The Effect of Feed Type and Diet Quality on Kinetics of Digestion.

The degradation properties of certain protein supplements, and the effect of concentrate supplementation and basal roughage quality on eating behaviour and particle passage from the rumen of sheep.

Natalie Dawn Gerber
B.Sc. Agric. (Natal)

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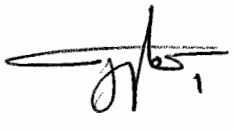
Declaration

I declare that this dissertation is my own work, except for assistance that is acknowledged or where due reference is made in the text. The results contained in this dissertation have not been submitted, in whole or in part, for a degree at any other university.

Natalie D. Gerber
December 2002

I, Dr. I. V. Nsahlai, chairperson of the supervisory committee approve release of this thesis for examination.



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"Burn down your cities and leave our farms, and your cities will spring up again as if by magic; but destroy our farms and the grass will grow in the streets of every city in the country"

William Jennings Byron

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List of Abbreviations

ADF	Acid detergent fibre
CF	Crude fibre
CP	Crude protein (Nitrogen x 6.25)
DM	Dry matter
DMI	Dry matter intake
ED	Effective degradability
LSmeans	Least Square means
NDF	Neutral detergent fibre
OM	Organic matter
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
VFI	Voluntary feed intake

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Abstract

The unique anatomical structure of the ruminant animals' digestive tract allows them to convert forages containing high levels of fibre into the valuable products of meat, milk and wool. At the same time, an interdependent relationship between the host animal and the rumen microbial population has evolved and a minimum requirement for dietary fibre by the ruminant was established. Where roughage intakes are high, intake is limited by rumen capacity and the rate of clearance of previously ingested material from the rumen. When animals are fed particularly poor quality roughage diets, high in fibre, as in sub-Saharan Africa, the problem of limited rumen capacity is compounded. The rate of passage of ingested material through the digestive tract will also influence the ability of the host animal to extract nutrients, whilst the degradation that a feed undergoes in the rumen will have a direct effect on the availability of nutrients to the host animal and, conversely, the availability of nutrients to the rumen microbes themselves. In sub-Saharan Africa, where readily available forage tends to be high in fibre and low in soluble nutrients (such as nitrogen) and readily digestible carbohydrates, the potential exists for the development of supplementation strategies that will enhance rumen microbial fermentation and microbial protein synthesis. This study comprised three separate, although interrelated investigations into aspects of rumen clearance and degradation parameters of eight nitrogen supplements. The supplements investigated were canola, copra, cottonseed oilcake, defatted maize germ, lucerne meal, lupin seed meal, soya oilcake and sunflower oilcake.

The first part of this study investigated the disappearance of dry matter (DM) and nitrogen from eight plant protein sources contained within nitrogen-free polyester bags incubated in the rumen of three fistulated Jersey cows. In addition, the protein sources were force-fed to adult cockerels to obtain an estimate of the intestinal digestion of the available protein by the host animal. The intercept values and time lags for DM degradation were highly significant ($P<0.001$), whilst potential degradability and rate of degradation were significantly different among protein sources ($P<0.05$). In terms of nitrogen degradation, the intercept values and rates of degradation were significantly different ($P<0.001$), while potential degradability and time lag were not significantly different among feeds. Effective degradability (ED) was calculated using different rates of passage for both solid particles (k) and liquid matter leaving the rumen (klq) for sheep, beef and dairy animals. Lupins had the highest ED for both DM (792 g kg^{-1} in sheep) and nitrogen (844 g kg^{-1} in sheep), while

canola showed the lowest effective DM degradability (433 g kg^{-1} in sheep) and copra the lowest effective nitrogen degradability (678 g kg^{-1} in sheep). Digestible rumen undegradable protein (RUP) was calculated as the difference between total digestible protein and effective nitrogen loss. Calculated in this manner, sunflower oilcake had the highest digestible RUP (126 g kg^{-1} nitrogen in sheep) while cottonseed oilcake had the lowest (83 g kg^{-1} nitrogen in sheep). Further consideration should be given to methods of determination of RUP described in this trial as well as determination of digestibility parameters for complete feeds rather than supplements alone.

The effect of increasing the nitrogen supply to the rumen microbes by improving the quality of roughage supplied to the host animal was investigated in the second phase of this work, by substituting the basal roughage (poor quality veld hay) with varying amounts of lucerne hay. Five treatments of 100% hay, 75% hay and 25% lucerne, 50% hay and 50% lucerne, 25% hay and 75% lucerne and 100% lucerne were used. In this way CP was increased as the lucerne proportion of the ration increased while the NDF concentration was decreased. The effect of varying roughage quality on the voluntary feed intake, feeding behaviour and rate of passage of both long and short particles from the rumen of South African Mutton Merino sheep was investigated, using 25 sheep blocked according to weight and randomly assigned to one of five treatments in a completely randomised block design. DM, CP, CF and NDF intakes increased significantly ($P<0.001$, $P<0.001$, $P<0.05$ and $P<0.05$, respectively) as lucerne inclusion level in the ration was increased. The individual effects of both diet and particle size on the rate constant (k1, indicative of the rate of passage of particles) were highly significant ($P<0.001$). The particle size and diet interaction was significant ($P=0.05$). Lucerne inclusion levels in the diet had no significant effect on ruminating time. Increasing the level of lucerne in the ration resulted in a significant decrease in total eating time and time spent ruminating per kilogram NDF consumed ($P<0.01$) and time spent eating per kilogram dry matter consumed ($P<0.05$). Sheep weight had a highly significant ($P<0.001$) effect on time spent eating per kilogram of dry matter consumed, time spent ruminating per kilogram of NDF consumed. There was a significant increase in ruminating time ($P<0.05$) as sheep weight increased. These results indicate that interactions among feed factors, such as particle size and feed composition, will affect the rate of clearance of ingested roughage particles from the rumen and, consequently, the level of roughage intake. The effect of animal factors and chewing effectiveness warrant further investigation.

The third trial conducted investigated the effect of increasing both basal roughage quality and level of concentrate supplementation on the intake of the basal roughage and the rate of passage of particles from the rumen (k1) of South African Mutton Merino sheep. Four roughage qualities were achieved by supplementing the basal roughage (poor quality veld hay) with varying amounts of lucerne hay such that lucerne contributed 20, 40, 60 or 80% of the diet. In this way CP, and ash contents were increased as the lucerne proportion of the ration increased and NDF and CF concentrations were decreased. A concentrate supplement was offered at levels of 90, 180, 270 or 360 g per sheep per day. Sixty-four sheep were blocked according to weight and sex and randomly assigned to one of the 16 treatments in a completely randomised block design. The interaction of lucerne inclusion level and concentrate supplementation was not significant. The relationship between level of concentrate supplementation and k1 is inversely quadratic and significant ($P<0.05$). Lucerne inclusion level had no effect on k1, but the effect of concentrate supplementation level on total dry matter intake (DMI) and total CP intake was highly significant ($P<0.001$), with DMI and CP intake increasing quadratically as the level of concentrate supplementation increased. NDF intake decreased quadratically ($P<0.05$) as lucerne inclusion level increased whilst CP intake increased quadratically ($P <0.001$) as lucerne inclusion level increased. These results indicate that below a certain level, the effect of concentrate supplementation on fibre digestion is not severe and that roughage and NDF intake is not affected. Total DM and CP intakes are increased as concentrate supplementation increases. The effect of concentrate supplementation on feeding behaviour warrants further investigation.

General Introduction

The unique anatomical structure of the ruminant digestive tract allows these animals to convert forages containing high levels of fibre into the valuable products of meat, milk and wool. At the same time, an interdependent relationship between the host animal and the rumen microbial population has been created and a minimum requirement for dietary fibre by the ruminant established. Where roughage contributes a significant proportion of the diet, intakes are limited by rumen capacity and the rate of clearance of previously ingested material from the rumen. This problem of limited rumen capacity is compounded when animals are fed particularly poor quality roughage diets, high in fibre, as is frequently the case in sub-Saharan Africa.

The rate of passage of ingested material through the digestive tract will also influence the ability of the host animal to extract nutrients, whilst the degradation that a feed undergoes in the rumen will have a direct effect on the availability of nutrients to the host animal and, at the same time, the availability of nutrients to the rumen microbes themselves. In sub-Saharan Africa, where readily available forage tends to be high in fibre and low in soluble nutrients (such as nitrogen) and readily digestible carbohydrates, the potential exists for the development of supplementation strategies that will enhance rumen microbial fermentation and microbial protein synthesis. However, it is important to remember that the rumen chamber is not simply a fermentation vat but encompasses a complex microbial ecosystem that is constantly changing and adapting to conditions within the rumen.

A pertinent example is that noted by Romney and Gill (2000) who stated that supplementation can be used as a means of increasing nutrient supply where animals are unable to consume sufficient forage as a result of physical limitations. However, although supplementation tends to have a positive effect on overall dry matter intake (DMI) it may have a positive or negative effect on the intake of the basal forage. Galyean and Goetsch (1993) noted that the effect of concentrate supplementation on roughage degradability is dependent on the amount and type of concentrate fed. A slight increase in the availability of soluble carbohydrates or nitrogen may increase rumen microbial fermentation (McDonald *et al.*, 1981) simply by virtue of the fact that there are more nutrients available for microbial growth. The increase in fermentation enables rumen microbes to better digest and utilise fibrous plant material such as that found in plant cell walls. On the other hand,

feeding higher levels of a high-energy supplement will result in a decrease in ruminal pH and a subsequent decrease in the growth of fibrolytic bacteria (Galyean and Goetsch, 1993) and thus a reduction in the amount of fibre digested by the rumen microbes (Webster, 1993). Interestingly, Hoover (1986), working with dairy cattle, noted that below levels of 300g of concentrate per kilogram of feed depression of ruminal fibre digestion is not severe.

Any increase in the rate and extent of fermentation will result in an increase in the availability of soluble nutrients, previously enclosed within cell walls, for use by both the rumen microbes and the host animal. There are a number of possible ways to supplement poor quality diets and thus meet the nutrient requirements of the rumen microbes. These include:

- supplementation with a readily degradable nitrogen source (Tamminga, 1993);
- supplementation with a readily degradable carbohydrate source (Tamminga (1982), cited by Owens and Goetsch (1986);
- supplementation with a combination of both a readily digestible nitrogen source and a readily degradable carbohydrate (Louw, 1979);
- substitution of a portion of the poor quality roughage with a roughage of better quality that will supply some readily degradable nitrogen as well as a slightly greater amount of more digestible carbohydrate than found in the poor quality roughage (Tamminga (1982), cited by Owens and Goetsch (1986).

All of the above means of supplementation will have some effect on the composition of the rumen microbial population and, thus, will affect the rate of breakdown and clearance rate of the ingested material. An increase in rate of clearance will allow a greater intake in those cases where intake was previously limited by rumen capacity. However, too great an increase in the clearance rate will lead to insufficient breakdown and loss of nutrients, otherwise potentially available to the ruminant.

From the above, it is clear that should the decision to supplement the basal roughage be taken, it is important to know what effect supplementation will have on the intake and digestibility of the basal roughage. Certain questions, which should be asked and preferably answered include:

- Is it possible to provide, relatively cheaply, a small amount of readily degradable carbohydrate and/or protein for use by rumen microbes and, thereby, increase microbial protein production and productivity of the host animal?
- What will be the effect of differing degrees of substitution of good quality roughage on the intake of the total diet, and how is this change in intake related to the rate of passage of particles from the rumen of the animal?

Physical characteristics of the roughage, such as particle size, will also influence the rate of passage of the feed. In addition, the amount of mastication required until feed particles are sufficiently small to leave the rumen will have an effect on the intake of the animal. Thus, will the substitution and/or supplementation of the poor quality roughage influence the eating behaviour of the animal and, thereby, influence the overall intake?

This study was comprised of three separate, although interrelated investigations into aspects of rumen clearance and degradation parameters.

This first part of this study was conducted to investigate the degradability parameters of eight protein rich feedstuffs commonly used in ruminant feeds in South Africa. Once known, these parameters could be used as guidelines when selecting a protein source as a supplement for a roughage diet. For instance, which of the protein sources currently in use are preferable as a source of readily degradable rather than by-pass protein?

In the second part of the study the effect of substitution of lucerne (a good quality roughage with a high nitrogen content and some readily degradable carbohydrate) on the intake of the total diet and the rate of passage of both long and short particles from the rumen of sheep was investigated. In addition, the effect of lucerne substitution on the eating behaviour of sheep fed a poor quality roughage diet was examined.

The third and final aspect of this study was an investigation of the effect of concentrate supplementation on the intake of the total diet and how this effect is influenced by the quality of the roughage diet fed as the basal diet to the animals.

Chapter 1

A review of the literature

1.1 Quality attributes of feeds

Modern animal production systems are intended to maximise the productivity of domestic animals, so feedstuffs are evaluated on the basis of their ability to elicit the desired response from the animal (Van Soest, 1994). Different feedstuffs do not have equal capacity to support the animal functions of maintenance, growth, reproduction and lactation, with energy and protein frequently the most limiting fractions of the food for ruminant production (Van Soest, 1994). As a result, energy and protein have received the most attention in feed evaluation systems (Van Soest, 1994). In addition, feed quality may be related to form rather than chemical composition (Van Soest, 1994) and the importance of physical characteristics of the feed should not be ignored (Beever, 1986). A typical example of such physical factors is particle size, which has an important effect on the passage rate of digesta from the rumen of the animal (Lee and Pearce, 1984). In short, the production response of an animal to a given feed is dependent on the complex interactions between diet composition, preparation and consequent nutritive value (Van Soest, 1994).

Traditionally, the nutritive value of a feed is classified in terms of digestibility, the amount of feed consumed and energetic efficiency (Raymond, 1969) and the more recent definitions of feed quality still use these measures as the basis of feed evaluation. Ulyatt (1981) cited by Akin (1989), defined roughage quality in terms of the digestibility or fermentation of plant constituents and in terms of the amount of feed that ruminant animals consume. Jarrige (1989) gave the feeding value of a forage, or the potential of the forage for ruminant production, as "the quantity of digestible organic matter or net energy consumed by the animal when the forage is fed *ad libitum* as the sole food". Thus, the nutritive value of any given feed is dependent not only on the apparent digestibility of organic matter, but also on the voluntary intake characteristics of the feed (Jarrige, 1989). Any feed evaluation system attempting to describe the potential production from a feed should include descriptions of potential feed intake, energy content, protein content, fat content, carbohydrate composition, physical structure, mineral and vitamin content and the content of specific anti-nutritional components (Madsen *et al.*, 1997).

1.1.2 Apparent versus true digestibility of nutrients

The digestibility of a feed or nutrient refers to the portion of the feed that is absorbed from the gastro intestinal tract of the animal and not excreted in the faeces (McDonald *et al.*, 1981). However, in addition to undigested feed, the faeces contain endogenous losses and metabolic products that are not a part of the feed. The metabolic excretions are composed mostly of microbial matter, while endogenous secretions comprise unfermented substances derived from sloughed off cells and animal waste products (Van Soest, 1994). Thus, the difference in dry matter (DM) between the feed and the faeces is defined as *apparent digestibility*. For example, Webster (1993) gives the formula for the calculation of apparent digestibility of organic matter (OM) as:

$$\text{Apparent digestibility of OM} = \frac{\text{OM in food} - \text{OM in faeces}}{\text{OM in food}}$$

As a result of the presence of nutritive factors from endogenous losses and microbial matter in the faeces, apparent digestibility gives a lower estimate of digestibility than does *true digestibility*, where the true digestibility of a feed is calculated as apparent digestibility minus the endogenous metabolic component (Van Soest, 1994). Webster (1993) thus describes the true digestibility of OM as:

$$\text{True digestibility of OM} = \frac{\text{OM in food} - (\text{faecal OM} - \text{endogenous faecal OM})}{\text{OM in food}}$$

1.1.3 Feed intake

The *ad libitum* intake of a feed is the most important factor of feed quality affecting animal response and in particular efficiency of production (Van Soest, 1994). The *ad libitum* intake of any given diet may vary considerably as a result of a number of factors, which are discussed in more detail in section 1.3.

1.2 Forage quality

Van Soest (1994) suggested that forage quality is one of the most important factors affecting ruminant productivity, regardless of whether animals are in a feedlot or grazing. Because ruminant animals have the ability to utilise low quality fibre, it may be financially worthwhile for producers to replace highly digestible feedstuffs, which are frequently more expensive, with bulky foods of low digestibility (Campling and Lean, 1983). Furthermore, ruminant livestock production relies heavily on forages, particularly those derived from grasslands, rangelands, forage crops and crop residues (Jarrige, 1989). It has been estimated that, worldwide, forages (i.e. the vegetative portions of plants) provide more than 90% of the feeds consumed by domesticated ruminants (Byerly *et al.*, 1978). It is therefore important, when dealing with the nutrition of ruminants, to have an understanding of forage quality.

Traditionally, feeds used in ruminant production systems can be divided into two main categories. Roughage (or forage) comprises those feeds with a high fibre content such as herbages, conserved green forages and cereal straws. The group of feeds typically termed concentrates comprises cereals, legume seeds, oil seeds and the high carbohydrate, protein and lipid containing by-products of the processing thereof (Campling and Lean, 1983).

Although quality of a forage is largely dependent on the amount of dietary fibre the forage contains, the precise quantification of forage quality is fairly complex (Van Soest, 1994). The unique pregastric system of fermentation in the rumen allows the ruminant animal to extract the maximum energy possible from fibrous feeds, but at the same time, this system has also created a dependency of the animal on the presence of fibre in its diet in order to stimulate normal rumen function (Mertens, 1993). Fibre represents the bulk of plant material that must be processed in the digestive tract and in addition acts as a source of energy for rumen microbes (Van Soest, 1994). Although the lignified component of dietary fibre is indigestible it is an important component of the diet because un-lignified material would not elicit adequate rumination. At the same time, it is essential that the forage provide sufficient energy for microbial growth (Van Soest, 1994). Forage quality is therefore a trade-off among several opposing factors; the supply of plant cell wall, its optimal digestibility and the rate of digestion (Van Soest, 1994). The amount of food energy available per unit time is dependant on the rate of digestion (Van Soest, 1994).

The majority of forages consumed by ruminants are angiosperms (Van Soest, 1994), with grasses and legumes comprising the bulk of grazed forages (Wilson and Kennedy, 1996). The anatomy or plant structure of these two distinctive groups has been shown to have an effect on the fragmentation of particles, occurring during ingestive and ruminative chewing, the particle breakdown characteristics and the digestive properties of each specific group (Akin, 1989; Wilson and Kennedy, 1996). Compounding these distinct differences in attributes exhibited by grasses and legumes, as a result of plant structure, is a further broad division between tropical and temperate grasses. These differences in quality also extend to parts of the plant itself with, for example, leaves being more digestible than stems (Minson, 1990). The stage of maturity at harvesting has been shown to have an effect on the quality of the forage, with a decrease in quality occurring as forages mature. This is a result of a corresponding decrease in the digestibility of plant cell wall components (Hatfield, 1993).

1.3 Voluntary feed intake

The nutrient requirements of any animal are satisfied by the distribution of nutrients in the feed and, in order to calculate the amount of feed the animal requires, it is necessary to have a good prediction of the nutritive value of the feed (Dulphy and Demarquilly, 1994). At the same time, it is generally recognised that the voluntary intake of a given feed is just as important in determining the nutritive value of the feed as the nutrient composition of the feed itself (Crampton *et al.*, 1960; Dulphy *et al.*, 1989; Jarrige, 1989; Beever, 1993; Madsen *et al.*, 1997).

Pienaar (1993) stated that the control of voluntary feed intake (VFI) in ruminants is multifaceted and in order to fully understand the potential intake of a feed, it is important to understand those factors involved in the control of VFI. In ruminants, intake is regulated by both short and long-term mechanisms. Long-term mechanisms adjust supply to animal requirements in order to maintain body weight (Dulphy and Demarquilly, 1994). However, feed intake regulation is achieved primarily over the short-term, with feeding activity regulated fairly accurately, both within a meal and within a day, by a simple physical mechanism (Dulphy and Demarquilly, 1994). Once the rumen has filled to a particular level, ingestion will stop and daily intake is then determined by the rate of digestion of digestible material and the transit rate of indigestible material, i.e. the rate of clearance of

ingested material from the rumen. Thus, as the quantity of feed in the rumen decreases, the animal may resume consumption (Balch and Campling, 1962).

When concentrate diets are fed, the potential intake of the diet exceeds the physiological capacity of the animal to metabolise the available nutrients and thus the concentration of nutrients in the diet can be used to calculate intake and therefore potential animal production from the diet (Pienaar, 1993). Although several metabolic and sensory factors are known to affect meal size and frequency (Campling and Lean, 1983), restrictions on feed intake may also occur as a result of chemical or physical characteristics of the feed or the anatomy or physiology of the ruminant animal (Conrad *et al.*, 1964). As a result, an accurate estimate of intake becomes an important consideration in the formulation of diets where forages are fed *ad libitum* or where roughages are included in the diet, as the regulation of intake will fall under physical control by way of rumen capacity (Conrad *et al.*, 1964; Dulphy and Demarquilly, 1994). The physical regulation of feed intake becomes an even more important consideration in the tropics (especially sub-Saharan Africa) where ruminants are fed roughages with lower digestibility than those fed in temperate regions (Madsen *et al.*, 1997).

1.3.1 Factors affecting intake

A number of factors are known to affect the VFI of any given animal (Broster *et al.*, 1981; McDonald *et al.*, 1981). These factors can be subdivided into restrictions arising either as a result of the anatomy or physiology of the ruminant animal or as a result of the chemical or physical characteristics of the feed (Conrad *et al.*, 1964).

1.3.2 Animal factors

It was originally thought that feed intake in ruminant animals, as in monogastric animals, is controlled by centres in the hypothalamus, situated beneath the cerebrum in the brain (McDonald *et al.*, 1981; Ramalho Ribeiro, 1989). This theory postulates that feeding is initiated by the feeding centre or lateral hypothalamus and continues until inhibited by the satiety centre or ventro-medial hypothalamus, which receives signals from the body as a result of feed consumption (McDonald *et al.*, 1981; Ramalho Ribeiro, 1989). However, it has been shown that these are not the only control mechanisms involved in intake

regulation (Ramalho Ribeiro, 1989). Theories of chemostatic, lipostatic and thermostatic regulation of feed intake have also been proposed (McDonald *et al.*, 1981), but the actual neurochemical events that regulate intake are not well understood (Ramalho Ribeiro, 1989). Furthermore, these methods of regulation will only come into play when physical limitations to intake do not intervene (Broster *et al.*, 1981) as is likely to be the case when diets contain large amounts of fibre.

