage of the HE sheep from weeks 8-15 was statistically insignificant. Once the wool growth of the HE and LE sheep had equilibrated with Diet B, it was apparent that the efficiency of LE sheep had been

Figure 4.2 a) Changes in mean WGR (gd^{-1}) of 5 LE sheep and 6 HE sheep offered Diet B for 15 weeks. Mean WGR change of the uniform intake group is included.

b) The mean efficiency (WGR per nitrogen intake) of groups LE and HE on Diet B for 15 weeks.

Significant levels refer to differences between HE and LE sheep.

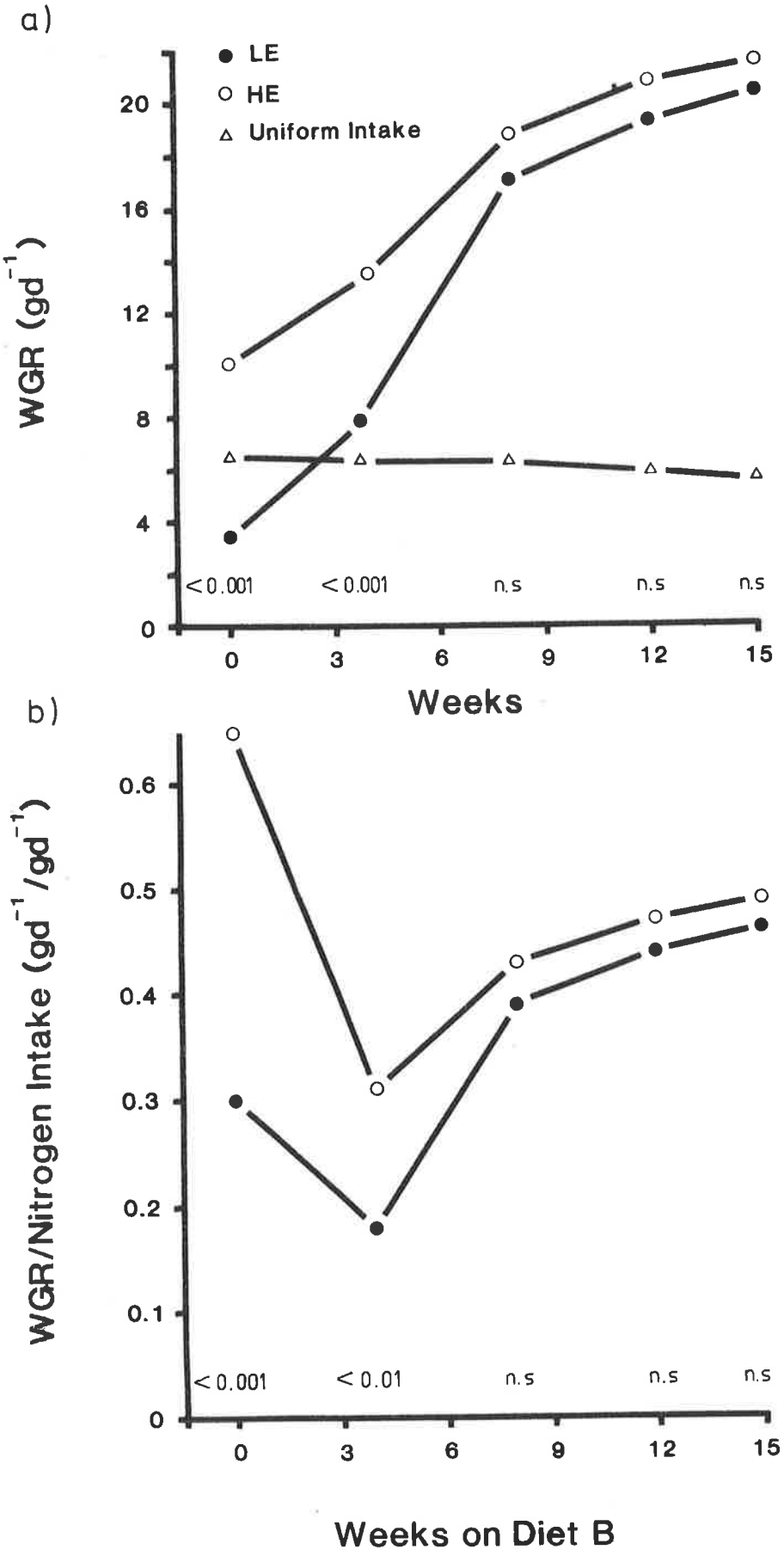

a)
LE
HE
Uniform Intake
WGR (gd^{-1})
20
16
12
8
4
0
< 0.001
< 0.001
n.s
n.s
n.s
0
3
6
9
12
15
Weeks
b)
WGR/Nitrogen Intake (gd^{-1}/gd^{-1})
0.6
0.5
0.4
0.3
0.2
0.1
0
< 0.001
< 0.01
n.s
n.s
n.s
0
3
6
9
12
15
Weeks on Diet B

Table 4.3 The relative WGR changes of LE and HE groups with time on 1000gd^{-1} diet B. Values represent the percentage of the total change, which had occurred at each wool harvest.

GROUP	WEEKS										Total WGR change (gd^{-1})
	0		4		8		12		15		
LE	0	***	27	***	81	**	94	ns	100		17.0±1.9
HE	0	***	30	***	76	ns	93	ns	100		11.5±2.7

*** P<0.001

** P<0.01

ns not significant

substantially improved ($P<0.01$) while HE sheep had used nitrogen less efficiently for wool production on Diet B than on Diet A (compare weeks 0 and 15 in Fig. 4.2.b). The significance of this finding must be considered in the light of the relationship observed between WGR and nitrogen intake, which is discussed in section 4.3.1.3.

The time sequence of wool growth responses to Diet B are presented in Table 4.3, as percentages of the total WGR change. Both groups approached equilibrium 12 weeks after the dietary change, and the pattern of wool growth change was very similar for LE and HE sheep, despite considerable differences in the absolute WGR change. It is also apparent from this table that the period of greatest increase in WGR was from weeks 4-8, during which time LE sheep increased wool growth from 7.9 to 17.1 gd^{-1}. Indivdual WGR and intake data are tabulated in Appendices 4.1 and 4.2

4.3.1.2 The repeatability of wool growth response to Diet A

Figures 4.3 and 4.4 show quite clearly how the differences in WGR and efficiency between groups at the end of Experiment 1 were rapidly eradicated within 8 weeks of feeding Diet B, only to become re-established when Diet A was fed for a subsequent 14 weeks. LE sheep in Experiment 1 were about 50% less efficient than HE sheep. When Diet A was reintroduced this difference was reduced to 20%. Although there was a tendency for the groups to resume their relative positions, the repeatability of individual response was poor, as evidenced by the low correlation coefficient between times ($r = 0.31$, $n = 8$). The ranking of individual efficiencies changed after the intervening period of high protein feeding (Table 4.4).

Figure 4.3 Changes in mean WGR (gd^{-1}) of 4 LE and 4 HE sheep which consumed Diet B for 8 weeks and Diet A for 14 weeks. Significance levels are indicated.

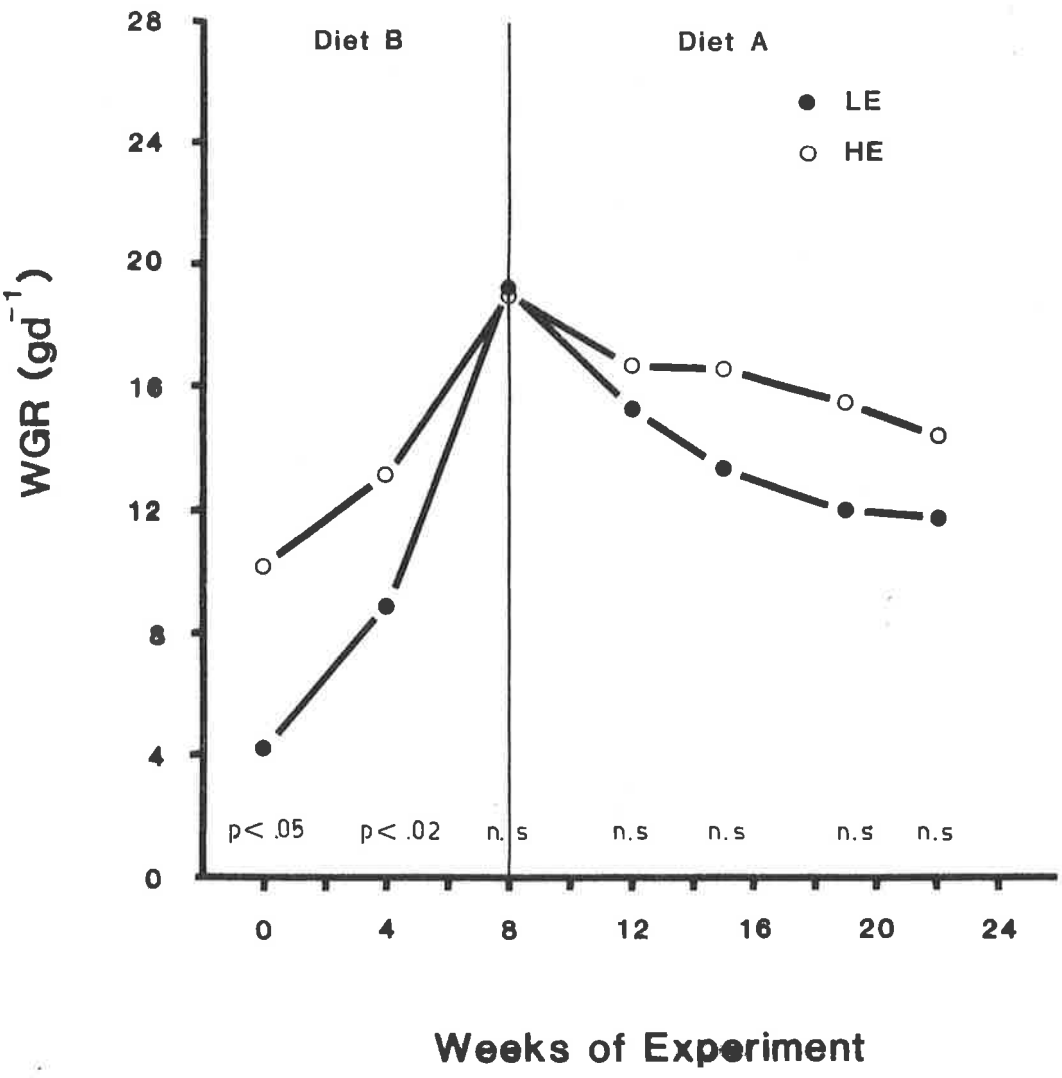

Diet B
Diet A
LE
HE
WGR (gd^{-1})
28
24
20
16
12
8
4
0
p< .05
p< .02
n.s
n.s
n.s
n.s
n.s
0
4
8
12
16
20
24
Weeks of Experiment

Figure 4.4 Changes in mean wool growth efficiency (g wool per g nitrogen) ± S.E., with time, for 4 LE sheep and 4 HE sheep on Diet B (0-8 weeks) and Diet A (8-22 weeks). Significance levels are indicated.

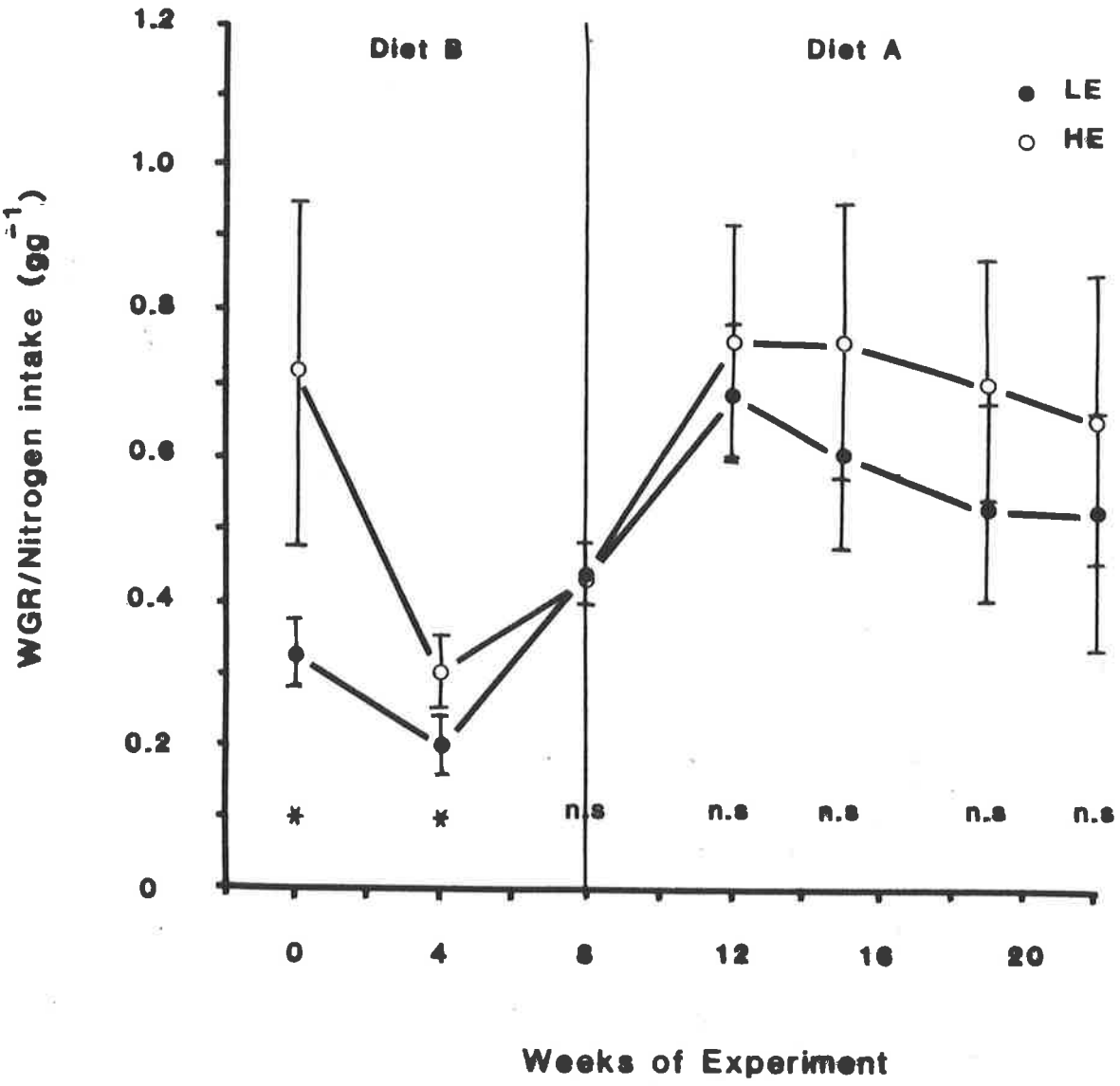

Diet B
Diet A
LE
HE
WGR/Nitrogen intake (gg⁻¹)
1.2
1.0
0.8
0.6
0.4
0.2
0
*
*
n.s
n.s
n.s
n.s
n.s
0
4
8
12
16
20
Weeks of Experiment

Table 4.4 Efficiency rankings for 8 sheep on Diet A in Experiment 1 and on Diet A in the present trial.

Group	Sheep No.	Experiment 1	Current Trial
HE	19	1	3
	17	2	4
	44	3	1
	13	4	8
LE	47	5	7
	14	6	6
	3	7	5
	41	8	2

From Fig 4.4 it is also evident that the between sheep variance of WGR reduced from 51.8%(C.V.) to 9.0% by diet B feeding, was once again increased by diet A (34.7%).

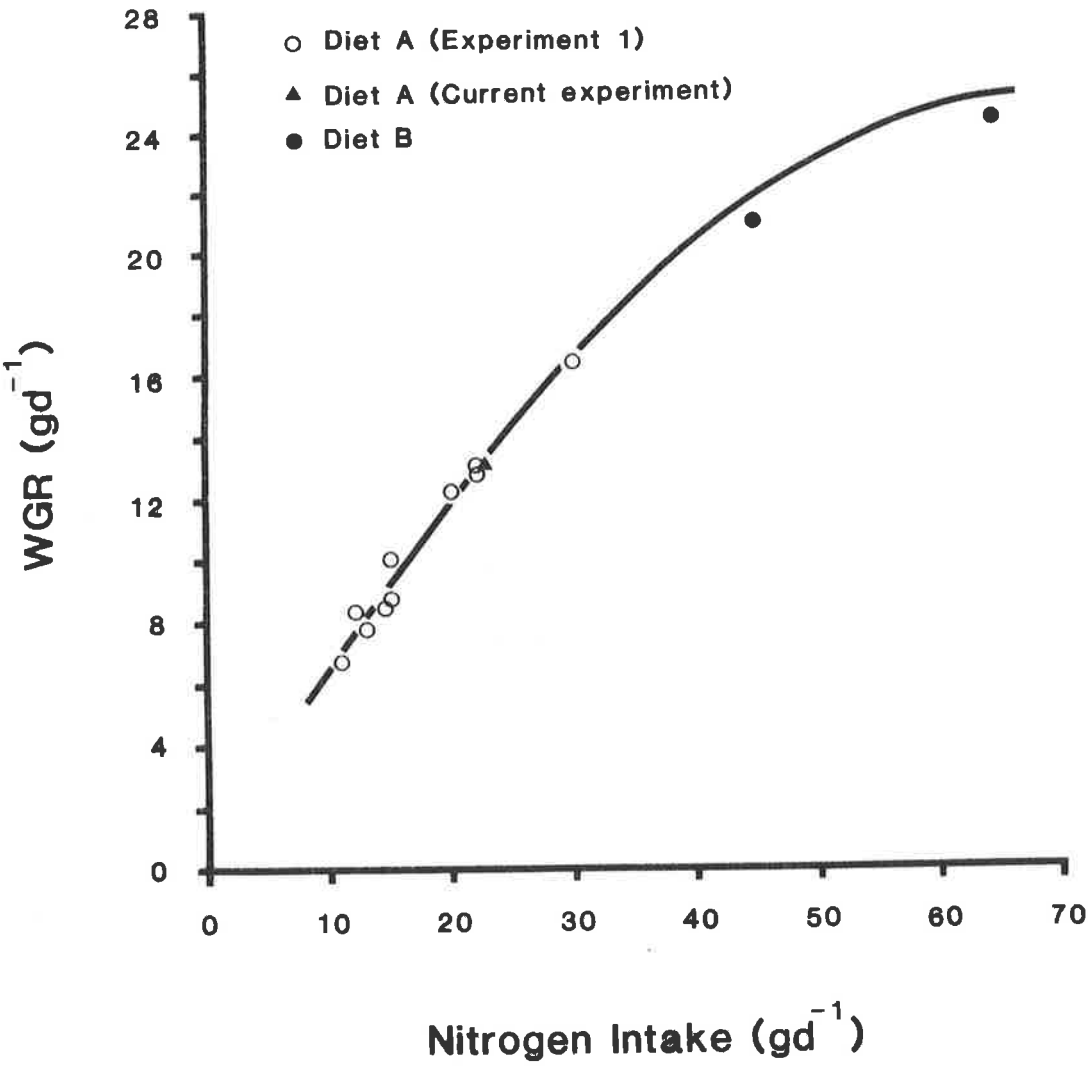
4.3.1.3 The relationship between WGR and nitrogen intake

When sheep received Diet B at a level of 1500gd^{-1} they produced 24.64±2.00g wool per day. The mean value for the 3 LE sheep was 25.4gd^{-1} and for the 2 HE sheep was 23.6 gd^{-1}, indicating that both groups were not constrained by an inherently low wool producing capacity.

The efficiency of wool production was lower at 1500gd^{-1} Diet B than at 1000gd^{-1} Diet B in the same sheep ($P<0.001$), and when data from the present experiment and Experiment 1 were analysed, it was apparent that there was a curvilinear relationship between WGR and nitrogen intake from 12-63gd^{-1}. Fig 4.5 was derived from data obtained for Diets A and B. It might therefore be suggested that lower efficiency on Diet B was a consequence of poorer nitrogen

Figure 4.5 Relationship between nitrogen intake (gd^{-1}) and WGR (gd^{-1}). Values are means for groups of sheep (n = 4-11) consuming Diet A in Experiment 1 and Diets A and B in the current experiment. The relationship is described by the following equation:

$WGR = -0.19 + 0.71(NI) - 0.005\ (NI^2)$ $\quad r^2 = 0.99$


Diet A (Experiment 1)
Diet A (Current experiment)
Diet B
WGR (gd^{-1})
Nitrogen Intake (gd^{-1})
0
4
8
12
16
20
24
28
0
10
20
30
40
50
60
70

utilisation on this ration (e.g. poor quality protein for wool growth) and not due to a diminishing response at high nitrogen levels. That this was not the case can be inferred from the depressed efficiency at the higher compared to the lower intake of Diet B. A diminishing response at such high intake levels (390g crude protein day^{-1}) is not surprising, as the WGR of sheep on this ration was approaching the genetic maximum for this genotype.

4.4 Discussion

The ability of the LE sheep to respond to a high-protein ration provides conclusive evidence that these animals were not genetically constrained in their capacity to produce wool in response to a high nutritional input. Rather, efficiency was related to the composition of the diet. Such an interaction between diet and wool growth efficiency has not been previously reported, and the results of this trial represent the first indication that the ranking of wool growth rates of individuals in a group are not absolute, but can, in fact, be altered by diet.

The two diets employed in this experiment contained a similar amount of metabolisable energy, but differed markedly in the concentration of crude protein (Table 4.2). Wool production responded to the additional protein in Diet B, the increase in LE sheep being substantially greater than that in the HE sheep, so that after 8 weeks of feeding there was no difference in WGR between the groups. The efficiency of wool growth of the HE sheep was reduced on Diet B but this was not a reflection of poorer protein quality or availability on this ration, but rather of reduced efficiency at high intakes of protein (Fig. 4.5). A

diminishing returns-type curve has been demonstrated for WGR and nitrogen supplied postruminally (Reis 1969), and many studies have revealed a depression of efficiency with increasing intake, although some of these have not allowed WGR to equilibrate with diet (1.1.4). Furthermore, few studies have achieved the substantial WGR's apparent in the current trial when sheep were fed diet B. WGR values obtained at the highest level of diet B ($24.64gd^{-1}$) are the highest recorded for this genotype and are most certainly close to the maximum genetic potential (Hogan et al. 1979). In contrast to the response of the HE sheep, the "low efficiency" group actually increased their wool growth efficiency despite the substantially greater rate of nitrogen intake (Fig 4.2b). One can only conclude that had the comparison between diets been made on an isonitrogenous basis, then the increase in efficiency of LE sheep would have been greater still. Clearly, some constraint to production operating in the LE sheep was alleviated when the high nitrogen diet was offered, but there was little suggestion that constrained productivity on the grain concentrate diet was genetically determined. The ranking of individual wool growth efficiencies was thus altered when this diet was reintroduced, although the number of sheep used in the repeatability trial was too low to make an unequivocal statement regarding any interaction between individual and diet. It is tentatively concluded that wool growth performance on a concentrate ration is not a highly repeatable character.

That the high variability in efficiency of wool growth on diet A demonstrated in this trial and in Experiment 1 is

indicative of responses on concentrate rations in general, is supported by the results of Schinckel (1960), Piper and Dolling (1969a) and Hutchinson (1961). These authors fed diets containing more than 50% cereal grain and noted large wool growth variation between individuals.

The results of the present experiment have important implications for the choice of rations for wool growth studies and for selection of high wool producing genotypes. High repeatability of wool growth efficiency under different nutritional conditions is essential if genotypes selected for high wool growth under one particular dietary regime are to be high producers on a different regime. There is considerable evidence that the diet x sheep interaction is small (Dolling and Moore 1961; Dunlop et al. 1966; Williams 1966; Dolling and Piper 1968), so that pen trials and field trials have produced similar efficiency rankings for individual sheep (Weston 1959). In contrast, the present results indicate a poor relationship between efficiency in the field, (estimated as WGR per unit bodyweight (Weston 1959)), and efficiency on diet A (r^2 = 0.004). Moreover, efficiency at one time on diet A was poorly related to efficiency measured at another (r^2 = 0.01). The latter result renders removal of error variance on this type of diet by covariance analysis, unsuitable. Efficiency of individuals on diet B, on the other hand, was closely related to field efficiency, the correlation coefficient of 0.84 being similar to that recorded by Dolling and Moore (1961) and Weston (1959).

4.5 Conclusions

In this experiment a significant diet x sheep interaction in relation to efficiency was demonstrated, but the response of an individual to the high grain diet was not repeatable. The interaction does not appear to be genetically determined. Such a finding is consistent with the hypothesis that the pathways of metabolism in the rumen are in a delicate state of balance when sheep are fed high grain diets, and that factors such as rate of feed and water consumption, the morphology of the rumen, and the composition of the rumen microflora at the initiation of feeding, could establish patterns of rumen fermentation that lead to substantially different flows of protein to the intestines. The dependence of wool growth on postruminal flow would then be reflected in gross differences in WGR. This concept is examined in the following Chapter.