Long-term regulation of feed intake is thought to be related to the animal's energy balance (Ramalho Ribeiro, 1989). In fact, the long-term preservation of a relatively constant body weight, combined with the animal's desire to return to that body weight should it be altered by either force-feeding or starvation, suggests that some agent associated with energy storage acts as a signal for the long-term regulation of food intake (Ramalho Ribeiro, 1989). Broster *et al.* (1981) stated that, if no physical limitation to intake exists, the cow will consume as much energy as she can utilize, not only for growth, maintenance and milk production, but also for the deposition of body fat. This implies that the physiological status of the animal will have an impact on food intake as a result of the demand for energy (McDonald *et al.*, 1981). This theory of long-term regulation of feed intake as a result of energy balance is known as the lipostatic theory of intake regulation. Thermostatic regulation of intake may also play a role in long-term intake regulation. For example, ruminants respond to prolonged changes in environmental temperatures, increasing intake during periods of decreased temperature while decreasing intake during warmer periods (McDonald *et al.*, 1981, Ramalho Ribeiro, 1989). When diets high in concentrate feeds are fed, chemostatic regulation of intake may play a role. However, it is unlikely that a glucostatic mechanism of intake control, such as is thought to exist in monogastric animals, could apply to ruminants. This is because relatively small amounts of glucose are absorbed from the digestive tract of the ruminant and blood glucose levels show little relation to feeding behaviour (McDonald *et al.*, 1981). However, numerous fatty acids are produced during rumen fermentation and it is possible that these may be involved in a chemostatic mechanism of intake regulation (McDonald *et al.*, 1981).

The size of the animal has been shown to play an important role in the regulation of feed intake. This is largely a result of the direct relationship between animal size, abdominal capacity and gut capacity, which increase with increasing animal size (Broster *et al.*, 1981; McDonald *et al.*, 1981).

1.3.3 Food factors

Although, the importance of animal factors in the control of feed intake is acknowledged, it is generally accepted that the most important factor limiting feed intake by animals fed diets containing large amounts of roughage is the physical fill of undigested feed residues in the rumen (Madsen *et al.*, 1997). Beever (1993) suggested that the VFI of a ruminant is influenced by the rate at which previously ingested feed is removed from the rumen by the competing processes of degradation and passage. Similarly, Wilson and Kennedy (1996) stated that the relief of rumen load by clearance of plant fibre residues occurs by processes of digestion and passage to the post-ruminal tract.

The clearance of DM present in ingested feed particles from the rumen may occur as a result of either solubilisation, microbial digestion or propulsion of digesta from the reticulo-rumen into the omasum following comminution (Kennedy and Doyle, 1993). As the probability of particle passage from the reticulo-rumen is an inverse function of particle size (Wilson and Kennedy, 1996), it would be expected that any feed factor that increases the intrinsic rate of breakdown of large particles would result in an increase in the rate of passage of small particles from the rumen.

The rate of breakdown of feed particles in the rumen can be directly attributed to certain aspects of the forage itself. Kennedy and Doyle (1993) suggested that, from a ruminant perspective, plant material comprises cell walls and cell constituents, with important differences existing between structural cells of low digestibility and highly digestible photosynthesising (storage) cells. The content of non-cellulosic polysaccharides relative to cellulose is generally higher in grasses than in legumes and in stem tissue than in leaf tissue (Kennedy and Doyle, 1993). Stage of maturity will also affect the amount of non-cellulosic polysaccharides present in plant tissues (Kennedy and Doyle, 1993). Thus any factor that affects forage quality as described in previous sections may affect the rate of breakdown of feed particles and the rate of passage of small particles from the rumen.

The physical processing (milling, grinding, chopping or pelleting) of diets containing large amounts of cellulose has been shown to increase the daily feed intake of ruminant animals (Walker, 1984). This increase in intake may be the result of an increased feed density (Walker, 1984), in addition to a decrease in the chewing time required to reduce ingested

material to a size suitable for microbial colonisation and digestion (Walker, 1984) or passage of particles from the rumen (Beever, 1993).

It should be noted that the evolutionary ruminal adaptations which makes the digestion of high fibre feedstuffs possible is dependent on the retention of ingesta and fermentation of the same by micro-organisms present in the rumen (Van Soest *et al.*, 1988). Microbes present in the rumen act on ingested feed, hydrolysing plant celluloses, hemicelluloses, pectins, fructosans, starches and other polysaccharides, along with any other simple sugars, to give various products which may undergo further microbial action (Hobson, 1997). Consequently, in order to achieve optimal performance when formulating ruminant diets, it is essential to consider not only the nutrient demands of the host animal, but also those of the rumen microbes (Leng, 1986). Thus, feed formulation for ruminant animals is a fine balance between the supply of sufficient fibre to make the diet economical whilst at the same time supplying sufficient protein and energy for the rumen microbes.

The rumen microbial population is considered to consist of a wide range of anaerobic organisms, which include bacteria, protozoa and fungi, and the sensitivity of these microbes to substrates varies greatly (Stewart *et al.*, 1997). For example, Illius and Gordon (1991) showed that minor changes in the microbial population in the pool of microbial matter could provoke large changes in the intake of roughage diets. Thus, it is important to determine what effect alterations in the diet will have on the microbial population and what effect a given change in microbial population will have on digestive interactions occurring among dietary components (Nocek and Russell, 1988).

1.4 Clearance of ingested feed material from the rumen

The rate of clearance of ingested material from the rumen has been shown to depend on a number of factors, including the chemical and physical composition of the feedstuff, rate of microbial digestion, particle size and pH in the rumen (Sutherland, 1988). Campling *et al.* (1961) showed that cows fed a diet of medium quality hay consumed more than double the amount consumed by cows fed a diet of poorer quality oat straw. These researchers showed that this difference in intake is directly linked to the relative rates of disappearance from the reticulo-rumen of digesta derived from the two feeds.

The true outlet from the rumen is considered to be the reticulo-omasal orifice, although the rumen wall allows some flow of gases, water and solutes between the rumen contents and blood supply, while fermentation gases may also leave the rumen via the oesophagus (Czerkawski, 1986). Thus, as noted previously, there are three ways by which ingested material may leave the reticulo-rumen; solubilisation, microbial digestion or propulsion of digesta from the rumen into the omasum following comminution (Kennedy and Doyle, 1993).

Because digestion is a time-dependent process, the rate of digestion relative to the rate of passage is a critical dynamic property affecting digestibility (Mertens and Ely, 1982; Mertens, 1993). The time period over which ingested feed particles are retained in the rumen is usually sufficient to allow the fibrolytic micro-organisms to digest an appreciable amount of the potentially digestible plant cell walls, thereby meeting the energy demand of the animal through the production of volatile fatty acids (Kennedy and Doyle, 1993). However, increasing plant maturity results in a decrease in the content of soluble material, the proportion of potentially degradable cell walls and the supply of substrates to animal tissues. Consequently, the rate of digestion of plant material decreases with increasing stage of plant maturity (Kennedy and Doyle, 1993).

The digestion of feed consumed can be divided into two phases: (i) microbial interaction and attachment, and (ii) chemical breakdown, while the passage of undigested material can be separated into: (i) particle size reduction, (ii) escape from the rumen, and (iii) movement of particles through the tract (Mertens and Ely, 1982).

1.4.1 The microbial digestion of ingested feed material

The dynamic process of digestion can be divided into three conceptual components, which may vary among forages. These components, as described by Mertens and Ely (1982), are the size of the potentially degradable fraction, the rate of digestion and the digestion lag.

Potentially digestible fraction: Wilkins (1969) defined the potential digestibility of a forage as "the maximum digestibility attainable when the conditions and duration of fermentation are not limiting". Tamminga (1993) suggested that the size of the indigestible fraction (or, inversely, the potentially degradable proportion) can be considered as an

intrinsic characteristic of the feed. Mertens (1977) and Mertens and Ely (1978 a and b; cited by Mertens and Ely, 1982), showed that the size of the potentially digestible fraction has a greater effect on the digestibility of forages, than the other components of the digestion model (that is, rate of digestion and digestion lag). The potential extent of degradation may be affected by chemical factors, plant morphology and crystallinity (Mertens and Ely, 1982).

The digestibility of a forage is largely dependent on the amount of fibre in the forage, as shown by Smith *et al.* (1972), who found the potential extent of degradation to be most closely related to the chemical composition of the forage. These researchers found correlations of greater than 0.78 between lignin and the 72-h cell wall indigestibility used to predict potential digestibility. Plant morphology will influence the potential extent of digestion, as certain tissues in plants are totally indigestible (Akin *et al.*, 1974; cited by Mertens and Ely, 1982; Akin and Amos, 1975; cited by Mertens and Ely, 1982). These indigestible tissues are high in lignin and the effect of morphology on potential digestion is more likely to be a result of their lignin content than their morphology *per se* (Mertens and Ely, 1982).

Rate of digestion: Van Soest (1994) defined rate of digestion as the quantity of feed that can be digested per unit of time. This rate of digestion may be affected by the composition of the diet, its quality and deficiencies, excesses and availability of nutrients. In addition, Mertens and Ely (1982) hypothesised that microbial and animal factors will also alter fractional rates of digestion.

Cell wall polysaccharides such as cellulose and hemicellulose are slowly digested in the rumen (3 to 9% per hour) as compared to starch and protein, which are digested at rates of between 10 and 20% per hour. Thus, it follows that feed composition will impose a limit on the extent to which a feed can be digested (Jung *et al.*, 1996). Although some studies conducted on isolated polysaccharides have shown them to be completely digestible given sufficient time for fermentation (Jung *et al.*, 1996), maximum potential rates of polysaccharide digestion are seldom achieved with forages and so the chemical structure of the polysaccharide itself cannot be considered to limit potential digestibility (Jung *et al.*, 1996). However, it is likely that these chemical and physical factors limit the access of rumen bacteria and/or their enzymes to the cell wall polysaccharide (Jung *et al.*, 1996).

Furthermore, different maximum rates of digestion have been observed for different polysaccharides. For example cellulose, the most abundant polysaccharide in forages, has a maximum rate of digestion by rumen bacteria of only 10% per hour, while pectins, which comprise a large proportion of legume cell walls, are broken down at rates greater than 20% per hour (Hatfield and Weimer, 1995; cited by Jung *et al.*, 1996).

Digestion Lag Time: Mertens and Ely (1979) defined lag time as the time required for the colonisation and multiplication of bacteria attached to substrates present in the rumen. Digestion lag time has the greatest impact on the ruminal digestion of feeds that have a rapid rate of passage, as these feeds will leave the rumen prior to sufficient digestion by microbes (Mertens and Ely, 1982). The extent of lag time is dependent on a number of factors as demonstrated by Allen *et al.* (1981), who modelled lag time using a number of factors, including the rate of microbial penetration of the plant epidermal layer, rate of particle hydration, removal of chemical and physical inhibitors, dietary composition, rate of microbial attachment, microbial species and time for increasing numbers of bacteria and amounts of enzymes. The effect of diet on lag time was further demonstrated by Owens and Goetsch (1986) who illustrated that it is possible to alter lag time, by changing a number of dietary factors. For example, adding small amounts of soluble carbohydrate to a forage diet, may improve fibre digestion by enhancing the attachment of bacteria to ingested feed particles (Tamminga, 1982; cited by Owens and Goetsch, 1986). The effect of specific factors may differ with the composition of the basal diet (Tamminga, 1982; cited by Owens and Goetsch, 1986). In addition, the chemical and physical fibre structures may alter the degree of microbial adherence and, as a result, affect lag time (Owens and Goetsch, 1986). Poppi *et al.* (1981a) showed the lag time before significant digestion occurred to be considerably greater in unchewed rather than chewed material.

1.4.2 The passage of ingested feed material

Poppi *et al.* (1980) showed that the probability of escape of feed particles from the rumen is an inverse function of particle size, although particles of macroscopic size still leave the reticulo-rumen at a rate less than that observed for soluble markers (Sutherland, 1988; Bosch and Bruining, 1992). Wilson and Kennedy (1996) further suggested that the probability of passage of particles of the same size is irrespective of forage diet, although forage form has a significant effect on the rate of clearance of fibre from the rumen. This

would seem to indicate a marked difference among feeds in the physical characteristics of their particles, even when of the same size. In general, feed particles are usually categorised as either small or large particles (Wilson and Kennedy, 1996), where those particles retained on a screen of 1 to 1.18 mm mesh are classified as large particles (Wilson and Kennedy, 1996) and have a low probability of escape from the rumen (Poppi *et al.*, 1981b). The large particle pool is thus usually comprised of those particles with a low probability of digestion and which, as a result, will require ruminative chewing in order to decrease their size (McLeod and Minson, 1988).

Rate of passage of fine particles: Those particles that escape from the rumen leave in aqueous suspension (Sutherland, 1988). However, as mentioned earlier in this section, it has been shown that the rate of passage of fine particles from the rumen is not the same as that of soluble markers. Thus, the rate of passage of fine particles from the rumen must be influenced by, and therefore be a function of, a number of other factors and not only the rate of liquid passage from the rumen (Sutherland, 1988). The following equation, explaining the degree to which the passage of particles of a certain size from the rumen is impeded, was proposed by Sutherland (1988):

$$k_p = P_1 \times P_2 \times P_3 \times \ldots\ldots \times P_n \times k_w$$

where

k_p and k_w = the rate constants governing the rate of passage from the reticulo-rumen of particles and water, respectively

$P_1, P_2, P_3, \ldots, P_n$ = probabilities that measure the effect of individual mechanisms on the flow of fine particles as compared to liquid molecules. These escape coefficients may vary for particles of different sizes, although, as yet, it is not possible to assign values for these coefficients.

Mertens and Ely (1982) proposed several factors, which may influence the passage rate of fine particles. These include forage digestibility, rate of digestion, rumination efficiency, form and composition of diet and digestion lag time. Rate of hydration and specific gravity also affect the rate of passage of particles from the rumen (Van Soest, 1994).

1.4.3 Factors affecting the rate of passage of fine particles

Flow of water and saliva: The fluid content of the rumen makes up approximately 15 percent of total body water. During a 24-hr period, roughly 30 percent of the total body water flows into the rumen as saliva but, at the same time, the passage rate of fluid from the rumen into the omasum is in the same range (Engelhardt, 1970). Thus, although there may be a considerable flow of water through the rumen wall (Engelhardt, 1970), the flow of water from the rumen will have a direct effect on the flow of rumen digesta out of the rumen (Czerkawski, 1986). In addition, the flow of water in the rumen may have an effect on the osmotic pressure and acidity of the rumen, while salivary input may increase buffering capacity of the rumen or may provide specific nutrients (Czerkawski, 1986).

Particle size and rate of particle breakdown: Lee and Pearce (1984) suggested that one of the major factors influencing the rate of passage of digesta from the reticulo-rumen is its physical breakdown into particles sufficiently small to pass through the reticulo-omasal orifice. Numerous studies have shown that although the reticulo-rumen of sheep normally contains a large proportion of long fibrous material, few particles greater than 1 mm in length pass beyond the reticulo-omasal orifice (Waghorn *et al.*, 1986; Sutherland, 1988). Considerable particle comminution must therefore occur prior to passage from the reticulo-rumen and occurs mainly as a result of ingestive and ruminative chewing (Balch and Campling, 1962; Kennedy and Doyle, 1993).

The rate of particle comminution is thought to be affected by the structural organisation of plant organs and their constituent tissues and this varies greatly, both within and between species (Wilson, 1993). In addition, although ingestive and ruminative chewing are the most important factors involved in particle size reduction, microbial fermentation and ruminal detrition also play a role (Lee and Pearce, 1984). Therefore, if particle size reduction is an important factor in feed intake regulation, then the anatomical structure of the plant material ingested is potentially a major contributor to establishing feed quality (Akin, 1989).

Reduction of particle size by ingestive chewing: Any physical process, such as ingestive chewing, which will facilitate the adherence and penetration of cellulolytic enzymes during the digestive process may enhance fibre digestion (Tamminga, 1993). Plant tissue

fragmentation caused by ingestive mastication results in a large array of particle sizes and particles of diverse tissue types (Pond *et al.*, 1984). The crushing and crimping of ingested plant material which occurs during initial mastication, although not accompanied by a decrease in forage particle size, is probably just as important as the decrease in particle size (Pond *et al.*, 1984) since the physical disruption of barrier tissues such as cuticle and vascular bundles allows for the penetration of microflora (Pond *et al.*, 1984). Kennedy and Doyle (1993) also suggested that the mechanical process of chewing allows for the infiltration of saliva and removes the cuticle from the forage. Probably more important, however, is the release of digestible cell components previously enclosed in indigestible "barrier" tissues (Pond *et al.*, 1984; Kennedy and Doyle, 1993).

McLeod and Minson (1988) used four forages covering a range of physical, chemical and nutritive values, to show that primary mastication or ingestive chewing is responsible for 25% of the reduction in large particle size. These observations compared favourably with results obtained in previous studies. For example, ingestive chewing was found to be responsible for 9 to 39% of large particle size reduction observed for tropical forages (Hendricksen *et al.*, 1981; Poppi *et al.*, 1981b) and 30 to 40% observed in temperate forages (Lee and Pearce, 1984). However, reduction rates of up to 50% of large particles have been reported by various authors for temperate forages (Gill *et al.*, 1966; Reid *et al.*, 1979, cited by Kennedy and Doyle, 1993; Ulyatt, 1983, cited by Kennedy and Doyle, 1993). McLeod and Minson (1988) suggested that this variation might be a result of variations in fibre content and digestibility of forages. This theory is supported by the observations of Bailey *et al.* (1990) that particle size reduction, occurring as a result of ingestive chewing, may be dependent on forage species as well as forage particle size prior to ingestion. Hooper and Welch (1983) suggested that larger animals are more efficient chewers, while Kennedy and Doyle (1993) postulated that other animal factors such as mouth size, teeth area, grinding action, rate of jaw movement and rate of ingestion may account for the variation in particle size distribution among animals of the same species. Still other researchers have suggested that the efficiency of mastication is related to the level of feed intake. For example, both Troelson and Bigsby (1964) and Van Soest (1966), cited by Lee and Pearce (1984), noted a direct relationship between feed intake and particle size distribution.

In addition, ingestive chewing helps to fulfil another important function, in forming the feed into a bolus that may be easily swallowed (Kennedy and Doyle, 1993). Furthermore, ingestive chewing may play a role in the rate of passage of fine particles, as there is considerable evidence to suggest that the passage rate of small particles is accelerated during eating (Kennedy and Doyle, 1993). This is probably a result of enhanced ruminal contractions which occur during eating and which force small particles to move out from the rumen faster than normally occurs during rumination or resting (Moseley and Jones, 1984; Aitchison *et al.*, 1986; Girard, 1990). Campling and Freer (1966) calculated that digesta loss from the rumen during eating increases to 8 to 10 g/min from the 6 g/min found to occur between meals, whilst Pond *et al.* (1989) found the faecal appearance of a marker dosed into the rumen at the start of a meal to be 42% faster than when dosed at the end of the meal.

Reduction of particle size by ruminative chewing and rumination efficiency: As with ingestive chewing, chewing during rumination has a positive effect on fibre digestion, with the majority of large particle breakdown (>75%) occurring as a result of ruminative chewing (Kennedy, 1985; McLeod and Minson, 1988; Kennedy and Doyle, 1993). In comparison to the reduction in particle size achieved during eating, Ulyatt *et al.* (1986) showed that ruminative chewing is probably more important for particle size reduction, with 12 to 16% of fresh forage and 27 to 39% of hay reduced to below the critical particle size by ruminative chewing.

Modelling of the process of particle size reduction occurring during rumination is slightly more complex than that occurring during ingestion. This is because ruminative chewing involves selection of particles of a given size to be regurgitated as well as the comminution of particles (Lowrison (1982), cited by Kennedy and Doyle (1993). This is emphasised by the large particle content of the up-bolus, which is an indication of the large particle content of the relaxed reticulum but not necessarily of the rumen and may vary from 55 to 96% of that in the reticulo-rumen of cattle and sheep (McLeod, 1986; cited by Kennedy and Doyle, 1993; Ulyatt *et al.*, 1986; Chai *et al.*, 1984).

Within a second or two of the aspirated bolus arriving in the mouth, excess unchewed digesta (the "tail" bolus) is swallowed and ruminative chewing begins (Kennedy and Doyle, 1993). After about a minute, the bolus is returned to the reticulo-rumen, although

small amounts of the chewed digesta (up to three boluses) may be swallowed prior to this (Kennedy and Doyle, 1993). Thus, the large particle content of regurgitated material retained in the mouth is enhanced by up to 18% in sheep (Ulyatt *et al.*, 1986) and up to 47% in cattle (Kennedy, 1985) as a result of concentration and possible selection of particulate matter in the pharynx, mouth and cheeks during the swallowing of the tail bolus (Kennedy and Doyle, 1993).

1.4.4 Stratification or entrapment of particles in the fibrous mat of rumen ingesta

Although plant material ingested by the ruminant undergoes microbial digestion in the rumen, there is invariably a portion of the material that is indigestible. This indigestible material must be cleared from the rumen in order to relieve the pressure of fill and thereby maintain a sufficient level of intake (Van Soest, 1994). Considerable variation in the size of undigested fibrous particles passing through the gastrointestinal tract and appearing in the faeces of the animal has been observed (Welch and Smith, 1978). It has been suggested that a critical particle size exists, above which particles are prevented from leaving the rumen (Poppi *et al.*, 1980). However, Evans *et al.* (1973) reported that a large proportion of the DM in the rumen is composed of particles smaller than 1 mm in size and, as such, should not be restricted, by the reticulo-omasal orifice, from leaving the rumen. Further evidence of this was reported by Welch and Smith (1978). They conducted an experiment using polypropylene ribbon, that is resistant to microbial digestion and which may only be cleared from the rumen once sufficient comminution as a result of chewing has occurred. Although smaller particles were cleared from the rumen in greater amounts than large particles, it was possible for particles of 2.0 cm in cattle, and 1.0 cm in sheep, to leave the rumen. Welch and Smith (1978) clearly illustrated the importance of particle size reduction. However, these results, along with the results of Evans *et al.* (1973) raise the question of why the average size of particles leaving the rumen is so much smaller than the size at which it becomes possible for them to pass through the reticulo-omasal orifice. Numerous other investigations (Balch and Campling, 1962; Uden and Van Soest, 1982; Waghorn *et al.*, 1986) have also indicated that the passage of large particles is inhibited before they reach the omasum and not simply as a result of the sieving effects of the reticulo-omasal orifice alone.