"Ruminants have complex stomachs to
compensate for their deficient teeth" (Aristotle)

CHAPTER 5 Rumen fermentation pattern and the efficiency of wool production of sheep fed a concentrate ration

5.1 Introduction

Extensive variation in the amount of wool produced at any intake of a grain-roughage diet, was a feature of the results obtained in Experiment 1 of this thesis. Further studies revealed that this phenomenon was a characteristic of the diet, and that efficiency of wool production on the barley-lucerne ration was neither an inherent characteristic of an individual, nor was it related to production on a ration of different composition.

High starch rations are known to be involved in the development of metabolic disorders such as grain bloat, but less is known of the consequences of rumen instability on the nutrition of the host when a more chronic situation prevails (Dirksen 1969). Wool growth is dependent mainly on the supply of protein to the duodenum, the postabsorptive utilisation of these amino acids being mediated by the availability of energy substrates (Black et al. 1973). There is evidence that rumen fermentation patterns could alter both the flow of protein and the utilisation of that protein in several ways. Firstly, the turnover rate of substances in the rumen influences the microbial species present, the efficiency with which they synthesise protein, the fluid pH, and the amount of dietary protein escaping ruminal degradation (Chamberlain & Thomas 1980). Secondly, the

composition of the fermentation products (particularly V.F.A.) is determined by the microbial species present (Briggs et al. 1957), the latter being principally governed by diet composition. The molar proportion of propionate may be of particular relevance to the rate of degradation of absorbed amino acids and hence, the quantity available for wool growth (Leng et al. 1967). Thirdly, digestive malfunctioning as a result of the high starch input, may impair the overall digestibility of protein and/or energy in the whole tract.

The experiment reported in this section, while not seeking to define the factors that initiate such changes in rumen function, was designed to test the postulate that the extensive variation in wool production of sheep on the diet used in Experiment 1 was the outcome of variation in the amounts of protein flowing to the small intestines, and that these quantitative differences were related to the fermentation and metabolism patterns in the rumen. This hypothesis was developed after a review of the literature pertaining to those factors likely to be of greatest importance in influencing postruminal protein flow. In this review, presented below, special emphasis was placed on the ruminal events induced by "concentrate" feeding, particularly variability among sheep on the same diet, a "concentrate" diet being one in which more than half the ration comprises cereal grain.

5.2 Literature review

Of the many interacting facets of ruminal digestive processes, those associated with the efficiency of protein and energy digestion are summarised in Figure 5.1, together

Figure 5.1 Some interactions between key parameters of ruminal metabolism associated with the efficiency of energy and protein digestion.

Key: 1. Crawford *et al.* (1980 b).

2. Crawford *et al.* (1980 a).

3. Sutherland (1976)

4. Bull *et al.* (1979).

5. Christiansen *et al.* (1964); Isaacson *et al.* (1975); Sutherland (1976); Bergen and Yokoyama (1977); Czerkawski and Breckenridge (1977).

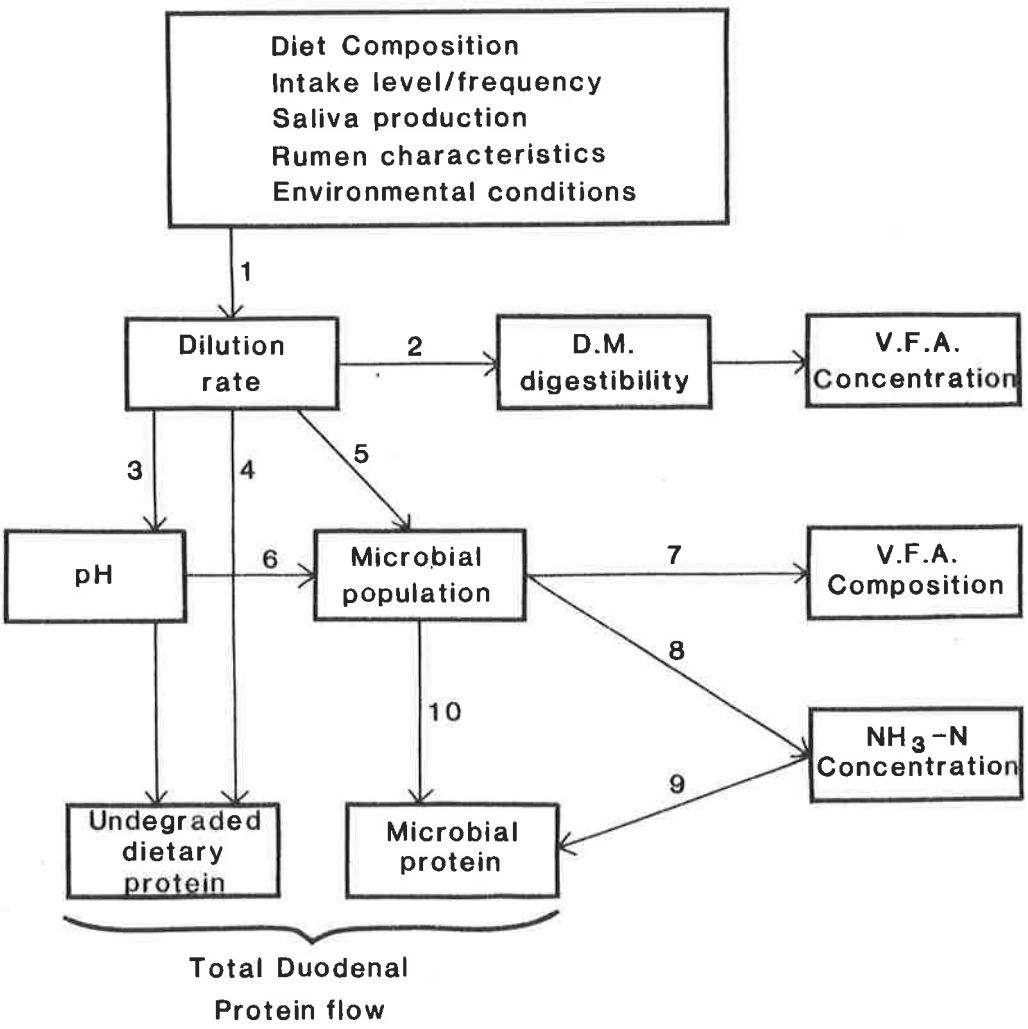
6. Briggs *et al.* (1957); Eadie *et al.* (1970).

7. Klopfenstein *et al.* (1966).

8. Abe *et al.* (1973).

9. Lewis and Annison (1974).

10. Leng (1976).


Diet Composition
Intake level/frequency
Saliva production
Rumen characteristics
Environmental conditions
1
Dilution rate
2
D.M. digestibility
V.F.A. Concentration
3
4
5
pH
6
Microbial population
7
V.F.A. Composition
8
10
NH3-N Concentration
9
Undegraded dietary protein
Microbial protein
Total Duodenal Protein flow

with a list of the main contributors to the understanding of each process. While each parameter is now considered separately, these interactions need to be kept in mind when assessing the performance of the whole system.

5.2.1 The turnover rate of rumen contents

Turnover rate, dilution and clearance rate are synonymous terms for the proportion of the total ruminal volume leaving the rumen each hour, typical values ranging from 0.02 to 0.33 per hour (Hyden 1961; Tulloh et al. 1965). While turnover usually refers to the fluid portion of the digesta, Bull et al. (1979) emphasise the importance of considering the outflow of solids as well, because the two are not always associated. It is clear from Fig 5.1 that the rumen fluid dilution rate (D) is closely linked to the pattern of metabolism having direct relationships with rumen pH, microbial protein, dry matter digestibility and the amount of undegraded dietary protein arriving at the duodenum. The fact that D is lower and more variable on concentrate diets than on forage diets (Sutherland 1976; Cole et al. 1976, Thomson et al. 1978; Chamberlain and Thomas 1979), may have important consequences for the protein nutrition of the host. Further, Fig. 5.1 indicates that the dilution rate is influenced by a wide variety of factors and "there is an almost infinite number of combinations of ration components, characteristics, and levels of feeding which may result in a response in turnover of liquid and/or solids" (Bull et al. 1979). For example, differences in the rate of feed intake between sheep in Experiment 1 may well have influenced D.

For any diet there is an optimal partitioning of

digestion between the stomach and intestines. Changes in D can influence the residence time of dietary components in the rumen and hence the extent of digestion in the stomach. Table 5.1 indicates the marked effect of D on the proportion of dietary protein escaping ruminal degradation.

Table 5.1 The influence of D on the extent of ruminal degradation of proteins from different sources (from Zinn unpubl. - cited by Bull et al. 1979).

Feeding level (xM)	1.2	1.6	
D(hr^{-1})	0.091	0.110	
Soybean meal	85	82	% ruminally degraded
Cottonseed meal	76	39	
Corn gluten meal	54	39	

Not only is the extent of ruminal degradation altered by D, but also the efficiency with which the microbial population synthesises protein from ammonia-nitrogen (NH_3N) and free amino acids. The majority of trials on this subject have achieved alterations in D in the rumen by infusion of artificial saliva or saliva salts (Harrison et al. 1974, 1975; Thomson et al. 1975, 1978; Chamberlain and Thomas 1980), or by inclusion of mineral salts in the diet (Berger et al. 1980). Alternatively in vitro continuous fermentation systems have been employed (Hobson 1965; Isaacson et al. 1975; Crawford et al. 1980 a, b). In these studies enhanced microbial efficiency was invariably induced by increased D

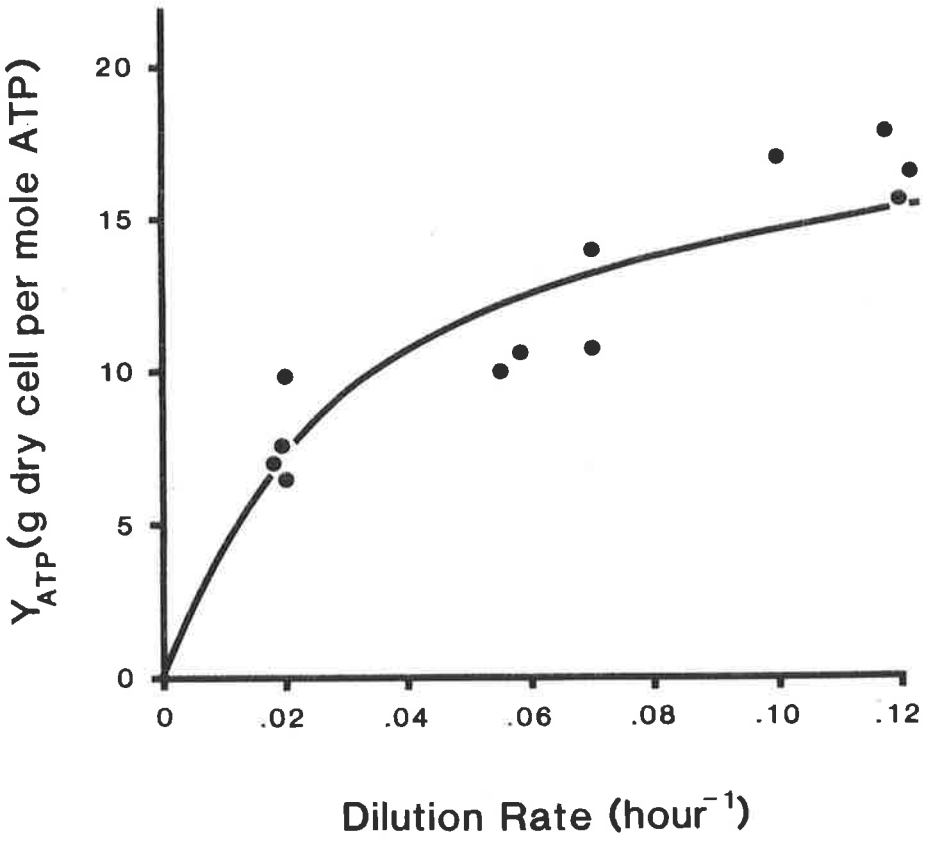
(Harrison et al. 1975; Isaacson et al. 1975; Cole et al. 1976; Bergen and Yokoyama 1977). The relationship between D and YATP from the in vitro study of Isaacson et al. (1975) is presented in Fig. 5.2a. Microbial efficiency, in this context, is expressed as either the molar growth yield (YATP = g dry microbial cells per mole ATP) or, more crudely, as g of microbial crude protein flowing from the rumen per unit of organic matter disappearing in the rumen. The dependence of microbial efficiency on D is thought to be a consequence of the effect of D on the microbial maintenance requirement. Maintenance functions include a) motility, b) turnover of cell macromolecules, c) synthesis of extracellular enzymes, proteins and carbohydrates, d) active transport, e) energy losses in energetic uncoupling and f) resynthesis of cells after cell lysis (Hespell 1979). The major factor in influencing the maintenance requirement is the energy lost between ATP production from catabolism and that available for synthesis (described in e). Limiting nutrients other than energy have an important bearing on this process. (e.g. NH_3N, branched chain V.F.A.) (Hespell 1979).

Because the maintenance requirement of the bacterial population is a time function (mmol ATP/g dry cells/hour), cell yields also depend on the bacterial growth rate. As growth rate of the population increases, a lower proportion of available ATP is used for maintenance (Hespell 1979). Bacterial growth rate and fluid turnover rate are synonymous, otherwise the population would change to assume a new steady state (Owens and Isaacson 1977). The importance of the above considerations to the flow of microbial and total protein from the rumen is illustrated in Fig 5.2b,

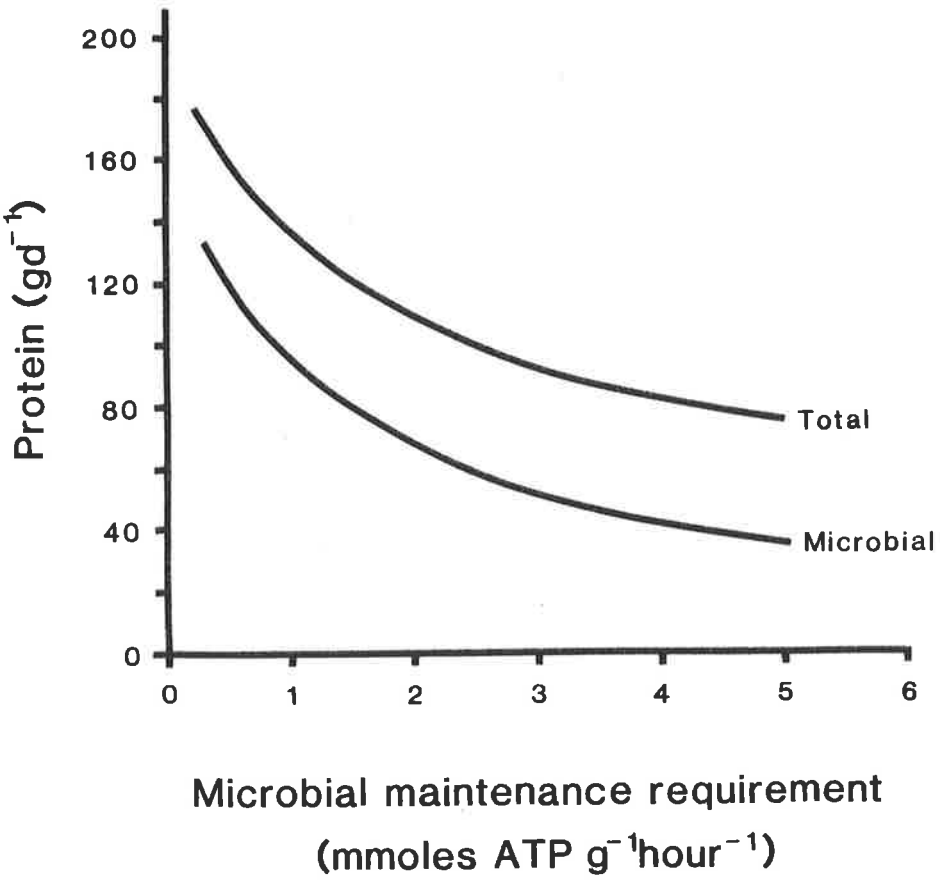
Figure 5.2 a) The influence of rumen fluid dilution rate (h^{-1}) on the molar growth yield (YATP) of microbes (from Isaacson *et al.* (1975))

b) Simulated effect of the microbial maintenance requirement on microbial and total postruminal protein flow (gd^{-1}). (from Faichney and Black 1979).

a)


Y_{ATP}(g dry cell per mole ATP)
20
15
10
5
0
0
.02
.04
.06
.08
.10
.12
Dilution Rate (hour^{-1})

b)


Protein (gd^{-1})
200
160
120
80
40
0
0
1
2
3
4
5
6
Total
Microbial
Microbial maintenance requirement
(mmoles ATP g^{-1}hour^{-1})

derived from the model of rumen function compiled by Faichney and Black (1979). From Figures 5.2 and 5.2b it is apparent that as D increases the microbial maintenance requirement decreases, and the microbial yield and microbial protein flow increase.

Not only is microbial yield altered by D, but there has also been speculation that the optimum D value is "species-dependent" because of differential growth rates among the bacterial species in the rumen (Sutherland 1976). As D changes, a redistribution of species might occur, presumably with concomitant shifts in the metabolite pattern. Moreover, fluid pH, osmotic pressure and ionic composition would be affected and, in turn, would influence organism selection (Sutherland 1976).

In this regard the decline in protozoal numbers as D increases (Bergen and Yokoyama 1977: Czerkawski and Breckenridge 1977; Crawford et al. 1980a) is of interest, and may be a reflection of the effects of low pH (Eadie et al. 1970) or of the relatively slow rate of protozoal growth (Christiansen et al. 1964).

Such shifts in microbial populations coincident with D changes might be expected to influence the pattern of metabolite production. Indeed, turnover rate and the V.F.A. composition are related (Fig 5.3). As D increases, the propionate molar proportion decreases, although, not surprisingly, the precise nature of the relationship has varied widely between studies. Some workers have observed no relationship at all, while others report a positive association (Isaacson et al. 1975). These discrepancies probably reflect differences in diets, feeding patterns and

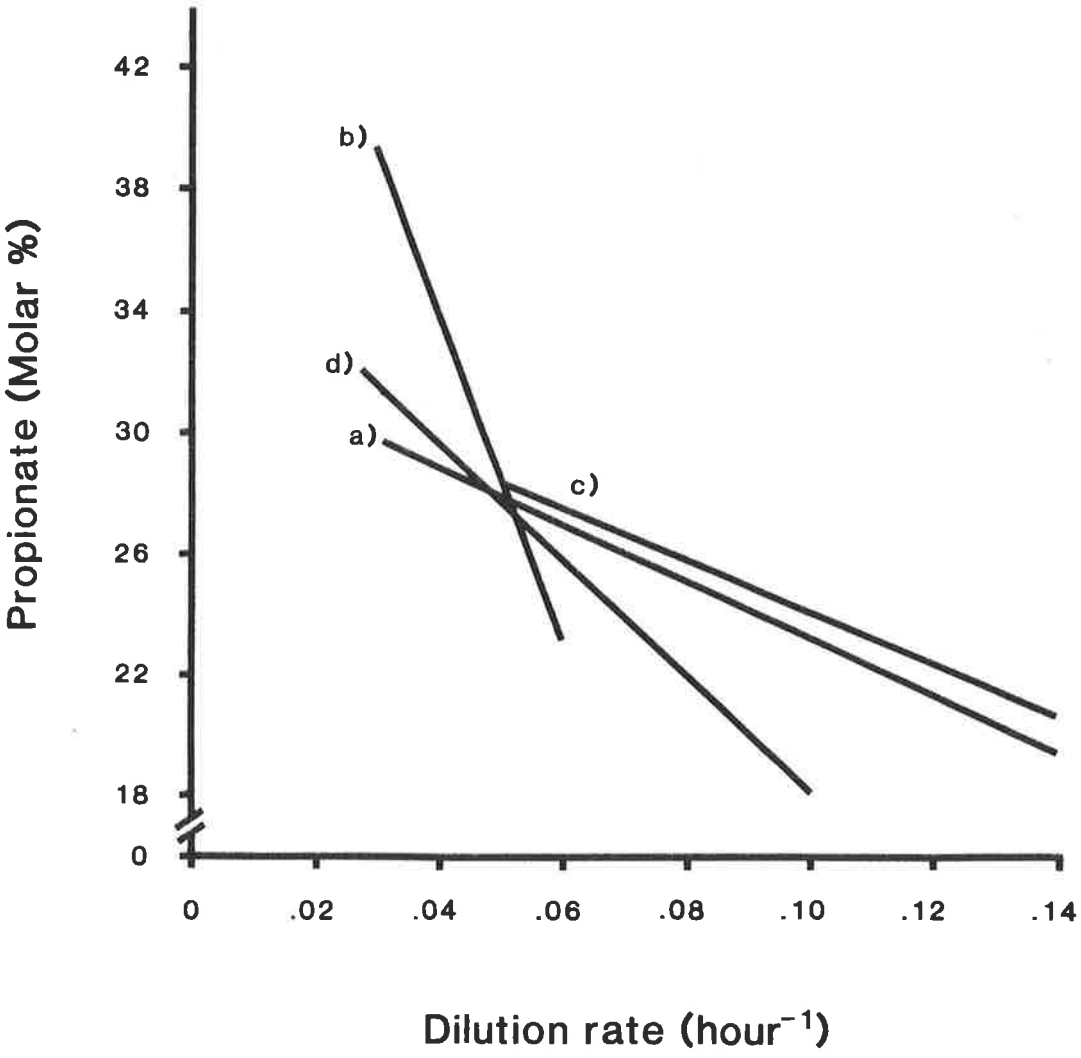
Figure 5.3 Published relationships between rumen fluid dilution rate (h^{-1}) and the molar proportion of propionate.

a) Harrison *et al.* (1975).

b) Thomson *et al.* (1978).

c) Harrison *et al.* (1974).

d) Hodgson and Thomas (1972).


Propionate (Molar %)
42
38
34
30
26
22
18
0
b)
d)
a)
c)
0
.02
.04
.06
.08
.10
.12
.14
Dilution rate (hour^{-1})

periods of adaptation. (Chamberlain and Thomas 1980). Overall, however, there is sound evidence that the V.F.A. end-products are influenced by D, a result in accord with changes in microbial species or the pattern of metabolism of existing species (Hobson 1965).

A negative association between D and propionate proportion, and a positive effect of D on microbial efficiency implies that "propionate" fermentations are inefficient in terms of microbial synthesis. Several authors have, in fact, reported higher bacterial yields when "acetate" fermentations have been induced on grain diets (McMeniman et al. 1974; Harrison et al. 1975, 1976; Thomson et al. 1975, 1978). Data of Harrison et al. (1976) in which D was modified by artificial saliva infusion, is summarised in Table 5.2. As D increased, the propionate % decreased and microbial efficiency and duodenal nitrogen flow were enhanced.

Similarly, when diets containing from 0-100% concentrate were fed, a decline in bacterial efficiency as the concentrate proportion increased, coincided with a high propionate proportion (Chamberlain and Thomas 1979).

In contrast to these findings, other workers have reported enhanced microbial efficiency in the presence of "propionate-type" fermentations (Ishaque et al. 1971; Jackson et al. 1971). In these trials, two distinct fermentation patterns were evident when sheep were fed a diet of ground barley, maize and hay at hourly intervals. Those with a high rumen fluid propionate proportion had lower NH_3N concentrations and higher duodenal nitrogen flows than those with a ruminal metabolism characterised by high

Table 5.2 The relationship between dilution rate (D), propionate %, microbial synthetic efficiency, and total duodenal nitrogen flow, when sheep were infused with artificial saliva (4 1/d). (adapted from Harrison et al. 1976).

	CONTROL	INFUSED	SIGNIFICANCE
$D(hr^{-1})$	0.032	0.075	$P<0.001$
Propionate (%)	31.6	20.0	$P<0.01$
Amino acid synthesised (g) per mole hexose fermented	25.4	29.8	$P<0.01$
Total duodenal N flow(gd^{-1})	11.65	13.25	$P<0.01$

acetate and butyrate. Moreover, diaminopimelic acid nitrogen flow, an indicator of bacterial protein flow (Hutton et al. 1971), was positively related to the propionate concentration. At present no satisfactory explanation can be made for the disparity between trials relating V.F.A. composition to efficiency of microbial protein synthesis and postruminal nitrogen flow. Other factors of importance in regulating the nature of the ruminal interactions will now be briefly discussed.

5.2.2 Rumen fluid pH

The pH of rumen fluid is determined by the balance between the buffering capacity of the fluid and the acidity/alkalinity of the fermentation products (Chalupa 1977).

For the majority of diets the value falls within the range 6.0-7.0 (Monroe and Perkins 1939; Olson 1941; Hunt et al. 1943), but when concentrates are fed, low saliva flows (Balch and Rowland 1957; Reid et al. 1957) coupled with rapid V.F.A. production, can induce pH values as low as 4.1 (Phillipson 1952; Briggs et al. 1957; Kezar and Church 1979). Lowest pH are recorded in the presence of lactic acid (Briggs et al. 1957).

There is little doubt that alterations in the pH of rumen fluid play an important role in the continual state of flux of rumen metabolism which allows a wide variety of feeds to be efficiently digested. It has long been established that pH and the metabolic end products of microbial digestion are related (Briggs et al. 1957; Slyter et al. 1966; Esdale and Satter 1972). Carefully controlled media inoculation experiments have confirmed that pH per se

has profound effects on the relative competitive abilities of different microbial species (Hobson 1972; Russel et al. 1979). Figure 5.4 from Hobson (1972) illustrates how the growth rates of two bacterial species varied with pH.

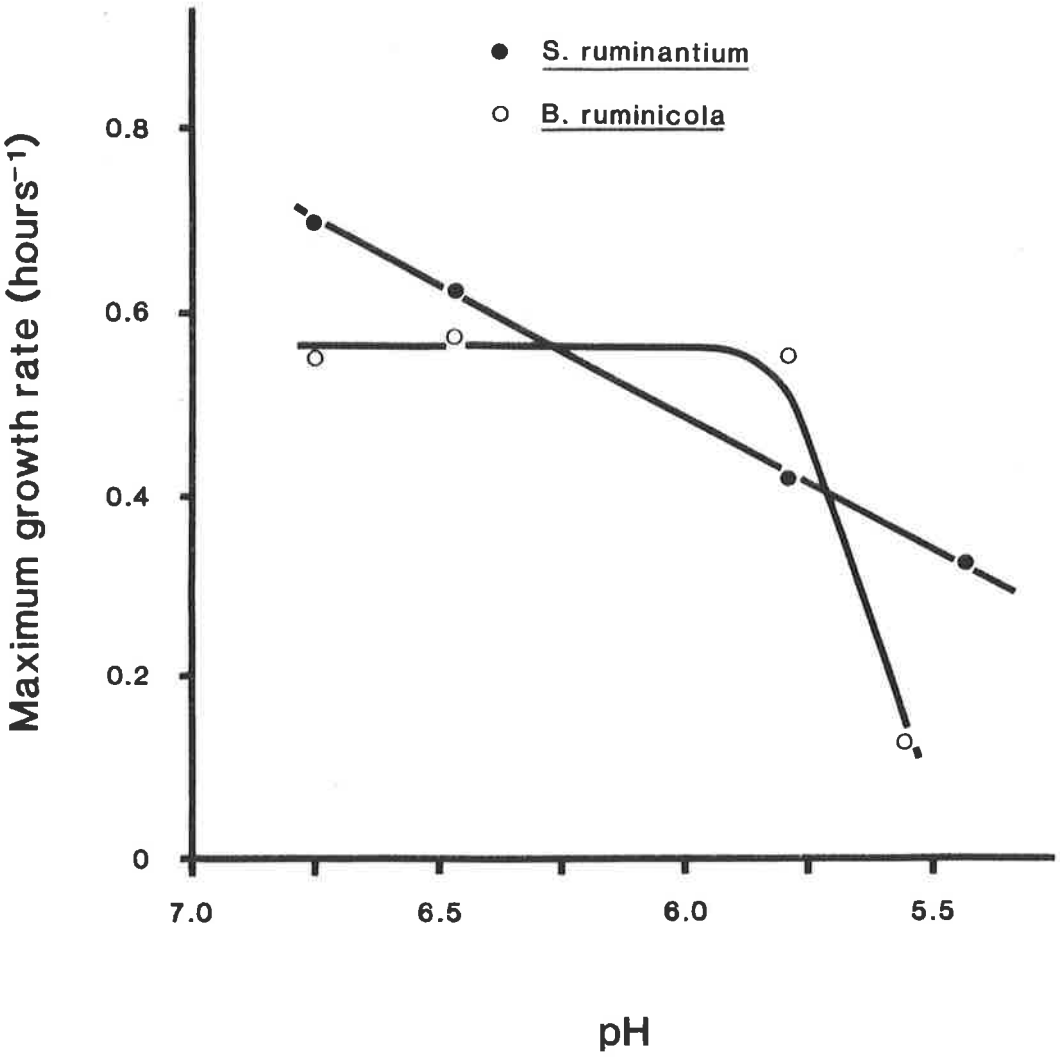
Pronounced shifts in the metabolic end products as pH changes would be anticipated following the redistribution of species. Adaptation of propionate-producing organisms to low pH was thus concluded as being responsible for the high propionate concentration in the trial of Briggs et al. (1957). However, compensating changes with pH in the metabolism of existing species also occurs (Hobson 1972). Microbes vary their metabolism with growth rate, a factor previously shown to be pH-dependent (Fig 5.4). For instance at low growth rates, Selenomonas ruminantium produces predominantly acetate and propionate while at high growth rates, lactate comprises a large proportion of the products (Hobson 1965; Hobson and Summers 1966).

Thus, ruminal pH, which is influenced strongly by concentrate feeding, can affect fermentation products by altering both the types of micro-organisms present and the biochemical pathways operating in these. Further alterations to metabolism are also noted when the pH during any part of the feeding cycle falls below 5.0-5.5, because massive loss of protozoa occurs under these conditions (Eadie 1962; Eadie et al. 1967 and 1970; Schwartz and Gilchrist 1975). The nutritional consequences of disturbances to the protozoal population are considered in Section 5.2.4.

5.2.3 Ruminal ammonia-nitrogen concentration

Microbial activity in the rumen provides the host with a supply of protein of good biological value (Bergen et al.

Figure 5.4 The growth rates of two bacterial species as influenced by inocula pH (from Hobson 1972).


S. ruminantium
B. ruminicola
Maximum growth rate (hours⁻¹)
0.8
0.6
0.4
0.2
0
7.0
6.5
6.0
5.5
pH

1968) derived from dietary proteins, peptides and non-protein nitrogen. Such nitrogenous substances are degraded by extracellular enzymes to NH_3, the resulting concentration of which is normally between 0 and 130mg/100 ml, and influenced by bacterial utilisation rate, metabolism in the rumen wall, absorption into the portal vein, and passage to the omasum (Tillman and Sidhu 1969).

The advantage of the ruminant system of microbial protein synthesis from non-protein, is most apparent when the protein content of the feed consumed is low. Under such conditions, a net gain of nitrogen can occur between the mouth and the intestines, because the microbes can capture recycled nitrogen (Nolan and Leng 1972).

In contrast, substantial nitrogen losses are recorded when the protein content of the diet is high, when the diet spends a long time in the rumen, or when the dietary protein is highly soluble (Hogan and Weston 1967a; Ferguson 1972), so that "the extent of rumen ammonia production from different protein sources was found to be inversely correlated with nitrogen retention". (quoted by Ferguson 1972; see also Lewis and Annison 1974). The diurnal pattern of ammonia production is likewise of importance, as production peaks may exceed bacterial incorporation rates so that NH_3 is absorbed across the rumen wall and excreted as urea.

Generally, the ruminal NH_3-N concentration reflects the NH_3 production rate in the same way that V.F.A. concentration is related to V.F.A. synthesis (Leng et al. 1968). The rumen fluid NH_3 concentration is important because this best describes the immediate microbial

environment, compared, for instance, to total rumen NH_3 levels. Because NH_3 is an obligatory substrate for most bacteria (Allison 1970) there is a lower limit of NH_3 concentration below which microbial synthesis is impaired. There is, however, no unanimity amongst workers on this "optimum" value, estimates ranging from as low as 2.2mg/100ml (Slyter et al. 1979) to almost 30mg/100ml rumen fluid (Miller 1973). (see Table 5.3). The criteria used to define optimum concentration varied between these authors, the value for maximal protein synthesis being somewhat lower than that for maximum fermentation rate and protein flow to the abomasum. Baldwin and Denham (1979) suggest that the low and high concentrations recorded in Table 5.3 can be reconciled by consideration of the two enzymes involved in ammonia utilistion by microbes, namely glutamate dehydrogenase and glutamine synthetase. The former has a low affinity for NH_3 (Km = 5mM) while the latter has a high affinity (Km = 0.2mM), implying two distinct concentrations of NH_3 for maximum enzyme saturation.

Alternatively, it has been suggested that the limiting NH_3 concentration varies with the population of microbes present, and with their growth rates (Allison 1970). Microbial species may differ in their abilities to concentrate ammonia and possibly their rate of ammonia assimilation (Buttery 1977). Pronounced effects of concentrate diets, therefore, on the microbial species distribution (Briggs et al. 1957; Schwartz and Gilchrist 1975), and their growth rates (Hobson 1972), may well be of importance in altering the nitrogen kinetics of the host. In accord with this concept is the observation that maximal

Table 5.3 Optimum mean ruminal ammonia concentration for microbial synthesis.

Author	Optimum (NH_3N)(mg/100ml)	Criterion
Mehrez *et al.* (1977)	23.5	Max fermentation rate
Slyter *et al.* (1979)	2.2	Max MP[1] synthesis
Satter & Slyter (1974)	5.0	" " " (*in vitro*)
Bryant & Robinson (1961)	6.0	" " " " "
Hume *et al.* (1970)	8.8-13.3	" " " (*in vivo*)
Miller (1973)	28.9	Unknown
Okorie *et al.* (1977)	8.5	Max MP synthesis
Allen & Miller (1976)	19.4-26.9	Abomasal NAN[2] flow

1 MP = Microbial protein

2. NAN = Non-ammonia nitrogen

microbial protein synthesis is only achieved at high NH_3 levels (up to 50mg/100ml) when sheep are fed purified diets (Hume et al. 1970), rolled barley diets (Orskov et al. 1972) and high energy diets (Bartley and Deyoe 1977; Okorie et al. 1977). Moreover, the optimum concentration for cell synthesis would vary with the rumen turnover rate if the rate of NH_3 assimilation was limiting (Kempton and Nolan 1978). The possibility exists, therefore, that the requirement of microbes for NH_3 is increased by concentrate feeding and that this may contribute to differences between sheep in N utilisation efficiency.

Between-sheep variability in ruminal ammonia concentration when sheep are fed concentrate diets

When sheep are fed diets containing a high proportion of cereal grain the pattern of ruminal fermentation varies widely between sheep and at different times in the same animal, a reflection of the instability in metabolism induced by such rations (Barry et al. 1977). Ishaque et al. (1971) were among the first workers to demonstrate this variability when sheep were fed a mixed diet of ground barley, ground hay and flaked maize, and it has since been corroborated by the studies of Hodgson and Thomas (1972; 1975) and Chamberlain and Thomas (1979). While these authors have related the alterations observed in microbial synthetic efficiency and duodenal protein flow to differences in short-chain fatty acid production (hence the previously discussed debate regarding the efficiency of "acetate" versus "propionate" versus "butyrate" fermentations), a consistent feature of the different metabolisms has been the differences in ammonia concentration. For instance, Ishaque

et al. (1971) recorded an inverse relationship between NH_3 concentration and duodenal nitrogen flow as indicated below:

NH_3 concentration:	29.9±2.8	11.3±1.7
N flow (as % of N intake):	57.5±2.3	104.1±3.5

Similarly, Hodgson & Thomas (1972) observed distinctly different fermentation patterns on the same diet (NH_3-N= 5.5 cf 21.6mg/100ml) and related these to dilution rate effects. High dilution rate is associated not only with altered V.F.A. mixtures (see Section 5.2.1) but also with reduced NH_3 concentration and increased duodenal N flow (Harrison et al. 1976).

While it is tempting to conclude from these studies that variations in postruminal nitrogen flow when sheep consume concentrate feeds are a consequence of variations in the extent of dietary nitrogen losses from the rumen as NH_3, the possibility remains that NH_3 concentration may be related to some other aspect of the efficiency of the system. In this regard the observation that NH_3 concentration is related to the presence or absence of protozoa (Abe et al. 1973) may be of importance.

5.2.4 The influence of protozoa on pattern of fermentation and nutrient availability

Relatively high concentrations of protozoa, up to 10^7/ml, have been recorded in the rumen fluid of sheep fed at restricted intakes of concentrate diets (Christiansen et al. 1964; Eadie et al. 1970, Slyter et al. 1970), in contrast to their virtual disappearance at ad libitum intakes of the same rations (Eadie et al. 1970). Low pH, induced by high grain intakes, apparently eliminates the

ciliate population (Eadie 1962). The contribution, therefore, of protozoa to the total microbial protein pool is of importance. Diet, frequency of feeding and level of intake determine this contribution (Klopfenstein et al. 1966; Eadie et al. 1970), and protozoa can comprise up to 50% of the total microbial biomass (Leng 1976).

Recently, considerable interest has arisen in the role of protozoa in ruminant digestion, after it was established that they were selectively retained in the rumen (Weller and Pilgrim 1974; Bird et al. 1979).

Incorporation of dietary protein into protozoal protein would depress the availability of protein to the host (Leng 1976; Bird 1978) because the hydrolysis of protozoal protein and subsequent recovery of the NH_3 by bacteria would involve losses of nitrogen as well as energy. Moreover, the fluctuation in protozoal numbers observed on highly fermentable diets would similarly depress the microbial protein available at the duodenum (Leng 1976). Removal of protozoa from the rumen has resulted in a reduction in dry matter digestibility (Lindsay and Hogan 1972), altered V.F.A. patterns (Eadie et al. 1970; Males and Purser 1970), reduced ruminal NH_3 concentrations (Christiansen et al. 1965; Klopfenstein et al. 1966; Luther et al. 1966) and increased protein availability (Bird et al. 1979). The latter workers attribute additional protein flow to reduced predation of bacteria by protozoa (Coleman 1975), greater bacterial outflow as a result of reduced recycling of microbial protein, or a greater proportion of the digestible crude protein leaving the rumen.

5.2.5 Conclusions

It is apparent from the literature review that sheep consuming a similar amount of a concentrate ration can differ widely in the quantity of protein arriving for digestion in the small intestine. While the source(s) of such differences in nutrient flow have not yet been clearly identified, the responses are associated with variability between sheep in microbial protein synthetic efficiency, the dilution rate of rumen fluid, and the production of endproducts of metabolism (V.F.A. and NH_3). The protozoal density and state of flux of the ciliate population are also likely to be of prime importance in generating variability of response to high grain diets. As yet no studies have investigated the nutritional consequences of the rumen fermentation patterns and postruminal nutrient flow responses induced by concentrate feeding.

5.3 Experiment 4: Objective

This study was undertaken to test the hypothesis that the variation in WGR observed on the high grain diet used in Experiment 1 was the outcome of a series of events associated with rumen digestion and metabolism that influenced the amount of dietary protein arriving at the intestines for absorption, and was not a function of the processes between absorption and the wool follicle.

To test this hypothesis the postruminal protein flows and ruminal digestive patterns of 13 sheep surgically prepared with ruminal and abomasal cannulae, were studied. These characters were compared when sheep were fed two diets of different composition, the first being a roughage diet of lucerne, and the second being a concentrate diet of similar

composition to that used in the studies reported earlier in this thesis. The roughage ration was fed for 14 weeks and the concentrate ration for 16 weeks, during which time rumen pH, ammonia concentration, V.F.A. concentration and composition, and diet digestibility were examined in relation to variations in the flow of protein to the abomasum determined for each animal. It was postulated that protein flows and rumen metabolism would be similar among sheep on the roughage diet but variable and divergent among the experimental animals when the concentrate ration was fed and that in both groups the postruminal flow of protein would be closely related to the observed variations in wool production.

5.4 Materials and Methods

5.4.1 Animals and surgery

Fourteen sheep were selected from the original 48 sheep used in Experiment 1, so that a wide range of wool production efficiencies measured on the experimental ration was represented (Table 5.4).

Table 5.4 The WGR (gd^{-1}), DMI (gd^{-1}) and efficiency of wool growth (WGR/DMI) of the 13 sheep selected for the experiment.

Sheep No.	WGR(1) (gd^{-1})	DMI(2) (gd^{-1})	Efficiency (gd^{-1}/kgd^{-1})
36	8.7	902	9.6
10	9.3	638	14.6
11	8.2	805	10.2
44	17.8	911	19.5
31	10.9	457	23.9
25	15.8	814	19.4
5	7.5	884	8.5
41	4.8	662	7.3
23	6.9	872	7.9
27	16.5	773	21.4
13	19.0	913	20.8
17	12.4	639	19.4
18	13.7	768	17.8
19	17.4	909	19.1
Mean± SEM			15.67± 5.58

(1) These data refer to performance at the end of Period II in Experiment 1. WGR are final values and DMI are means for the whole period.

(2) Differences between sheep are not a reflection of

voluntary intake differences. Sheep were offered different levels of feed in Experiment 1.

Surgical Procedures

Simple cannulae were inserted into both the rumen and abomasum of each of the experimental sheep in the following manner.

Anaesthesia was induced with sodium pentobarbitone (Nembutal), and maintained via endotracheal tube with cyclopropane. An incision 5-6cm long was made in the anterodorsal portion of the flank, the muscles separated by blunt dissection and the peritoneum cut and secured with Mosquito forceps. A part of the dorsal sac of the rumen was secured with bowel clamps and sutured to the fascia. The rumen was then cut and the exposed rumen walls held with hemostats while a flexible cannula (see Hecker 1974 p.111) was inserted. An external flange and rubber stopper completed the preparation. There was very little digesta leakage from the fistula because the laparotomy was done as high as possible on the dorsal flank, and a tight seal was maintained between the external and internal flanges.

Abomasal cannulae were inserted immediately after the rumen cannulation. A lateral incision was made about 4cm behind the last rib. The muscle layers were parted by blunt dissection and the peritoneum exposed, clamped with Mosquito forceps and cut.

A suitable area close to the pylorus was selected and 2 rings of Murphy's sutures, about 1.5cm in diameter, placed in the serosal layer of the abomasal wall using chromic 4/0 gut. A tube, about 7cm long and 1cm in diameter, was then inserted into a slit made through the abomasal wall within

these sutures. The ends of the suture were drawn up firmly and tied. The free end of the tube was passed out through a stab wound, and the peritoneum, muscle layers and skin were sutured.

Routine post-operative care was carried out including a course of antibiotics for 3 days. Feed intake returned to normal in all sheep within one week of surgery.

The experiment did not commence until 4 weeks post-surgery. During this period the sheep received a ration of lucerne chaff ($1000gd^{-1}$) and were treated for parasites. Vitamins A, D and E were also administered. Sheep numbers 44, 25 and 27 were operated on 6 weeks after the trial commenced to replace nos. 41 and 18, two sheep from which it proved difficult to obtain abomasal samples. The data for these 3 replacement sheep are omitted for the period of roughage feeding.

5.4.2 Design and Feeding

The experiment spanned a period of 30 weeks which was divided into 2 sub-periods. For the first 14 weeks sheep were offered a roughage diet (Diet R) comprising hammermilled and pelleted lucerne chaff at $1100gd^{-1}$ airdry. A concentrate ration (diet C) prepared precisely as for the initial studies of this thesis, was offered at $900gd^{-1}$ airdry for the remaining 16 week period. This diet comprised 60% barley (cv Clipper) and 40% lucerne chaff, which were hammermilled and pelleted. Adaptation to this high grain ration was achieved by gradually increasing the daily intake from $500gd^{-1}$ (1 week) to $700gd^{-1}$ (2 weeks) and then $900gd^{-1}$ for the remainder.

Feeding was carried out once daily at 0.800 hours after

the residue from the previous day was collected and weighed. The two rations were fed at levels estimated to be isocaloric so that effects of energy intake per se on rumen function were eliminated. The composition of the diets is given in Table 5.5

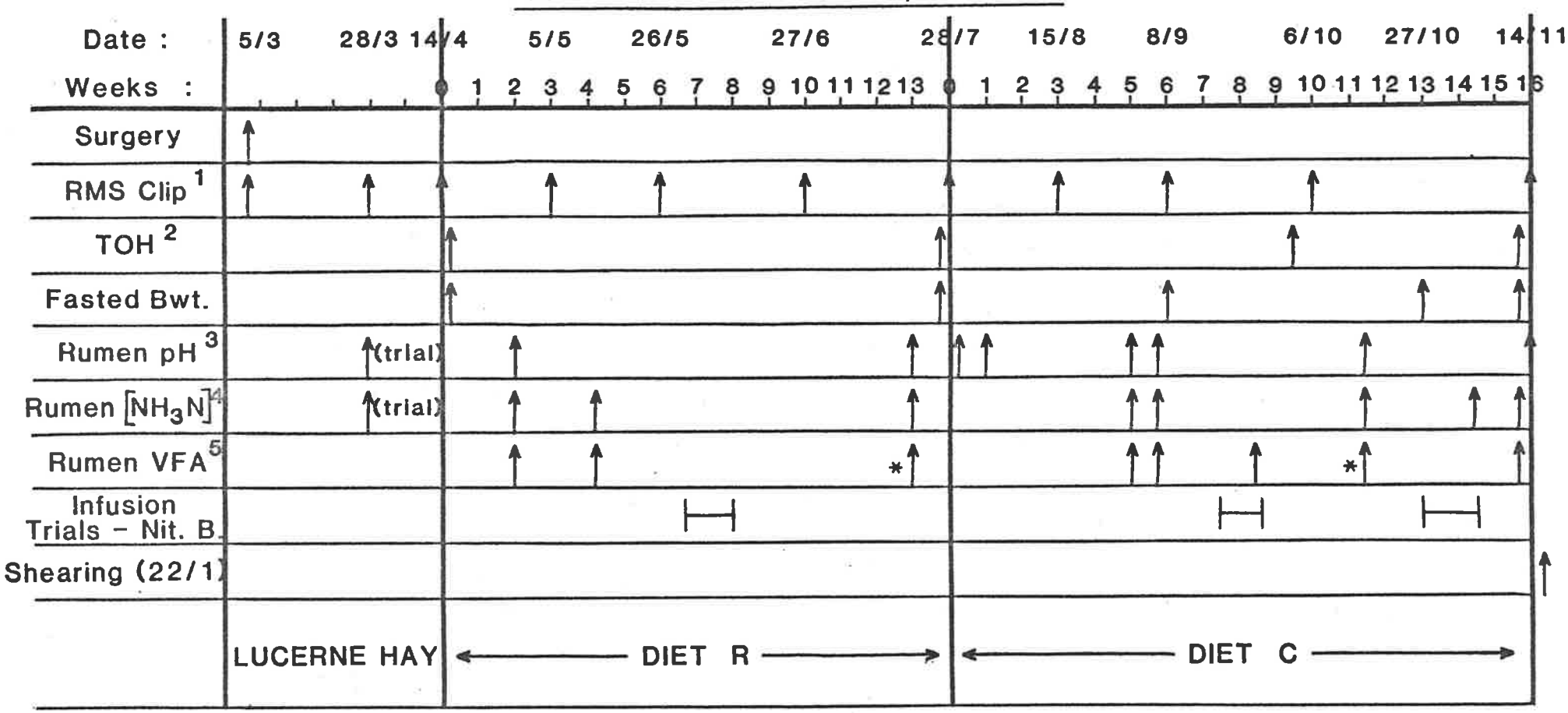
Table 5.5 The dry matter (D.M.%), nitrogen (%), energy ($MJkg^{-1}$), sulphur (%) and phosphorus (%) content of diets R and C. Dry matter digestibility (DMD) (%) is also shown. (Mean ± S.E.M.)

	Diet R	Diet C
D.M. (%)	88.21 (0.16)	89.57 (1.01)
Nitrogen (%D.M.)	3.18 (0.26)	2.28 (0.08)
ME($MJkg^{-1}d.m$)	8.72 (0.26)	10.92 (0.39)
D.M.D. (%)	64.2 (1.9)	79.2 (2.7)
Phosphorus (%DM)	n.d	0.324
Sulphur (%DM)	n.d	0.231

n.d = not determined

The experimental protocol is presented in Table 5.6. The design allowed estimation of digestive efficiency of individual sheep fed firstly, a diet which would presumably induce a "stable" fermentation pattern (Barry et al. 1977), and secondly the concentrate diet. To characterise the prevailing rumen digestion processes on each ration, rumen pH, ammonia concentration and V.F.A. concentration and composition were estimated as described in Section 5.4.3 at intervals throughout each period. Flows of organic matter, dry matter and non-ammonia-nitrogen (NAN) were measured in one infusion trial on diet R(7-8 weeks after the diet was

Table 5.6. The outline of Experiment 4.



Key : 1 : Right midside patch wool harvest.

2 : Tritiated water space.

3 : pH - at 4 h. intervals.

4 : Ammonia - nitrogen concentration - diurnal estimation at 4 h. intervals.