The principal theory put forward to explain this inhibition of small particle escape is one of entrapment in the fibrous mass present in the rumen (Welch and Smith, 1978; Sutherland, 1988; Kennedy and Doyle, 1993). On ingestion, the majority of feed particles, with the exception of grains, are coarse and buoyant and because of this buoyancy, tend to float in the dorsal sac of the rumen, forming a three dimensional particulate raft, unless constrained by mixing contractions (Kennedy and Doyle, 1993). Although Hooper and Welch (1985) suggested that particle buoyancy may be reduced on hydration, it is regained once microbial digestion begins, because of gas production during fermentation (Kennedy and Doyle, 1993).

Czerkawski (1986) proposed a mechanism to explain how the solid mass of digesta present in the rumen is incubated and processed in a controlled manner, and the concentration of soluble substances is reasonably uniform within the rumen. In this scheme, rumen movement and rumination cause the solid digesta to move slowly clockwise towards the caudal region of the raft, enabling the removal of boli, their transfer to the mouth and subsequent mastication. The liquid digesta present is filtered through this particulate mass. Large particles floating in the liquid digesta become trapped in a mass of solid material as a result of the filtration and become part of the matrix. Consequently, they are carried forward, regurgitated, chewed and broken down. Small particles may be trapped and then released during alternating liquid flows or may escape entrapment altogether. Either way, they will eventually leave the rumen because of flow of fluid through the reticulo-omasal orifice. The proportion of particles leaving or re-entering the rumen after re-swallowing of the ruminated bolus is dependent on their density.

This mechanism is similar to the one described by Kennedy and Doyle (1993), who suggested that particles from a newly ingested forage bolus arriving in the reticulo-rumen enter a dorsal digesta stream and flow in that stream until such time as a progressive tendency to sink deflects them into a ventral stream, which subsequently carries them to the cranial ventral sac. Oscillating contraction and relaxation of the cranial ventral sac and reticulum eventually propel the particles into the reticulum. Once in the reticulum, particles sort themselves by buoyancy. Denser particles have the opportunity to pass into the omasum through the reticulo-omasal orifice during the second contraction of a strong double contraction of the reticulum. An additional contraction of the reticulum occurs

during rumination when a relatively large volume of digesta is propelled from the reticulum through the oesophagus and into the mouth for subsequent chewing.

1.4.5 Rate of hydration and functional specific gravity of particles

Ehle (1984) suggested that, although little information was available, the results of a number of studies indicate that the density of feed particles has an effect on their rate of passage from the rumen. However, some reports are contradictory. For example, Evans *et al.* (1973) showed that particle size and density varied according to sampling location in the rumen as well as with sampling time and level of feeding. However, Balch and Kelly (1950) found little difference between specific gravity of particles taken from different sites within the rumen. The results of the study conducted by Evans *et al.* (1973), indicated that particles from the dorsal rumen were larger and lighter than those sampled from the ventral reticulo-rumen.

1.4.6 Animal size

It has been shown (Balch and Campling, 1962; Troelsen and Campbell, 1968; Reid *et al.*, 1979, cited by Kennedy and Doyle, 1993; Poppi *et al.*, 1980; Poppi *et al.*, 1985) that the probability of a particle leaving the rumen decreases exponentially as particle size increases. However, although a smaller particle has a higher probability of leaving the rumen, there are differences in the fractional passage rates of particles among sheep, cattle and even camels, with a critical particle size of 1 to 2 mm accepted for sheep and cattle and 3 mm for camels (Lechner-Doll and Engelhardt, 1989; cited by Lechner-Doll *et al.*, 1991).

1.5 Discussion and conclusions

In order to be cost effective, a large proportion of ruminant production systems, particularly those found in sub-Saharan Africa, rely heavily on high fibre forages as the basis of diets fed. A unique pregastric system of fermentation makes it possible for these high fibre forages to be efficiently utilised by the ruminant animal.

The nutritive or feeding value of a feed can be characterised in terms of the nutrient composition of the feed and the amount of feed the animal consumes. A good estimate of

feed intake is just as essential as nutrient composition when determining the ability of any given forage or feed to meet the nutrient requirements of the animal (Crampton *et al.*, 1960; Dulphy *et al.*, 1989; Jarrige, 1989; Beever, 1993; Dulphy and Demarquilly, 1994; Madsen *et al.*, 1997). When concentrate diets with a high nutrient density are fed, the animal's requirements for essential nutrients are usually met before intake becomes limiting and feed intake is thought to be controlled by neurochemical mechanisms (McDonald *et al.*, 1981; Ramalho Ribeiro, 1989). However, in the case of ruminant animals fed diets high in fibre, with a low nutrient density, a simple mechanical mechanism relating to available rumen volume will operate first (Balch and Campling, 1962).

The pregastric system of rumen fermentation is dependent on a dynamic rumen microbial population and, in order for the host animal to obtain maximum benefit from the forage, it is important that the rumen microbial population is adapted to the diet fed. Furthermore, it is important that rumen microbes are allowed sufficient time to attach to and digest those components of the feed that would be indigestible by the host animal alone. Consequently, a fine balance is necessary to ensure that adequate time is allowed for sufficient microbial digestion of ingested feed material, while clearance rate of the same feed is great enough to allow satisfactory levels of intake.

Clearance of ingested material occurs in one of three ways; namely passage from the rumen through the reticulo-omasal orifice, microbial digestion or solubilisation. Thus, any process, which serves to increase the rate of disappearance of ingested feed from the rumen, by any means, will help to increase the potential for feed intake.

Chapter 2

Ruminal degradability and intestinal digestion of eight plant protein sources used in ruminant feeds

2.0 Abstract

This study investigated the disappearance of dry matter and nitrogen from eight plant protein sources contained within nitrogen-free polyester bags incubated in the rumen of three fistulated Jersey cows. In addition, the protein sources were force-fed to adult cockerels to obtain an estimate of the intestinal digestion of the available protein by the host animal. Plant protein sources used were canola, copra, cottonseed oilcake, defatted maize germ, lucerne meal, lupin seed meal, soya oilcake and sunflower oilcake. The intercept values and time lags for dry matter degradation were significantly different ($P<0.001$), whilst potential degradability and rate of degradation differed ($P<0.05$) among protein sources. In terms of nitrogen degradation, the intercept value and rate of degradation were significantly different ($P<0.001$), while potential degradability and time lag were not significantly different among feeds. Effective degradability was calculated using different rates of passage for both solid particles (k) and liquid matter leaving the rumen (klq), for sheep, beef and dairy animals. Lupins had the highest effective degradability for both dry matter (792 g kg^{-1} in sheep) and nitrogen (844 g kg^{-1} in sheep), while canola showed the lowest effective dry matter degradability (433 g kg^{-1} in sheep) and copra the lowest effective nitrogen degradability (678 g kg^{-1} in sheep). Digestible rumen undegradable protein (RUP) was calculated as the difference between total digestible protein and effective nitrogen loss. Calculated in this manner, sunflower oilcake had the highest digestible RUP (126 g kg^{-1} nitrogen in sheep) while cottonseed oilcake had the lowest (83 g kg^{-1} nitrogen in sheep). Further consideration should be given to methods of determination of RUP described in this trial as well as determination of digestibility parameters for complete feeds rather than supplements alone.

Keywords: protein, supplementation, ruminant, roughage, digestible undegradable protein, rumen degradable protein

2.1 Introduction

Cronjé (1990) defined supplementary feeding as the addition of catalytic amounts of strategic nutrients to the basal diet, in order to increase the efficiency of feed utilisation. More importantly however, supplementary feeding strategies can only be effective if the deficiencies of the feed can be related to the requirements of the animal (Cronjé, 1990).

Supplementation of roughage diets is aimed primarily at alleviating nutritional deficiencies in the rumen and thereby enhancing the utilisation of roughage (Nsahlai *et al.*, 1998) and a readily degradable source of nitrogen or a combination of readily degradable nitrogen and carbohydrate are a commonly used means of supplementation, aimed at enhancing roughage utilisation (Cronjé, 1990).

Increasing the efficiency of rumen fermentation has a number of benefits. Firstly, there may be an increase in the potential supply of energy to the animal through increased volatile fatty acid production resulting from the fermentation of low quality roughage (Czerkawski, 1986). The increased rate of degradability allows a more rapid movement of roughage through the gut and, thus could lead to an increased intake and thirdly, the protein supply to the lower digestive tract may be increased as a result of a greater outflow of microbial biomass from the rumen (Cronjé, 1990). Preston and Leng (1987) further suggested that any improvement in microbial efficiency will increase the protein to energy ratio of digesta flowing into the small intestine and this may increase the efficiency with which acetate is utilised by body tissue.

Numerous systems are currently in use world-wide for the calculation of protein requirements (Erasmus *et al.*, 1990). Although these systems vary slightly, they are all based on the concept that the protein requirements of ruminant animals can best be met by supplying sufficient nitrogen for rumen microbes (rumen degradable protein (RDP)), as well as additional protein which will pass undegraded through the rumen (rumen undegradable protein (RUP)). The RUP is provided in order to meet any deficiency existing between the supply of microbial protein synthesised in the rumen and tissue requirements for intestinally absorbed amino acids (Erasmus and Prinsloo, 1988). Quantitative estimates of degradability are therefore extremely useful, as the extent of degradation determines both the degradable protein available for the rumen microbes and

the undegraded protein available for host animal enzymatic digestion (Ørskov and McDonald, 1979).

Numerous methods of assessing protein degradability in feedstuffs have been proposed. However, the *in situ* technique as described by Mehrez and Ørskov (1977) is currently the standard method used for determining protein degradation in the USA, UK and Nordic countries (Van der Honing and Alderman, 1988; Beever and Mould, 2000) even though there are problems associated with this method (Beever and Mould, 2000). The method described by Mehrez and Ørskov (1977) is the method used in this trial.

The potentially degradable DM or nitrogen component of a feed (PD) comprises a rapidly degradable fraction (a) which can be assumed to be completely degraded in the rumen, and a slowly degradable fraction (b) which disappears at a constant fractional rate (c) per unit time (Ørskov and McDonald, 1979). The potential degradability of a supplement is an indication of how digestible the feed is, while the soluble DM component or rapidly degradable fraction is a measure of the DM or nitrogen immediately available to rumen microbes for digestion. This gives an indication of how readily fermentable the protein supplement is. The slowly degradable fraction is available for degradation by rumen microbes, although the extent of degradation is dependent on the time spent in the rumen.

Henning *et al.* (1989) noted that, when formulating ruminant feeds, it is important to know what portion of protein fed to the animal is undegraded dietary protein that can be digested in the lower gastro-intestinal tract of the animal and what portion of the dietary protein is rumen degradable. The undegraded dietary protein will act as a supplement to the amino acids of microbial origin and thereby contribute to meeting the amino acid requirements of the tissues (Henning *et al.*, 1989).

Rooke (1985) proposed a method that could be used to determine the digestibility of the RUP fraction. This method requires the insertion of small polyester bags in the proximal duodenum. These are then recovered from the faeces and nitrogen disappearance from the bags determined. Hvelplund and Weisbjerg (2000) reviewed the methods currently in use for the determination of post ruminal digestibility of undegraded feed protein. They noted that intestinal digestibility of rumen-undegraded protein has only been investigated in a few *in vivo* experiments and that results obtained using the mobile bag technique can be

regarded as equal to estimates of *in vivo* digestibility. However, the mobile bag technique requires the use of animals fitted with a duodenal cannula, the initial cost and maintenance of which is high. Henning *et al.* (1989) further noted that the use of mobile bags placed in the duodenum of sheep poses some practical problems and that bags tend to get stuck. In addition, many factors, including bag cloth characteristics, pepsin-HCl pre-treatment of samples and place of bag recovery, will influence the disappearance of protein from the bag and thus the estimate of post ruminal undegraded protein digestibility (Hvelplund and Weisbjerg, 2000). Thus, the development of an easy and reliable technique for determining the digestibility of rumen-undegraded protein would be of benefit (Henning *et al.,* 1989).

It may be possible to estimate by calculation, using results obtained for digestibility in poultry, the digestibility of RUP. In order to do this, two assumptions need to be made, namely, (a) post ruminal protein digestion occurs in the same manner as protein digestion in the digestive tract of the chicken, and (b) potentially RDP and RUP undergo the same extent of digestion in the small intestine of the chicken. Henning *et al.* (1989) proposed a method for determining the digestibility of RUP using cockerels. A method similar to that described by Henning *et al.* (1989) was used in this trial. Whereas Henning *et al.* (1989) first exposed diets containing one of five protein rich feed supplements to ruminal degradation and then fed them to adult cockerels, in this trial complete diets were not investigated and the protein supplements used were not exposed to ruminal degradation prior to digestion by chickens. Henning *et al.* (1989) thus evaluated the degradation and digestibility of the protein portion of complete diets whilst in this trial the digestibility of the undegraded protein supplement *per se* was evaluated and not as part of a complete ration.

This study was carried out to determine the rumen degradation parameters and intestinal digestion in poultry of eight plant protein supplements commonly used in ruminant rations.

2.2 Materials and methods

Animals and Diet: The disappearance of DM and nitrogen from protein supplements contained in nitrogen-free polyester bags incubated in the rumen of three fistulated non-lactating Jersey cows (434.1 ± 43.1kg live weight) was investigated using the method described by Mehrez and Ørskov (1977).

Each cow was fitted with a rumen cannula (8.0 cm internal diameter). The animals were allowed *ad libitum* access to a basal diet of Coast Cross 2 hay (K11) (a hybrid of *Cynodon dactylon* (Tainton *et al.*, 1976)). The basal diet had 451 g CF and 59 g CP per kilogram of DM. Animals were allowed a 10-day adaptation period to the basal diet.

Protein sources: Eight plant protein sources, lucerne meal (*Medicago sativa*), lupin seed meal (*Lupinus alba*) and solvent extracted canola meal (*Brassica napus*), copra meal (*Cocos nuciferay*), cottonseed oilcake (*Gossypium barbadense*), defatted maize germ meal (*Zea mays*), soya oilcake (*Glycine max*) and sunflower oilcake (*Helianthus annuus*), which are routinely included in ruminant feeds and which are readily available in South Africa, were selected for this trial. The protein sources were milled through a 2.0-mm screen using a laboratory mill, thoroughly mixed and then stored in glass jars at room temperature until required.

Incubation procedure: Nitrogen-free polyester bags (5 x 10 cm) (Ankom Technology Corporation, New York, USA), with an average pore size of 50 ± 15 µm and seams sealed with a heat sealer, were used. Bags were labelled S1 through to S100. Approximately 8 g of a sample was accurately measured out and placed in a bag, the number and weight of which was recorded. The bags were tied to a round stainless steel disc (100 g, 6 cm diameter, 4 mm thick) with 10 evenly spaced small holes drilled around the outside of the disc. The disc was tied to a nylon string and secured to the rumen cannula.

One bag per sample was placed in the rumen of each of the three cows for each incubation period. Each sample (protein source) was thus replicated three times for each time period. Samples were incubated in the rumen for 3, 6, 12, 24, 48, 72 and 96 hours. The 96-hour bags were placed in the rumen first, followed by the 72-hour bags and so on. On completion of the incubation period, the bags were removed and rinsed under tap water until the water ran clear. The bags were placed in a plastic bag, sealed and then stored in a freezer at -20°C until required for analysis.

Incubations were carried out in two phases (four protein sources per phase) as a result of limitations imposed by rumen capacity. After completion of both phases, samples were defrosted and washed in a semi-automatic washing machine (using a constant volume of cold water) for five successive cycles of five minutes each. The water used to rinse the

bags was drained and replaced with fresh water after each cycle. The samples were further dried in an oven at 60°C for 48 hours. Zero-hour values were determined using three additional bags for each protein supplement. These bags were subjected to the same washing and drying procedures detailed above, although they had not been incubated in the rumen. Residues were analysed for DM and nitrogen.

Protein digestibility determined in adult cockerels: An adaptation of the rapid *in vivo* assay method for the determination of gross energy described by Sibbald (1976), allowed for the determination of the digestible amino acids and consequently, protein content of a given feed. A total of 54 birds were used in this trial. Water was available *ad libitum* throughout the experimental period. Six birds were randomly allocated to each feed sample and were fasted for a 24-hr period, after which they were force-fed 50 g of dextrose in 50 ml of water, by intubation. Each bird was subsequently fed by intubation a slurry containing 50 g of feed sample and 50 ml of water, 48-hr after the fasting period began. A clean tray was placed under each bird and excreta collected over a 48-hr period. The tray was then removed and the excreta collected, placed in a preweighed aluminium container and dried in a forced-draft oven at 65°C for 48-hr. The excreta was reweighed and the dry weight determined. The faeces collected from birds fed the same sample were then pooled and milled through a 1.0-mm sieve using a Rechtsmühle mill.

The remaining six birds were treated in the same manner, but fed 50 g of table sugar (sucrose) instead of a feed sample. These birds were included in the trial to allow for the determination of endogenous amino acid and protein losses, which cannot be attributed to the feed.

Laboratory analysis: DM and organic matter (ash) were determined according to the Association of Official Analytical Chemists, method 930.15 and method 942.05 respectively (AOAC, 1990). Nitrogen was analysed in a LECO FP2000 Nitrogen analyser using the Dumas Combustion (AOAC (1990), method 990.03). Fat (Ether Extract) was extracted according to the Soxhlett procedure using a Büchi 810 Soxhlett Fat extractor (Büchi Laboratoriums-Technik AG, Flawil, Switzerland).

Both feed and faeces samples were analysed for digestible amino acids using a high sensitivity protein hydrolysate analysis with a Beckman 6300 (Applications Data A6300-

AN-002, 1983). Samples were first hydrolysed in order to break peptide bonds and liberate constituent amino acids. A standard hydrolysis procedure was followed. Approximately 50 mg of sample was weighed into a glass test tube and 3 ml of 6M-Hydrochloric acid (HCl) added. The tube was then filled with nitrogen, which was bubbled through the HCl in order to displace any air present in the tube. The tube was then sealed and placed in an oven at 110°C for a period of 24 hours. An internal standard was added to each sample to provide a means of correcting for any losses that may occur during hydrolysis and sample preparation.

2.3 Data derivation and statistical analysis

The disappearance of DM or nitrogen from the nylon bags was described using SAS (1987) according to the exponential model described by Ørskov and McDonald (1979):

$$P = a + b\,(1 - e^{-ct})$$

where P is the DM or nitrogen disappearance at time t, a is the zero time intercept, b is the slowly degradable fraction and c is the degradation rate. Time lag (TL) was calculated as:

$$TL=\{(-1/c)\ \ln\ [1-((w-a)/b)]\}$$

where w is washing loss. Potential degradability (PD) of the protein sample is calculated as (a + b).

The effective degradability of the protein sources is a function of the rate of passage of feed through the rumen. Thus effective degradability, (ED), was estimated following the model:

$$ED\ (g\ kg^{-1}) = f\,(a) + (bc)/(c + k)$$

where k is the rate of passage of particles through the rumen and f (a) = a x 0.3/(0.3+klq), where 0.3 is assumed to be the rate of digestion of solubles (Van Soest, 1994) and klq is the liquid passage rate. As rates of passage vary between species and functional types of ruminant, different values of k (0.03, 0.05, 0.07) and klq (0.05, 0.07, 0.10) were assumed

(Offer and Dixon, 2000) when calculating the effective DM and nitrogen loss properties for sheep, beef and dairy cattle respectively.

The amount of each amino acid digestible by the chicken was calculated using the following equation:

$$\text{Digestible AA (g/g)} = (50 \times AA_{Feed} - ((E_{Feed} \times AA_{Excreta}) - (EAAL_{Excreta} \times EEL_{AA})))/50$$

where:

Digestible AA	= digestible amino acid concentration (%).
AA_{Feed}	= concentration of amino acid in a given feed sample (g).
E_{Feed}	= average dry mass of excreta from birds fed a given feed sample (g).
$AA_{Excreta}$	= concentration of amino acid in the dry excreta of birds fed a given feed sample (g).
$EAAL_{Excreta}$	= average dry mass of faeces from birds fed sugar (g).
EEL_{AA}	= concentration of amino acid in the dry faeces of birds fed sugar (g).

Total digestible protein was then derived from the digestible amino acids.

Once the total digestible protein and effective nitrogen loss after rumen incubation have been determined, it is possible to calculate, by subtraction, the digestible RUP portion, for example:

$$\text{Digestible Undegradable Protein (g kg}^{-1}\text{)} = \text{TD Protein (g kg}^{-1}\text{)} - \text{E Nitrogen Loss (g kg}^{-1}\text{)}$$

where

TD Protein (g kg^{-1}) =	Total Digestible Protein (g kg^{-1}) and
E Nitrogen Loss (g kg^{-1}) =	Effective Nitrogen Loss (g kg^{-1})

A one-way analysis of variance was performed on the data to determine statistical differences in terms of DM and N degradation parameters and calculated effective degradability among protein sources (SAS, 1987). A correlation analysis was performed using a spreadsheet package, Microsoft Excel (Microsoft Corporation, 1997), on some

components of the data to determine the relationships between components such as DM and nitrogen degradability.

2.4 Results

The chemical composition of the protein sources is given in Table 2.1. Appendix 1 gives the DM and nitrogen degradation characteristics over time. Table 2.2 gives the DM degradation properties of the eight plant protein sources. There is a highly significant difference ($P<0.001$) among sources with respect to soluble DM components, with lupins having the highest solubility (625 g kg^{-1}) and canola the lowest (271 g kg^{-1}). These protein sources also had different ($P<0.05$) rates of DM degradation and similar slowly degradable DM fractions ($P>0.05$). Numerically, lupins had the highest effective DM degradability whilst canola had the lowest.

The nitrogen degradation parameters of the eight protein sources and the effective nitrogen degradability (calculated for sheep, beef and dairy cattle) are given in Table 2.3. There were significant differences ($P<0.001$) among feeds in soluble nitrogen, slowly degradable nitrogen and the nitrogen degradation rate. For example, lupin nitrogen is about 94% soluble, followed by cottonseed oilcake and defatted maize germ with nitrogen solubility of 77% and 61%, respectively. Canola has the lowest nitrogen solubility at 36.4%. Sunflower oilcake, copra, lucerne meal and soya oilcake all had moderate nitrogen solubility. However, the slowly degradable nitrogen fraction followed the reverse order. The effective nitrogen loss was highest in lupins followed by sunflower oilcake, and lowest in copra. The correlation between effective DM and nitrogen degradabilities was poor ($r = 0.50$, $P = 0.19$).