5 : Volatile fatty acid concentration and composition estimated on samples taken 4 h. after feeding. * = diurnal V.F.A. estimation.

introduced) and twice when Diet C was fed (at 7-9 weeks and 13-15 weeks after diet introduction.) The digestibility of nitrogen, dry matter and organic matter was determined for each sheep on both diets during the infusion trials. Nitrogen balance was also measured at these times.

5.4.3 Methods

Wool Growth Rate

WGR was estimated from patch wool production as described in Section 2.2.4. The sheep were shorn 3 months prior to, and at the conclusion of the experiment.

Nitrogen balance and feed digestibility

Nitrogen retention was determined for each animal, once on Diet R and twice on Diet C. Sheep were put into metabolism crates 1-2 weeks prior to the collection period. Faecal and urinary nitrogen was measured as outlined in Section 2.2.4. Body composition estimation is described in Section 2.2.4.

Digesta flow rate

Animals in the trial were fed only once daily, so accurate estimates of flow rate could only be made by regarding the whole 24hr. feeding cycle as a steady-state unit as described by Faichney (1980). Samples of abomasal fluid were thus taken during the last 3 days of an 8 day infusion (Plate 5.2), so that each 2hr. period of the 24hr feeding period was represented. Equal quantities of whole digesta and of filtrate obtained by straining fluid thru terylene cloth, were bulked for each sheep.

In this study it was recognised that microbial responses to the diets used were of great importance and for this reason the radioactive elements of chromium (^{51}Cr) and

Plate 5.1 Sampling rumen fluid for estimation of pH, ammonia nitrogen concentration and volatile fatty acids.

Plate 5.2 Sheep in metabolism crates during an infusion period. The infusate and pump are housed near the roof, and infusion lines to each sheep can be seen. Apparatus for faeces and urine collection are also apparent.

ruthenium (^{103}Ru) were used to label EDTA and phenanthroline respectively because the detection limits of these markers are much lower than for the corresponding "cold markers". Smaller doses can thus be used, and the risk of altered metabolism decreased. In the final infusion period, however, cold markers were infused because of the difficulties involved in the preparation and handling of the gamma emitters.

Infusates

Ruthenium - labelled tris (1, 10-phenanthroline) chloride was prepared as described by Tan et al. (1971). ^{103}Ru-chloride and ^{51}Cr-EDTA were obtained from the Radiochemical Centre (Amersham Eng) and the AAEC (Lucas Heights, Syd) respectively. Cr-EDTA was prepared by complexing $CrCl_3$ with disodium EDTA.

The radioactive infusate comprised 1mCi ^{51}CrEDTA, 0.2mCi ^{103}Ru-P and 300mg Cr EDTA per litre (Faichney 1975). This solution was intraruminally infused at 80ml sheep $^{-1}d^{-1}$ for 8 days. The "cold" markers were infused at 5mg d^{-1} Ru-P and 50mgd^{-1}CrEDTA.

Sample preparation for gamma counting

A subsample of the bulked digesta and filtrate samples from each sheep was treated with a gel powder (Cab-O-Sil) at rates of 4% for whole digesta and 5% for filtrate and urine. Infusate standards were similarly prepared in unlabelled fractions.

The gamma counter (Packard Autogamma scintillation spectrometer Model 5120) was operated as follows:

	Lower Level	Window	Gain
Channel 1 (Cr^{51})	250	100	2
Channel 2 (Ru^{103})	350	150	3

Counting efficiencies thus obtained were approximately:

Cr^{51} Channel		Ru^{103} Channel	
Cr^{51}	Ru^{103}	Cr^{51}	Ru^{103}
7.9%	8.9%	0%	18.4%

That is, ^{103}Ru could be counted without ^{51}Cr interference but ^{51}Cr counts had to be corrected for ^{103}Ru counts in that channel.

The volume of sample counted was also important and was carefully standardised.

Flow of digesta and its constituents were calculated as described by Faichney (1975) after correction was made for the percentage of the daily ^{51}Cr dose excreted in urine. It was assumed that 43% of urinary Cr was absorbed from the stomach (Faichney pers. comm.). Rumen volume was determined (Faichney 1975) only in the second infusion trial on diet C, so dilution rate data are confined to this period.

"Cold" chromium for trial 2 on Diet C, was assayed by atomic absorption spectrophotometry using a Pye Unicam SP9 AAS machine. Abomasal, ruminal and faecal Cr concentrations were estimated by repeated centrifuging and washing of the pellet until all Cr was eluted (usually 3 washes was sufficient). Urine Cr was determined by direct aspiration of

centrifuged samples.

Considerable difficulty was encountered in quantifying "cold" ruthenium in this trial. Neither X-ray fluorescence spectrometry (Evans et al. 1977) nor atomic absorption using electrothermal atomisation (Megarrity and Siebert 1977) proved sufficiently sensitive for accurate estimation. Flow rates for this trial were therefore based on chromium data alone. Such an approach would not be admissible without justifying its use. For example Faichney (1980) demonstrated that nitrogen flow rates can be influenced greatly by the sampling errors associated with single marker flow estimates, but in the first infusion trial on the concentrate ration there was little sampling error apparent (Fig. 5.5). The finely divided particulate nature of this ration may produce a more homogeneous digesta than coarsely divided forages. On this basis the estimation of nitrogen flow from chromium dilution alone appears justified.

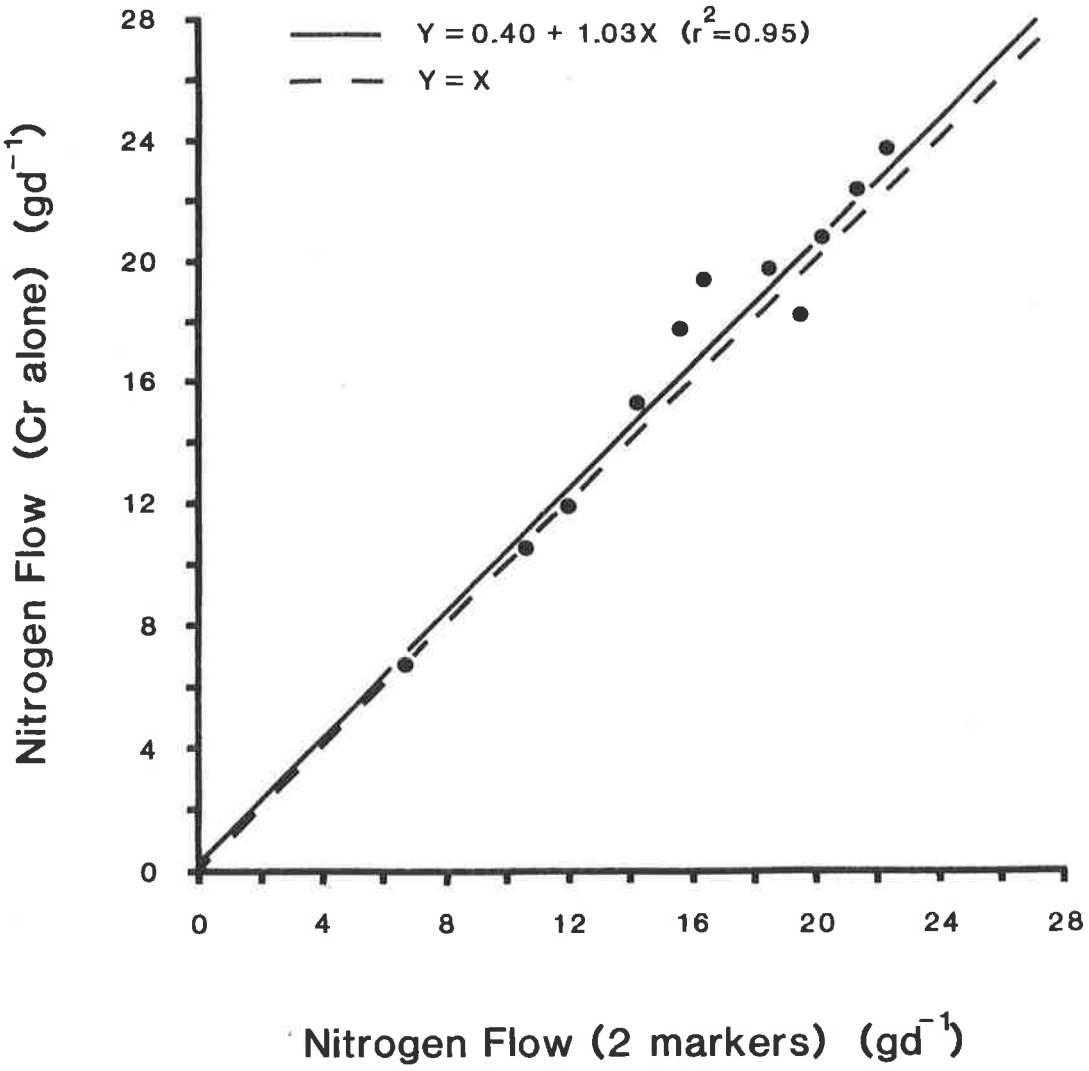
Measurement of rumen digestion variables

One of the problems of characterising the rumen digestion processes is that intermittent feeding induces non-steady-state conditions. To obtain realistic estimates of the dynamics of digestion in the present study, rumen samples were taken at 4-hourly intervals throughout the day. Ruminal pH, NH_3N and V.F.A. were measured on these samples for each animal by the following techniques.

Rumen pH

Samples of rumen liquor were obtained by inserting a plastic tube into various sections of the rumen and

Figure 5.5 Postruminal nitrogen flow (gd^{-1}) estimated from digesta flow data based on chromium dilution alone, or from digesta flow corrected for sampling errors by ruthenium dilution also.


Y = 0.40 + 1.03X (r^2=0.95)
Y = X
Nitrogen Flow (Cr alone) (gd^{-1})
Nitrogen Flow (2 markers) (gd^{-1})
0
4
8
12
16
20
24
28

siphoning 40ml aliquots into a 200ml glass tube which was stoppered when full (see Plate 5.1). The pH of the fluid was determined within 30 secs of sampling using an Anax pH meter with automatic temperature calibration. The samples, taken 0, 4, 8, 12, 16 and 20h after feeding were then filtered through terylene cloth and immediately stored at -17°C.

Ammonia-nitrogen concentration

Filtered ruminal and abomasal fluids were analysed for NH_3N by the Conway microdiffusion technique using $(NH_4)_2SO_4$ as the primary standard.

Volatile fatty fluids in rumen fluid

The following technique is based on that of Fennessy (pers. comm).

Rumen fluid, treated with a protein precipitant and a measured amount of an internal standard, was analysed by gas-liquid chromatography. The concentration of individual acids were estimated by comparing the ratio of acid to internal standard peak heights with the corresponding ratios measured on standard V.F.A. mixtures.

Reagents

Protein precipitant - 25% metaphosphoric acid (HPO_3)

Internal standard - 10.5ml n-Caproate in 21 H_2O

Stock V.F.A. solutions- Acetic, propionic and n-Butyric made to 1.0M. Valeric, iso-Valeric made to 0.1M. using AR grade reagents.

Stock VFA mixture - 10ml Acetic stock solution, 2.5ml propionic, 2.5ml Butyric, 5.0ml Valeric and 5.0ml iso-Valeric stock solutions, diluted to 100ml.

To produce standards containing reagents of approximately the same proportions as unknown samples, the following mixtures were made.

Std. No.	ml. Stock VFA Mixture	ml. H_2O	ml. protein Precipitant	ml. internal Standard
1	0	25	5	5
2	5	20	5	5
3	10	15	5	5
4	15	10	5	5
5	20	5	5	5
6	25	0	5	5

5ml. protein precipitant and 5ml. internal standard were added to 25ml. rumen fluid, mixed thoroughly and centrifuged. After thawing the samples were shaken, then allowed to stand for at least 1hr.

Separations were made on a Packard GLC (Model 7721) as follows:

Column: 10% AT1200 (Alltech) + 1% H_3PO_4 on Chromosorb W-AW (80-100 mesh) on a 2.4m x 3mm ID glass coil

Inlet Temp: 175°C

Outlet Temp: 180°C

Column Temp: 118°C

Detector Temp: 175°C

Sensitivity: $3x10^{-10}$ amps

Chart Speed: 10mm/min

Sample size: 3µl.

Carrier gas (N_2): 24ml/min

FID (H_2 : 63ml/min; Air : 300 ml/min)

5µl formic acid (4%) was injected on column after every 4th sample to minimise "ghosting" effects (Geddes and Gilmour 1970).

The above conditions provided good separation of the VFA, and the internal std. (C_6) had eluted within 5 mins.

Full calibrations with standard mixtures were made for each run, and checked with primary standards. This is necessary as peak height ratios vary with carrier gas flow and the FID mixture.

Efficiency of bacterial protein synthesis

An assessment was made of the contribution of bacterial protein to total postruminal protein flow by reference to the concentration of diaminopimelic acid (DAPA) in the abomasal fluid of sheep fed Diet C. DAPA was measured in hydrolysed (6N HCL at 110^{o}C under nitrogen) abomasal fluid on a Beckman 119 amino acid analyser under the conditions described below for other amino acids.

To convert DAPA-nitrogen to total bacterial nitrogen, it was assumed that 1mg DAPA-nitrogen was equivalent to 159mg of bacterial nitrogen. This conversion factor is a mean value from other trials (Hogan and Weston 1970; Hutton et al. 1971; Bird 1972; Ulyatt et al. 1975; Ling and Buttery 1978). It was recognised that this assumption may not be valid if the factor changes with bacterial species (Ling and Buttery 1978). Nevertheless, large differences in bacterial synthesis between sheep would reflect either grossly different bacterial populations or true synthesis differences.

The efficiency of bacterial synthesis was derived from bacterial protein flow rate and the quantity of organic matter which disappeared in the rumen (OMI-OM flow), and was thus expressed as gd^{-1} per $100gd^{-1}$.

Amino acid composition of proteins in abomasal digesta

It was considered that the WGR differences may not necessarily have been a consequence of protein flow differences per se, but may have been associated with the composition of the protein flowing postruminally.
Such a possibility was a very real one if there were large differences between sheep in the dilution rate of rumen fluid, and protozoal contribution to the total protein flow.

Proteins in the abomasal digesta of sheep fed Diet C (trial 2) were precipitated with an equal volume of 10% T.C.A., frozen overnight, thawed and centrifuged. The precipitate was then subjected to the following:

a) oxidation in performic acid (approx 1.5mg protein in 2ml performic acid) and hydrolysis in 6N HCl at 110°C under nitrogen reflux for 22h.

or

b) hydrolysis in HCL alone without prior oxidation.

The freeze-dried hydrolysates were then taken up in sodium citrate buffers (containing .25g/l tetra sodium EDTA to precipitate the chromium and ruthenium present), and injected onto a 420mm AA15 resin in a Beckman 119 AA system.

All amino acid data are expressed as grams of each acid per 100 grams total amino acids.

Statistical Analysis

Effects of diet, time of day, and the "diet x time" interaction for pH and ammonia-nitrogen concentration, were tested in a split plot analysis of variance using diet as the main plot, and time as the sub-plot.

The relationships between the various digestive parameters and between digestive parameters and WGR, were

determined by regression analysis. Multiple regression analysis was employed to derive relationships in which the independent variates were co-correlated, the partial regression coefficients indicating the effect of each variate corrected for its association with the other. Partial coefficients were tested for significance against the degrees of freedom used for total correlation testing, less the number of eliminated variates (usually 1). Coefficients significant at the 5-10% level are indicated, because some of the correlations were derived for a small number of animals (n=12).

It was recognised that correlation does not indicate causality. All relationships are discussed, therefore, in relation to the biology of the system in which the interactions take place.

Other comparisons in this trial were made in an analysis of variance; group means were tested using a simple "t-test".

5.5 Results

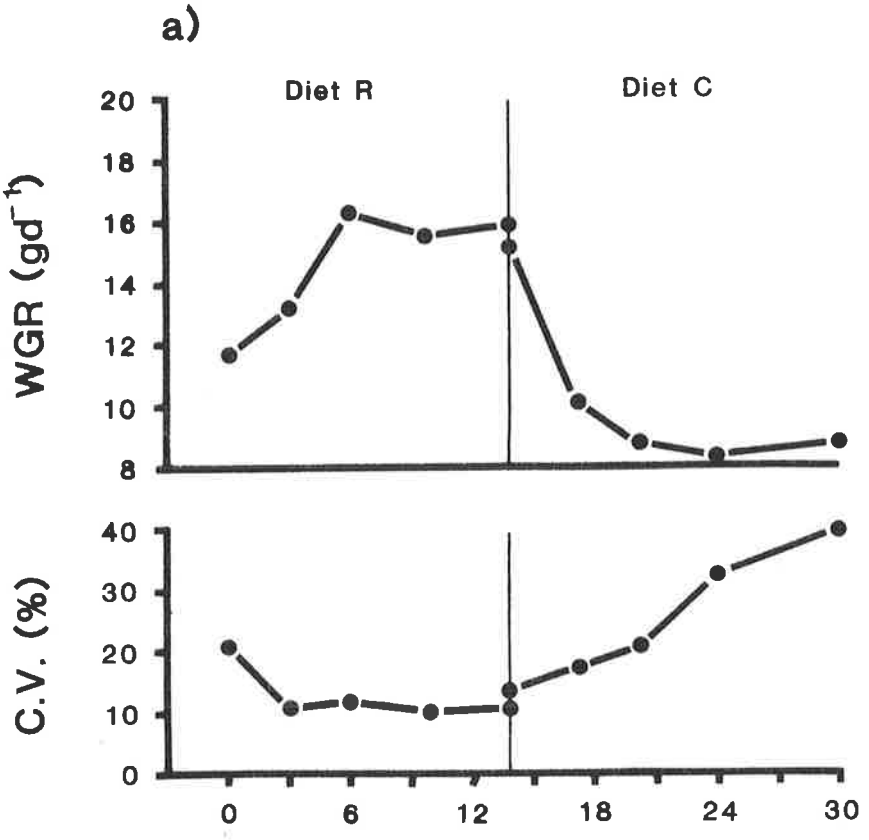
5.5.1 Wool growth

When sheep were transferred to Diet C after 14 weeks on diet R, the mean WGR declined substantially and the between-sheep variance of WGR increased (Fig 5.6a).

Depressed wool growth on diet C was anticipated in response to the lower protein content of this ration. Indeed, when wool production was expressed per unit of nitrogen intake (see Appendix 5.1 for intakes) the diet differences were eliminated (Fig. 5.6b). It is also evident from Figures 5.6a) and 5.6b) that the wool growth variance of sheep consuming diet R was low (CV 10.5%) while diet C generated a

Figure 5.6 a) Changes in mean WGR (gd^{-1}) and the coefficient of variation in WGR(%) with time on Diet R and Diet C.

b) Mean efficiency of wool growth (WGR per unit nitrogen intake) (± S.E.M.) on Diets R and C.


a)
Diet R
Diet C
WGR (gd⁻¹)
20
18
16
14
12
10
8
C.V. (%)
40
30
20
10
0
0
6
12
18
24
30
Weeks of Experiment

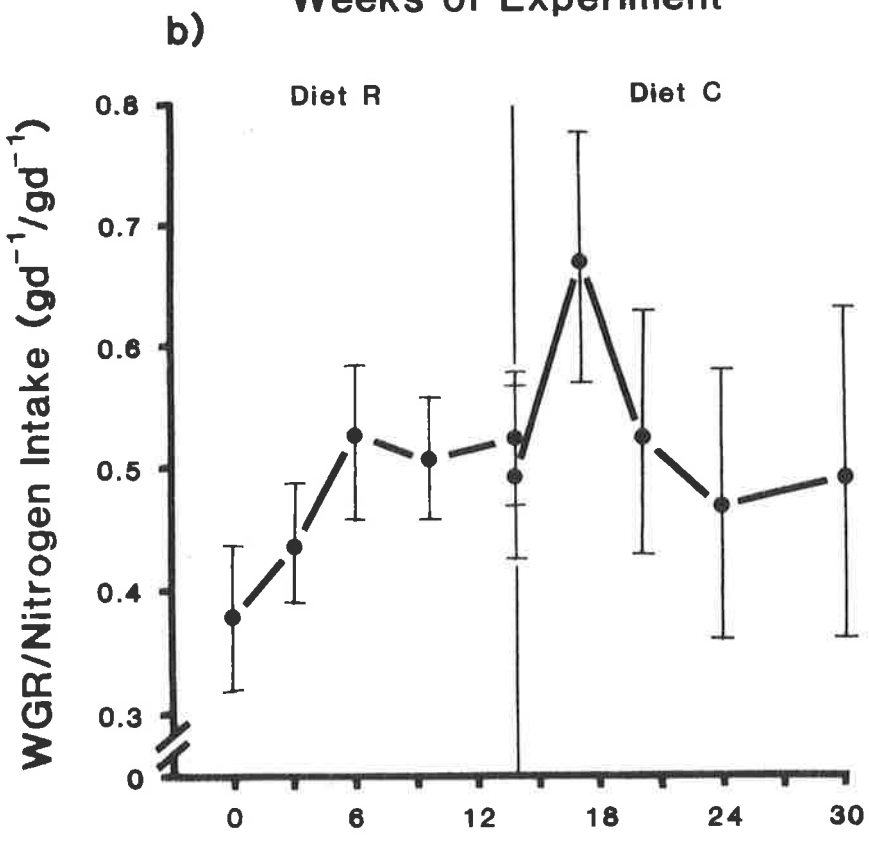
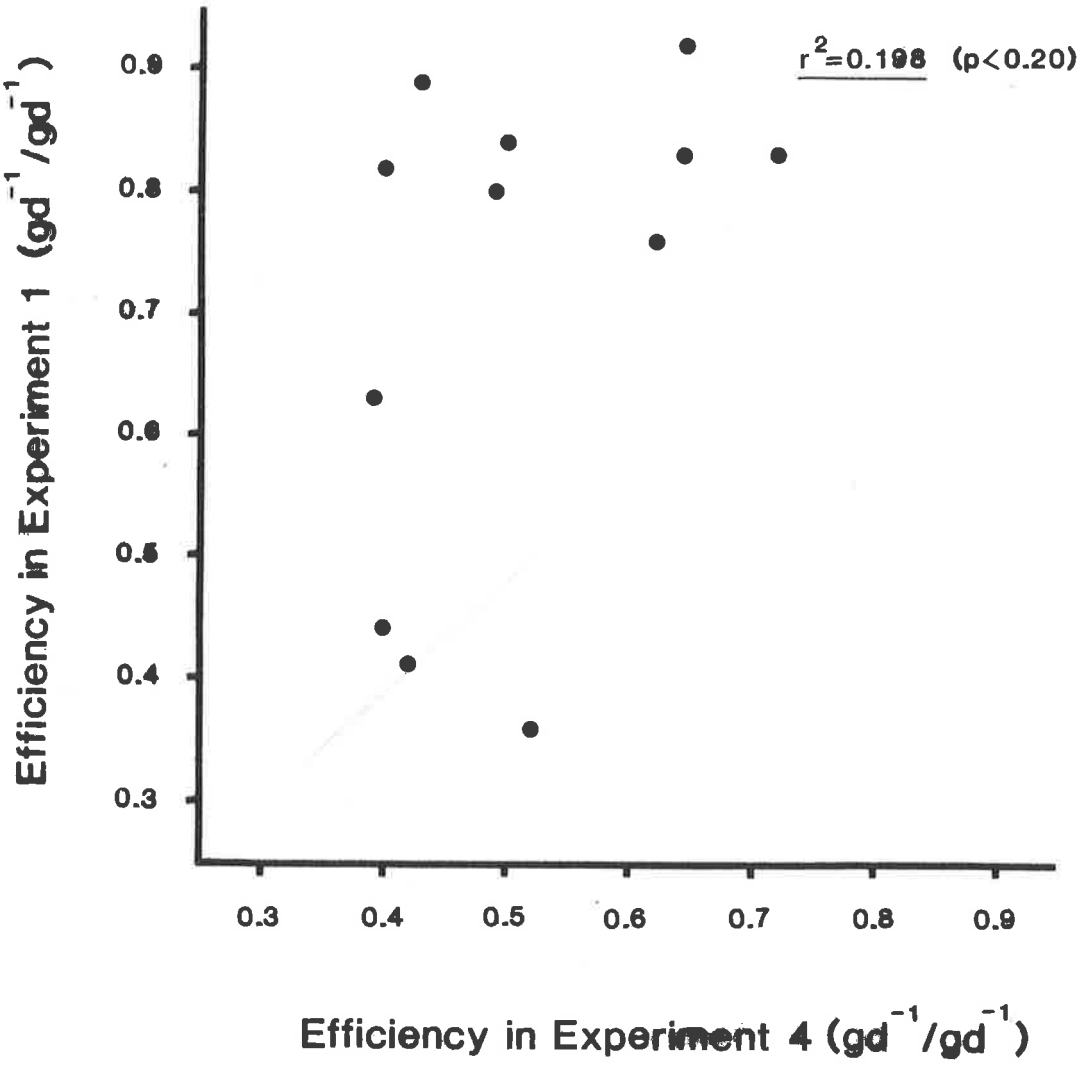

b)
Diet R
Diet C
WGR/Nitrogen Intake (gd⁻¹/gd⁻¹)
0.8
0.7
0.6
0.5
0.4
0.3
0
0
6
12
18
24
30
Weeks of experiment

Figure 5.7 The relationship between wool growth efficiency (WGR per nitrogen intake) of sheep on Diet C in Experiment 1 and Experiment 4.


r^2=0.198 (p<0.20)
Efficiency in Experiment 1 (gd^{-1}/gd^{-1})
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Efficiency in Experiment 4 (gd^{-1}/gd^{-1})

much wider array of wool growth rates and efficiencies (CV 31.5% and 22.6% respectively), indicating the same instability of production that had been noted in Experiment 1. Wool data for individual sheep are presented in Table 5.7, and individual wool responses with time on each ration in Appendix 5.2. Final efficiencies ranged from $0.44gg^{-1}$ to $0.62gg^{-1}$ on diet R, while on diet C the range was from $0.39gg^{-1}$ to $0.72gg^{-1}$ (Table 5.7).