The relationship between DM and nitrogen degradation parameters was further investigated using correlation analysis. The correlation coefficients for the corresponding DM and nitrogen degradation properties were 0.82 ($P = 0.014$), 0.78 ($P = 0.022$) and 0.91 ($P = 0.002$) for wash values, slowly degradable fraction and the rate of degradation, respectively.

Table 2.4 gives the calculated total digestible protein determined using adult cockerels for each plant protein supplement along with the digestible RUP calculated for sheep, beef and

dairy cattle. Copra meal had the lowest total digestible protein determined for cockerels whilst lupin seeds had the highest total digestible protein determined for cockerels. Dairy cattle have a higher digestible RUP for a given protein supplement than beef cattle, which in turn have a greater RUP than sheep. These observations can be directly attributed to differences in effective nitrogen degradability resulting from differences in the liquid flow rate and rate of passage of solid particles through the rumen.

2.5 Discussion

Rumen degradation: The correlation analysis showed a strong relationship between corresponding DM and nitrogen degradation parameters which suggests that it may be possible to obtain a reasonable estimate of the nitrogen degradation parameters for a given protein supplement simply by determining its DM degradation parameters. This relationship may help to decrease the costs of analysis incurred during degradability studies. However, if such a relationship were to be used, a greater sample number and range of samples would need to be analysed. A similar approach has been proposed and calibration equations developed for forage legumes (Nsahlai *et al.*, 1995).

Assuming that the soluble portion of the supplement undergoes degradation during passage, it is possible to estimate the effective degradability of the supplement, using a suitable estimate of passage rate. As a result of the differential rates of passage for both particulate matter and liquid through the rumen of sheep, beef and dairy cattle, the effective DM and nitrogen degradability differed considerably, although the same trends were exhibited. The effective degradability of a supplement is an indication of the amount of protein available for microbial digestion in the rumen. The results shown in Table 2.3 indicate that the protein sources investigated here are largely rumen degradable in sheep, but less degradable in the rumen of a dairy cow, simply by virtue of retention time. This study has demonstrated that although most plant proteins are predominantly degradable in ruminants, they could supply appreciable quantities of digestible RUP in dairy cattle as a result of increased rates of particle and liquid passage rates in larger ruminants.

Digestible undegradable protein: Recent protein evaluation systems require the partitioning of feed protein into a ruminally degradable fraction as well as digestible undegradable protein (Tamminga *et al.*, 1994; Beever, 1996). These systems take into

account the protein requirements of the rumen microbes as well as the contribution of these microbes to meeting the host animal's amino acid requirements. An accurate estimate of the RDP fraction, as well as the RUP fraction, is thus essential when formulating ruminant diets. As a result, various methods of estimating the undegradable protein fraction have been proposed as discussed previously, although the mobile bag method proposed by Rooke (1985) has a number of associated problems, which includes the cost of maintaining multi-cannulated sheep.

In an attempt to overcome the problems and costs associated with the mobile bag technique as described by Hvelplund (1985), cited by Henning *et al.* (1989), this study used adult cockerels to determine the digestibility of true protein. The method used in this trial, although modified somewhat, was similar to that described by Henning *et al.* (1989). In comparison to the work of Henning *et al.* (1989), protein supplements were fed to adult cockerels without being subjected to ruminal degradation. By difference the amount of RUP available for digestion in the lower digestive tract was then determined.

The validity of this method can be questioned for a number of reasons. Firstly, microbial digestion of fibre within the rumen could render encapsulated protein available for digestion in the lower gut. This protein would not be available for digestion by the chicken, thus reducing the estimated digestibility of the protein for the ruminant animal. Furthermore, even if this method were suitable for determining the digestibility of rumen undegraded protein in supplements such as those used in this trial or concentrate rations, this is unlikely to be the case where samples under investigation contain high levels of fibrous roughages.

Mgheni *et al.* (1994), cited by Hvelplund and Weisbjerg (2000), showed potential degradability of tropical roughages in the rumen to be greater than original protein digestibility in the small intestine as measured by the mobile bag technique. Volden and Harstad (1995), cited by Hvelplund and Weisbjerg (2000), showed this also to be the case with other feeds such as grains, whilst Vanhatalo and Varvikko (1995) also showed this to be a problem with protein-rich feeds with a relatively high fibre content such as rapeseed meal. As a result, Hvelplund and Weisbjerg (2000) recommended that when determining total digestibility, pre-incubation of mobile bags should take place in the rumen for approximately 16 hours. Thus, in retrospect, incubation of the protein supplements in the

rumen should have been carried out prior to digestibility determinations using chickens as described by Henning *et al.* (1989).

A second assumption made in using chickens to determine the digestibility of undegraded protein is that all protein, whether rumen degradable or undegradable, when fed to ruminants is digested in the same manner by chickens. For example, if certain proteins are highly rumen degradable whilst others are highly rumen undegradable, this difference, due to structural differences between the proteins *per se*, may be manifested in the digestibility of the protein in the gastro-intestinal tract of the chicken.

Although the applicability of this method is questionable, a strong correlation was observed in this trial between the digestibility of true protein (determined based on the digestibility of amino acids in poultry) and effective degradability of protein in the rumen ($r = 0.75$, $P = 0.03$). The results obtained as described above are similar to those obtained in research conducted by Henning *et al.* (1989). The five diets formulated by Henning *et al.* (1989) varied in UDP and each contained one of the protein-rich feedstuffs: sunflower oilcake, cottonseed oilcake, maize gluten meal and fish meal. Although there was a highly significant correlation between true nitrogen digestibility determined in chickens and that determined in the small intestine of sheep for four of the diets, this was not the case when the diet containing maize gluten was included in the analysis. Further consideration needs to be given to the evaluation of feed supplements *per se* or to the evaluation of these samples as part of a complete ration. Evaluation of supplements as part of a complete ration may pose problems as variation in the composition of the ration will affect the degradability of the supplement as the rumen microbial ecosystem adapts to different conditions. In contrast, evaluation of supplements *per se* may not be valid for the same reasons. The variation in basal diet will also have an effect on degradation and will influence the degradability parameters obtained.

The validity of this method to determine RUP may be questioned, although the results obtained indicate that it may be possible to use chickens to obtain a reasonable estimate of true protein digestibility in the absence of suitably prepared ruminants. However, the observations of Henning *et al.* (1989) regarding maize gluten as discussed above raise questions about the suitability of this method of undegradable protein determination for all protein sources.

Digestible RUP was calculated as the difference between the digestibility of true protein (in cockerels) and the effective degradability of protein in the rumen. Results indicate that most plant proteins are largely rumen degradable in sheep (and thus contain 2.8 to 12.6% of protein as digestible RUP), but most supply moderate levels of digestible RUP (17.1 to 27.9%) when fed to dairy cattle.

2.6 Conclusion and implications

This study showed that, among the protein sources investigated, there are significant differences in intercept values and time lag for dry matter degradation. Potential degradability and rate of degradation are also significantly different among protein sources. Intercept values and rates of degradation were significantly different for nitrogen degradation while potential degradability and time lag were not. Lupins had the highest effective degradability for both dry matter (792 g kg^{-1} in sheep) and nitrogen (844 g kg^{-1} in sheep) while canola showed the lowest effective dry matter degradability (433 g kg^{-1} in sheep) and copra the lowest effective nitrogen degradability (678 g kg^{-1} in sheep). Sunflower oilcake had the highest digestible rumen undegradable protein (126 g kg^{-1} nitrogen in sheep) while cottonseed oilcake had the lowest (83 g kg^{-1} nitrogen in sheep).

A reliable and easy method of post ruminal digestibility is still not available. The use of poultry as a means of estimating digestibility has been suggested, but some adaptations are needed to improve validity:

a) Exposing the plant protein supplements to rumen degradation for a given period of time, similar to that which the supplement would under normal circumstances spend in the rumen, prior to the determination of degradation in poultry.
b) Investigation of the rumen degradability and rumen undegradable protein digestibility in poultry of both the individual plant protein sources as well as investigation of these parameters for complete feeds comprising one or more of the plant protein supplements. At the same time the effect of the basal ration and correlated rumen parameters will also affect the extent of degradation of the plant protein supplements.
c) Where possible a comparison of the adapted method described in a) above, should be made with duodenally fistulated ruminant animals, in order to establish the reliability and accuracy of the adapted method which uses poultry to establish an estimate of the supply of digestible RUP to the host animal.

Table 2.1: The chemical composition (g kg^{-1} DM) of eight plant protein sources used in the degradability study.

Protein Supplement	Dry Matter	Organic Matter	Ash	Crude Protein	Fat
Canola meal	932.0	952.2	47.9	254.9	362.1
Copra meal	925.0	902.1	98.0	245.7	191.2
Cottonseed oilcake	938.0	933.7	66.3	394.1	87.1
Defatted maize germ meal	911.0	959.1	40.9	132.7	10.9
Lucerne hay	920.0	868.2	131.9	222.6	15.8
Lupin seeds	942.0	964.2	35.8	376.3	119.6
Soya oilcake	918.5	936.9	63.2	497.7	44.3
Sunflower oilcake	927.5	926.9	73.1	418.0	16.7

Table 2.2: The DM degradation parameters and effective DM degradability (ED_{DM}) of eight plant protein sources calculated for sheep, beef and dairy cattle.

	Wash Values	Degradation Parameters [a]					Effective Dry Matter Degradability ($g\ kg^{-1}$)		
	($g\ kg^{-1}$)	($g\ kg^{-1}$)			(h^{-1})	(h)			
Protein Supplement		*a*	*b*	*PD*	*c*	*TL*	**Sheep**	**Beef**	**Dairy**
Canola meal	28.16	271	630	901	0.0859	0.2	433	391	353
Copra meal	53.62	475	452	927	0.0366	4.0	656	576	511
Cottonseed oilcake	56.42	525	444	969	0.0215	5.2	636	559	498
Defatted maize germ meal	56.50	558	447	1005	0.0247	0.7	680	600	535
Lucerne hay	57.68	377	426	803	0.0578	11.0	604	534	475
Lupin seed	66.84	625	379	1004	0.0633	2.0	793	719	649
Soya oilcake	57.60	554	521	1075	0.0397	1.2	772	680	604
Sunflower oilcake	34.05	329	500	829	0.0598	0.5	615	539	477
SED [b]		18.50	1.66	2.17	2.11	3.90			
P-value [c]		***	NS	*	*	***			

[a] ***a***, intercept; ***b***, potentially degradable component; ***c***, rate of degradation; ***PD***, potential degradability; ***TL***, time lag.

[b] **SED** = standard error of difference.

[c] **P-value** : NS, P>0.05; *** P<0.001; ** P<0.01; * P<0.05.

Table 2.3: The nitrogen degradation parameters and effective nitrogen degradability (ED_N) of eight plant protein sources calculated for sheep, beef and dairy cattle on a dry matter basis.

	Wash Values	Nitrogen Degradation Parameters [a]					Effective Nitrogen Degradability		
	($g\ kg^{-1}$)	($g\ kg^{-1}$)			(h^{-1})	(h)	($g\ kg^{-1}$)		
Protein Supplement		***a***	***B***	***PD***	***c***	***TL***	**Sheep**	**Beef**	**Dairy**
Canola meal	33.40	364	576	940	0.1311	-0.4	781	712	649
Copra meal	55.53	518	435	953	0.0352	2.4	679	600	534
Cottonseed oilcake	83.27	774	247	1021	0.0245	-3.8	774	709	645
Defatted maize germ meal	60.26	614	337	951	0.0530	-0.6	741	671	606
Lucerne hay	54.62	543	440	983	0.0810	0.1	787	712	643
Lupin seed	94.18	937	61	998	0.0625	1.6	844	794	732
Soya oilcake	56.91	554	463	1017	0.0550	0.6	774	692	620
Sunflower oilcake	46.80	470	527	997	0.0807	-0.01	787	706	635
SED [b]		17.29	6.46	1.55	4.31	2.17			
P-value [c]		***	***	NS	***	NS			

[a] ***a***, intercept; ***b***, potentially degradable component; ***c***, rate of degradation; ***PD***, potential degradability; ***TL***, time lag.

[b] **SED** = standard error of difference.

[c] **P-value** : NS, P>0.05; *** P<0.001; ** P<0.01; * P<0.05.

Table 2.4: Total digestible protein determined using adult cockerels and digestible RUP for the eight plant protein sources, given on a dry matter basis.

Protein supplement	Total Digestible Protein[a] ($g\ kg^{-1}$)	Digestible RUP ($g\ kg^{-1}$)		
		Sheep	Beef	Dairy
Canola meal	870.3	89.3	157.9	221.5
Copra meal	781.6	103.2	182.3	248.0
Cottonseed oilcake	858.1	83.5	149.2	213.5
Defatted maize germ meal	854.5	113.5	183.8	249.3
Lucerne hay	814.6	28.5	102.7	171.7
Lupin seeds	931.5	87.5	138.3	200.4
Soya oilcake	873.0	98.6	181.3	253.8
Sunflower oilcake	913.7	127.0	207.5	279.3

[a] Total digestible protein was determined for adult cockerels

Chapter 3

The effect of basal roughage quality on the voluntary feed intake, feeding behaviour and rate of passage of long and short particles from the rumen of South African Mutton Merino sheep

3.0 Abstract

The effect of increasing the nitrogen supply to the rumen microbes by improving the quality of roughage supplied to the host animal was investigated in this trial by substituting the basal roughage (poor quality veld hay) with varying amounts of lucerne hay. Five treatments of 100% hay, 75% hay and 25% lucerne, 50% hay and 50% lucerne, 25% hay and 75% lucerne and 100% lucerne were used. In this way the crude protein (CP) was increased as the lucerne proportion of the ration increased and neutral detergent fibre (NDF) and crude fibre (CF) concentrations were decreased. The effect of varying roughage quality on the VFI, feeding behaviour and rate of passage of both long and short particles from the rumen of South African Mutton Merino sheep was investigated, using 25 sheep blocked according to weight and randomly assigned to one of five treatments in a completely randomised block design. Dry matter (DM), CP, CF and NDF intakes increased significantly ($P<0.001$, $P<0.001$, $P<0.05$ and $P<0.05$, respectively) as lucerne inclusion level in the ration was increased. The effects of both diet and particle size on the rate constant (k1, indicative of the rate of passage of particles) were highly significant ($P<0.001$). The particle size diet interaction was significant ($P=0.05$). Lucerne inclusion levels in the diet had no effect ($P>0.05$) on ruminating time. Increasing the lucerne inclusion level in the ration resulted in a significant decrease in total eating time and time spent ruminating per kilogram NDF consumed ($P<0.01$) and time spent eating per kilogram dry matter consumed ($P<0.05$). Sheep weight had a highly significant ($P<0.001$) effect on time spent eating per kilogram of dry matter consumed and time spent ruminating per kilogram of NDF consumed. There was a significant increase in ruminating time ($P<0.05$) as sheep weight increased. These results indicate that interactions among feed factors, such as particle size and feed composition, will affect the rate of clearance of ingested roughage particles from the rumen and, consequently, the level of roughage intake. The effect of animal factors and chewing effectiveness warrant further investigation.

Keywords: roughage quality, particle passage, particle size, feed intake, ruminant, protein, fibre

3.1 Introduction

Many animal production systems, in particular intensive systems, require high feed intakes to ensure high outputs of animal products (Campling and Lean, 1983). However, in order to be cost-effective the production of ruminant livestock relies heavily on highly fibrous diets containing large amounts of relatively cheap forages, particularly those derived from grasslands, rangelands, forage crops and crop residues (Jarrige, 1989). The potential of a forage for use in ruminant production is essentially a function of the quantity of digestible organic matter (OM) or net energy consumed when the forage is fed *ad libitum*, as the sole food (Jarrige, 1989). Feeding value therefore depends on the digestibility of OM and on the voluntary intake characteristics of the forage (Jarrige, 1989). However, the voluntary intake of diets containing a high proportion of roughage is frequently limited by the capacity of the reticulo-rumen and by the rate of disappearance of digesta from the reticulo-rumen (McDonald *et al.*, 1981), thus somewhat restricting the feeding value of the forage. The clearance rate of digesta depends, in turn, on the rate of breakdown of ingested feed material in the reticulo-rumen, by both mechanical and microbial processes (Campling, 1970). Supplementation with a protein source has been shown to enhance the utilisation of poor quality feeds through improvement of the rumen ecosystem (O'Donovan, 1983; cited by Abule *et al.*, 1995). Thus, where high roughage diets, inherently low in nitrogen are fed, supplementation with a protein-rich feed or a non-protein nitrogen source is intended to alleviate any inherent nitrogen deficiency and increase the intensity of microbial fermentation of digesta (Wilson and Kennedy, 1996).

Numerous studies have shown that particles greater than a certain size rarely leave the rumen and this has led to the idea of a critical particle size (Poppi *et al.*, 1980). Dulphy and Demarquilly (1994) suggested that only particles of less than approximately 1 mm in size can leave the rumen of sheep, whilst Wilson and Kennedy (1996) added that the probability of particle passage from the reticulo-rumen is an inverse function of particle size, regardless of the forage diet. Forage form has also been shown to affect the actual rate of clearance of fibre from the rumen (Wilson and Kennedy, 1996). However, any strategy

that serves to increase the rate of particle size reduction will also increase the rate at which digesta passes from the reticulo-rumen to the abomasum. For example, fibrous feeds of low digestibility are degraded slowly because the rate at which physical comminution occurs is slow (Wilson and Kennedy, 1996). Supplementation of a roughage diet with a concentrate will help to increase microbial activity and thereby particle degradation (McDonald *et al.*, 1981). However, as Galyean and Goetsch (1993) noted, the effect of concentrate supplementation on roughage degradability is dependent on the amount and type of concentrate fed. Below levels of 300 g of concentrate per kilogram of feed, depression of ruminal fibre digestion is not severe in dairy cattle (Hoover, 1986). However, feeding higher levels of a high-energy supplement will result in a decrease in ruminal pH and a subsequent decrease in the growth of fibrolytic bacteria (Galyean and Goetsch, 1993) and thus the amount of fibre digested by the rumen microbes (Webster, 1993).

The influence of roughage quality and, in particular NDF content, on the time spent ruminating will further influence the rate of particle breakdown and roughage quality is thus a critical factor in determining the rate of passage of ingested feed material through the digestive tract. Kennedy and Doyle (1993) noted that some diets have a high capacity to stimulate rumination, with rumination time increasing as particle size is increased. They further noted that most of the large particle breakdown in the rumen occurs as a result of ruminative chewing.

This study was designed to investigate the effect of basal roughage quality (in terms of crude protein (CP) and fibre content) on the rate of passage of ingested feed material from the rumen. Furthermore, a comparison between large and small particle passage rates would give an indication of the rate at which large particles are broken into smaller particles capable of leaving the rumen. It was expected that roughage quality would affect the length of time the animal spends ruminating, the total feed intake and the passage of ingested feed from the rumen. It was also expected that particle size of ingested feed would affect the time taken for ingested feed to exit the rumen.

3.2 Materials and methods

Animals and Diets: A total of 25 South African Mutton Merino sheep (mean body weight ± SD 43.6 ± 11.5 kg, ranging from 28.0 kg to 64.5 kg) and having two to four teeth were

blocked by weight into five groups. This large variation in animal size was deliberately intended to determine whether the body weight of the animal had an effect on the rate of particle outflow from the rumen. The sheep in each group were randomly assigned to five dietary treatments in a completely randomised block design. Animals were housed in individual pens (70 cm wide, 150 cm long and 90 cm high) with slatted wooden floors and allowed *ad libitum* access to both feed and water.

The five diets fed in this trial were designed to provide a range of roughage qualities, without introducing other factors, which might affect digestibility or passage rates. The diets thus consisted only of veld hay and lucerne hay, mixed in varying proportions. The hay to lucerne ratio and nutrient profiles of the five diets are given in Table 3.1.

Intake: The weight of feed offered to each sheep on a weekly basis was recorded and any remaining feed was weighed back at the end of the week. Weekly intake was determined by difference and averaged over the feeding period to give an average daily feed intake.

Passage rate studies (marker administration and sample collection): In order to determine the passage rate of long and short particles, veld hay was mordanted with chromium as per the method described by Uden *et al.* (1980).

Approximately two kilogram of sun-dried hay was weighed and then washed to remove dirt. This hay was the same as that used in Treatment 1. A representative sample from each of the five experimental diets was not used. Potassium dichromate ($K_2Cr_2O_7$), representing 33% of the sun-dried weight of the material to be mordanted, was spread on the material and enough water added to submerge the substrate. The container was covered, the lid secured, and then placed in an oven at 100°C for 48 hours. After baking, the mordanted material was thoroughly washed until the water was only faintly coloured. The mordanted feed was then suspended in water and ascorbic acid, equivalent to one half of the sun-dried weight of the fibrous material, added. The fibrous material suspended in ascorbic acid solution was left to stand for 1 hour. Finally, the mordanted material was washed several times in tap water and dried at 65°C for 48 hours, after which it was stored in a sealed polythene bag at room temperature until required.

One kilogram of the mordanted material was milled through a 2.0-mm sieve using a laboratory mill (Hippo Mill) and then sieved through a series of five different mesh sizes (>2.0, 2.0, 1.2, 1.0 and 0.5 mm). The particles remaining on the 1 mm screen size were collected and this material was used for the fine particle phase of the passage studies. The remaining kilogram of mordanted hay was not milled and was used to investigate the rate of passage of long particles.

Rate of passage of short particles: After a seven-day period during which animals were allowed to adapt to the respective experimental diets, each animal was fed 10 g of mordanted feed (1.0 mm < particle size < 1.2 mm) prior to consumption of the daily feed allowance. The mordanted material was mixed with a small amount of molasses in order to encourage consumption of the mordant within the first 30 minutes of offer. Mordanted material not consumed within 30 minutes was removed from feed bins and the animals allowed access to their feed. Collection of faeces samples began four hours later with grab samples being collected from the rectum at 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 61, 69, 77, 85, 95, 108, 120, 132 and 144 h after dosing. The collected faeces were dried at 60°C for 48 hours, ground for one minute using a laboratory mill (IKA cutting mill) and stored at ambient room temperature, in small plastic vials, pending chromium determination.

Rate of passage of long particles: After allowing a seven-day period for clearance of any remaining chromium from the gut of the sheep, each animal was fed 10 g of un-milled mordanted feed prior to the consumption of the daily feed allowance. The same procedure as described for the short particles was followed.

Feeding behaviour: The feeding behaviour patterns of the sheep were investigated in the period allowed for clearance of chromium from the gut of the sheep, i.e. between the two feedings of mordanted material. The measurements of feeding behaviour taken included duration of feeding, drinking and rumination. These measurements were taken on three different occasions for periods of 24 hours at a time. Each 24-hour period was divided into hour-long periods, which, in turn, were divided into five-minute segments, and the activity of individual sheep classified during these five-minute periods as one of the following activities: eating, drinking or ruminating.

Laboratory analysis: DM, OM, nitrogen and fat (ether extract) were analysed as described in Chapter 2.2. Crude fibre was determined using AOAC (1990) methods 978.10. Neutral detergent fibre (NDF) was determined according to the method described by Van Soest *et al.* (1991).

Faecal samples collected for the determination of particulate passage rate were analysed for chromium concentration by atomic absorption spectrometry according to the method described by Costigan and Ellis (1987).

3.3 Data derivation and statistical analysis

Intake studies: Variation in intakes of DM, CP, CF, and NDF as a result of diet were analysed using the GLM procedure (SAS, 1987) according to the model:

$$Y_{ij} = \mu + T_i + SWT_j + e_{ij},$$

where Y_{ij} is the individual observation, μ is the overall mean, T_i the average effect of the i^{th} treatment and SWT_j the covariate effect of the j^{th} sheep weight. The error term, e_{ij}, is assumed to be independently and normally distributed. The contrast option of the GLM procedure was used to determine whether there were significant linear and quadratic effects of the lucerne inclusion in the diets on intake. A spreadsheet package, Microsoft Excel (Microsoft Corporation, 1997), was used to determine regression coefficients.

Passage rate studies: Regression of the natural log of faecal chromium concentration in the decreasing phase of the chromium concentration was used to determine the rate constant (k1, indicative of the rate of passage of particles). The decreasing phase occurred 48 h after the feeding of the chromium-mordanted marker. The rate constant was determined for all sheep and for both long and short particle lengths.

Differences among diets were analysed using the GLM procedure (SAS, 1987) according to the model:

$$Y_{ijk} = \mu + T_i + Psize_j + T_i.Psize_j + SWT_k + e_{ijk},$$

where Y_{ijk} is the individual observation, μ is the overall mean, T_i the average effect of the i^{th} treatment, $Psize_j$ the average effect of the size of the j^{th} particle, $T_i.Psize_j$ the average effect of the interaction of the i^{th} treatment and the j^{th} particle size and SWT_k the covariate effect of the k^{th} sheep weight. The error term, e_{ijk}, is assumed to be independently and normally distributed. The contrast option of the GLM was used to determine whether the treatment by particle size interaction had a significant linear or quadratic effect. The least square means (LSmeans) for passage rate on each diet were plotted with the aid of a spreadsheet package, Microsoft Excel (Microsoft Corporation, 1997).

Feeding behaviour: Differences among diets were analysed using the GLM procedure (SAS, 1987) according to the model:

$$Y_{ij} = \mu + T_i + SWT_j + e_{ij},$$

where Y_{ij} is the individual observation, μ is the overall mean, T_i the average effect of the i^{th} treatment and SWT_j the covariate effect of the j^{th} sheep weight. The error term, e_{ij}, is assumed to be independently and normally distributed. Time was introduced as a repeated measure. The contrast option of the GLM procedure was used to determine the linear and quadratic effects of the lucerne inclusion levels on feeding behaviour. The Pdiff-option of the LSmeans statement of the SAS programme was used to perform a pair-wise comparison of the LSmeans. The LSmeans for feeding behaviour on each diet were plotted with the aid of a spreadsheet package, Microsoft Excel (Microsoft Corporation, 1997).

3.4 Results

Dietary compositions are given in Table 3.1 and, as expected, CP and ash contents increased as the proportion of lucerne in the diet increases. At the same time the DM, CF, NDF and fat content of the diets decreased as the proportion of lucerne in the diet increased.

Intake studies: Table 3.2 gives the average daily intake of DM, CP, crude fibre and NDF for each diet, for the duration of the passage rate studies. The proportion of lucerne in the

diet had a significant effect on DMI ($P<0.001$), with a linear increase in DMI of 5 g/day per unit increase in lucerne proportion of the diet. The effect of diet on CP intake was highly significant ($P<0.001$) with a linear increase of 2.450 g/day per unit increase in lucerne content of the diet. The effect of diet on crude fibre ($P<0.05$) and NDF ($P<0.05$) intake was also significant. Crude Fibre and NDF intakes increased by 1.2 g/day and of 2.4 kg/day, respectively per unit increase in the lucerne content of the diet.

The effect of sheep starting weight on all intake variables was highly significant ($P=0.001$), with a linear increase in intake of 36 g DM per kilogram sheep weight as sheep starting weight increased.

Passage rate studies: Table 3.3 gives the Least Square means (Lsmeans) for k1 (rate constant as per Grovum and Williams (1973)), determined for long and short particles in sheep on the five different diets. The effect of diet on the rate constant (indicative of the rate of passage of particles) was highly significant ($P<0.001$), as was the effect of particle size ($P<0.001$). The interaction of particle size and diet was significant ($P=0.05$). Figure 3.1 shows the relationship between particle size and lucerne proportion of the diet on the rate of passage of particles from the rumen. The effect of sheep weight on the passage rate of particles from the rumen was not significant.

Feeding behaviour studies: Table 3.4 gives the mean average daily intake (for the three days over which feeding behaviour was observed) of DM, CP, crude fibre and NDF for each of the five diets. The effect of treatment on the intake of CP was highly significant ($P<0.001$), with a linear increase in intake as the proportion of lucerne in the diet increased. A similar trend was noted for the intake of DM ($P<0.05$). There was no significant effect of diet on the crude fibre or NDF intake. As expected, the starting weight of the animals had a highly significant ($P<0.001$) effect on intake, regardless of which diet the animals were fed. Table 3.5 gives the mean and standard deviation of the five groups of sheep receiving the five different experimental diets.

The mean time that sheep spent eating and ruminating is given in Table 3.6. Treatment had no significant effect on ruminating time or time spent ruminating per kilogram of NDF consumed. However, treatment had a significant effect on total eating time ($P<0.01$) and on time spent eating per kilogram dry matter consumed ($P<0.05$). Total eating time decreased

by 1.064 minutes/day per unit increase in lucerne proportion of the diet. Eating time per kilogram dry matter consumed also decreased linearly by 1.5 minutes/kg per unit increase in lucerne proportion of the ration.

The starting weight of the animals had no significant effect on total eating time, although sheep weight had a highly significant ($P<0.001$) effect on time spent eating per kilogram of dry matter consumed and time spent ruminating per kilogram of NDF consumed. The effect of sheep weight on total ruminating time was also significant ($P<0.05$). The effects of diet on eating and ruminating time are shown graphically in Figures 3.2 and 3.3 respectively.

3.5 Discussion

Intake studies: The increase in feed (DM) intake observed as the proportion of lucerne included in the diet (feed quality) increased, is comparable to the observations of Campling *et al.* (1961), who showed that dairy cows given medium quality hay consumed more than double the amount consumed by cows fed a diet of poorer quality oat straw. In this trial, sheep fed a diet comprising 100% lucerne consumed on average 1.47 times as much DM as those fed a diet comprised solely of veld hay. CP intake was 5.97 times higher in the animals fed 100 % lucerne as compared to those fed veld hay, and crude fibre intake 1.26 times greater. Campling *et al.* (1961) further illustrated that this difference in intake is linked directly to the relative rates of disappearance from the reticulo-rumen of digesta derived from the two feeds. This relationship is to be expected, as an increase in the clearance rate of ingested material will allow a greater intake of feed, as more space becomes available in the rumen.

In contrast to the observations of Campling *et al.* (1961), rates of passage (of long and short particles) in this trial increased as the proportion of hay in the diet increased. In the trial conducted by Campling *et al.* (1961), passage rate increased when animals were fed good quality hay as compared to poor quality hay. The observed increase in rate of passage in this trial might be expected to lead to an increased rate of clearance and thereby increased feed intake as discussed above. Increased feed intake was not observed however, in those sheep fed high hay diets and which showed increased rate of passage of feed particles. Although unexpected, this observation can probably be explained when one

considers that passage of undigested material (solid particles) from the rumen is not the only manner by which ingested material leaves the rumen. If normal microbial digestion of the ingested feed material is occurring then, large portions of the highly digestible nutrients contained in the feed, such as CP and readily digestible sugars such as pectins (which are found in lucerne), may escape from the rumen by either solubilisation, or through the rumen wall as volatile fatty acids products of microbial digestion. In addition, lucerne contains some starch, which is also readily digestible. This starch, along with the higher levels of CP present in the lucerne, may result in greater microbial fermentation of the ingested fibrous feed material and this would mean that a smaller percentage of ingested fibre would require comminution and clearance from the rumen. Whatever the means of clearance from the rumen, this clearance would result in space becoming available in the rumen for increased feed intake. In this trial, therefore, it is possible to explain the unexpected increase in lucerne intake (in the light of decreased passage rate) as compared to hay, simply by the increased clearance of the nutritional components of lucerne from the rumen by absorption, fermentation and digestion.

Passage rate studies: It was expected that the mordanted material fed un-milled (as large particles) would take longer to pass through the rumen, as increased comminution would need to occur in comparison to the milled material. However, in Treatments 1 to 4, un-milled material was observed to pass through the rumen at a faster rate than milled material. This observation was unexpected, but can probably be explained by the fact that the mordanted material was offered as part of the feed and thus was ingested orally rather than dosed directly into the rumen of the animals via a rumen cannula. Mordanted feed particles, particularly un-milled particles, would probably undergo a considerable amount of ingestive chewing. Furthermore, long particles are likely to undergo considerably more ruminative chewing post-ingestion than smaller particles. Lowrison (1982), cited by Kennedy and Doyle (1993) noted that ruminative chewing involves selection of particles of a given size to be regurgitated, which is emphasised by the large particle content of the up-bolus (Kennedy and Doyle, 1993). As a result, large mordanted particles entering the rumen would more likely be aspirated for ruminative chewing than smaller ones and would thus also be ground finer than the originally small particles. It is then likely that, because of ingestive chewing, ruminative chewing and the process of mordanting (which could render the plant material unusually brittle), larger particles were rapidly broken into smaller ones capable of passing through the reticulo-omasal orifice. Those particles aspirated to the

mouth for rumination would then be swallowed as part of the down bolus. Wyburn (1980) suggested that the probability of passage of particles swallowed as part of the down bolus may be greater than that of particles remaining in the reticulo-rumen as they are deposited close to or in the reticulum. This may help to explain why the large mordanted feed particles moved through the digestive tract of the animals faster than the smaller particles.

The bulk density (mass: volume ratio) of particles passing through the rumen will also play a role in their rate of passage. Kennedy and Murphy (1988) illustrated that there is a close relationship between reticulo-rumen retention time and the specific gravity of various feedstuffs of different lengths. This was further demonstrated by Ellis *et al.* (1991), cited by Kennedy and Doyle (1993) who identified changes in particle buoyancy as the major process resulting in an increased retention time from that expected if mass action particle kinetics is applied. In contrast, Kennedy and Doyle (1993) noted that the functional specific gravity of particles is variable after ingestion and that the functional specific gravity of particles changes as they undergo communition, digestion and fermentation. In this trial, both the long and short particles were mordanted in the same manner and thus differences in density as described by Ehle (1984) should not apply. At the same time it is expected that the longer particles would become trapped more readily in the fibrous mat whilst the smaller particles would be able to move more freely through the rumen.

Another possible explanation for the observed anomaly in passage rates may be that a single mordant was used for all the diets under investigation, that is, the mordant used was simply a sample of ration one, which contained only veld hay. As a result the mordanted fibre particles used to study the rate of passage were not the same as the diets studied and conceivably could have exhibited a different pattern of motion within the rumen compared to the dietary roughages *per se*. For example, the likelihood of suspension of a hay particle with in the rumen may be different to that of a lucerne particle as a result of different densities of the particles themselves. This would mean that in rations two, three and four the passage rate of only one component of the basal roughage was investigated and this effect would have been compounded for the fifth ration, which was comprised solely of lucerne. However, Vega and Poppi (1997), using ytterbium labelled material, concluded that particles of legume or grass of the same size behave similarly within a diet type. An alternative option to be investigated in future, similar trial work would be to mordant samples taken from each basal diet and to feed these mordants according to the different

basal diets in use. This method would provide a better picture of the movement of particles representative of the complete ration, from the rumen. In this way, if lucerne particles are expelled from the rumen at slower rates than hay particles, traces of the lucerne mordant, would remain in the rumen longer than the hay mordant and vice versa. A second, and possibly more useful alternative, which would allow a more complete picture of the differences in legume and hay particle passage from the rumen, would be to label the two different forages (lucerne and hay) with different markers. For example hay could be labelled with chromium whilst the lucerne could be labelled with ytterbium (Vega and Poppi, 1997). The labelled forages could then be mixed in such a way as to match the composition of the basal roughage and fed in this manner. Collected faecal samples could then be analysed for both ytterbium and chromium and the different passage rates of the hay and lucerne for each of the basal roughages compared.

It was observed in this study that in the case of small particles the rate of passage decreased as the proportion of lucerne included in the diet increased. Previous experiments suggested that as lucerne proportion (and therefore quality) increases, rate of passage would also increase (Campling *et al.*, 1961). Notably though, Wyburn (1980) pointed out that most intensive studies conducted on movement of plant particles in the rumen have been carried out using ruminants fed good quality forages and that conclusions drawn as a result may need modification when considering highly fibrous forages. In this trial, rate of passage decreased as the proportion of lucerne in the diet increased.

A second compounding factor related to feed intake might also play a role in the rate of passage of the particles. Ehle (1984) and Tamminga (1993) noted that, as the level of feed intake increases, passage rate also increases, although the influence of feeding level on passage rate varies between diets and rumen ingesta components (Tamminga, 1993).

Wilson and Kennedy (1996) suggested that the animal has the capacity to control the two competitive processes of reduction in particle size and the rate of passage of small particles and these two processes have a direct effect on fermentation time. Wilson and Kennedy (1996) thus further suggested that the animal therefore has the ability to respond to different dietary situations. In this way an animal faced with a scarcity of feed may be able to increase the retention time of feed (by decreasing rumination or the force and frequency of ruminal contractions) and thereby maximise digestive recovery of nutrients. On the

other hand, an animal faced with a large supply of poor quality roughage may attempt to increase the daily yield of nutrients by clearing partially digested feed from the rumen at an increased rate through greater rumination effort. However, these researchers noted that the achievement of this optimal situation may be limited by the capacity of the animal to process feed particles. Taking these observations into account, it is possible that when fed the poor quality hay, the animals had the capacity to increase the clearance rate from the rumen in an attempt to improve their daily nutrient intake.

It may also be possible to explain the increased rate of passage of small particles in those animals fed larger amounts of hay as compared to those fed a greater proportion of lucerne, in terms of the fibrous raft and effective fibre. Physically effective fibre is considered to be that which stimulates ruminative chewing (Varga, 1997) and is often referred to as "scratch" in feedlot diets and is directly related to NDF (Varga, 1997). As the NDF proportion of the diet is decreased, so ruminative chewing decreases allowing for an increased fibrous raft within the rumen. This fibrous raft traps smaller particles and thus prevents or impedes their escape from the rumen. In addition, ruminative chewing is thought to enhance the peristaltic movements of the rumen (Sutherland, 1988) and this would aid the flow of small particles suspended in the rumen fluid through the rumen and into the lower digestive tract. Conversely, reduced ruminative chewing would result in a larger rumen mat, fewer peristaltic movements and a decrease in the rate of passage of small particles from the rumen. Although there was no significant difference in rumination time among the five diets, there was a significant effect of treatment on time spent ruminating per kilogram of NDF.

The use of a legume (lucerne) as a means of improving the quality of the veld hay may have complicated the results observed in this trial. Rumination patterns of legumes and grasses differ significantly as do the chewing and comminution patterns. Kennedy and Doyle (1993) noted that significant rumination is required to reduce the length of grass leaves whereas rubbing movements in the reticulo-rumen may cause comminution of legume leaves. Furthermore, these researchers noted that lucerne leaf particles accounted for less than 5% of the digesta particles ruminated into the mouth despite a dietary content of 43 to 65%. This is in contrast to observations by Kelly and Sinclair (1989), cited by Kennedy and Doyle (1993), who noted that the leaf component of the up bolus was only slightly less than that of the dietary material in animals fed a perennial ryegrass and

meadow hay (90% grass). These observations suggest that, when exposed to the same degree of ruminative chewing, lucerne would undergo a greater degree of comminution than would hay particles. Thus, although rumination time observed in this trial was not significantly affected by the diet, it is possible that the animals fed a diet of 100% lucerne were able to degrade and digest a greater proportion of the feed consumed than the animals fed a diet consisting entirely of veld hay simply as a consequence of the increase rate of fragmentation of lucerne hay particles as compared to those of veld hay.

In addition to the interactions between legumes and grasses discussed above and under the section on intake studies, using a combination of lucerne (a legume hay) and veld hay (a combination of grasses) may have brought additional factors into play, not only through differences in plant structure and morphology (i.e. legumes versus grasses) but may also have added an extra dimension as a result of nutrient composition of the forage. This could include the increase in fermentability and ruminal clearance as rumen microbes were provided with enhanced ruminal conditions (increased carbohydrate and nitrogen levels), which encouraged improved microbial fermentation. Lucerne, for example, has a high pectin content and this may have helped to increase the amount of readily fermentable carbohydrate available for microbial fermentation.

Feeding behaviour: It was originally expected that diet quality would affect both eating and ruminating time and considering the effect of basal roughage quality on the rate of passage of the fine particles, it was expected that ruminating time would be considerably increased as the proportion of hay and thus NDF in the diet increased. Table 3.6 illustrates significant decreases in time spent eating and time spent eating per kilogram of DM consumed. Whilst feeding activities were monitored intakes increased significantly as lucerne content of the diet increased. Contrary to expectations, eating time did not increase with the observed increase in intake. Again, this is probably a result of the different fragmentation patterns observed for lucerne and grass hay during ingestive chewing. As lucerne hay is more readily fragmented during ingestive chewing, less ingestive chewing is required prior to swallowing so that more feed is consumed per unit time. Ulyatt *et al.* (1986) summarised work relating to the contribution of chewing during eating to ruminal clearance. These researchers noted that, compared to other forages perennial ryegrass causes a higher number of chews per gramme of DM, a lower rate of eating and a higher proportion of particles retained on a 4.0 mm sieve. They attributed this to the greater

tensile strength of the perennial ryegrass leaves. In the same paper Ulyatt *et al.* (1986) presented a table from work by John and Reid (unpublished). Here various parameters relating to chewing during eating are detailed for three fresh herbages and two hays (lucerne hay and meadow hay). Although the lucerne hay causes a higher number of chews per gramme of DM and a lower intake rate per minute than meadow hay, total intake of lucerne was slightly greater than for meadow hay but the proportion of dietary DM retained on a 4.0 mm sieve was only 19.2 % as compared to 27.6 % for meadow hay. Of large particle DM 37.1% was reduced to less than 1.0 mm by chewing during eating in the case of lucerne as compared to 34.6 % in the case of meadow hay. Measurement of time spent eating was not categorised on the basis of ingestive chewing and actual consumption, nor were parameters such as those observed by John and Reid (unpublished) measured in this trial. It would be difficult to determine, simply by observation, the precise length of time spent on ingestive versus ruminative chewing but some means of quantification may be useful should a similar trial be conducted in future.

3.6 Conclusions and implications

This investigation showed that increasing the level of protein in the basal roughage through substitution of veld hay with lucerne increased the DM, CP, CF and NDF intakes significantly. It was further shown that both diet and particle size, have a significant effect on the rate of passage of particles from the rumen. Particle size by diet interactions was also significant.

Lucerne inclusion levels in the diet do not affect ruminating time. However, increasing the lucerne level resulted in a significant decrease in total eating time, time spent eating per kilogram dry matter consumed and time spent ruminating per kilogram of NDF consumed. Sheep weight has a significant effect on time spent eating per kilogram of dry matter consumed, time spent ruminating per kilogram of NDF consumed and total ruminating time.

These results indicate that interactions among feed factors will affect the rate of clearance of ingested roughage particles from the rumen and consequently the level of roughage intake. The effects of animal factors as well as chewing effectiveness warrant further investigation. Further work conducted should consider the compounding effect of

comparing legume hay to grass hay and the differences in the fragmentation patterns of the two. The effect of increased roughage quality or decreased NDF content on the rumen pH and the rumen microbial population has not been investigated in this trial, nor has there been extensive investigation into the effect of animal factors such as size and chewing effectiveness. These factors will not only have an effect on the rate of clearance of ingested material from the rumen, but will also be affected by the form and quality of the ingested forage. As such, these aspects warrant investigation. Supplementation of roughage diets is a common practice and the effect of high-energy concentrate supplementation on the rates of particle passage also warrants investigation.

Table 3.1: The physical composition, DM (g kg^{-1} as fed) and CP, CF, NDF, fat and ash (g kg^{-1} DM) content of the five experimental diets.

Diet	1	2	3	4	5
% Hay	100	75	50	25	0
% Lucerne	0	25	50	75	100
Dry Matter	915.8	911.8	907.9	903.9	900.0
Fat	26.8	23.2	19.6	16.0	12.4
Ash	59.8	65.9	71.9	78.0	84.1
Crude Protein	46.4	81.0	115.6	150.2	184.8
Crude fibre	420.9	403.9	386.9	369.9	352.8
NDF	786.7	757.9	729.1	700.3	671.5

Table 3.2: The mean average daily intake (over the entire trial period) of DM (g) and CP, CF and NDF (g DM) for each of the five diets.

Diet	% Lucerne	DM intake (g/day)	CP intake (g/day)	CF intake (g/day)	NDF intake (g/day)
1	0	965	49	443	829
2	25	1067	95	473	887
3	50	1225	156	522	984
4	75	1319	219	540	1022
5	100	1426	293	559	1064
SED		968	235	377	716
P-value		**	***	*	*
Linear effect		4.70***	2.45***	1.20**	2.42**
Live-weight		***	***	***	***

SED = standard error of difference.

P-value : *** P<0.001; ** P<0.01; * P<0.05; NS, P>0.05.