A comparison was made of the relationship between efficiency on the concentrate ration in Experiment 1 and efficiency in the current trial to re-examine the hypothesis that wool production on this type of ration is genetically determined. It will be recalled that there were only 8 sheep in the previous examination made in Experiment 3. The results of the current work confirmed the findings of that trial that the diet effect is not repeatable within an animal (see Fig. 5.7). Repeatability of response, as indicated by the low, non significant correlation coefficient (r^2=0.198, n=12) was poor, and it can be stated with confidence that the wool production of any individual sheep consuming this ration cannot be predicted from previous performance.

5.5.2 Flow of non-ammonia nitrogen in relation to wool production

The variations in wool growth rate on the roughage diet (R) and the concentrate-roughage diet (C) were closely associated with the within-group variations in the flow of NAN from the rumen. On the roughage diet the responses of all sheep were uniform in terms of wool growth and NAN flow and it was not possible to distinguish a relationship

Table 5.7 Final WGR(gd^{-1}) and wool growth efficiency (gd^{-1} per g nitrogen d^{-1}) for sheep on Diets R and C.

Sheep No.	Final WGR(gd^{-1})		Final efficiency (gd^{-1}/gd^{-1})	
	Diet R	Diet C	Diet R	Diet C
36	15.6	4.0	0.506	0.416
10	13.8	6.8	0.448	0.385
11	15.6	7.0	0.506	0.395
44	(12.6)	9.1	(0.409)	0.500
31	19.1	8.9	0.620	0.485
25	(11.3)	12.4	(0.366)	0.671
5	15.9	7.9	0.516	0.520
23	15.5	-	0.552	-
27	(13.5)	12.3	(0.438)	0.665
13	15.7	7.4	0.509	0.427
17	15.9	13.4	0.516	0.724
18	18.8	11.4	0.610	0.617
19	13.7	6.0	0.444	0.400
Mean	15.96	8.88	0.523	0.517
±SD	1.68	2.80	0.055	0.117

( ) these 3 sheep were replacements and had lower WGR because they were on Diet R for a shorter time than the other sheep. Data for these sheep on Diet R are thus omitted.
The WGR for each sheep throughout the experiment are presented in Appendix 5.2.

between the two variables. However, when sheep were offered diet C, the variance of protein flow between sheep increased substantially, the coefficient of variation rising from 8.2% on diet R to 23.9% and 32.5% in infusion trials 1 and 2 on diet C (Table 5.8). The relations of WGR to the postruminal NAN flow are presented in Fig. 5.8. The top graph, Fig. 5.8a), illustrates the values for sheep on diets R and C for the first two infusion studies, in which the radioisotopes of chromium and ruthenium were used to estimate digesta flow rate. Two regression lines have been fitted. One relates to diet C only and is statistically significant (r^2 = 0.53, $P<0.02$). The other, in which data from the two diets are pooled, is more highly significant (r^2 = 0.77, $P<0.001$). As mentioned earlier there was little variation in wool production or NAN flows among the sheep on diet R, and no significant relation between these characters was observed on that diet.

Figure 5.8b) depicts the relationship between WGR and NAN flow in the second infusion trial on diet C, in which cold ruthenium was used as the second marker but could not be quantified. The responses on diet R are also included. Once again the within group WGR/NAN flow of sheep on diet C was linear and highly significant (r^2 = 0.67, $P<0.01$) the regression involving all sheep being curvilinear (r^2 = 0.85, $P<0.025$). Similarly the third graph (Fig. 5.8c), which represents the mean of both infusion trials on diet C, shows a significant linear relation between NAN and WGR among sheep on diet C (r^2 = 0.78, $P<0.01$), and a curvilinear effect where all data were pooled (r^2 = 0.86, $P<0.001$). There was a strong correlation (r = 0.73, $P<0.05$) between

Figure 5.8 WGR (gd^{-1}) as influenced by postruminal NAN flow (gd^{-1}) on Diet R (one trial only) and

a) Diet C - Trial 1

b) Diet C - Trial 2

and c) Diet C - mean of both trials

The regression equations describing these relationships are presented in the text.

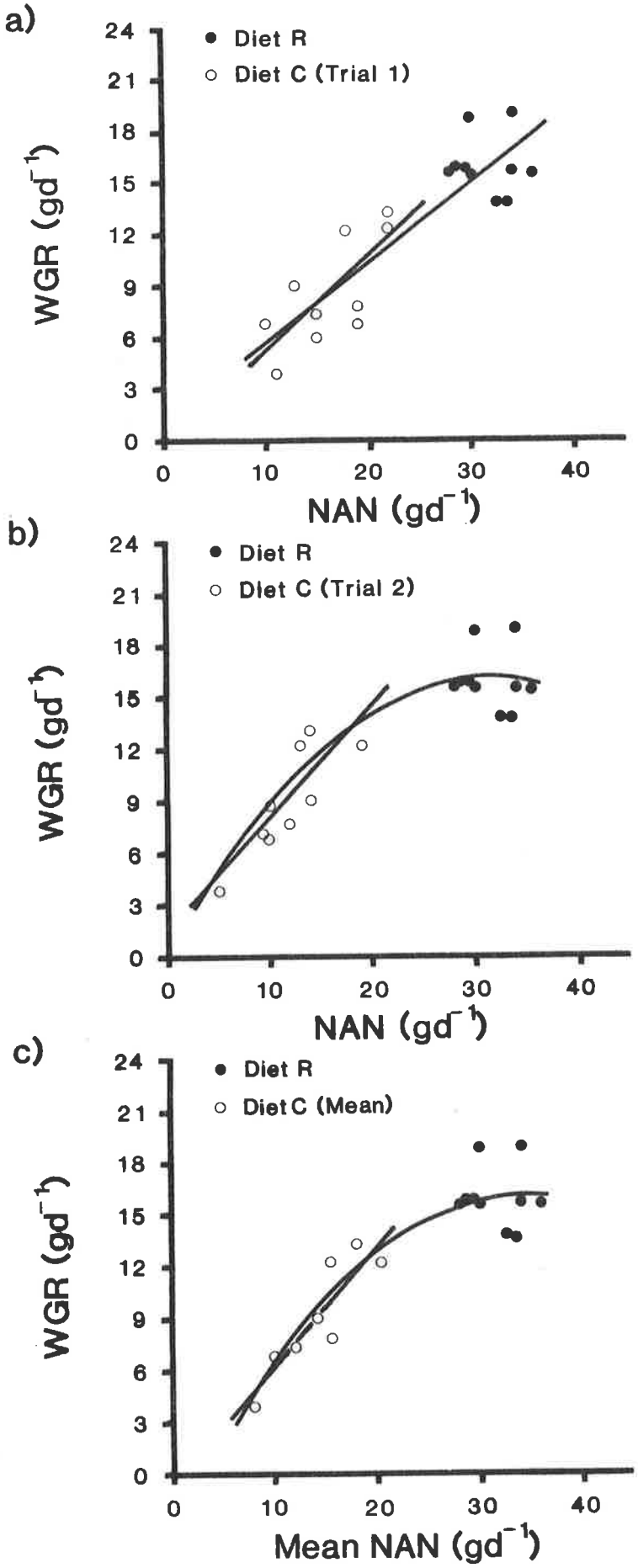

a)
Diet R
Diet C (Trial 1)
WGR (gd⁻¹)
NAN (gd⁻¹)
b)
Diet R
Diet C (Trial 2)
WGR (gd⁻¹)
NAN (gd⁻¹)
c)
Diet R
Diet C (Mean)
WGR (gd⁻¹)
Mean NAN (gd⁻¹)
0
3
6
9
12
15
18
21
24
0
10
20
30
40

Table 5.8 Nitrogen intake (gd^{-1}), NAN flow (gd^{-1}) and P/E ratio (gd^{-1}/MJd^{-1}) for sheep on Diets R and C.

Sheep No.	Diet R			Diet C					
				1			2		
	NI	NAN	P/E[a)]	NI	NAN	P/E	NI	NAN	P/E
36	30.0	27.9	13.9	17.0	11.2	5.5	9.9	5.0	3.9
10	30.0	32.5	16.8	15.7	18.6	10.5	17.8	+	
11	30.0	30.3	15.0	17.3	10.2	5.0	17.8	9.6	4.3
44	-	-	-	18.9	13.2	5.9	17.7	14.4	6.8
31	30.0	33.7	16.7	18.7	+		17.8	9.8	4.5
25	-	-	-	18.9	17.8	8.6	17.8	13.0	6.1
5	29.4	29.5	15.1	18.9	18.8	8.8	16.1	12.1	6.0
23	30.0	36.1	18.6	-	-	-	-	-	-
27	-	-	-	18.9	21.8	10.6	17.8	19.1	9.3
13	30.0	34.2	16.7	18.9	15.2	6.9	17.8	8.7	3.9
17	30.0	28.5	14.3	18.9	21.7	10.7	17.8	13.8	6.6
18	30.0	30.3	15.5	-	-	-	-	-	-
19	30.0	33.5	17.3	18.8	14.7	6.8		+	
Means	29.9	31.7	16.0	18.2	16.3	7.9	16.7	11.7	5.7
S.E.M.	0.2	2.6	1.4	1.1	3.9	2.1	2.5	3.8	1.7

+ flow rate not determined due to unsuccessful infusion (e.g. irregular pump rate).

a) P/E = NAN flow to the abomasum x 6.25, divided by the estimated metabolisable energy intake (derived from digestibility data).

the NAN flow of a sheep measured in the first and second infusion trials on diet C indicating that once a pattern was established, it was sustained.

The point of greatest significance in Fig. 5.8 is that a large proportion of the variation in WGR among sheep offered similar amounts of a concentrate-roughage diet could be accounted for by the flow of NAN from the rumen. While there were some refusals of feed on diet C resulting in a small variation in nitrogen intake (Table 5.8) this was not related to the extensive NAN flow differences also depicted in this table. Moreover, NAN flow accounted for more of the differences in WGR than did nitrogen intake. For example, in the first infusion trial when the double-marker was used and diet refusals were least, the relation of N intake to WGR was non significant (r^2 = 0.30), whereas NAN and WGR were closely related. When averaged over both infusion trials NAN flow removed 0.78 of the differences in WGR while N intake accounted for only 0.54 of the variance.

The following equations describe the relationships between final WGR(Y) and NAN flow (X) illustrated in Figures 5.8a, b and c.

Equation 5.1.... Diet C (Trial 1):

$Y = -0.44+0.556X$ $\quad r^2=0.53$ $(P<0.02)$

Equation 5.2.... Diet C (Trial 2):

$Y = -1.80+0.169X$ $\quad r^2=0.67$ $(P<0.01)$

Equation 5.3.... Diet C (mean) :

$Y = -0.80+0.709X$ $\quad r^2=0.78$ $(P<0.01)$

Regression equations best describing the relationship when results for both diets were combined were as follows:

Equation 5.4.... Diet C (Trial 1)+Diet R:

$Y = 1.25+0.460X$ $\quad r^2=0.77 \quad (P<0.001)$

Equation 5.5.... Diet C (Trial 2)+Diet R:

$Y = -0.86+1.08X-0.017X^2$ $\quad r^2=0.85 \quad (P<0.025)$

Equation 5.6.... Diet C (Mean) +Diet R:

$Y = -5.24+1.35X-0.022X^2$ $\quad r^2=0.86 \quad (P<0.001)$

Ruminal factors associated with high and low abomasal N flow were examined in this experiment, and the results are discussed in the following sections. Firstly, however, the relation of wool growth to diet digestibility is considered, as is the impact of the NAN flow differences generated on diet C on body nitrogen balance.

5.5.3 Digestibility and wool production on diet C

The dry matter digestibilities (DMD) of individual sheep are tabulated below (Table 5.9). The higher metabolisable energy content of diet C is apparent, the DMD for this diet being some 15% units higher than for the roughage ration. It is also evident that the DMD differences observed between sheep on diet C in trial 1 were maintained when a second estimate was made. As for NAN, there was a tendency for sheep to maintain their relative rankings between trials suggesting that different patterns of digestion were maintained throughout the entire period of diet C feeding. The possibility existed, therefore, that wool growth differences were related to digestive efficiency

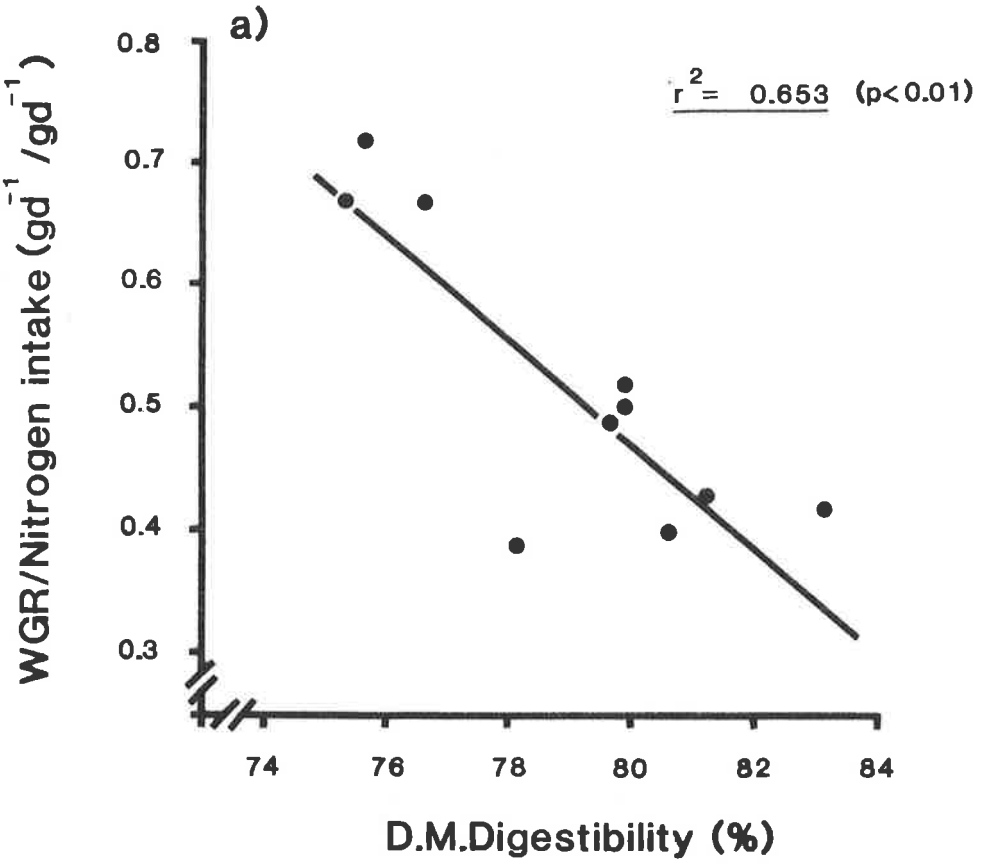
Table 5.9 Dry matter digestibilities (%) for each sheep fed Diet R (1 estimate) and Diet C (2 estimates)

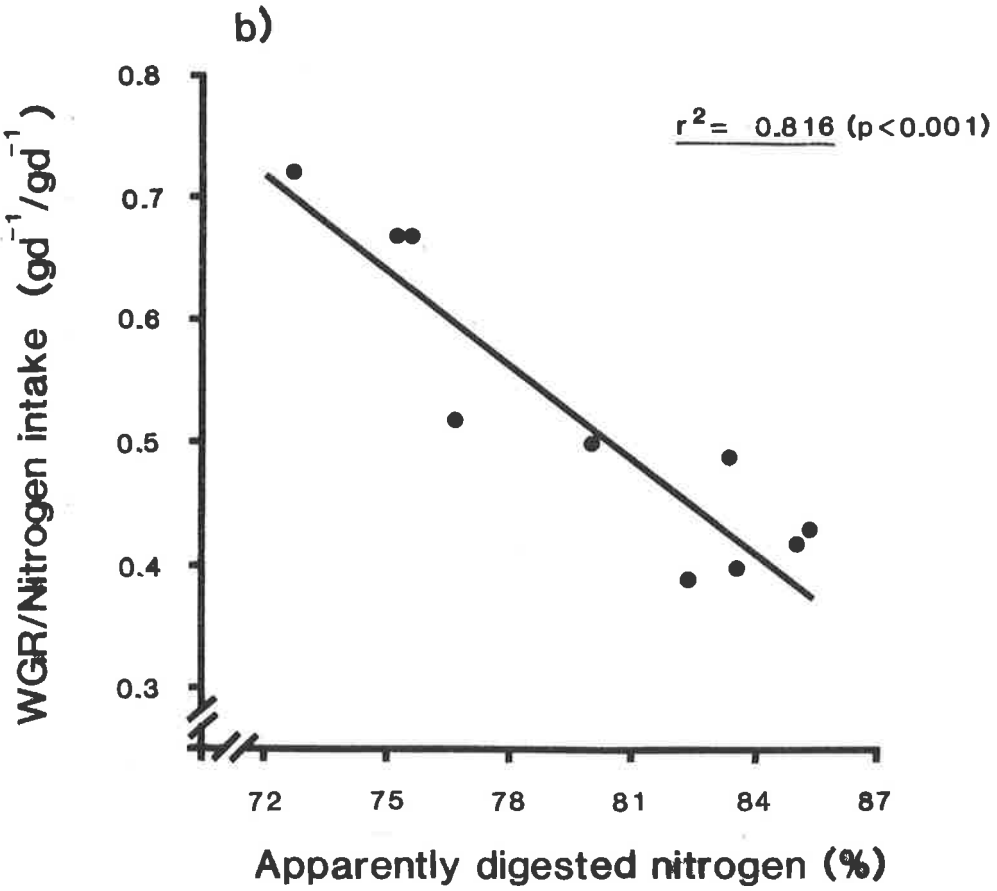
Sheep No.	Diet R	Diet C	
		1	2
36	64.7	83.0	83.1
10	63.1	78.1	78.0
11	65.5	80.9	80.2
44	-	82.4	77.3
31	63.6	79.9	79.3
25	-	76.5	76.7
5	60.8	78.9	80.9
23	62.5	-	-
27	-	75.5	75.0
13	66.3	80.9	81.4
17	65.6	75.0	76.1
18	63.5	-	-
19	62.6	79.7	-
Mean±S.E.M.	63.8±1.6	79.2±2.5	78.8±2.5

on this ration. The relationships between wool growth efficiency (WGR per NI) and both dry matter digestibility (%) and apparently digested nitrogen (%) are depicted in Figures 5.9 a) and b). In contrast to what might be expected, sheep which digested the dry matter and nitrogen fractions of the feed to the greatest extent, were in fact the least efficient wool producers.

Clearly, the digestibility and NAN flow data indicate that the site of protein digestion is of much greater nutritional significance than is the extent of digestion in the whole gastro intestinal tract.

Figure 5.9 The relationship between a) dry matter digestibility (%) and b) apparently digested nitrogen (%), and the efficiency of wool growth (WGR per nitrogen intake) when sheep were consuming Diet C. Digestibility data are the means of 2 estimates; WGR's are the final values recorded on that diet and nitrogen intakes are the means for the whole period.


a)
r^2= 0.653 (p<0.01)
WGR/Nitrogen intake (gd^{-1}/gd^{-1})
0.8
0.7
0.6
0.5
0.4
0.3
74
76
78
80
82
84
D.M.Digestibility (%)


b)
r^2= 0.816 (p<0.001)
WGR/Nitrogen intake (gd^{-1}/gd^{-1})
0.8
0.7
0.6
0.5
0.4
0.3
72
75
78
81
84
87
Apparently digested nitrogen (%)

5.5.4 NAN flow and body nitrogen retention

The variability in NAN flow among sheep on the concentrate/roughage ration was reflected not only in wool growth responses, but also in the retention of nitrogen in body tissues, although the relationships were less precise in the latter case. Nitrogen retention data for individual sheep are presented in Table 5.10. Nitrogen balance minus nitrogen retained in wool tissues (NB-WN) was highly variable between sheep on both diets, and NAN flow accounted for only a small, non significant portion of this variance on diet C in trials 1 and 2 (r^2 = 0.13 and 0.16). However there was a significant linear relation of NAN(X) and body nitrogen retention (Y) (estimated by faeces and urine collection) when the means over both periods were examined (Equation 5.7):

Equation 5.7.... $Y = -1.16 + 0.258X$ $\quad r^2 = 0.55$ ($P<0.05$)

Similarly, when nitrogen retention was determined for the whole period for diet C feeding by reference to changes in body composition estimated by tritium dilution (Table 5.6), it was significantly related to the mean NAN flow for both infusion trials (r^2 = 0.61, $P<0.05$).

Consideration of the urinary nitrogen (UN) output data in Table 5.10 reveals a considerable range of N excretion values on diet C in comparison to the diet R values. In trial 1 on diet C, UN ranged from 7.44 to 13.27gd^{-1}, a substantial variance when the similarity of nitrogen intakes is considered. Similarly, for infusion trial 2 there were large between-sheep differences, so that when urinary N

SHEEP	Diet R						Diet C (Trial 1)						Diet C (Trial 2)					
	FEED N (gd^{-1})	FAECES N (gd^{-1})	URINE N (gd^{-1})	NB (gd^{-1})	NB-WN (gd^{-1})	ADN(%)	FEED N (gd^{-1})	FAECES N (gd^{-1})	URINE N (gd^{-1})	NB (gd^{-1})	NB-WN (gd^{-1})	ADN(%)	FEED N (gd^{-1})	FAECES N (gd^{-1})	URINE N (gd^{-1})	NB (gd^{-1})	NB-WN (gd^{-1})	ADN(%)
36	29.97	6.41	18.11	5.45	2.97	78.6	16.97	2.33	10.85	3.79	2.68	86.3	9.92	1.62	5.54	2.76	1.65	83.7
10	29.97	7.17	20.87	1.93	-0.22	76.1	15.74	2.99	12.25	0.50	-0.58	81.0	17.83	2.91	11.79	3.13	1.97	83.7
11	29.97	6.53	18.14	5.30	2.99	78.2	17.30	2.62	13.27	1.41	0.09	84.9	17.83	3.20	12.55	2.08	0.76	82.1
44							18.90	2.86	13.27	2.77	1.36	86.2	17.68	4.63	11.54	1.51	0.10	73.8
31	29.97	6.26	20.17	3.54	0.68	79.1	18.74	3.50	10.27	4.97	3.38	81.3	17.83	2.16	12.02	3.65	2.06	87.9
25							18.90	4.36	9.72	4.82	3.10	76.9	17.83	4.58	9.70	3.55	1.83	74.3
5	29.39	6.53	21.80	1.60	-0.65	77.8	18.90	3.82	9.75	5.33	4.08	79.8	16.07	3.31	8.90	3.86	2.61	79.4
23	29.97	6.88	21.25	1.84	-0.69	77.0	omitted due to inappetance											
27							18.90	5.06	7.44	6.40	4.68	73.2	17.83	4.03	7.36	6.44	4.72	77.4
13	29.97	5.57	17.35	7.05	4.64	81.4	18.90	3.35	12.83	2.72	1.51	82.3	17.83	2.08	11.07	4.68	3.47	88.3
17	29.97	5.70	17.42	6.85	4.34	81.0	18.90	4.92	9.46	4.52	2.42	74.0	17.83	5.11	6.17	6.55	4.45	71.3
18	29.97	6.22	20.10	3.65	0.81	79.3	omitted due to cannula failure											
19	29.97	7.03	20.31	2.63	0.60	76.5	18.83	3.60	9.29	5.94	4.78	80.9						
MEAN	29.91	6.43	19.55	3.98	1.55	78.50	18.27	3.58	10.76	3.93	2.50	80.62	16.85	3.36	9.66	3.82	2.36	80.19
± SEM	0.17	0.50	1.56	1.95	1.91	1.68	1.04	0.86	1.82	1.79	1.68	4.25	2.37	1.14	2.43	1.59	1.41	5.63

Table 5.10 Nitrogen balance data for sheep fed Diets R and C.

output was expressed as a percentage of N intake the coefficient of variation was about 20% on diet C and only 8% on diet R (Table 5.11). Some sheep on diet C excreted the equivalent of nearly 80% of the ingested nitrogen in the urine and others only 40%. When these data were examined statistically NAN flow was observed to be associated with these differences in urinary nitrogen output, but part of this was associated with differences in nitrogen intake (NI). The relationship derived between NI, NAN and UN when data for both trials on diet C were examined are presented in Equation 5.8. The partial correlation coefficient of NAN flow against UN is also shown.