Table 3.3: The rate constant (k1, indicative of the mean rate of passage of particles through the rumen) for sheep fed diets differing in lucerne proportion.

Diet	% Lucerne	k1	
		Short particles	Long particles
1	0	0.019^{a}	0.023^{ab}
2	25	0.020^{a}	0.025^{ab}
3	50	0.011^{b}	0.027^{ab}
4	75	0.012^{b}	0.020^{b}
5	100	0.011^{b}	0.010^{c}
LS Mean		0.015	0.021
SED		0.0008227	

SED = standard error of difference.

^{abc} Treatments, in the same column, with different superscripts are significantly different at the 5% level.

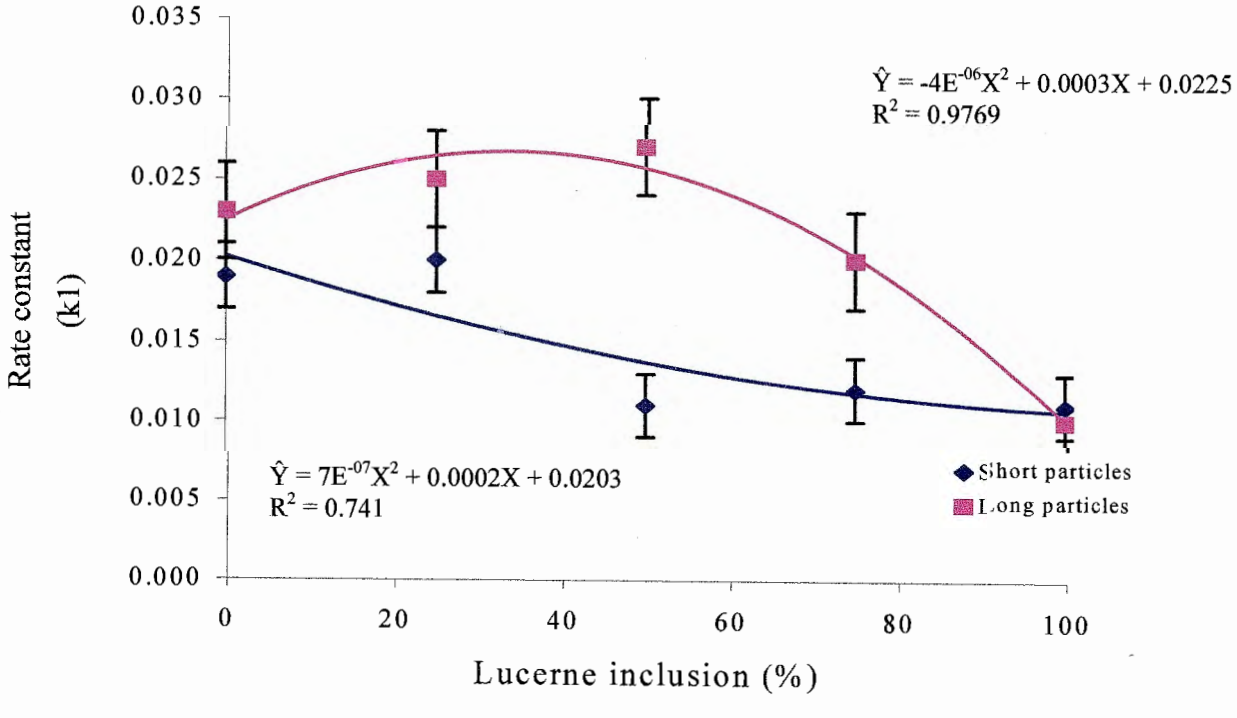

Figure 3.1: The relationship between particle size and lucerne proportion of the diet and the rate constant (k1) indicative of the rate of passage of particles through the rumen.

Table 3.4: The mean average daily intake (for the three days of the feeding behaviour studies) of DM (g) and CP, CF and NDF (g DM) for each of the five diets.

Diet	% Lucerne	DM intake (g/day)	CP intake (g/day)	CF intake (g/day)	NDF intake (g/day)
1	0	1098	56	505	944
2	25	1246	111	552	1035
3	50	1406	179	599	1129
4	75	1370	228	561	1062
5	100	1594	327	625	1189
SED		150	31	60	115
P-value		*	***	NS	NS
Linear effects		4.46^{**}	2.642^{***}	0.996^{NS}	2.07^{NS}
Live-weight		0.0363^{***}	0.00521^{***}	0.0152^{***}	0.0287^{***}

SED = standard error of difference.

P-value : *** P<0.001; ** P<0.01; * P<0.05; NS, P>0.05.

Table 3.5: The mean starting weight and standard deviation of the five groups of sheep fed the five experimental diets.

Group	Lucerne %	Mean starting weight (kg)	Standard Deviation
1	0	43.6	12.1
2	25	43.6	12.1
3	50	43.6	11.8
4	75	43.6	14.3
5	100	43.62	12.5

Table 3.6: The mean time spent eating (ET) or ruminating (RT) by sheep fed diets of varying roughage quality.

Diet	Lucerne (%)	ET (min day^{-1})	ET/kg DM consumed (min kg^{-1})	RT (min day^{-1})	RT/kg NDF consumed (min kg^{-1})
1	0	404	390	571	650
2	25	357	302	617	610
3	50	326	261	562	561
4	75	283	251	596	669
5	100	308	228	546	508
SED		29.809	45.386	32.821	82.496
P-value		**	*	NS	NS
Linear effects		-1.064^{**}	-1.5^{**}	-0.284^{NS}	-0.9^{NS}
Live-weight		0.2949^{NS}	-6.8808^{***}	2.9438^{**}	-12.6963^{***}

SED = standard error of difference

P-value : *** P<0.001; ** P<0.01; * P<0.05; NS, P>0.05.

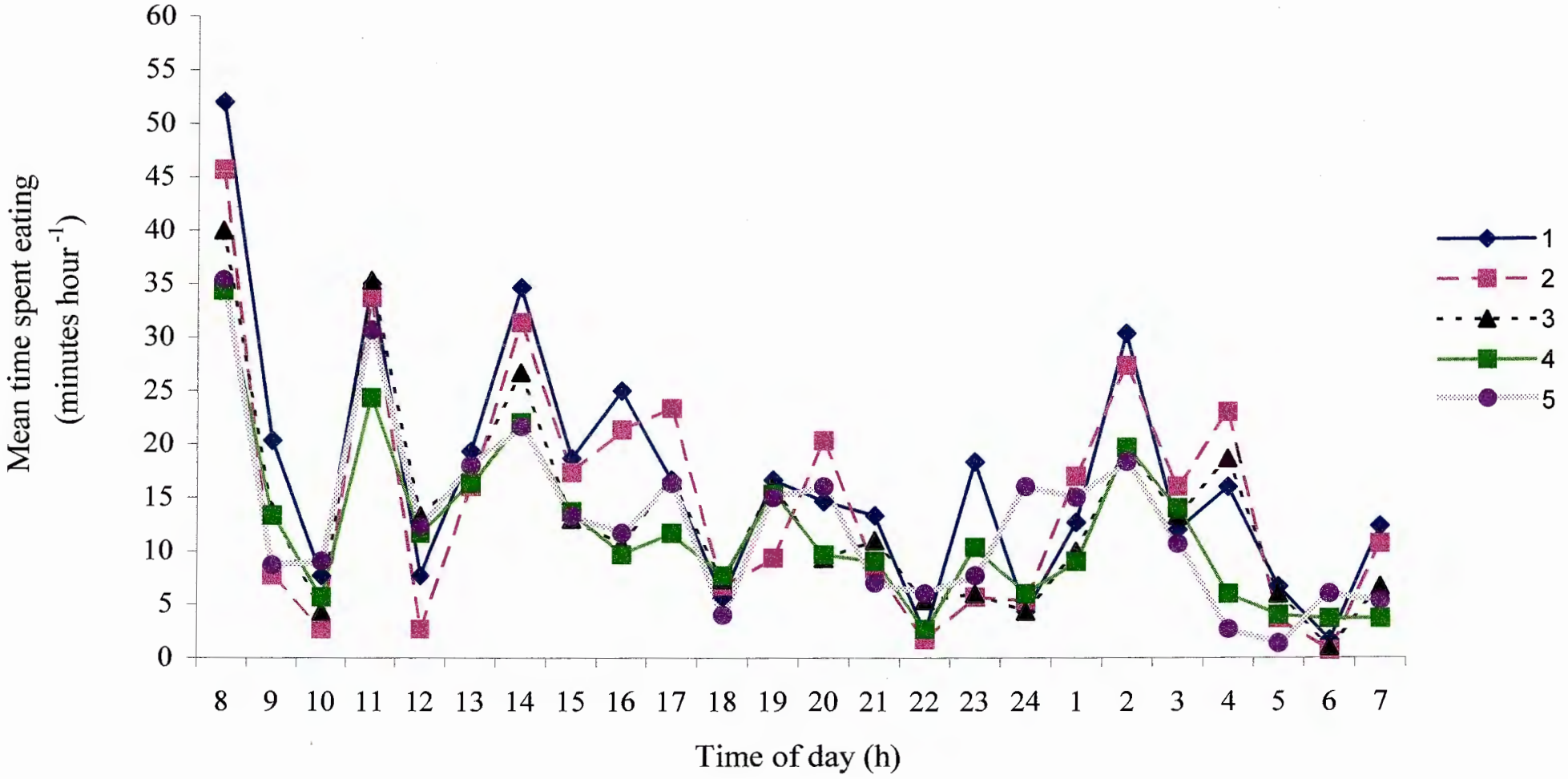

Figure 3.2: The effect of lucerne inclusion rate (roughage quality) on the time sheep spent eating.

Explanation of key: Treatment 1: 100% hay, 0% lucerne, Treatment 2: 75% hay, 25 % lucerne, Treatment 3: 50% hay, 50% lucerne,

Treatment 4: 25% hay, 75% lucerne, Treatment 5: 0% hay, 100% lucerne

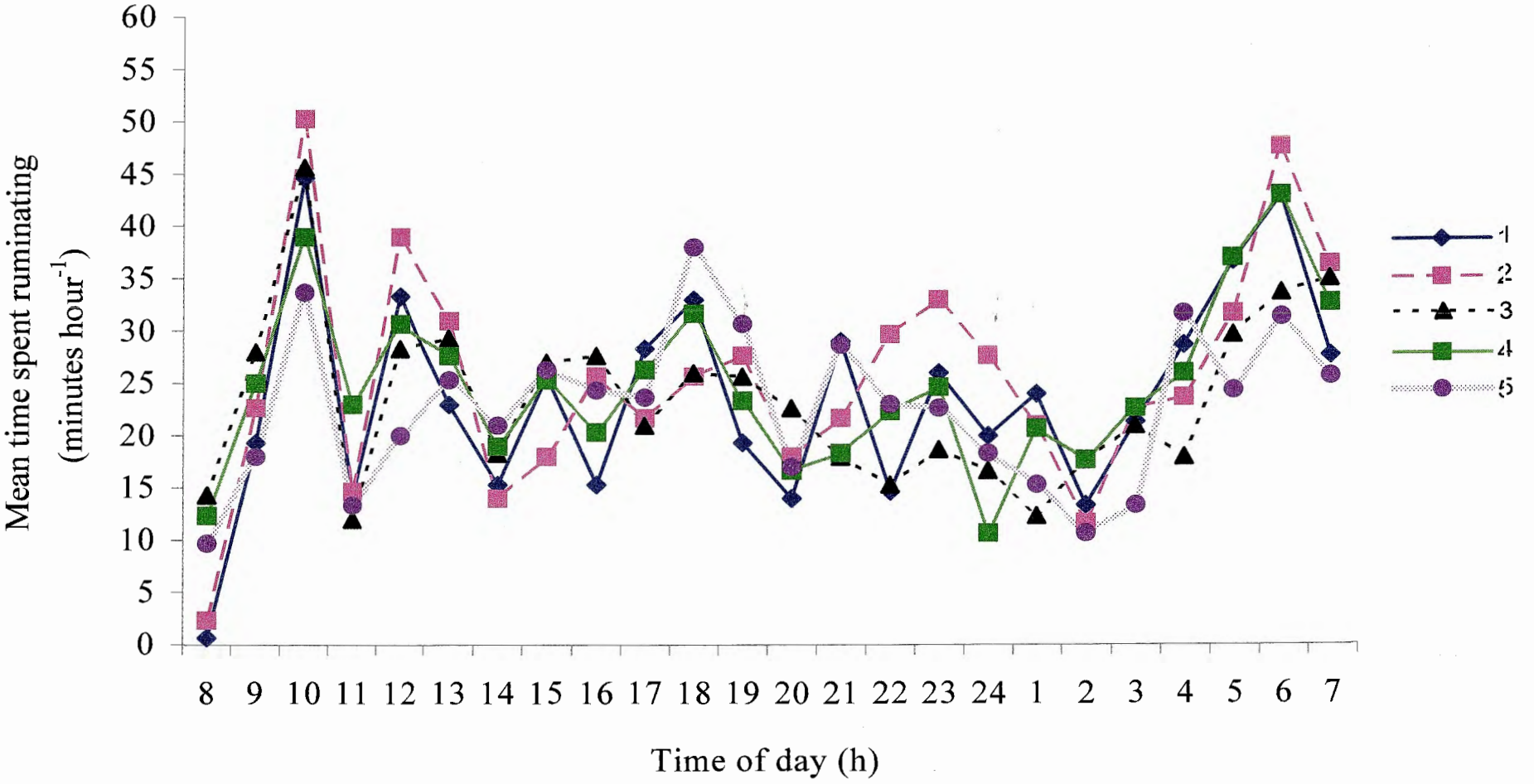

Figure 3.3: The effect of lucerne inclusion rate (roughage quality) on the time sheep spent ruminating.

Explanation of key: Treatment 1: 100% hay, 0% lucerne, Treatment 2: 75% hay, 25 % lucerne, Treatment 3: 50% hay, 50% lucerne Treatment 4: 25% hay, 75% lucerne, Treatment 5: 0% hay, 100% lucerne

Chapter 4

The influence of roughage quality and concentrate proportion on the rate of passage of particles from the rumen and feed intake

4.0 Abstract

The effect of increasing both basal roughage quality and level of concentrate supplementation on the intake of the basal roughage and the rate of passage of particles from the rumen (k1) of South African Mutton Merino sheep was investigated. Four roughage qualities were achieved by supplementing the basal roughage (poor quality veld hay) with varying amounts of lucerne hay such that lucerne contributed 20, 40, 60 or 80% of the diet. In this way crude protein (CP), and ash were increased as the lucerne proportion of the ration increased and neutral detergent fibre (NDF) and crude fibre (CF) concentrations were decreased. A concentrate supplement was offered at levels of 90, 180, 270 or 360 g per sheep per day. Sixty four sheep were blocked according to weight and sex and randomly assigned to one of the 16 treatments in a completely randomised block design. The interaction of lucerne inclusion level and concentrate supplementation was not significant. The relationship between level of concentrate supplementation on k1 is inversely quadratic and significant ($P<0.05$). Lucerne inclusion level had no effect on k1, but the effect of concentrate supplementation level on total dry matter intake (DMI) and total crude protein (CP) intake was highly significant ($P<0.001$) with DMI and CP intake increasing quadratically as the level of concentrate supplementation increased. NDF intake decreased quadratically ($P<0.05$) as lucerne inclusion level increased whilst CP intake increased quadratically ($P <0.001$) as lucerne inclusion level increased. These results indicate that below a certain level, the effect of concentrate supplementation on fibre digestion is not severe and that roughage and NDF intakes are not affected. Total DM and CP intakes are increased as concentrate supplementation increases. Further investigation into the effect of concentrate supplementation on feeding behaviour is warranted.

Keywords: roughage quality, concentrate supplementation, particle passage rate, feed intake, ruminant, protein

4.1 Introduction

The interaction between the high feed intakes required and the use of cost effective forages in ruminant production systems has been discussed in Chapter Three. The addition of a readily degradable concentrate supplement to a roughage diet is considered a means of increasing the nutrient supply to ruminant animals whose intakes are restricted by physical limitations (Romney and Gill, 2000). However, although total dry matter intake (DMI) is increased, this supplementation may have a positive or negative effect on the intake of the basal roughage (Romney and Gill, 2000). In addition, even though microbial activity and thereby particle degradation (McDonald *et al.*, 1981) may be increased as a result of supplementation, as Galyean and Goetsch (1993) noted, the effect of concentrate supplementation on roughage degradability is dependant on the amount and type of concentrate fed. Hoover (1986) noted that below levels of 300g of concentrate per kilogram of feed in dairy cow rations, depression of ruminal fibre digestion is not severe, whilst Doyle (1987) cited by Galyean and Goetsch (1993), noted that low levels of concentrate supplementation may slightly increase fibre digestion. This is likely to be a result of increased microbial activity and the synthesis of bacterial glycocalyces for attachment to fibrous digesta (Demeyer, 1981; cited by Galyean and Goetsch, 1993; Hiltner and Dehority 1983; cited by Galyean and Goetsch 1993). However, decreased cell wall degradation has long been recognised as a side effect of high inclusion levels of starch rich concentrates in mixed feeds (Sutton, 1986; cited by Givens and Moss, 1995; Webster, 1993). This decrease in cellulose degradation may be initiated as a result of a decline in ruminal pH to values less than 6.2. This in turn results in a decreased number or growth rate of cellulolytic bacteria, a decreased rate at which cellulases are synthesised and a decrease in enzyme activity (Givens and Moss, 1995). In addition many fibre-degrading organisms will preferentially digest starch, which results in an increased lag time for fibre digestion (Mertens and Loften, 1980).

Beever *et al.* (1988) and Doyle (1987) cited by Galyean and Goetsch (1993) showed that as forage quality is decreased, depression in forage digestion is increased. This is likely to be a result of the increased contribution of fibre digesting microbes to the digestion of poor quality forages as compared to good quality forages (Akin, 1989). Conversely, depression in forage intake is relatively greater for high quality forages, such as legumes, supplemented with grain, than for poorer quality forages, such as grasses (Jarrige *et al.*,

1996; Minson, 1990). This effect is likely to be a consequence of the greater potential intake of the high quality forage with the animal decreasing the intake of the latter so as to maintain a similar intake of nutrients (Jarrige *et al.*, 1996).

This study was designed to investigate the effect of supplementing a basal roughage (of varying quality) with a readily digestible carbohydrate and nitrogen source. The effect on feed intake and the rate of passage of digesta from the rumen were investigated.

4.2 Materials and methods

Animals and Diets: Sixty-four two-tooth South African Mutton Merino lambs (mean ± SD body weight 21.3 ± 2.6 kg, ranging from 16.5 to 31.0 kg) were blocked by weight and sex into four groups and within groups randomly assigned to the 16 dietary treatments in a completely randomised block design. As in Chapter Three, the large variation in sheep body weight was deliberately intended to determine whether body weight had an effect on particle passage rate. Animals were housed in individual pens (70 cm wide, 150 cm long and 90 cm high) with slatted wooden floors and allowed *ad libitum* access to both feed and water.

Four different roughage diets were fed in this trial and were mixed to obtain a gradient in roughage quality as described in Chapter Three. Lucerne to veld hay ratios of 20:80, 40:60, 60:40 and 80:20 were used in this trial. The hay to lucerne ratio and nutrient profiles of the four roughage diets are given in Table 4.1. In addition varying amounts of a concentrate diet (45, 90, 135 and 180 g) were offered on a twice-daily basis to each sheep in order to create a range of roughage to concentrate ratios. Total concentrate intakes per sheep per day were thus 90, 180, 270 and 360 g depending on the treatment. The ingredient and analysed nutrient composition of the concentrate diet are given in Table 4.2.

Passage rate studies (marker administration and sample collection): In order to determine the rate of passage of feed particles from the rumen, veld hay was mordanted with chromium as per the method described by Uden *et al.* (1980). The method used is the same as described in Chapter Three. The mordanted material was fed unmilled.

After a 14-day adaptation period each animal was fed 10 g of mordanted feed (particle size > 1 < 1.2 mm) prior to the consumption of the daily feed allowance. The same method of administration of the mordant as described in Chapter Three was used. Collection of faeces samples began 48 hours after the administration of the mordant, with further rectal grab samples being collected from the rectum at 60, 72, 96 and 120 h after dosing. The collected faeces were dried, milled and stored pending chromium analysis as described in Chapter Three.

Intake studies: The amount (by weight) of roughage offered to each sheep over the period of the faecal collection was recorded and any remaining feed left over at the end of the collection period weighed back in order to determine average daily intake by difference.

Laboratory analysis: DM, OM, nitrogen, fat (ether extract), CF and NDF were analysed as described in Chapter Three.

Faecal samples for the determination of particulate passage rate were analysed for chromium according to the method described by Costigan and Ellis (1987) as described in Chapter Three.

4.3 Data derivation and statistical analysis

Passage rate studies: Regression of the natural log of chromium concentration in the faeces in the decreasing phase of the chromium concentration was used to determine the rate constant (k1, as per Grovum and Williams (1973)). The decreasing phase occurred 48-hr after the feeding of the chromium-mordanted marker.

Differences among diets for all variables were analysed using the GLM procedure (SAS, 1987) according to the model:

$$Yij = \mu + Li + Cj + eij,$$

where Y_{ij} is the individual observation, μ is the overall mean, L_i the average effect of the i^{th} lucerne inclusion level and C_j the average effect of the j^{th} level of concentrate supplementation. The error term, e_{ij}, is assumed to be independently and normally

distributed. The least square means (LSmeans) for passage rate, for each treatment, were plotted with the aid of a spreadsheet package, Microsoft Excel (Microsoft Corporation, 1997).

4.4 Results

The nutrient composition of the roughage diets is given in Table 4.1. The CP, fat and ash contents of the rations increase as the proportion of lucerne in the diet increases. At the same time the DM, CF and NDF contents of the diets decrease as the proportion of lucerne in the diet increases. The nutrient composition of the concentrate feed is given in Table 4.2.

Passage rate studies: Table 4.3 gives the LSmeans for k1 determined for the four different concentrate supplementation levels. Concentrate supplementation level had a significant effect on k1 ($P<0.05$) as shown graphically in Figure 4.1. Both lucerne inclusion level and the interaction between lucerne inclusion level and concentrate supplementation level did not significantly affect k1. Table 4.4 gives and Figure 4.2 depicts the LSmeans for k1 determined for the four different lucerne inclusion levels. Appendix 2 gives the rate constant (k1) for each of the 16 different treatments.