Equation 5.8.... $NAN = -5.8+1.59NI - 0.78UN \qquad (R^2=0.45)$

R^2 after correction for NI = 0.40 ($P<0.10$)

The partial correlation coefficient approached significance ($P<0.10$), and with the small degrees of freedom involved, the likelihood of this occurring by chance is slight. The evidence suggests therefore that the observed NAN flow variance on the concentrate ration was in part, a consequence of differences between sheep in urinary nitrogen excretion. High urinary nitrogen output was associated with low NAN flow.

5.5.5 Patterns of ruminal fermentation and their relationship to postruminal NAN flow

It should be emphasised at this stage that the design of the experiment did not set out to establish the factors that initiated a particular pattern of metabolism in each of the sheep under study. Rather, it sought to examine whether

Table 5.11 Urinary nitrogen output (gd^{-1}) as a percentage of nitrogen intake (gd^{-1}) for sheep fed Diet R and Diet C.

SHEEP	DIET	DIET C	
	R	1	2
36	60.4	63.9	55.9
10	69.6	77.8	66.1
11	60.5	76.7	70.4
44	-	70.2	65.3
31	67.3	54.8	67.4
25	-	51.4	54.4
5	74.2	51.6	55.4
23	70.9	-	-
27	-	39.4	41.3
13	57.9	67.9	62.1
17	58.1	50.1	34.6
18	67.1	-	-
19	67.8	49.3	-
Mean	65.38	59.37	57.29
± S.E.	5.44	12.00	11.06

the variations in ruminal metabolic processes reflected, or were associated with, the variations observed in NAN flow and WGR on the two diets.

Of all the rumen variables measured in this trial, the concentration of ammonia-nitrogen (NH_3N) in the rumen fluid appeared to be a most important characteristic defining dietary differences and, more importantly, differences between sheep consuming diet C. The diet effect and diurnal pattern of NH_3N were most pronounced and are illustrated in Fig. 5.10. The influences of diet, time of day, and the "diet x time" interaction on NH_3N, as depicted in this graph, were statistically highly significant when analysed in a split plot analysis of variance (Table 5.12). Values tested by this means represented 3 sampling periods for diet R and 4 for diet C.

The diurnal NH_3N pattern on diet R was relatively consistent both between sampling times and between sheep (Note the standard deviations around the mean estimates in Fig 5.10). A peak mean concentration of 33mg/100ml fluid occurred 4h after feeding, followed by a decline to 16h and a second, smaller peak at 20h. This latter peak was apparent at each of the 3 sampling periods for diet R, and regularly coincided with a period of rumination by most animals.

In contrast, a much higher variability was apparent on diet C (see S.D. values in Fig. 5.10) with no distinct diurnal pattern of NH_3N. Nevertheless, the diet effect was significant, the mean NH_3N on diet C of about 46mg/100ml being substantially higher than that on diet R (22mg/100ml). Initially, this result appears to be inconsistent with the protein intake differences on the two rations (Table 5.10)

Figure 5.10 Diurnal patterns of mean ruminal ammonia-nitrogen concentration (mg/100ml) for Diets R and C. Values in parentheses are standard deviations about the mean; dashed lines represent diet means. Values for each time were calculated for all sheep on Diets R and C from 3 and 4 sampling periods respectively.

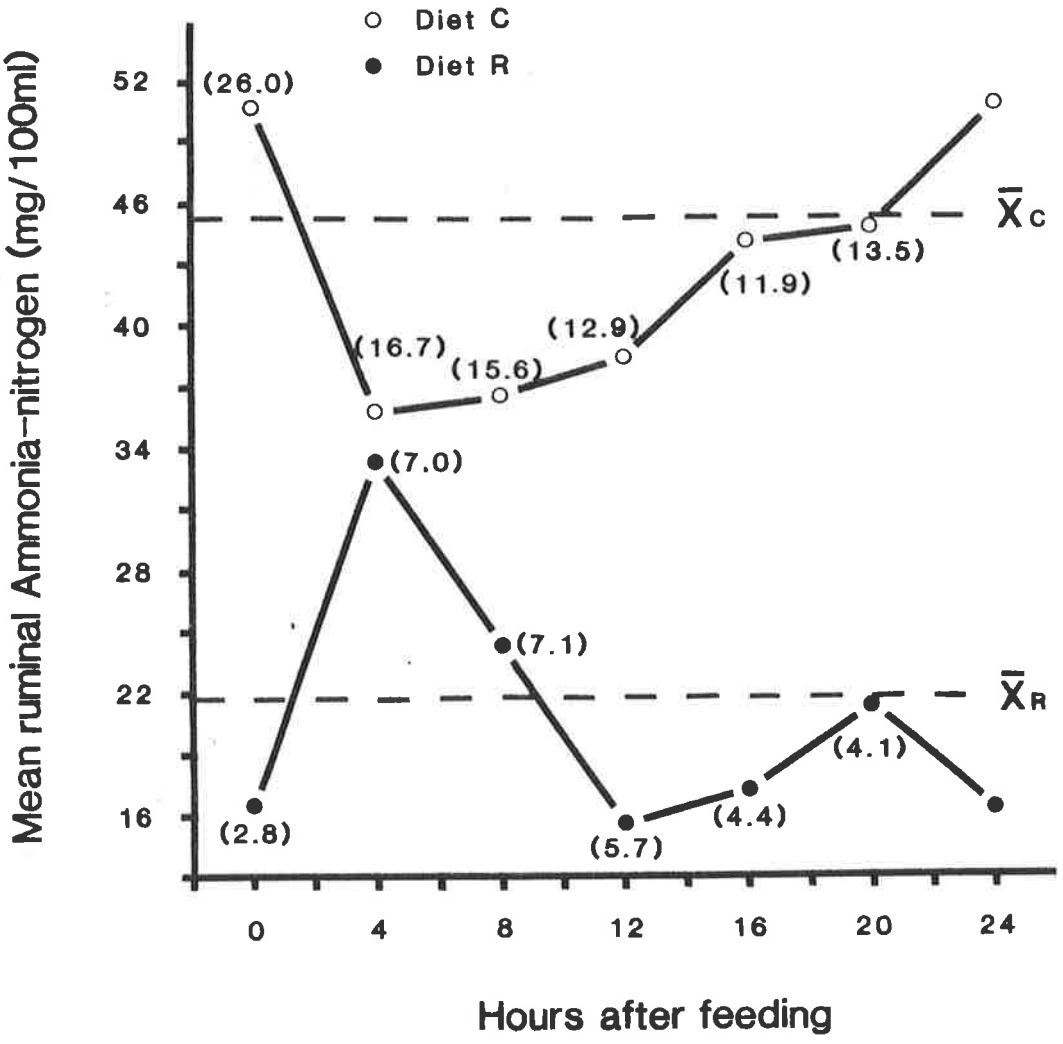

Diet C
Diet R
Mean ruminal Ammonia-nitrogen (mg/100ml)
52
46
40
34
28
22
16
(26.0)
(16.7)
(15.6)
(12.9)
(11.9)
(13.5)
(7.0)
(7.1)
(2.8)
(5.7)
(4.4)
(4.1)
$\bar{X}_C$
$\bar{X}_R$
0
4
8
12
16
20
24
Hours after feeding

Table 5.12 Split plot analysis of variance in NH_3-N concentration in the rumen fluid of sheep fed Diet R and Diet C.

Main plot	n-1	SS	MS	F	Significance
Diet	1	8716.67	8716.67	34.43	P<0.001
Error	20	5063.39	253.17		
Total (a)	21	13780.06			
Split plot					
Time	4	1253.29	313.32	15.35	P<0.001
Diet x time	4	2874.49	718.62	35.21	P<0.001
Error	80	1632.81	20.41		
TOTAL	109	19540.65			

with diet R having considerably more protein than diet C. However it must be recognised that NH_3N is a concentration estimate, and differences in rumen volume on the two rations could account for the anomaly.

Mean daily NH_3N concentrations for individual sheep on the two diets at different sampling times are presented in Table 5.13, and the diurnal NH_3N concentration pattern for each sheep on diet C in Appendix 5.3. Values are included in Table 5.13 for the adaptation period to diet C (27/8, 3/9) but the means for diet C are calculated only for times when the sheep were offered the full ration ($900gd^{-1}$). Once again, the mean daily NH_3 data between-sheep (i.e. down columns) indicate wider variability on diet C than on diet R. An important feature of these data is that while mean daily NH_3N for each sheep changed with sampling date on diet C, there was a strong tendency for the relative rankings of sheep to remain unaltered. This can be seen in Table 5.14 which indicates the magnitude of the correlation coefficients between mean NH_3N concentration observed in sheep at different sampling times. In all comparisons but one, there was a significant repeatability of NH_3N with time, further indication that the between-sheep fermentation pattern differences generated by this diet were sustained for the whole period. The establishment of a mean NH_3N concentration for each sheep thus appeared reasonable, estimates ranging from 21.7 ± 5.8 to 56.4 ± 11.2mg/100ml (Table 5.13).

These NH_3N concentration differences between sheep on diet C were related to both urinary nitrogen output and NAN flow when these three parameters were concurrently

Table 5.13 Mean daily NH_3N concentration for sheep on Diets R and C.

Sheep No	DIET R					DIET C							
	29/4	14/5	22/7	$\bar{X}_R$	(a) CV(%)†	27/8	3/9	26/9	16/10	4/11	19/11	$\bar{X}_c$*	CV(%)†
36	17.3	24.1	23.7	21.7	14.4	42.0	52.3	54.0	21.8	47.1	49.5	43.1	29.1
10	19.8	22.4	24.4	22.2	8.5	13.9	30.3	34.0	38.9	53.1	63.2	47.3	24.4
11	21.7	23.0	30.7	25.1	15.8	43.7	44.0	57.2	38.7	69.6	59.9	56.4	19.9
44	-	-	22.8	22.8	-	30.9	32.2	43.3	30.6	48.3	51.9	43.5	18.5
31	17.8	26.0	24.7	22.8	15.8	35.6	18.1	32.0	32.5	60.8	61.4	46.7	35.7
25	-	-	19.0	19.0	-	19.1	18.0	23.3	12.6	43.5	53.9	33.3	48.8
5	22.0	22.9	23.4	22.8	2.5	39.0	38.2	44.1	32.3	39.3	49.6	41.3	15.4
23	17.1	17.0	20.9	18.3	9.9								
27	-	-	14.5	14.5	-	19.6	20.3	23.2	12.3	28.1	23.3	21.7	26.7
13	20.2	19.7	25.3	21.7	11.6	27.1	42.8	50.9	35.8	58.7	56.2	50.4	17.6
17	15.9	21.0	29.2	22.0	24.9	15.4	19.6	20.6	12.8	28.4	31.1	23.2	30.8
19	20.4	20.1	28.4	23.0	16.7	33.1	46.4	47.9	37.1	-	-	42.5	12.7
$\bar{X}$	19.1	21.8	23.9	21.3		29.0	32.9	39.1	27.8	47.7	50.0	40.9	
	2.1	2.5	4.3	2.7		10.2	12.0	12.5	10.3	12.8	12.4	10.3	
CV(%)†	11.0	11.5	17.9	12.6		35.2	36.5	32.0	37.2	26.9	24.7	25.1	

* $\bar{X}_c$ is the mean concentration averaged over dates when sheep were offered $900gd^{-1}$ Diet C (i.e. excluding the adaption periods 27/8, 3/9).

† Coefficients of variation along rows represent "between samplings-within sheep" variances. CV's down columns represent "between-sheep" variance at any particular sampling.

Table 5.14 The correlation coefficients for the relationships between mean NH_3^+N concentration at different sampling times on Diet C. Sample 1 = 26/9; 2 = 16/10; 3 = 4/11; 4 = 19/11.

Comparison	Correlation coefficient (r)	Significance of r
1 x 2	0.677	P<0.05
1 x 3	0.663	P<0.05
1 x 4	0.514	n.s.
2 x 3	0.805	P<0.01
2 x 4	0.780	P<0.01
3 x 4	0.871	P<0.01

determined in trials 1 and 2 on diet C (Fig. 5.11). High mean daily NH_3N concentration was associated with high UN and low NAN flow in both trials. The outlier in both regressions of trial 2 data was an animal whose intake was depressed during that trial, with subsequently low UN and NAN, independent of NH_3N. To account for the effects of the small intake differences between sheep on UN and NAN, the data were also analysed using multiple regression techniques.

High significant partial correlation coefficients of NH_3N against both UN and NAN (Table 5.15) confirm the relationships derived between these variables (Fig. 5.11).

Because NH_3N and NAN flow were significantly related when measured concurrently, and because NH_3N was measured more frequently than NAN flow, further evidence that NAN flow was the major determinant of the WGR differences on diet C would be obtained if mean NH_3N for the whole period were inversely related to WGR. The multiple regression equation of mean nitrogen intake (NI) and mean NH_3N with final wool growth rate (WGR) is presented in Equation 5.9.

Equation 5.9.... $WGR = 5.08+0.65(NI)-0.18\ (NH_3N) \quad (R^2=0.93)$

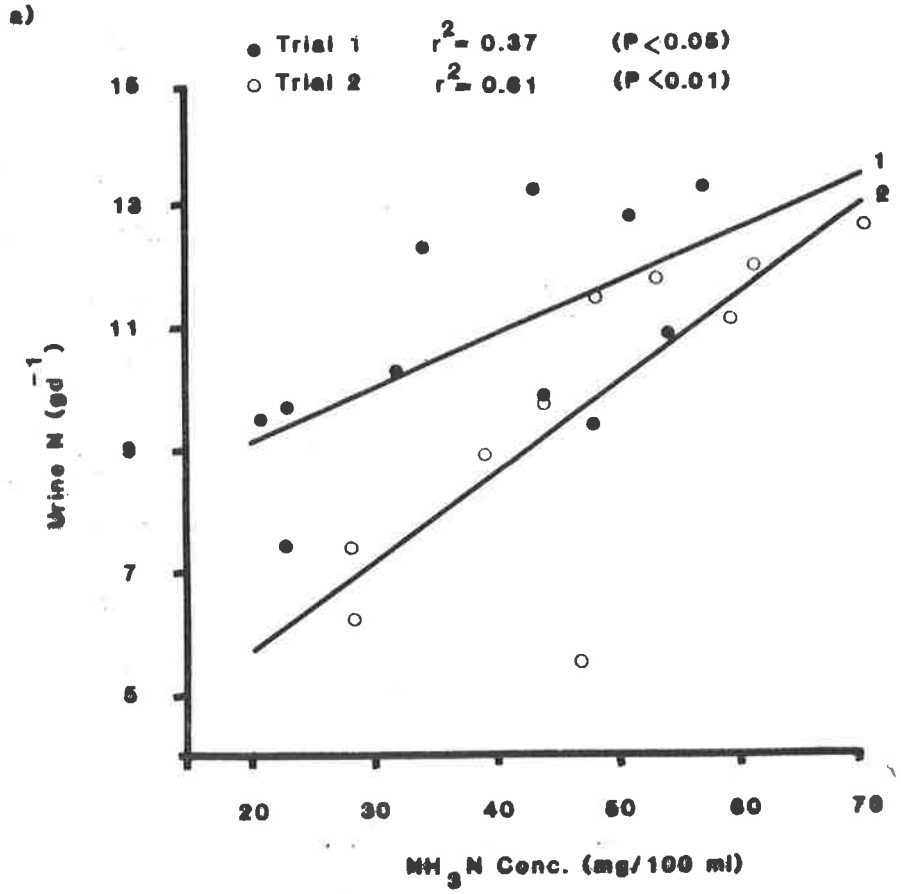
The partial correlation coefficient of NH_3N with WGR was -0.92 ($P<0.001$). If the relationship between NH_3N and NAN flow noted in the two trials on diet C, was maintained throughout the whole period, then there is little doubt that postruminal NAN was the major variable inducing wool growth

Figure 5.11 The relationship between mean daily ammonia-nitrogen concentration (NH_3 mg/100ml) and

a) Urine nitrogen output (gd^{-1})

and b) Non-ammonia nitrogen (NAN) flow (gd^{-1}) during infusion trials 1 and 2 on Diet C.

a)


● Trial 1 r^2= 0.37 (P<0.05)
○ Trial 2 r^2= 0.61 (P<0.01)
Urine N (gd^{-1})
15
13
11
9
7
5
20
30
40
50
60
70
1
2
NH_3 N Conc. (mg/100 ml)

b)

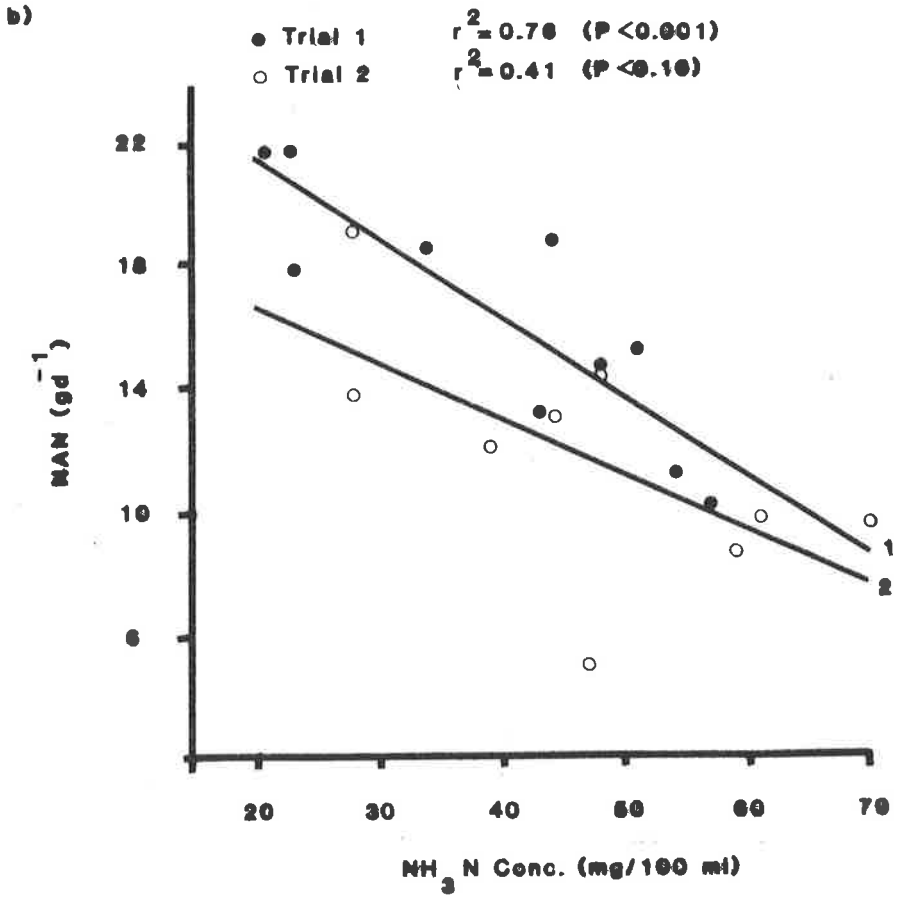

● Trial 1 r^2= 0.76 (P<0.001)
○ Trial 2 r^2= 0.41 (P<0.10)
NAN (gd^{-1})
22
18
14
10
6
20
30
40
50
60
70
1
2
NH_3 N Conc. (mg/100 ml)

Table 5.15 Multiple regression analysis of data relating to nitrogen intake (NI), urinary-nitrogen output (UN), non-ammonia-nitrogen flow (NAN) and ammonia-nitrogen concentration (NH_3N) for sheep fed Diet C.

Trial	Independent Variates		Dependent Variate	Partial Correlation Coefficients[2]		Multiple Correlation Coefficient
	1	2	Y	$R^2_{1y.2}$	$R^2_{2y.1}$	R^2
1	NI	NH_3-N	UN	-0.11	0.32	0.44
2	NI	NH_3-N	UN	0.76^{**}	0.85^{**}	0.91^{**}
Both	NI	NH_3-N[a]	UN[b]	0.32^{*}	0.46^{**}	0.53^{**}
1	NI	NH_3-N	NAN	0.02	-0.74^{**}	0.77^{**}
2	NI	NH_3-N	NAN	0.70^{**}	-0.72^{**}	0.83^{**}
Both	NI	NH_3-N[a]	NAN[c]	0.52^{**}	-0.70^{**}	0.80^{**}

where a)= mean daily ammonia-nitrogen conc^{n} in each collection period

b)= mean daily urinary-nitrogen output in each collection period

and c)= mean daily non-ammonia nitrogen flow in each collection period

* $P<0.05$

** $P<0.01$

differences on this ration, the indication being that variations in nitrogen losses from the rumen and in the urine were of importance.

The efficiency of bacterial protein synthesis on diet C

Estimated values for bacterial protein flow rates (gd^{-1}), the quantity of organic matter (OM) disappearing from the rumen (gd^{-1}) and the efficiency of bacterial protein synthesis derived from these data (g crude protein per day/100gm OM per day) are presented in Table 5.16 for individual sheep on diet C. Dilution rates (h^{-1}) determined only in the second infusion trial on this ration, are also included.

The range of estimated bacterial protein flow rates between sheep was substantial (5.0 to 118.1 gd^{-1}) while the fermentation rates, as estimated by organic matter digestion, were much less variable. Consequently, the rate of bacterial synthesis per unit organic matter "digested" ranged from as low as 1.0g per $100gd^{-1}$ to 35.4g per $100gd^{-1}$, indicating either grossly different bacterial populations with attending variance of DAPA-nitrogen to total bacterial-nitrogen ratios, or true synthetic efficiency differences between sheep. That the latter is the case is supported by significant relationships between bacterial efficiency and other rumen parameters. For instance, when data from the two infusion trials were combined, a significant negative correlation between NH_3N and bacterial efficiency was apparent ($r^2 = 0.25$, $P<0.05$). Further, sheep with a high fluid dilution rate had a higher synthetic efficiency than those with a low fluid turnover rate in trial 2 ($r^2 = 0.65$, $P<0.01$).

Table 5.16 Bacterial protein efficiency estimated from bacterial protein flow rate and the amount of organic matter disappearing in the rumen (OMDR). Rumen fluid dilution rate measured in trial 2 is also tabulated.

	Trial 1			Trial 2			
Sheep No.	Bacterial protein flow (gd^{-1})	OMDR (gd^{-1})	Efficiency (g/100g/d)	Bacterial protein (gd^{-1})	OMDR (gd^{-1})	Efficiency (g/100g/d)	Dilution (h^{-1})
36	5.0	454	1.1	19.4	369	5.2	.0261
10	95.6	309	30.9	Flow estimate unsuccessful			
11	22.5	421	5.3	37.5	486	7.7	.0304
44	72.5	506	14.4	118.1	482	24.6	.0399
31	Flow estimate unsuccessful			20.6	481	4.3	.0242
25	40.6	435	9.4	80.6	457	17.7	.0367
5	98.1	401	24.5	59.4	453	13.1	.0268
27	nd	375	nd	100.6	302	33.4	.0369
13	nd	442	nd	47.5	511	9.3	.0249
17	89.4	302	29.6	58.1	480	12.1	.0235
19	105.6	299	35.4	nd	nd	nd	
Mean S.D.	66.2±35.9	391±74	18.8±12.1	60.2±32.2	447±64	14.2±9.1	.0299±.0059

nd = not determined; missing samples

The contribution of estimated bacterial protein flow to total postruminal protein flow was low and highly variable (Table 5.17).

Table 5.17 The proportion of total protein flow (gd^{-1}) contributed by bacterial protein (gd^{-1}) (%). Diet C, Trials 1 and 2.

Sheep No.	Infusion Trial 1	Infusion Trial 2
36	6.8	59.7
10	65.9	-
11	35.7	58.2
44	80.0	123.9
31	-	31.2
25	33.1	94.6
5	67.7	75.8
27	-	81.9
13	-	82.0
17	65.0	65.4
19	109.7	-
Mean±S.D.	58.0±29.8	74.7±24.5

The two values greater than 100 are clearly erroneous. Overall, bacterial protein accounted for 67±28% of total protein flow from the rumen.

The amino acid profiles of proteins in the abomasal digesta of sheep on diet C

The high variability of bacterial/total protein flow between sheep on diet C, raised the possibility that absolute protein flow was not the only factor inducing wool growth differences on this ration. If bacterial, protozoal

and feed proteins were of substantially different composition, the supply of specific amino acids to the intestines of individual sheep may have differed. The data in Table 5.18 indicate that this was not the case. The total sulphur-amino acid content varied between 1.84 and 2.61% of the total amino acids in different sheep, but was not related to wool production on this ration. Other essential amino acids comprised a similar proportion of total acids in each animal, and the profile of free amino acids in the abomasal digesta did not appear to be a significant determinant of wool growth response (Appendix 5.4).

The pH and V.F.A. content of rumen fluid

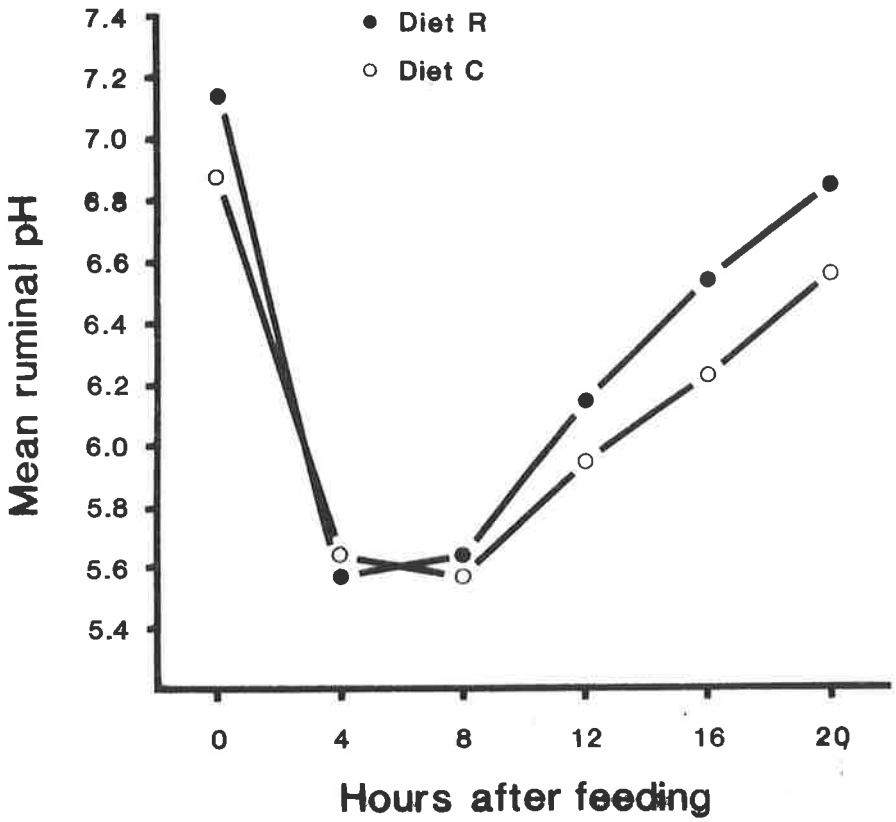
The diurnal pattern of ruminal pH for both diets is shown in Fig. 5.12a, the main determinant of fluid pH being the total V.F.A. concentration (Fig. 5.12b). Overall the mean pH on diet C (6.15) was significantly less than that for diet R (6.31), but the "diet x time" interaction was non-significant (Appendix 5.5). Mean daily pH for each sheep are presented in Table 5.19. Unlike the other characters examined in this study, the within group variation on diet C was similar to that of the roughage diet. However, the lowest recorded pH values were 5.2-5.3 for diet R, and 4.5-4.9 for some sheep fed diet C, there being substantial variation at the 4-8h samplings (Appendix 5.6).

An examination of the impact of the mean minimum daily pH on ruminal digestive pattern was warranted in the light of the pronounced effects of low pH on microbial populations (see Section 5.2.2). Table 5.20 shows the strength of the relationships between mean minimum pH and digestibility of dry matter (DMD), NH_3N and NAN flow. There was a significant

Figure 5.12 a) Diurnal patterns of mean ruminal pH for Diets R and C.

b) The relationship between V.F.A. concentration (mM) and rumen pH on Diets R and C.

a)



Std. deviations :

Diet R	.09	.14	.18	.20	.17	.09
Diet C	.22	.49	.46	.29	.23	.19

b)

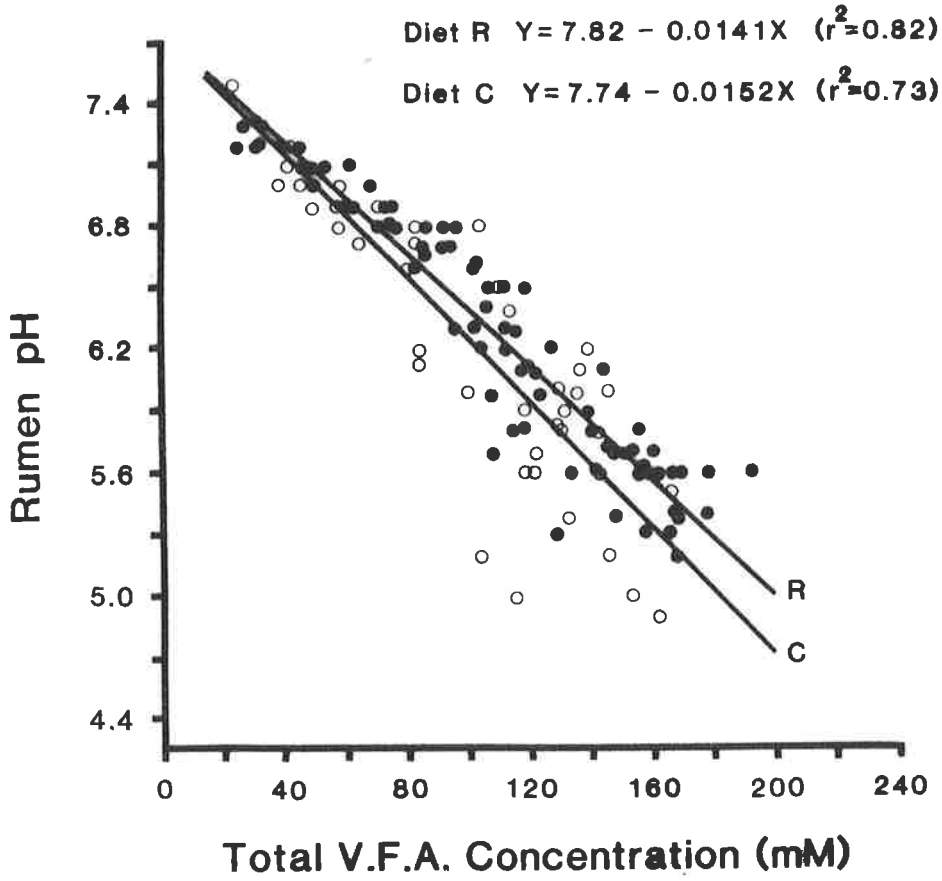


Table 5.18 Amino acid content of proteins precipitated from the abomasal fluid of sheep on Diet C (concentrations expressed as g/100g total amino acids).

Sheep: A.A.	27	17	44	11	5	13	31	25	36	F†
ASP	11.3	11.3	11.7	11.9	11.4	13.1	11.0	12.5	13.0	9.9
THR	5.1	4.9	5.3	5.3	5.3	5.5	5.3	5.2	5.2	3.7
SER	4.8	4.7	4.7	5.0	4.8	5.0	5.2	5.1	5.4	4.9
GLU	15.1	15.4	15.2	13.5	15.4	13.2	14.7	13.9	13.3	23.0
PRO	4.6	4.7	4.0	4.2	3.7	4.1	4.4	4.2	4.0	9.3
GLY	6.1	6.0	6.3	6.4	6.3	6.6	6.3	6.4	6.4	4.7
ALA	8.0	7.6	7.5	7.1	7.8	6.8	7.1	7.2	6.6	5.2
VAL	5.8	5.8	6.4	5.9	6.7	5.6	5.8	5.5	5.5	4.8
MET	1.3	1.2	1.3	1.4	1.2	1.1	1.6	1.2	0.9	1.2
ILE	4.7	4.9	5.2	5.5	5.1	5.4	5.6	5.1	5.3	3.7
LEU	8.0	8.0	7.8	8.1	7.5	8.2	8.1	8.4	8.2	7.6
TYR	4.5	4.8	5.1	5.2	5.2	5.0	4.7	5.0	5.1	3.8
PHE	5.3	5.9	5.8	6.1	5.5	6.0	6.3	5.9	6.0	5.2
HIS	2.0	1.9	1.9	1.9	1.8	1.9	1.9	1.9	1.9	2.0
LYS	7.0	6.7	7.2	7.0	7.1	7.3	6.9	6.9	7.5	4.5
ARG	5.1	5.1	4.8	4.4	4.1	4.4	4.5	5.0	4.8	5.7
CYS	1.4	0.9	0.8	1.2	1.2	0.7	1.0	0.8	1.0	0.9
TOT.S-A.A.	2.61	2.13	2.10	2.55	2.38	1.84	2.53	1.97	1.89	2.09

†F = feed sample

Table 5.19 Mean ruminal pH for individual sheep on Diet R and Diet C.

	DIET R			DIET C						
Sheep No	1	2	$\bar{X}$	1 500–700gd^{-1}	2 700gd^{-1}	3 900gd^{-1}	4 900gd^{-1}	5 900gd^{-1}	6 900gd^{-1}	$\bar{X}$ (only 900gd^{-1} i.e. 3-6)
36	6.44	6.43	6.44	6.25	6.36	6.18	6.27	6.83	6.49	6.44
10	6.42	6.31	6.37	6.08	6.37	6.48	6.13	6.26	6.16	6.26
11	6.41	6.07	6.24	6.06	6.15	6.00	5.92	6.28	5.83	6.01
44	–	6.37	6.37	6.29	6.29	6.20	6.10	6.30	5.90	6.13
31	6.32	6.16	6.24	5.28	6.20	6.11	6.28	6.14	5.90	6.11
25	–	6.09	6.09	5.86	5.85	5.85	5.80	6.05	5.87	5.89
5	6.41	6.48	6.45	6.57	6.32	6.26	6.47	6.46	6.18	6.34
23	6.52	6.52	6.52							–
27	–	6.32	6.32	4.73	6.33	6.04	5.96	6.42	5.75	6.04
13	6.42	6.30	6.36	6.19	6.30	6.25	6.22	6.39	5.91	6.19
17	6.22	6.12	6.17	6.26	5.96	5.87	5.86	6.15	6.08	5.99
19	6.19	6.09	6.14	5.03	6.65	6.49	5.94	6.18	–	6.20
Mean	6.37	6.27	6.31	5.87	6.25	6.16	6.09	6.32	6.01	6.15
S.D.	0.10	0.16	0.13	0.56	0.20	0.20	0.20	0.20	0.21	0.16

positive relationship between pH and both DMD and NH_3N, and a negative relation of pH to NAN flow. These relationships do not necessarily indicate causality but rather may reflect co-correlation with other variables.

Table 5.20 Simple linear regression coefficient estimates between mean minimal pH and DMD, NH_3-N and NAN flow on Diet C.

Variate 1	2	r^2	Significance
pH	DMD	0.70	P<0.01
pH	NH_3-N	0.59	P<0.01
pH	NAN	0.76	P<0.01

V.F.A. composition

The mean V.F.A. molar proportions estimated on rumen samples taken during peak fermentation (4h after feeding) are presented in Table 5.21. Here again, the coefficients of variation on diet C were similar to diet R values, but there were differences in the acetate, butyrate and valerate proportions (P<0.001) between diets. The propionate percentage on diet C was not related to production performance on that ration, and there was no relationship between propionate % and fluid dilution rate in infusion trial 2 (r^2= 0.05). In contrast, the butyrate % of sheep on diet C was positively related to NH_3N in trial 1 (r^2= 0.56 P<0.01) and negatively to NAN flow (r^2= 0.68 P<0.01 trial 1 only).

Table 5.21 Mean V.F.A. molar proportions at 4 hrs. for each sheep fed Diets R and C.

	DIET R				DIET C			
Sheep No.	Ac.	Pr.	Bu.	Val.*	Ac.	Pr.	Bu.	Val.*
36	64.1	21.4	10.8	3.8	62.2	19.0	16.2	2.7
10	63.6	22.3	10.7	3.6	56.1	22.1	18.1	3.7
11	68.6	20.1	9.5	2.0	58.4	17.9	20.2	3.4
44	66.0	22.5	9.2	2.4	64.1	16.6	14.2	5.1
31	69.9	16.3	11.9	2.0	58.9	24.3	14.8	2.0
25	65.9	21.9	9.8	2.6	59.5	17.8	16.2	6.5
5	62.6	21.6	12.5	3.4	59.0	20.3	16.7	3.9
27	71.0	18.4	9.6	1.0	64.2	17.1	13.6	5.1
13	68.0	19.6	9.9	2.6	55.1	22.1	18.9	3.8
17	61.0	25.5	11.2	2.5	58.7	19.5	14.6	7.3
Mean	66.07	20.96	10.51	2.59	59.62	19.67	16.35	4.35
± S.E.M.	3.11	2.39	1.05	0.80	2.89	2.38	2.05	1.57
C.V.(%)	4.71	11.40	10.00	30.91	4.86	12.11	12.57	36.00

* includes n - valeric and iso - valeric

The postruminal disappearance of NAN

It was postulated that suboptimal pH in the abomasum and small intestine may have impaired digestion and absorption of protein in some sheep on Diet C. The proportion of NAN flowing from the rumen which was excreted in the faeces was thus examined. The results are presented in Table 5.22.

Table 5.22 The ratio of faecal nitrogen output (gd^{-1}) to abomasal NAN flow (gd^{-1}) in sheep on Diets R and C (%).

Sheep No	Diet R	Diet C (Trial 1)	Diet C (Trial 2)
36	22.9	20.5	32.0
10	22.2	16.1	-
11	21.5	25.5	33.3
44	-	22.0	31.9
31	18.7	-	22.5
25	-	24.7	35.4
5	22.0	20.2	27.3
23	19.1	-	-
27	-	23.4	20.9
13	16.4	22.4	24.1
17	20.0	22.6	37.0
18	20.5	-	-
19	20.9	24.5	-
	20.4±1.9	22.2±2.6	29.4±5.5

It is clear from this table that the apparent digestion/absorption processes in the small intestine and hindgut did not vary widely between sheep on either diet, nor did consideration of the extent of nitrogen disappearance postruminally, improve the relation of NAN to WGR previously demonstrated.

5.6 Discussion

The results of this trial regarding the influence of diet composition on the WGR variance between individual sheep, confirm a conclusion drawn earlier in this thesis that the feeding of high-grain diets induces a substantially greater range of WGR than is apparent when predominantly roughage diets are fed. The coefficient of variation for wool growth efficiency was 10.5% when Diet R was offered, and 22.6% for concentrate-fed sheep, but there was no evidence that WGR on the grain diet was genetically determined (Fig 5.6). A similar finding was reported earlier in this thesis and it can be unequivocally concluded that the source(s) of wool growth differences acts independently of genotype.

The efficiency of digestion of energy and protein nutrients in the whole gastrointestinal tract varied widely betwen sheep on Diet C, but was not responsible for generating the high WGR variance. In fact, sheep with the lowest apparent digestion coefficients produced more wool per unit dietary intake than those with a high disappearance of nutrients from the tract. Hutchinson (1961), in a study of similar sheep to those used in the present trial, noted large differences in both wool growth efficiency and the digestibility of a concentrate ration. The two were not

related, and he concluded that the variance in efficiency was a reflection of genotype differences in the efficiency of post absorptive metabolism, thereby corroborating earlier observations that genotypic wool growth differences were not related to digestive efficiency per se (Weston 1959; Dunlop et al. 1966; Piper and Dolling 1969b). The results of the present trial allow more appropriate interpretation of the Hutchinson data. Firstly, the variance in wool growth efficiency he observed was probably not a function of genotype at all, but rather related to the diet effect demonstrated currently. Secondly, while total digestibility was unrelated to efficiency, the current study clearly demonstrates that the site of digestion of concentrate rations is the more important determinant of wool growth.

The wide range of NAN flow rates generated in sheep on the concentrate ration, had a pronounced effect on wool growth (Fig. 5.8), a result in accord with previously demonstrated responses to protein protected from ruminal degradation (Ferguson 1972) or infused postruminally (Reis 1969). Comparison of Figures 5.8 and 5.9 substantiates the contention that the site of nutrient digestion is of much greater importance than total nutrient digestibility, in terms of wool production. The chain of events in the rumen leading to variations in NAN flow and WGR of sheep receiving the same diet, appear to be directly linked to the effects of rumen retention time on nutrient availability. Thus metabolisms characterised by high rumen NH_3N, high diet digestibility, high butyrate concentration, and low fluid dilution were inefficient in terms of bacterial protein synthesis and NAN flow. Moreover, dry matter digestibility in ruminant is

positively related to ruminal residence (Balch and Campling 1965), and the positive association between DMD and NH_3N (r^2= 0.43, P 0.01) is probably a reflection of their relation to residence time in the rumen. High NH_3N concentrations, in turn, were recorded when urinary-nitrogen output was also high (Fig 5.11a) and NAN flow was low (Fig 5.11b). It appears that the negative association of urine-N excretion and NAN flow (Equation 5.8) indicative of a shift in the site of digestion from the rumen to the intestines of some sheep. Subsequently the availability of protein at the intestines (P) relative to energy availability (E) varied widely between sheep because P and E were inversely related.

To speculate at length would be both unjustified and unrewarding since the experiment was not designed to assess the causal factors underlying the variations in metabolism. However, some of the relationships observed warrant mention.

Firstly, the ruminal NH_3-N concentration was a factor of some importance in distinguishing between high and low producers on Diet C. In contrast, when sheep were offered Diet R, the NH_3-N concentration differed little between sheep and the diurnal pattern was consistent. The smaller peak in NH_3 concentration at 16-24h. after feeding was associated with rumination and may have been a result of an input of a large quantity of salivary urea, some of which would have been derived from NH_3 absorbed immediately post feeding. High variability of NH_3-N on Diet C is in accord with results obtained by other workers using concentrate rations (Ishaque et al. 1971; Hodgson and Thomas 1972; Chamberlain and Thomas 1979), the NH_3-N level being

positively related to protozoal numbers (Abe et al. 1973; Christiansen et al. 1975), and the butyrate concentration (Ishaque et al. 1971). In the latter experiment, high NH_3 and high butyrate were associated with low duodenal nitrogen flow, a feature of the results of the present trial. Ishaque et al. (1971) concluded that a "butyrate fermentation pattern" was generated by microorganisms of low efficiency, although no estimate of bacterial efficiency was made in that trial.

Bacterial efficiency in the present study was low and highly variable on Diet C, in comparison to values normally recorded for forage diets (19-23g/100g OMDR) (Hogan and Weston 1967b). For barley and maize-based diets, on the other hand, the efficiency range is 10-21g/100g OMDR (Chamberlain and Thomas 1979; 1980) when sheep are fed at regular intervals.

While current efficiency estimates (18.8±12.1g/100g OMDR and 14.2±9.1g/100g OMDR) fall within this range, the variability was substantially greater, possibly a consequence of once-daily feeding with its attending gross fluctuation in the pattern of ruminal fermentation (Fig 5.10, Fig 5.12a). Alternatively, high between-sheep variance may have resulted from poor conversion of DAPA-nitrogen to total bacterial nitrogen (Ling and Buttery 1978), a real possibility if different bacterial populations existed in different sheep. That the bacterial synthetic data are valid is strongly supported by the significant positive relationship between rumen fluid dilution rate and efficiency (r^2 = 0.65 $P<0.01$), a result also recorded by Harrison et al. (1976) when D was increased by infusion of artificial saliva. More efficient

bacterial protein production is generated when the rate of fluid turnover in the rumen is enhanced, but unlike the data of Harrison et al. (1976) this occurred in the present study without any alteration in the propionate molar proportion. Again this may be a result of infrequent feeding but it does indicate that bacterial efficiency changes need not be associated with changes in metabolic end-products. The contrasting reports in the literature concerning the V.F.A. metabolism pattern which is most efficient in terms of bacterial synthesis (McMeniman et al. 1974; Chamberlain and Thomas 1979) are in accord with this hypothesis.

If the bacterial protein estimates are correct, then a large quantity of either undegraded dietary protein, or protozoal protein, was flowing from the rumen of some sheep, because only 67±28% (n = 17) of the total postruminal protein flow comprised bacterial protein. This value is lower than that recorded by Chamberlain and Thomas (1980) for a similar diet.

Low bacterial efficiency on concentrate rations has been attributed to low ruminal pH (Hobson 1972), low dilution rate (Harrison et al. 1976), high protozoa populations (Lindsay and Hogan 1972) or limiting nitrogen supply (Satter and Slyter 1974). In the current trial there was no evidence of a positive relationship between NAN flow and minimum pH, nor was there any suggestion that bacterial efficiency was related to pH. That ruminal nitrogen supply was limiting bacterial efficiency is also considered unlikely because at no time did the NH_3-N concentration fall below levels regarded as optimum for microbial synthesis (see Table 5.3 for optimal values). The possibility remains that higher

optima are required when sheep are fed high-starch diets (Orskov et al. 1972; Okorie et al. 1977; Bartley and Deyoe 1977). However sheep with the highest NH_3 levels in the present study were the least efficient (r^2 = 0.25 $P<0.05$). This may be due to an elevation of bacterial maintenance requirements with increasing NH_3 concentration, so that efficiency is depressed (Isaacson et al. 1975), but it is considered more likely that inefficient bacterial synthesis was induced by the presence of a large or fluctuating ciliate population (Leng 1976). This contention is supported by the known effects of protozoal populations on the NH_3 and butyrate levels in the rumen (Abe et al. 1973) and the efficiency of bacterial synthesis (Jackson et al. 1971; Ishaque et al. 1971). The status of the protozoa in the rumen could well have been important in the current experiment for the following reasons:

a) Restricted intakes of high grain rations can induce a high density of protozoa (Eadie et al. 1970).

b) The ruminal pH of some animals often fell below that required to sustain an active ciliate population (Eadie and Mann 1970).

c) The bacterial protein flow of some sheep was only a small proportion of total protein flow.

d) The fermentation pattern induced in some sheep was characteristic of that associated with the presence of protozoa, and sheep with such metabolisms had low bacterial efficiency.

An analysis was made of the amino acid (AA) composition of the proteins flowing to the abomasum of each sheep consuming Diet C, because the contribution of bacterial

protein to total protein flow varied widely between sheep. If the AA composition of bacterial, protozoal and feed proteins differed, then alterations in their relative contribution to total postruminal protein flow would be reflected in changes to the abomasal protein composition. Differences between sheep were small and probably not nutritionally significant (Table 5.18). WGR, then, varied from sheep to sheep in response to total protein availability and not as a result of the AA composition of that protein. Moreover, the disappearance of nitrogen between the abomasum and the faeces was relatively constant between sheep (Table 5.22) and there was no evidence that digestion and absorption processes postruminally were impaired in poor performers.

Suffice it to say that at this stage the relationships between rumen variables when sheep are consuming concentrate diets, need much closer study. Such studies are warranted because diets containing a high proportion of grain are commonly used both as experimental rations and for drought feeding. Extensive cereal grain feeding, moreover, is frequently practised under the more intensive production systems. As manipulation of rumen fermentation processes becomes more widely examined as a mean of improving productive efficiency (Chalupa 1977), knowledge of the type of rumen interactions described in this chapter are clearly important.

The results of the present experiment also relate to the choice of diet for selection of genotypes for wool growth potential. Poor extrapolation of wool growth efficiency from the concentrate ration used in Experiment 1

to other diets, including grazing, is of significance particularly to studs which feed diets of high nutritional quality and select rams accordingly. If these diets contain a high cereal-grain proportion the accuracy of selection must be questioned.

Consideration of the rumen processes and postruminal protein flows of sheep fed the wheat diet in the study of Reis and Tunks (1974), may provide an explanation for the depressed wool growth observed when these sheep received methionine supplements intra abomasally. These authors predicted the "probable" protein flow to the intestines from previous work, but in the light of the present results such prediction is likely to be imprecise. The decline in WGR of control sheep on the wheat diet and the variability of individual sheep to methionine supplementation (Reis & Tunks 1974) support the contention that digestion of the basal ration is a determinant of response.

A clear application of the studies reported in this Chapter lies in the characterisation of digestive processes when sheep consume concentrate rations. A large number of animals would be required, for instance, in a study such as that described by Chamberlain & Thomas (1979), when the ruminal nitrogen metabolism and passage of amino acids to the duodenum of sheep receiving diets containing hay and concentrates in various proportions, was examined.

Finally, the choice of rations for wool growth experiments must be carefully made, in the light of the present results. While cereal grains provide a readily definable energy source and a means of manipulating liveweight responses, the disturbances to ruminal nitrogen

metabolism in some sheep are such that wool growth is adversely affected.