Intake studies: The interaction between lucerne inclusion level and concentrate supplementation level was not significant for total DM, total CP or NDF intake. The effect of concentrate supplementation level on total DM intake and total CP intake was highly significant ($P<0.001$). Roughage and NDF intakes were not significantly affected by concentrate inclusion level. Figure 4.3 illustrates the effect of concentrate supplementation level on total DM and CP intakes. Lucerne inclusion level significantly affected NDF ($P<0.05$) and total CP intake ($P<0.001$). Roughage intake and total DMI were not significantly affected by lucerne inclusion level. Tables 4.3 and 4.4 give the LSmeans for roughage intake, NDF intake, total DM intake and total CP intake determined for the four different concentrate supplementation and lucerne inclusion levels respectively. Figure 4.2 illustrates the effect of lucerne inclusion level (%) on NDF and CP intakes.

Appendix 3 gives the mean average daily intake of DM and NDF derived from the roughage diet for each of the 16 treatments while Appendix 4 gives the mean average total

DM and CP intake derived from both the roughage and concentrate supplement for each of the 16 treatments.

4.5 Discussion

The rates of passage (k1) observed in this trial are comparable to those obtained for the long particles reported in Chapter Three. Concentrate inclusion level had a significant effect on k1 ($P<0.05$) and an inverse quadratic relationship was observed between the level of concentrate supplementation and the rate of passage of particles from the rumen ($r = 0.84$). This relationship is illustrated graphically in Figure 4.1. There is a significant decrease in the rate of passage as the level of concentrate supplementation is increased from 90 to 180 g/day. This significant decrease in k1 at supplementation levels of 180 g, as compared to the other concentrate treatments, was unexpected (Colucci *et al.*, 1990; Valdes *et al.*, 1999) and is difficult to explain. However, Napoli and Santini (1989) using steers grazed on oat or ryegrass and clover pasture, with or without supplementation, concluded that concentrate supplementation did not markedly affect rumen environment or protein and fibre digestion kinetics.

The inverse quadratic relationship observed in this trial may be the result of a combination of factors. A small amount of concentrate supplementation may result in an increase in the amount of fibre digestion, particularly in cases where the forage is of a particularly poor quality and deficient in nutrients such as nitrogen. Hoover (1986), using dairy cattle, noted that suppression of fibre digestion as a result of supplementation is not severe below 300 g of concentrate per kilogram of diet, whilst Mould *et al.* (1983) observed increased celluloytic bacterial counts when 250 g per kilogram of a hay diet were replaced with barley.

Thus, although it is known that the digestion of fibre is decreased with increasing concentrate supplementation, it is possible that a minimum percentage of the total ration could be supplied in the form of concentrate before this effect is observed. In this trial, 10.8 % of the total DMI is made up by the concentrate supplement when only 90 g of concentrate per sheep per day is offered. This increases to an average of 20.6, 27.4 and 32.9 % of the total DMI when 180, 270 and 360 g respectively, of concentrate is offered per sheep per day.

Another aspect of this trial, which should be noted, is that the concentrate supplement was offered in two equal proportions each day, and this would mean that the decrease in rumen pH at the time of feeding would not be as severe, as is likely to be the case if the entire amount of supplement was offered in one feeding. It is likely, therefore, that the levels of concentrate supplement offered in this trial might have elicited only small changes in the rumen microbial population and subsequently only a small effect on fibre digestion.

The quadratic relationship may be a result of the increased rate of passage when 90 g of supplement per sheep per day was fed. As observed in Chapter Three, ruminating time increases with NDF intake. When considered in terms of the total ration, NDF levels are considerably greater in this ration than in the other rations fed during this trial and these increased levels of NDF will directly affect the level of effective fibre in the ration and will stimulate increased rumination and rate of particle breakdown.

The voluntary feed intake of high fibre diets by ruminants is constrained largely as a result of physical fill (Romney and Gill, 2000) and the cause and consequences of this are discussed in more detail in Chapter One. However, ingested material is comprised of an indigestible fraction and one, or more, potentially digestible fractions degraded at different rates (Tamminga, 1993). The extent of digestion of the degradable fractions depends on the balance between the rate of degradation of the ingested material and its rate of passage from the rumen (Tamminga, 1993). Material not digested must be cleared from the rumen in order to allow further intake of feed. Thus, as discussed in Chapter One, any factor, which serves to increase the rate of clearance of particles from the rumen will have a positive effect on the overall feed intake of the animal. Concentrate supplementation may, as discussed earlier in this chapter, stimulate some improvement in the rate and extent of fibre digestion (McDonald *et al.*, 1981; Hoover 1986; Doyle (1987), cited by Galyean and Goetsch (1993); Galyean and Goetsch, 1993), as a result of increased microbial activity (Demeyer (1981), cited by Galyean and Goetsch, (1993)). Whilst an increase in digestion will be observed as a decrease in rumen contents, any increase in the rate of degradation of fibre will serve to increase the proportion of small particles capable of passing out of the reticulo-omasal orifice and will in turn result in an increase the rate of clearance of the rumen and greater feed intake (Beever, 1993).

It is possible that at 90g of concentrate supplement per day, the level of supplementation is sufficiently large enough to cause an increase in the rate of microbial breakdown of

particles in the rumen, but not large enough to decrease ruminal pH to a degree that would reduce the number and efficiency of the fibrolytic micro-organisms present in the rumen. It is possible that although the addition of concentrate to a roughage diet is likely to result in a decrease of forage intake, there are situations in which it might have no effect. The increasing concentrate supplementation levels, as expected, significantly affected both total DM and total CP intakes as a result of the high DM and protein content of the concentrate. The level of feed intake has also been shown to have an effect on the rate of passage of particles from the rumen. Colucci *et al.* (1990) found rates of passage of both liquid and solid particles to be faster at high feed intakes as compared to low feed intakes for both sheep and cattle.

Although, it is unlikely that CP was limiting in any of the treatments as the lowest CP intake observed was 95 g per sheep per day, there may have been a compounding effect of increasing CP intakes as the level of supplementation increased. The effect of concentrate supplementation level on CP intake was significant and CP intake increased as concentrate supplementation level increased. Oldham and Smith (1982), cited by Tamminga (1993), reported an increase in digestibility of 10 g per kilogram of DM, for each additional 10 g of CP per kilogram of DM, over the range of 80 to 160 g of CP per kilogram of DM. In this trial CP intakes ranged from 95 to 164 g per sheep per day.

The nutrient composition of the concentrate and the rumen degradability of the protein source used in the concentrate ration warrant some discussion. Tamminga (1993) reviewed the effect of concentrate composition on fibre digestion and suggested that concentrates containing ingredients with a high fibre content have a stabilizing effect on rumen fermentation and prevent the depression of fibre digestion. Tamminga (1993) further suggested that the benefits of fibrous concentrates are increased the more concentrate is included in the diet. In this trial cottonseed oilcake, wheat bran and molasses meal together comprise almost half the concentrate. As a result, this concentrate is likely to have a high fibre content and it is possible that the benefits described by Tamminga (1993), would have come into play in this trial.

3.6 Conclusions and Implications

The available literature indicates that the effect of concentrate supplementation, as either a means of meeting animal requirements not met by the basal roughage, or to enhance the potential supply of nutrients from the roughage, is complex and not simply a function of the basal roughage and the concentrate alone. Rumen microbial synthesis is affected by the level of supplementation, in particular the supply of nitrogen and carbohydrate. However, the initial dry matter intake and rate of degradation of the carbohydrate and nitrogen sources in the feed amongst other factors may also come into play.

The results presented here indicate that an inverse quadratic relationship exists between the rate of passage of particles from the rumen of sheep and the level of concentrate supplementation. Thus, as the rate of concentrate supplementation increases there is an initial decrease in the rate of passage of particles from the rumen. However, a point is reached where any additional supplementation results in an increase in the rate of passage of feed through the rumen, and thus a decrease in the time that the feed may undergo fermentation.

Table 4.1: The physical composition, DM (g kg^{-1} as fed), CP, CF, NDF, fat and ash (g kg^{-1} DM) content of the four experimental diets.

Diet	1	2	3	4
% Hay	80	60	40	20
% Lucerne	40	20	60	80
Dry Matter	922.5	916.0	914.5	906.0
Fat	13.7	14.9	19.7	18.1
Ash	81.1	100.2	92.2	99.4
Protein	66.7	103.7	126.8	156.5
Crude fibre	371.6	333.5	337.6	317.0
NDF	648.5	589.1	541.8	486.2

Table 4.2: The ingredient and chemical composition of the concentrate diet fed.

Ingredient	**Quantity (kg as fed)**
Yellow Maize meal	471
Wheat Bran	100
Molasses Meal	50
Cottonseed oilcake	341
Limestone	30
Vitamin-Mineral Premix	1
Mono-calcium Phosphate	7
Nutrient	**Analysed Value (g kg^{-1} as fed)**
Dry Matter	891.0
Fat	2.72
Ash	6.45
Crude Protein	20.02

Table 4.3: The effect of concentrate supplementation level on ruminal rate of passage (k1), roughage intake, NDF intake, Total DM intake and CP intake.

Variable (g sheep^{-1} day^{-1})	Concentrate level (g sheep^{-1} day^{-1})				SED	P-value
	90	180	270	360		
k1	0.027^{a}	0.018^{b}	0.025^{a}	0.031^{a}	0.006	*
Roughage intake	659	623	637	652	71	NS
NDF intake	411	390	395	404	45	NS
Total DM intake	738^{a}	781^{a}	874^{b}	968^{c}	71	***
CP intake	103^{a}	117^{b}	142^{c}	164^{d}	9	***

SED = standard error of the difference.

***P*-value** : *** P<0.001; ** *P*<0.01; * *P*<0.05; NS, *P*>0.05.

^{abc} Treatments, in the same row, with different superscripts are significantly different at the 5% level.

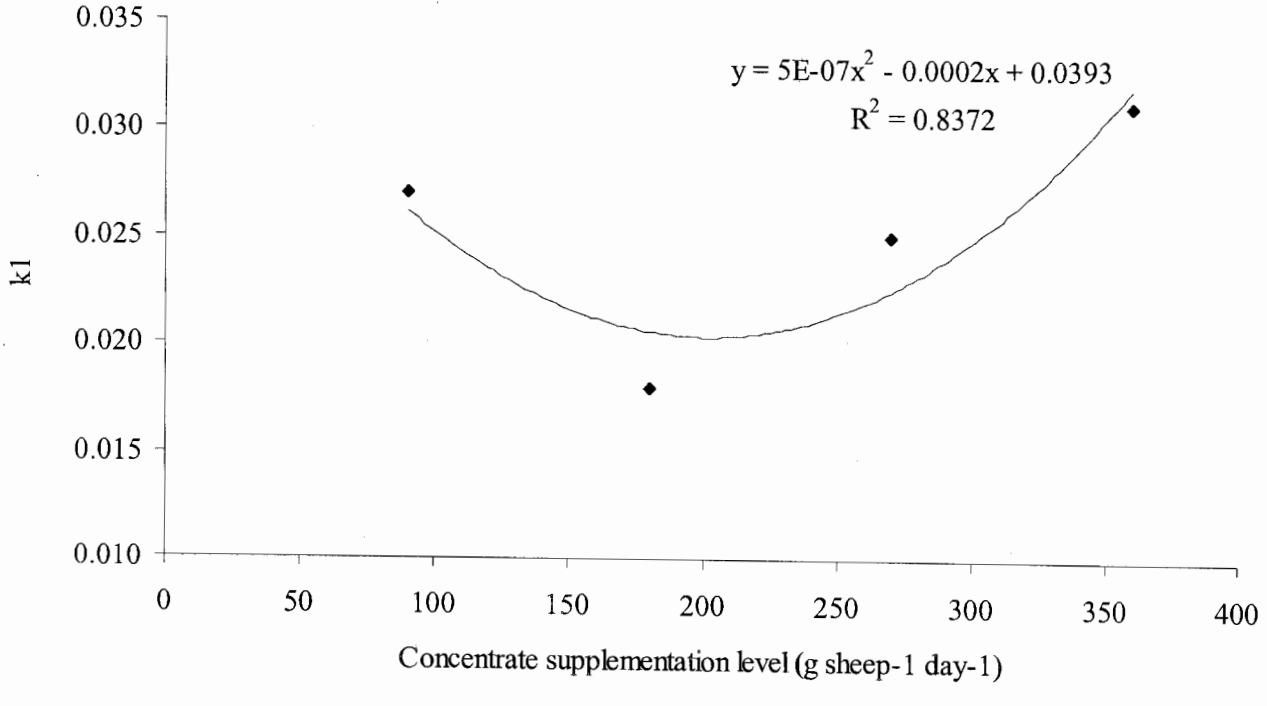

Figure 4.1: The effect of concentrate supplementation level (g sheep^{-1} day^{-1}) on ruminal rate of passage (k1).

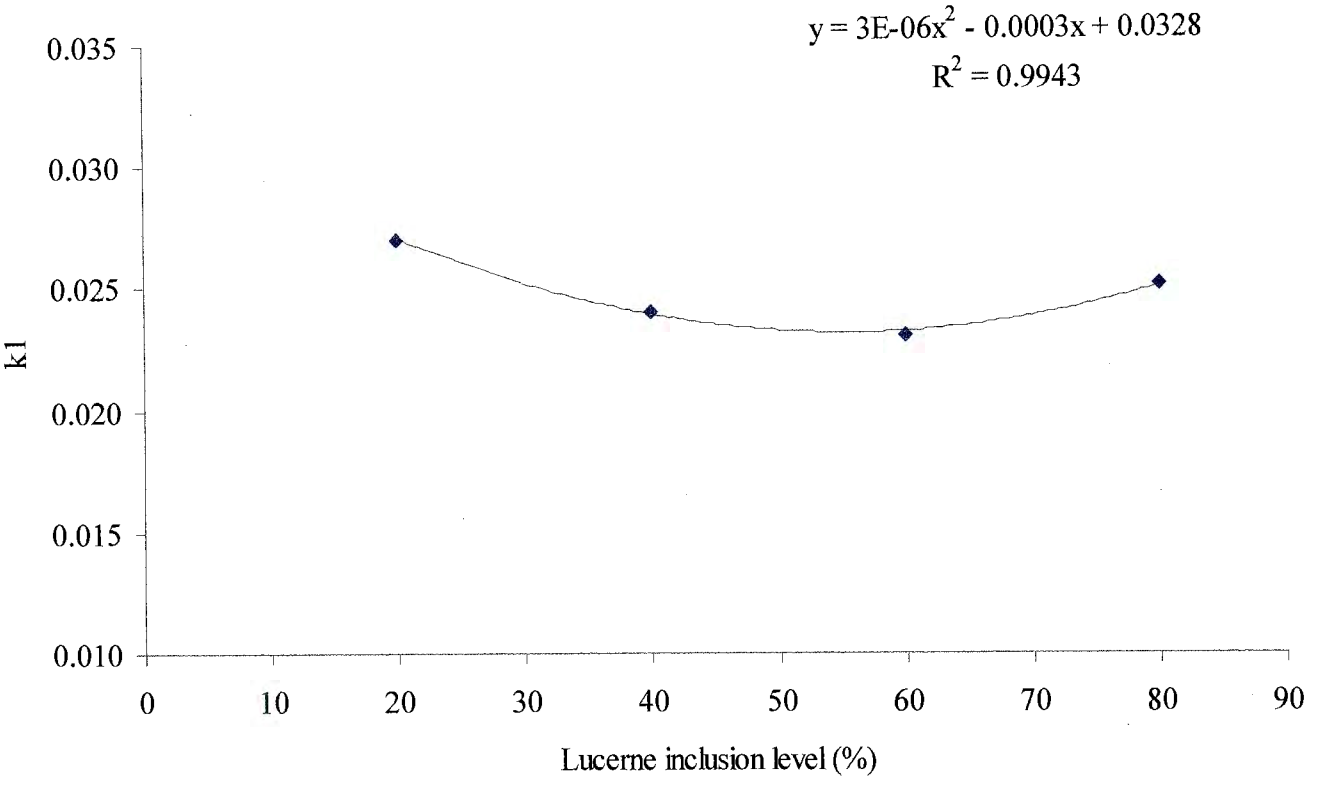

Figure 4.2: The effect of lucerne inclusion level (%) on ruminal rate of passage (k1).

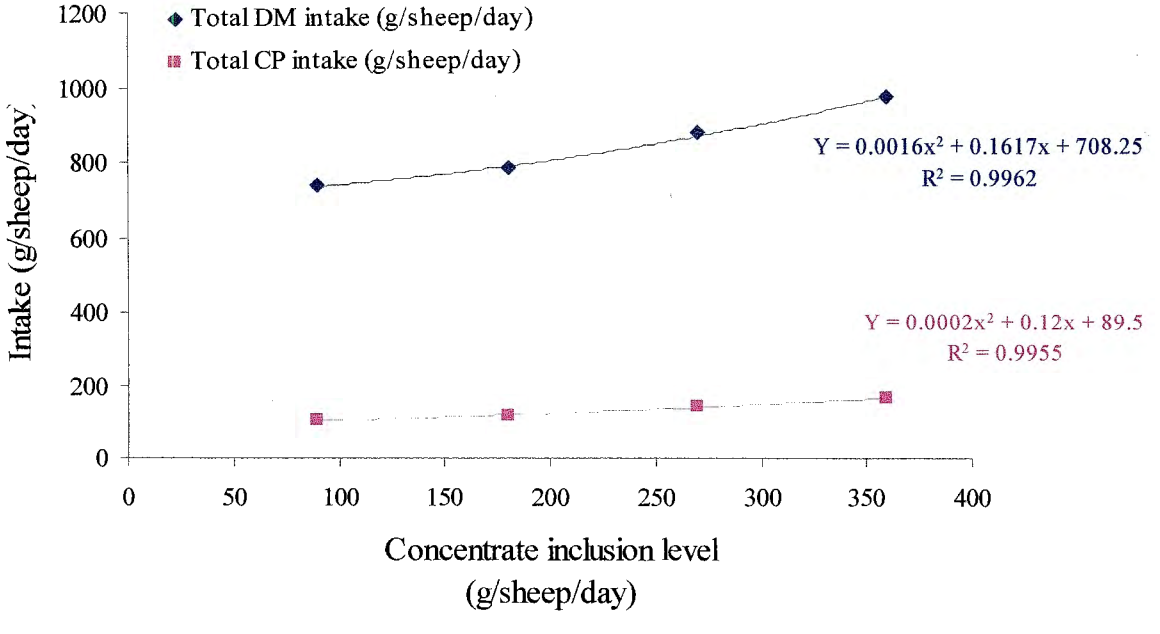

Figure 4.3: The effect of concentrate supplementation level (g/sheep/day) on total DM and CP intakes (g/sheep/day).

Table 4.4: The effect of lucerne inclusion level on ruminal rate of passage (k1), roughage intake, NDF intake, Total DM intake and CP intake.

Variable (g sheep^{-1} day^{-1})	Lucerne inclusion level (%)				SED	P-value
	20	40	60	80		
k1	0.027	0.024	0.023	0.025	0.006	NS
Roughage intake	611	644	661	654	71	NS
NDF intake	432^{a}	422^{a}	394^{ab}	353^{b}	45	*
Total DM intake	809	842	858	852	71	NS
CP intake	95^{a}	125^{b}	143^{c}	164^{d}	9	***

SED = standard error of the difference.

***P*-value** : *** P<0.001; ** *P*<0.01; * *P*<0.05; NS, *P*>0.05.

^{abc} Treatments, in the same row, with different superscripts are significantly different at the 5% level.

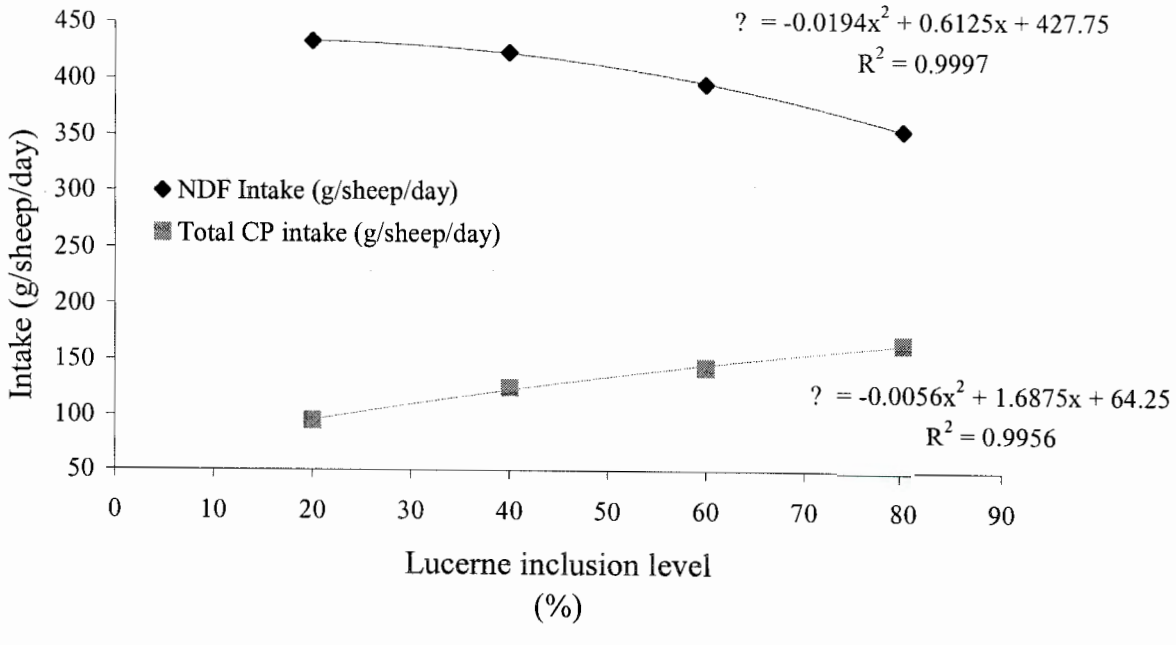

Figure 4.4: The effect of lucerne inclusion level (%) on NDF and total CP intakes (g/sheep/day).

Summary and Conclusions

The use of forages as part of the basal diet makes up an intrinsic part of many (if not most) ruminant production systems and the importance of forages is well known and accepted (Adesogan, 2001). However, there are a wide variety of factors, which influence the nutrient composition of the forage and subsequently the degradation characteristics of the forage in the rumen and thus the nutritional value of the forage. These factors include, amongst others, plant species, variety, physiological maturity, regrowth, season, time of harvest, cutting height and fertilisation.