5.6 Conclusions

The experiment confirmed the hypothesis that the high WGR variance of sheep on a concentrate ration was related to the quantity of protein supplied postruminally and not a consequence of differences between sheep in nutrient digestibility, absorption or post-absorptive utilisation. Protein flow to the intestines was governed by the pattern of fermentation prevailing in the rumen; fermentations characterised by high NH_3 concentration, high butyrate molar proportion, and low dilution rate, were associated with low bacterial efficiency and low postruminal NAN flow. Differences in AA composition of the abomasal proteins were small and not related to wool production. The results are consistent with the postulated effects of protozoa on nutrient digestion in the rumen.

Results of this experiment have important application, firstly, to the selection of diet for wool growth studies and for estimating wool growth potential, and secondly for the characterisation of digestive processes when ruminants are fed high grain diets.

APPENDICES

Appendix 2.1 Fleece free liveweight changes (gd^{-1}) in Periods II and III of Experiment 1. (Estimated from initial and final weights in each period)

Group	Sheep No.	Bodyweight change (gd^{-1}) Period II	Period III
Ad lib. B_1	1	99	14
	7	143	-40
	15	107	11
	42	123	-22
	Mean (SD)	118 (17)	-9 (23)
AA_1	21	81	-7
	33	105	-25
	40	42	51
	45	111	9
	Mean (SD)	85 (27)	7 (28)
AB_1	13	143	-20
	25	114	-6
	39	144	-6
	44	132	-1
	Mean (SD)	133 (12)	-8 (7)
AC_1	20	141	-22
	30	108	-13
	37	158	nv
	41	65	-43
	Mean (SD)	118 (36)	-26 (13)
AD	4	77	13
	5	105	-19
	6	137	-47
	9	109	-57
	11	81	-34
	14	132	-22
	19	126	-23
	23	97	-23
	32	132	-9
	35	91	12
	36	138	-25
	43	119	-31
	Mean (SD)	112 (21)	-22 (20)

Group	Sheep No.	Bodyweight change (gd^{-1}) Period II	Period III
BB_1	8	84	27
	18	104	4
	27	97	10
	34	81	35
	(Mean (SD)	92 (9)	19 (13)
CC_1	10	62	-27
	17	69	32
	26	74	19
	28	-45	34
	Mean (SD)	40 (49)	15 (25)
DA_1	3	14	40
	46	38	55
	47	24	51
	48	26	55
	Mean (SD)	26 (9)	50 (6)
DB_1	12	8	49
	22	13	37
	31	29	54
	38	11	46
	Mean (SD)	15 (8)	47 (6)
DD	2	28	19
	16	31	11
	24	27	16
	29	21	-9
	Mean (SD)	27 (4)	9 (11)

n.v. = no value for this sheep

Appendix 2.2 The changes in body protein (kg) with time after intake change in Period II (0-18 weeks) and Period III (0-9 weeks). Group means ± S.E.

Group		WEEKS - PERIOD II				WEEKS - PERIOD III	
		0	4	8	18 (0)	(3)	(9)
Ad lib.	B_1	5.24 (.27)	5.33 (.21)	5.63 (.20)	6.67 (.33)	5.97 (.25)	5.71 (.20)
	AA_1	4.26 (.21)	4.09 (.02)	4.39 (.15)	4.60 (.55)	4.42 (.40)	4.12 (.31)
	AB_1	3.90 (.19)	4.40 (.31)	4.82 (.30)	5.80 (.18)	5.25 (.20)	4.91 (.22)
	AC_1	3.91 (.07)	4.52 (.17)	4.81 (.33)	5.19 (.79)	5.08 (.40)	4.42 (.60)
	AD	3.93 (.29)	4.22 (.34)	4.69 (.33)	5.16 (.54)	4.90 (.33)	4.43 (.43)
	BB_1	4.06 (.12)	4.33 (.47)	4.44 (.45)	5.08 (.42)	4.93 (.51)	4.84 (.30)
	CC_1	3.84 (.30)	4.03 (.29)	4.27 (.35)	4.35 (.72)	4.14 (.72)	4.30 (.62)
	DA_1	4.13 (.19)	3.71 (.26)	4.05 (.30)	3.90 (.27)	4.21 (.10)	4.07 (.20)
	DB_1	4.10 (.08)	3.90 (.11)	3.94 (.16)	4.21 (.20)	4.24 (.21)	4.36 (.10)
	DD	4.19 (.10)	3.96 (.18)	3.91 (.25)	4.41 (.29)	4.15 (.20)	4.16 (.10)

Appendix 2.3: Indivdual WGR (gd^{-1}) with time in Experiment 1 (Chapter 2).

		WEEKS											
		PERIOD I		PERIOD II					PERIOD III				
Group	Sheep	4	8	0 12	4	8	12	18 0	3	7	11	15	20
Ad lib. B_1	1	11.3	16.2	16.6	16.8	nd	18.5	15.2	8.4	7.0	7.0	5.8	5.1
	7	nd	14.9	16.2	17.5	nd	22.1	20.9	17.6	11.4	11.6	8.3	8.2
	15	10.6	10.8	10.9	7.0	nd	9.1	9.0	7.9	6.5	6.2	4.2	2.9
	42	15.0	21.1	20.0	21.8	nd	14.1	14.6	12.9	6.9	5.9	4.2	5.0
	Mean	12.3	15.8	15.9	15.8	-	13.5	14.9	11.7	8.0	7.7	5.6	5.3
	S.E	1.9	3.7	3.3	5.4	-	5.5	4.2	3.9	2.0	2.3	1.7	1.9
AA_1	21	10.4	9.3	7.4	5.8	9.4	11.1	13.2	11.4	9.1	6.3	6.6	8.9
	33	9.7	7.5	6.6	5.3	5.5	5.8	5.9	4.8	4.4	4.3	3.1	2.7
	40	8.8	7.3	5.4	6.8	8.7	7.6	7.4	4.0	4.4	5.7	3.8	3.6
	45	12.7	11.1	8.1	7.6	8.2	9.0	11.7	8.2	7.3	6.5	5.1	6.0
	Mean	10.4	8.8	6.9	6.4	8.0	8.4	9.6	7.1	6.3	5.7	4.7	5.3
	S.E	1.4	1.5	1.0	0.9	1.5	1.9	3.0	2.9	2.0	0.9	1.3	2.4
AB_1	13	7.4	6.1	5.9	7.1	13.5	15.1	19.0	15.6	13.8	12.6	7.4	5.7
	25	10.9	9.2	8.1	6.5	8.4	12.4	15.8	13.3	11.6	12.3	10.1	9.7
	39	9.7	8.8	6.6	7.0	15.0	16.1	18.0	16.6	13.3	10.8	7.5	6.6
	44	8.0	8.3	7.8	10.7	16.8	17.1	17.8	14.2	11.3	9.2	6.7	6.5
	Mean	9.0	8.1	7.1	7.8	13.4	15.2	17.7	14.9	12.5	11.2	7.9	7.1
	S.E	1.4	1.2	0.9	1.7	3.1	1.8	1.2	1.3	1.1	1.4	1.3	1.5

AC_1	20	8.5	6.2	5.6	5.9	7.1	7.9	8.6	7.9	7.8	8.2	6.3	6.1
	30	11.2	9.4	9.2	9.1	14.1	14.9	16.8	13.8	10.9	10.4	8.7	8.4
	37	7.6	7.9	6.1	8.8	14.0	14.3	14.6	12.5	8.9	5.4	-	-
	41	11.0	9.5	7.8	7.3	6.2	6.8	4.8	5.4	5.0	2.8	1.4	1.0
	Mean	9.6	8.3	7.2	7.8	10.4	11.0	11.2	9.9	8.2	6.7	5.5	5.3
	S.E	1.6	1.3	1.4	1.3	3.7	3.7	4.8	3.4	2.1	2.9	3.0	3.0
AD	4	11.1	10.6	6.2	4.7	8.8	14.6	15.1	11.5	7.4	6.7	6.2	5.0
	5	10.2	8.5	7.3	7.3	7.6	7.5	7.5	5.5	4.1	3.1	2.1	2.2
	6	9.8	9.0	7.0	5.7	7.9	9.0	10.1	7.1	4.8	4.3	2.9	3.0
	9	9.2	10.3	5.2	4.8	4.1	5.0	6.0	6.4	5.3	3.4	2.4	1.8
	11	14.4	11.6	9.7	8.7	9.7	9.7	8.2	6.7	5.6	4.9	2.9	3.5
	14	11.0	9.4	8.9	5.7	6.8	7.3	6.3	7.2	5.7	4.9	4.2	4.5
	19	12.2	11.0	10.1	10.8	14.8	16.2	17.4	13.4	10.0	9.9	8.4	8.4
	23	8.5	9.8	7.2	5.0	10.6	7.4	6.9	5.1	3.9	3.3	2.0	1.4
	32	7.9	9.8	8.6	9.0	13.9	15.2	15.6	11.1	8.2	8.0	7.2	7.1
	35	10.1	8.7	5.5	5.8	7.5	6.7	7.4	7.3	5.6	4.0	2.9	3.1
	36	12.8	10.5	8.8	6.5	7.5	7.7	8.7	8.2	5.1	4.6	3.9	4.1
	43	9.2	9.4	8.5	9.7	15.3	14.8	15.2	10.2	8.3	8.0	6.6	6.3
	Mean	10.5	9.9	7.8	7.0	9.1	10.1	10.4	8.3	6.2	5.4	4.3	4.2
	S.E	1.8	0.9	1.5	2.0	3.3	3.8	4.0	2.5	1.8	2.1	2.1	2.1

		PERIOD I			PERIOD II				WEEKS	PERIOD III			
Group	Sheep	4	8	0 12	4	8	12	18 0	3	7	11	15	20
BB_1	8	7.5	7.7	7.2	4.1	4.7	4.9	5.0	5.4	6.1	6.1	4.1	3.8
	18	12.0	9.9	6.7	7.8	9.4	11.6	13.7	14.6	14.7	15.1	11.5	10.6
	27	12.2	12.3	11.1	12.5	16.0	16.0	16.5	15.1	13.0	13.3	12.2	11.5
	34	9.3	8.7	5.5	5.8	7.5	6.7	7.4	7.3	5.6	4.0	2.9	3.1
	Mean	10.3	9.7	7.6	7.6	9.4	9.8	10.7	10.6	9.9	9.6	7.7	7.3
	S.E	2.0	1.7	2.1	3.1	4.2	4.3	4.6	4.3	4.1	4.7	4.2	3.8
CC_1	10	9.7	9.7	7.6	5.8	7.8	8.6	9.3	7.1	6.2	5.6	4.4	4.5
	17	11.2	10.0	9.0	8.9	10.9	12.0	12.4	11.9	11.0	12.1	9.7	10.0
	26	13.4	9.5	5.8	6.5	8.2	8.8	8.2	6.3	7.2	7.6	6.3	5.6
	28	6.9	4.6	4.1	3.6	2.6	2.0	1.0	0.6	0.4	1.2	2.1	2.0
	Mean	10.3	8.5	6.6	6.2	7.4	7.9	7.7	7.3	6.2	6.6	5.6	5.5
	S.E	2.4	2.2	1.9	1.9	3.0	3.6	4.2	3.6	3.8	3.9	2.8	2.9
DA_1	3	11.9	11.2	10.7	8.2	5.8	6.1	6.9	6.8	7.0	5.4	4.2	4.9
	46	11.0	8.3	7.4	6.2	6.3	6.2	6.4	7.2	8.4	9.4	7.8	6.8
	47	9.8	8.3	7.1	6.1	5.9	6.4	6.0	5.2	5.6	5.9	4.6	4.4
	48	9.3	9.2	8.8	7.1	7.0	7.3	8.2	9.3	10.8	12.2	10.7	11.4
	Mean	10.5	9.3	8.5	6.9	6.3	6.5	6.9	7.1	8.0	8.2	6.8	6.9
	S.E	1.0	1.2	1.4	0.9	0.5	0.5	0.8	1.5	1.9	2.8	2.6	2.8

DB_1	12	12.3	11.0	10.7	8.9	8.7	8.4	8.6	9.8	9.6	9.4	6.4	5.9
	22	12.7	7.3	7.6	5.8	5.9	4.9	3.9	5.9	7.8	9.1	7.7	4.8
	31	10.8	11.0	11.3	9.9	10.5	10.2	10.9	11.6	12.4	14.0	11.7	12.5
	38	11.1	9.6	8.5	7.5	5.6	5.9	7.4	8.7	10.4	11.7	10.0	9.8
	Mean	11.7	9.7	9.5	8.0	7.7	7.4	7.7	9.0	10.1	11.1	9.0	8.3
	S.E	0.8	1.5	1.5	1.5	2.0	2.1	2.5	2.1	1.7	2.0	2.1	3.1
DD	2	11.0	8.0	7.3	6.5	5.8	7.0	7.2	7.2	7.9	7.7	6.1	6.2
	16	10.2	11.4	10.9	10.1	9.8	10.0	9.6	9.8	9.9	9.8	7.0	7.8
	24	12.9	13.8	11.5	10.0	9.4	9.9	10.7	8.6	10.2	11.2	7.8	8.2
	29	8.9	7.2	6.0	4.9	4.1	4.9	4.4	4.1	3.7	3.0	2.0	2.2
	Mean	10.8	10.1	8.9	7.9	7.3	8.0	8.0	7.4	7.9	7.9	5.7	6.1
	S.E	1.5	2.7	2.3	2.3	2.4	2.1	2.4	2.1	2.6	3.1	2.2	2.4

Appendix 4.1 Dry matter intakes (gd^{-1}) of sheep in Experiment 3 (Chapter 4).

a) Mean daily DMI (gd^{-1}) for the 6 HE sheep and 5 LE sheep offered diet B for 15 weeks after diet A feeding.

SHEEP	DIET A	DIET B
LE		
5	445	855
11	411	855
12	393	855
23	445	855
36	445	855
	428±22	855±0
HE		
4	445	855
18	578	855
25	578	855
38	578	855
39	578	855
48	622	855
	563±55	855±0

b) Mean daily DMI (gd^{-1}) for 4 HE and 4 LE sheep offered diet A, then diet B for 8 weeks, then diet A again for 14 weeks.

SHEEP	DIET A	DIET B	DIET A
LE			
3	622	855	846
14	445	855	829
41	418	855	873
47	622	855	760
	527±96	855±0	827±42
HE			
13	578	855	862
17	533	855	728
19	445	855	845
44	578	855	845
	534±54	855±0	820±54

Appendix 4.2 a) WGR(gd^{-1}) of sheep in Experiment 3 (Chapter 4) from groups LE and HE, and the maintenance intake group (M).

GROUP	SHEEP NO.	WEEKS 0	4	8	12	15
LE	3	5.0	10.9	21.1)		
	14	4.2	7.6	18.4) Transferred to Diet A		
	41	1.5	6.5	16.9) (See App. 4.2b)		
	47	6.2	10.6	20.4)		
	5	3.8	9.1	16.2	18.5	19.4
	11	3.4	9.4	20.0	22.4	23.9
	12	4.0	6.2	16.1	18.7	20.0
	23	1.8	6.0	16.3	18.1	19.5
	36	3.8	8.7	16.8	18.8	19.3
HE	13	6.5	12.4	19.1) Transferred to Diet A		
	17	12.6	16.7	21.6) (See App. 4.2b)		
	19	12.5	12.5	16.6)		
	44	9.1	11.0	18.5)		
	4	6.0	8.4	16.7	18.9	20.2
	18	11.8	15.5	21.2	23.0	22.6
	25	12.0	15.9	18.4	19.8	19.8
	38	11.4	15.1	20.7	22.3	22.9
	39	7.0	11.3	18.6	21.4	22.7
	48	12.3	15.1	17.1	19.1	21.6
M	2	6.6	5.6	6.5	6.7	5.9
	16	7.8	6.8	5.5	5.6	5.8
	24	8.8	10.1	9.5	8.6	8.7
	29	2.6	3.2	3.7	2.8	2.2

Appendix 4.2 b) WGR(gd^{-1}) of sheep in Experiment 3 from groups HE and LE on diet A (after diet B) for weeks 8-22.

		WEEKS			
GROUP	SHEEP NO.	8	12	15	19
LE	3	21.1	18.5	14.3	11.3
	14	18.4	13.6	9.4	9.0
	41	16.9	13.7	18.7	18.0
	47	20.4	15.2	11.3	9.8
HE	13	19.1	14.9	11.1	10.4
	17	21.6	20.4	19.3	13.3
	19	16.6	14.7	16.0	17.2
	44	18.5	16.6	19.8	21.1

Appendix 5.1 The mean daily dry matter intakes (gd^{-1}) of sheep fed Diet R and Diet C in Experiment 4. (averaged over the last 13 weeks of each period).

Sheep No	Diet R	Diet C
36	970±2	523±184
10	970±2	766±71
11	970±2	777±34
44	970±2	793±27
31	970±2	770±81
25	970±2	807±6
5	966±12	748±66
23	970±2	-
27	970±2	808±6
13	970±2	795±27
17	970±2	808±6
18	970±2	801±14
19	970±2	703±85
	970±1	758±77

Appendix 5.2 WGR (gd^{-1}) for sheep fed Diets R (0+14 weeks) and C (14-30 weeks) in Experiment 4.

Weeks / Sheep	0	3	6	10	0 14	3 17	6 20	10 24	16 30
36	12.8	14.3	16.4	15.7	15.6	9.9	7.9	5.5	4.0
10	9.6	11.9	14.0	13.8	13.8	8.3	5.9	6.4	6.8
11	12.5	13.2	16.7	16.2	15.6	11.5	8.4	7.7	7.0
44	-	-	-	10.8	12.6	9.1	9.5	7.7	9.1
31	13.3	14.9	18.4	18.2	19.1	12.1	10.4	9.5	8.9
25	-	-	-	10.5	11.3	8.5	10.0	10.6	12.4
5	9.3	10.7	14.1	14.4	15.9	8.5	7.5	7.5	7.9
23	8.6	13.8	17.7	16.2	15.5				
27	-	-	-	13.1	13.5	10.3	10.0	9.6	12.3
13	8.5	12.8	15.3	15.7	15.7	9.0	8.1	6.4	7.4
17	12.3	14.3	17.0	15.5	15.9	13.7	11.9	13.4	13.4
18	13.1	14.8	19.1	17.7	18.8	12.1	9.7	9.8	11.4
19	9.1	10.8	13.0	12.8	13.7	9.6	8.0	6.9	6.0
original 9 sheep (WGR	10.91	13.15	16.17	15.62	15.96				
original 9 sheep (± S.D.	1.93	1.49	1.92	1.56	1.68				
9 + 3 replacements (WGR					15.13	10.22	8.94	8.42	8.88
9 + 3 replacements (± S.D.					2.21	1.68	1.54	2.14	2.80

Appendix 5.3 Ruminal fluid ammonia-nitrogen concentration (mg/100ml) with time after feeding for individual sheep fed Diet C ($900gd^{-1}$) in Experiment 4 (Means of 5 estimates ± SE).

	HOURS AFTER FEEDING				
Sheep	0	4	8	12	16
36	42.5 (12.5)	45.6 (13.7)	46.0 (13.4)	38.7 (9.1)	43.2 (9.7)
10	34.6 (15.1)	32.0 (12.6)	30.5 (11.7)	35.7 (15.9)	38.9 (15.1)
11	49.2 (14.7)	46.3 (8.5)	52.3 (10.2)	48.5 (11.5)	55.3 (13.1)
44	39.9 (13.1)	39.1 (6.5)	33.0 (7.0)	33.6 (6.4)	36.7 (5.1)
31	32.8 (19.8)	35.6 (14.9)	34.9 (17.6)	38.5 (13.9)	38.9 (10.4)
25	30.1 (15.0)	16.9 (6.2)	20.4 (12.5)	25.5 (13.1)	30.1 (10.8)
5	39.0 (8.2)	37.8 (4.5)	37.8 (4.1)	40.8 (2.5)	41.2 (2.6)
27	17.9 (3.4)	19.0 (6.9)	18.3 (5.3)	20.9 (4.1)	28.8 (5.7)
13	42.7 (17.0)	43.8 (10.1)	43.0 (14.7)	40.1 (10.8)	42.7 (5.5)
17	26.5 (5.9)	13.5 (4.3)	12.3 (4.2)	17.7 (4.3)	26.7 (7.2)
19	36.1 (4.3)	40.6 (8.9)	38.2 (4.3)	43.1 (5.9)	45.3 (8.3)
Mean (SD)	35.6 (8.3)	33.7 (11.3)	33.3 (11.7)	34.8 (9.2)	38.9 (7.9)

Appendix 5.4 The amino acid composition of free amino acids in the abomasal fluid of sheep fed Diet C, Experiment 4.

Sheep	11	17	31	44	13	25	5	27	36
A.A.									
Asp	3.0	4.4	0.6	1.7	3.8	5.5	2.1	3.9	4.3
Thr	2.3	0.9	1.1	1.1	1.3	1.1	0.7	1.5	1.6
Ser	3.5	1.6	2.3	2.2	2.1	2.0	1.2	2.1	2.5
Glu	2.0	22.0	9.3	12.5	8.8	13.0	7.2	8.7	8.5
Pro	0.8	0.2	1.5	0.8	1.0	0.6	0.5	1.1	1.2
Gly	3.0	1.5	2.2	2.4	1.8	2.1	1.8	2.5	3.1
Ala	12.6	.8	6.2	5.7	5.1	4.1	2.9	7.9	8.3
Val	6.7	6.1	6.0	6.1	5.9	5.2	4.9	5.2	9.9
Met	2.8	4.6	3.9	4.4	4.6	4.9	4.9	3.3	7.3
Ile	5.4	1.8	4.0	3.0	3.2	3.0	2.2	3.4	5.6
Leu	12.1	12.3	13.9	12.5	11.7	17.1	12.0	14.2	18.3
Tyr	24.5	12.0	17.8	20.5	25.6	19.1	26.3	24.1	10.8
Phe	12.6	24.9	24.4	20.8	17.0	18.5	26.1	16.0	9.1
His	1.0	0.7	1.6	1.6	1.5	1.0	3.2	1.5	3.9
Lys	6.6	3.0	3.2	3.2	4.2	2.5	2.9	2.5	3.9
Arg	0.9	1.0	1.8	1.3	2.1	0.4	0.9	2.0	0.9
Cys acid	0.3	0.5	0.3	0.3	0.3	0.2	0.2	0.2	0.7

Apendix 5.5 Split plot analysis of variance of ruminal pH for each sheep fed Diets R and C.

Main Plot	n-1	SS	MS	F	Significance
Diet	1	1.15	1.15	5.99	P<0.025
Error (a)	22	4.22	0.192		
Total (a)	23	5.37			
Split Plot					
Time	5	45.72	9.144	203.2	P<0.001
Date x Time	5	0.310	0.062	1.38	n.s.
Error (b)	110	4.900	0.045		
Total	143	56.30			

Appendix 5.6 Ruminal fluid pH with time after feeding for individual sheep fed Diet C ($900gd^{-1}$) in Experiment 4. (Means of 4 estimates ± S.E.).

	HOURS AFTER FEEDING					
Sheep	0	4	8	12	16	20
36	6.90 (.08)	6.08 (.46)	6.21 (.34)	6.38 (.26)	6.24 (.26)	6.66 (.17)
10	6.79 (.27)	5.97 (.23)	5.91 (.24)	6.11 (.14)	6.25 (.11)	6.46 (.24)
11	6.75 (.24)	5.70 (.22)	5.55 (.15)	5.73 (.22)	5.96 (.27)	6.36 (.24)
44	6.88 (.22)	5.68 (.28)	5.55 (.22)	5.90 (.16)	6.11 (.24)	6.49 (.20)
31	6.82 (.16)	5.76 (.37)	5.53 (.21)	5.78 (.21)	6.23 (.21)	6.52 (.16)
25	6.97 (.13)	5.09 (.19)	4.98 (.00)	5.64 (.27)	6.14 (.20)	6.55 (.13)
5	6.99 (.18)	5.84 (.12)	5.77 (.15)	6.25 (.18)	6.47 (.30)	6.73 (.23)
27	7.31 (.10)	4.82 (.20)	4.98 (.23)	5.85 (.48)	6.44 (.35)	6.88 (.20)
13	6.88 (.05)	5.71 (.10)	5.71 (.25)	6.02 (.33)	6.25 (.28)	6.57 (.16)
17	7.04 (.04)	5.09 (.09)	5.09 (.08)	5.78 (.30)	6.28 (.25)	6.66 (.13)
19	6.77 (.07)	6.10 (.36)	5.99 (.48)	5.98 (.29)	6.04 (.19)	6.41 (.09)
$\bar{X}$ (S.D)	6.92 (.15)	5.62 (.41)	5.57 (.39)	5.95 (.22)	6.24 (.16)	6.57 (.14)

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