Basal forages used in ruminant production systems, particularly in sub-Saharan Africa, tend to be low in readily degradable carbohydrates and nitrogen and supplementation of the basal forage with nutrients (for example, a readily degradable carbohydrate or nitrogen source), which are known to be lacking in the roughage, is a common practice aimed at overcoming the intrinsic nutrient deficiency. However, as a consequence of the pregastric system of rumen fermentation, the effect of supplementation with any given nutrient is not simply to increase the amount of the specific nutrient available for digestion, absorption and metabolism by the host animal. Rather the impact of supplementation is expressed firstly, as a change in the rumen microbial population, which changes degradation pattern of fibre in the rumen along with the post ruminal microbial protein supply. Such changes are particularly evident where the basal roughage is of poor quality (high in fibre) and where a large amount of a supplement, containing a readily degradable source of carbohydrate or nitrogen, is fed.

Although one would expect that an increase in the supply of nutrients to the host animal would result in increased performance in terms of meat, milk or wool production, it is important to note that one of the important factors contributing to the success (in terms of productivity) of the ruminant animal population is their ability to extract maximum nutritional benefit from low quality roughages. The ability of the ruminant animal to make use of such high fibre forages that cannot be digested by monogastric animals thus places them in a unique ecological position and provides man kind with an efficient means of converting, otherwise valueless, low quality forages into the valuable outputs of meat, milk and wool.

However, as long as the possibility exists to improve the output of meat, milk or wool by some means of supplementation, it is important to be able to estimate the potential benefits of supplementation in relation to the cost of the supplement. As discussed previously, the effect of supplementation on the ruminal degradation of forages may be either positive or negative and are a result of complex changes in the microbial population dynamics within the rumen ecosystem. Of considerable importance in determining the effect of a supplement on the rumen microbial population is the degradation pattern of the supplement itself and it is fundamental that this aspect is not overlooked.

Therefore, in order to effectively formulate rations that optimise animal performance, the nutritional characteristics of the forage and nutritional deficiencies inherent in the forage need to be correctly identified (Adesogan, 2001). At the same time, it is essential to have a good understanding of the degradation characteristics of the proposed supplement. This will allow the nutritionist to formulate a ration, which combines two or more forages and/or supplements and where a combination of feed ingredients provides a ration, which taking account of the ruminal ecosystem, meets, more precisely, the nutritional requirements of the animal, than any of the single ingredients alone. Once the degradation characteristics of the supplement are known, an estimate of its effect on the rumen microbial population can be made.

To this end, Chapter Two reports an investigation into the degradation patterns of eight plant protein supplements commonly used in ruminant rations in Southern Africa. The basis of the method used in this trial was that described by Ørskov *et al.* (1980). In addition, an attempt was made to determine the post ruminal protein degradability of the plant protein supplement by means of digestion in adult cockerels. The study clearly illustrated the wide range in the both the dry matter and nitrogen degradation parameters and hence further reinforces the need to correctly determine the degradation characteristics of a supplement when attempting to formulate a ration, which will optimise animal performance.

Although the method described by Ørskov *et al.* (1980) is one of the few techniques that allows the description of rumen degradation characteristics and now forms the basis of describing the nitrogen requirements of ruminant animals in the feeding systems of several

countries (Adesogan, 2001), certain problems with the reproducibility and repeatability of these measures do exist and have been reviewed by several authors (Nocek, 1985; Ørskov, 2000). In addition, as discussed previously, a reliable and easy method for determining post ruminal digestibility is still not available and thus, Chapter Two further reports an investigation into a proposed method of determination using adult cockerels. However, some adaptations to the proposed method are required in order to improve the validity of the method and these are discussed in Chapter Two. Nevertheless, the study showed significant differences in parameters associated with protein and dry matter degradability among the eight supplements investigated and consequently, there appears to be potential for the proposed method of evaluating the post ruminal protein supply for a given protein source. Further investigation into the usefulness of this method, with the implementation of those adaptations noted in Chapter Two, is certainly warranted.

Once the decision to use some form of supplementation has been taken and the degradation pattern of the supplement is known, it is important to be able to estimate what effect supplementation will have on the intake and digestibility of the basal roughage, as presented in Chapters Three and Four. In this way the optimum use can be made of both the available basal roughage and the potential protein and/or carbohydrate supplements. Although Chapters Three and Four describe the effect of changes in basal roughage quality and/or supplementation on the rate of passage of particles from the rumen, the significance of variation in the rate of passage is clearly illustrated in the estimation of effective degradability for the eight plant protein supplements investigated in Chapter Two. Although a range of k1 values was used in order to estimate ED for the different functional ruminants, the effect of increasing (or decreasing) the residence time in the rumen on the availability of nutrients to the host animal is clear.

From the evidence presented above it is obvious that a reasonable estimate of the length of time a feedstuff spends in the rumen is required, if one is to calculate as accurately as possible the ED of the feedstuff. Although the calculations presented in Chapter Two used estimates of k1 (rate constant as per Grovum and Williams, 1973) and klq (rate of passage for liquid matter leaving the rumen), the effect of altering the basal roughage quality and supplementation on the rate of passage of roughage particles from the rumen was investigated in trials reported in Chapters Three and Four. However, no similar trials were carried out to determine what effect an alteration in the basal ration or a combination of

supplements would have on the rate of passage of those feeds investigated in Chapter Two. In retrospect, this may have been useful in determining the ED of the feedstuffs. The use of a constant k1 and klq value for different feedstuffs may be simplistic and the effect of supplementation with one of these feedstuffs alone may result in considerable changes in rumen kinetics. The effect of a combination of these feedstuffs, as part of a concentrate type supplement, on rumen kinetics may also warrant further investigation. Another aspect that should be given further consideration in this regard is the observation that different particles may behave differently in the rumen and consequently, particles with a low specific gravity will react differently to ones which have a greater specific gravity, even if they are of the same size. On the other hand, larger particles such as those derived from straw or hay, will react very differently to heavier more compact particles, such as grains which form part of a concentrate. Although these aspects are not considered here for the supplements described in Chapter Two, *per se*, some consideration should be given to these factors when determining effective degradability of a feedstuff.

The work described in Chapters Three and Four, clearly illustrates that the effect of changing basal roughage quality alone and/or in combination with concentrate supplementation will have a complex effect on the rate of passage of particles from the rumen and consequently, will affect the effective dry matter degradability of the different protein sources as described in Chapter Two and further discussed above. A change in the quality and/or composition of the basal roughage will have an effect on the size and composition of the rumen microbial population, with a subsequent effect on the degradability and rate of passage of any supplementation offered. At the same time the addition of any form of feed supplement will have a further effect on the composition of the rumen microbial population and consequently the digestibility and degradation parameters of the basal roughage.

The extent of the complexity of the interactions between feed components (both basal and supplemental) and other factors associated with the feed and the host animal are clearly illustrated in both Chapters Two and Three. In Chapter Two the effect of the animal species on the effective degradability of the supplement as a consequence of retention time is noted. This observation should be considered when formulating rations for different species as it will have implications on the availability of nitrogen and/or protein, contained in the supplement and/or basal roughage, for use by the rumen microbes and further on the

amino acids available for post ruminal digestion by the host animal. In Chapter Three the anomalous results obtained when lucerne in the basal diets replaced hay, in an attempt to increase the quality of the basal roughage diet, clearly illustrated that there are a number of other factors that will influence the digestion and degradation of the feed in the rumen and that these factors are not necessarily related simply to the composition or efficiency of the rumen microbial population. For example, the inclusion of lucerne, rather than simply increasing the available crude protein levels of the basal ration, may have had an effect on the composition of the fibrous mat in the rumen and consequently the entrapment of fine particles. Furthermore, the effect of the different fragmentation patterns and the effect of stimulation of rumination (as a result of effective fibre composition of the ration), on the particle degradation patterns observed for a given ration cannot be underestimated, as illustrated by the eating and ruminating behaviour discussed in Chapter Three. At the same time including lucerne in the basal roughage altered not only the quality and quantity of protein available to the rumen microbes (as was the intention) but may have improved the supply of readily digestible sugars and probably the supply of other nutrients (e.g. readily fermentable pectins and minerals) to ruminal microbes. The addition of these nutrients alone, would have had a significant impact on the efficiency and productivity of the rumen organisms, with a corresponding change in the degradability of both roughages and concentrates in the rumen. Thus, even a small change, such as changing the type of forage, will have considerable impact on many of the parameters associated with the rumen microbial ecosystem, the movement of ingested feed materials through the rumen and subsequently the productive performance of the animal in question.

The findings of this work, when considered as a whole, would support the view that the effect of changing basal roughage quality and concentrate supplementation on rumen kinetics and, in particular, rates of passage, is complex. The effects of supplementation, even with a single feedstuff, may have a profound effect on rumen kinetics. The results would suggest that when feeding a very poor quality basal roughage, there is a window of opportunity in which some form of readily degradable protein supplementation might provide sufficient nitrogen for increased rumen microbial activity. Where roughage quality is extremely poor, it is suggested (although there is no evidence provided within the framework of this thesis) that additional supplementation of the most limiting nutrients, including protein, readily fermentable carbohydrates and even minerals, may help to improve rumen productivity. The single aspect of the work conducted, which most

warrants further investigation, is the potential for the use of poultry in determining the post ruminal availability of undegraded protein.

A number of conclusions can be drawn from this study

- Amongst the protein sources investigated in Chapter Two, there are significant differences in intercept values and time lag for dry matter degradation. Potential degradability and rate of degradation are also significantly different among protein sources.

- There is a considerable effect of the estimated rate of passage on the effective dry matter and protein degradability of supplements as illustrated by the estimated ED for the different species.

- Although a reliable and easy method of post ruminal digestibility is still not available, the method described in this trial, along with the adaptations described warrants further investigation.

- Studies described in Chapter Three showed that by increasing the level of protein in the basal roughage DM, CP, CF and NDF intakes could be increased significantly.

- Both diet and particle size, have a significant effect on the rate of passage of particles from the rumen and particle size by diet interactions also plays an important role.

- Ruminating time is not affected by lucerne inclusion level in the diet. However, improving basal roughage quality leads to a decrease in total eating time, time spent eating per kilogram dry matter consumed and time spent ruminating per kilogram of NDF consumed.

- The livemass of the animal has a significant effect on time spent eating per kilogram of dry matter consumed, time spent ruminating per kilogram of NDF consumed and total ruminating time.

- The results of Chapter Four showed that interactions among feed factors will affect the rate of clearance of ingested roughage particles from the rumen and consequently the level of roughage intake.

- The effects of animal factors as well as chewing effectiveness warrant further investigation. Further work conducted should consider the compounding effect of comparing legume hay to grass hay and the differences in the fragmentation patterns of the two.

- The results presented in Chapter Four indicate that an inverse quadratic relationship exists between the rate of passage of particles from the rumen of sheep and the level of concentrate supplementation.

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Appendix 1: The DM and nitrogen degradation characteristics of eight plant protein sources incubated in the rumen of Jersey cows fed a basal diet of K11 hay.

	Dry Matter Degradation Parameters ($g\ DM\ (kg\ DM)^{-1}$) Incubation Time (hours)								Nitrogen Degradation Parameters ($g\ N\ (kg\ DM)^{-1}$) Incubation Time (hours)							
	3	6	12	24	36	48	72	96	3	6	12	24	36	48	72	96
Canola meal	442	527	653	821	826	953	865	873	609	693	782	898	898	977	930	940
Copra meal	568	510	557	772	806	826	860	872	626	583	593	762	805	829	869	895
Cottonseed oilcake	570	547	612	670	711	800	882	905	755	787	836	893	937	967	978	983
Defatted maize germ meal	614	727	675	737	804	881	940	950	698	798	771	860	934	922	961	953
Lucerne meal	577	472	560	728	730	762	793	922	739	653	791	950	951	963	964	988
Lupin seeds	785	755	887	935	986	985	989	992	969	952	978	992	996	995	995	996
Soya oilcake	803	680	702	883	941	958	982	1048	813	680	694	890	950	963	985	1008
Sunflower oilcake	673	595	486	739	750	758	826	782	782	749	626	956	968	979	986	982
P-value [a]	NS	NS	***	***	***	**	***	NS	NS	*	**	***	**	*	**	**
SED [b]	0.90	1.33	2.64	4.36	3.89	2.18	3.02	1.55	1.08	1.86	2.98	3.26	2.26	1.65	2.02	2.19

[a] **P-value** : NS, $P>0.05$; *** $P<0.001$; ** $P<0.01$; * $P<0.05$.

[b] **SED** = standard error of difference

Appendix 2: The rate constant (k1, indicative of the mean rate of passage of particles through the rumen) for sheep fed diets differing in lucerne content.

Roughage diet	Lucerne (%)	Concentrate level (g sheep^{-1} day^{-1})	k1
1	20	90	0.027 ^{abcd}
1	20	180	0.025 ^{abcd}
1	20	270	0.028 ^{abcd}
1	20	360	0.028 ^{abcd}
2	40	90	0.023 ^{abcd}
2	40	180	0.014 ^{d}
2	40	270	0.025 ^{abcd}
2	40	360	0.034 ^{a}
3	60	90	0.032 ^{abc}
3	60	180	0.016 ^{d}
3	60	270	0.019 ^{abcd}
3	60	360	0.028 ^{abcd}
4	80	90	0.028 ^{abcd}
4	80	180	0.017 ^{cd}
4	80	270	0.026 ^{abcd}
4	80	360	0.033 ^{ab}
SED			0.0067
P-value			NS

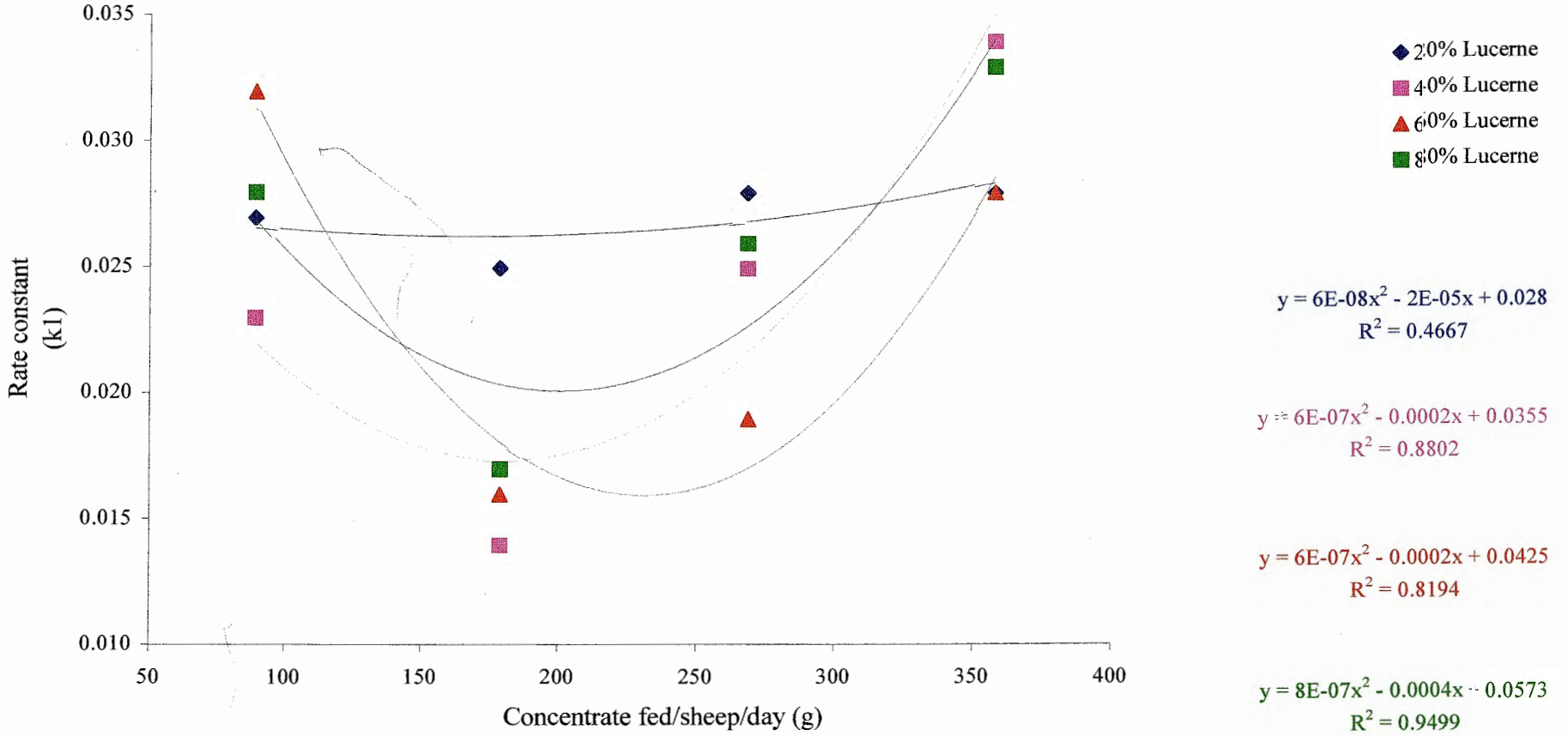
^{abc} Treatments with different superscripts are significantly different at the 5% level.

Appendix 3: Mean values of average daily intake of DM and NDF derived from the basal roughage for each of the 16 dietary treatments.

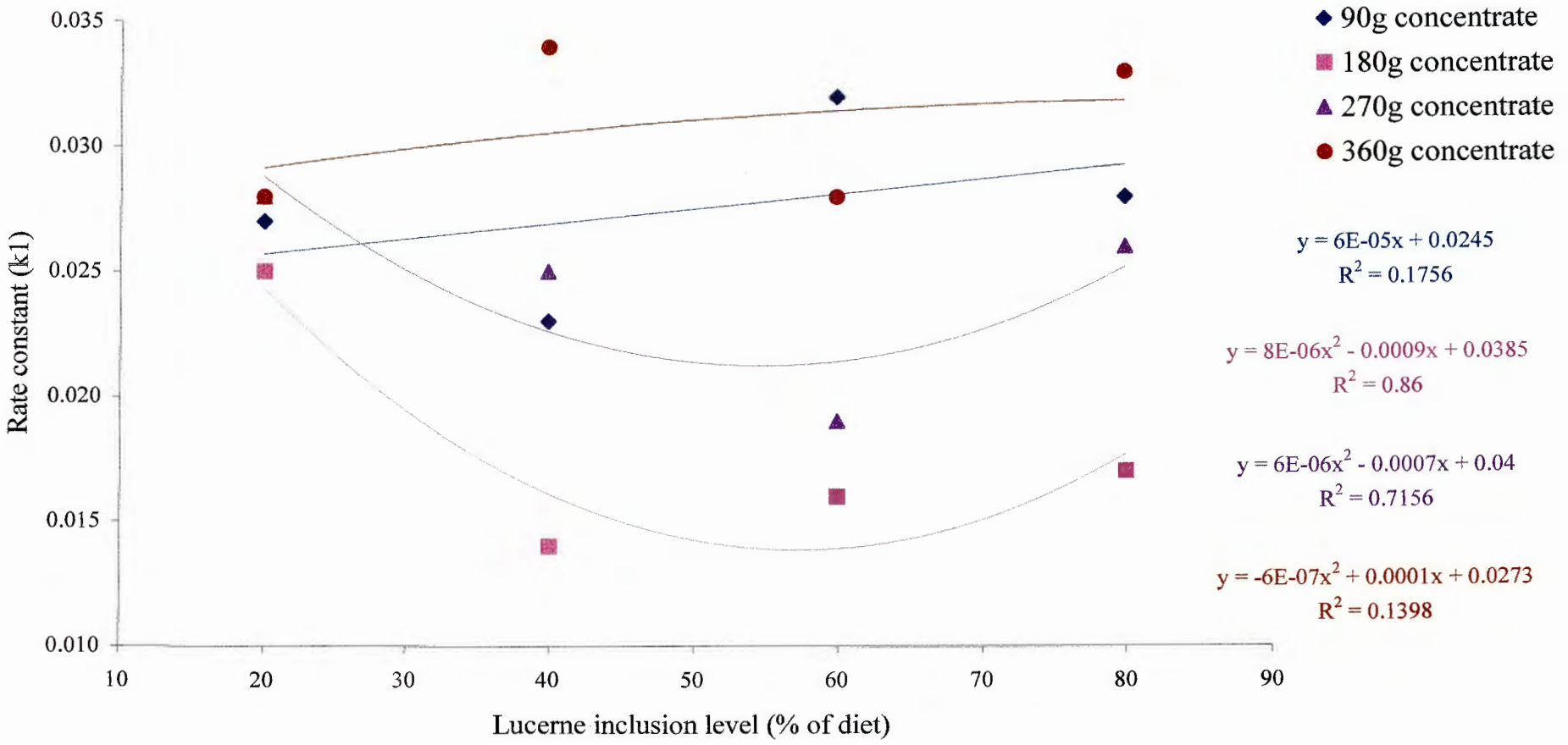
Roughage diet	Lucerne (%)	Concentrate level (g sheep^{-1} day^{-1})	DM intake (g day^{-1})	NDF intake (g day^{-1})
1	20	90	633	467
1	20	180	640	452
1	20	270	595	420
1	20	360	578	408
2	40	90	688	450
2	40	180	664	435
2	40	270	588	385
2	40	360	636	417
3	60	90	670	400
3	60	180	613	366
3	60	270	642	383
3	60	360	717	428
4	80	90	643	347
4	80	180	572	309
4	80	270	724	391
4	80	360	678	366

Appendix 4: Mean values of average total daily intake of DM and CP from both the basal roughage and the concentrate supplement for each of the 16 dietary treatments.

Roughage diet	Lucerne (%)	Concentrate level (g sheep^{-1} day^{-1})	DM intake (g day^{-1})	CP intake (g day^{-1})
1	20	90	712	66
1	20	180	798	87
1	20	270	832	104
1	20	360	894	123
2	40	90	767	99
2	40	180	822	117
2	40	270	825	128
2	40	360	952	154
3	60	90	749	114
3	60	180	772	126
3	60	270	879	150
3	60	360	1032	181
4	80	90	722	132
4	80	180	730	140
4	80	270	961	186
4	80	360	994	199



Appendix 5: The effect of concentrate inclusion level on the rate constant (k1), for each of the four different basal roughage rations fed.



Appendix 6: The effect of lucerne inclusion level on the rate constant (k1), for each of the four different concentrate levels fed.