THE NUTRITIVE VALUE OF SAINFOIN (ONOBRYCHIS VICIIFOLIA), SHEEPS' BURNET (SANGUISORBA MINOR) AND LUCERNE (MEDICAGO SATIVA).

BY

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DECLARATION

I declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in the University of Pretoria. It has not been submitted before for any degree or examination at any other university.

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ABSTRACT

The nutritive value of sainfoin (Onobrychis viciifolia), sheep's burnet (Sanguisorba minor) and lucerne (Medicago sativa).

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The aim was to assess the nutritive value of sainfoin and sheep's burnet using lucerne as a reference or control. The following indices of nutritive value determined on pasture or with sheep were employed: dry matter (DM) yield and leaf to stem ratios, chemical composition, digestibility and voluntary intake of organic matter (OM) of the forages, post-ruminal disappearance of non-ammonia nitrogen (NAN) and degradation of the forage proteins in the rumen of sheep.

The study was conducted in two phases with Phase I covering mid-summer to mid-autumn and with only sainfoin and sheep's burnet as treatments. Phase II covered late autumn to early summer with lucerne included as a treatment.

There were no significant ($P \leq 0,05$) differences in DM yield of sainfoin and sheep's burnet in Phase I, whereas both sainfoin and sheep's burnet had significantly higher yields than lucerne in Phase II. There were significant differences in leaf to stem ratios between the three forages in both phases with sainfoin generally having a higher proportion of leaf material followed by lucerne and sheep's burnet in that order.

There were significant differences between the three pastures in both phases with respect to crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF). However, all three forages contained adequate CP for animal production purposes. Sainfoin had considerably higher lignin contents compared to the other two forages. Calcium, P and Mg contents in the three pastures exceeded the optimum values quoted in the literature.

There were significant differences in OM digestibility between the three pastures in both phases with lucerne having the highest digestibility, followed by sheep's burnet and sainfoin in that order. There were no significant

differences in the intake of OM by sheep between sainfoin and sheep's burnet in Phase I. In Phase II, however, there was a significantly higher OM intake on sainfoin compared to sheep's burnet and lucerne which did not differ significantly from each other.

There were significant differences in NAN disappearance in the small intestine of sheep in both phases with sainfoin having the highest disappearance followed by sheep's burnet and lucerne in that order. There were also significant differences in the digestibility of NAN postruminally in both phases with sainfoin having a higher digestibility than sheep's burnet in Phases I and II but not differing significantly from lucerne in Phase II. Sheep's burnet had a significantly lower NAN digestibility than lucerne.

Incubation of samples of the forages in nylon bags in the rumen resulted in significant differences in predicted degradation of feed crude protein with lucerne having the highest degradation, followed by sainfoin and sheep's burnet in that order. An analysis for tannins confirmed the presence of condensed tannins in sainfoin and most probably hydrolysable tannins in sheep's burnet. This probably accounted for the low crude protein degradation of the two pastures in the rumen and the higher availability and disappearance of NAN in the small intestine.

SAMEVATTING

Die voedingswaarde van sainfoin (Onobrychis viciifolia), skaapburnet (Sanguisorba minor) en lusern (Medicago sativa).

deur

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Die doel van die ondersoek was om die voedingswaarde van sainfoin en skaapburnet te bepaal deur lusern as verwysing of kontrole te gebruik. Die parameters van voedingswaarde wat op die weidings en met skape bepaal is, is droë materiaal (DM) opbrengs, blaar tot stingel verhouding, chemiese samestelling, verteerbaarheid en vrywillige inname van die organiese materiaal (OM) van die gewasse, verdwyning van nie-ammoniak-stikstof (NAN) in die laer spysverteringskanaal en degradeering van gewasproteïen in die rumens van skape.

Die studie is in twee fases uitgevoer. Fase I is van toepassing op middel-somer tot middel-herfs, met slegs sainfoin en skaapburnet as behandelings. Fase II het laat herfs tot vroeë somer gedek, hierdie keer met lusern ingesluit.

Geen betekenisvolle verskille ($p \leq 0,05$) is in DM opbrengs tussen sainfoin en skaapburnet vir Fase I gevind nie. Daar was wel in Fase II betekenisvolle verskille, met sainfoin en skaapburnet wat groter opbrengste gelewer het as lusern. Daar was betekenisvolle verskille in blaar tot stingel verhouding tussen die drie gewasse in albei fases, waar sainfoin oor die algemeen 'n hoër proporsie blaarmateriaal gehad het, gevolg deur lusern en skaapburnet met die kleinste verhouding.

Die drie gewasse het in albei fases betekenisvolle verskille getoon met betrekking tot ruproteïen (RP), suurbestande vesel (SBV) en neutraalbestande vesel (NBV). Alle gewasse het voldoende RP waardes vir diereproduksie getoon. Sainfoin het heelwat meer lignien gehad in vergelyking met die ander twee gewasse. Volgens die literratuur was Ca-, P- en Mg-inhoud van alle gewasse deurgaans hoër as behoefte.

Daar was betekenisvolle verskille tussen die OM verteerbaarhede van al drie gewasse in albei fases. Lusern het die hoogste verteerbaarheid gehad gevolg deur skaapburnet en dan sainfoin. In Fase I was daar tussen sainfoin en skaapburnet geen betekenisvolle verskille met betrekking tot OM inname nie. Daar was wel in Fase II 'n betekenisvol hoër OM inname vir sainfoin in vergelyking met skaapburnet en lusern. Laasgenoemde twee gewasse het nie betekenisvol van mekaar verskil nie.

Sainfoin het betekenisvol hoër NAN verdwyning in die laer spysverteringskanaal van skape in albei fases gehad, gevolg deur

skaapburnet en lusern, in dié volgorde. Die verteerbaarheid van NAN in die laer spysverteringskanaal het ook betekenisvol verskil in albei fases. Sainfoin het 'n hoër NAN verteerbaarheid as skaapburnet in albei fases gehad, maar die verteerbaarheid het nie betekenisvol van lusern in die tweede fase verskil nie. Die NAN verteerbaarheid van skaapburnet was betekenisvol laer as dié van lusern.

Inkubasie van die gewasse in nylonsakkies in die rumen het betekenisvolle verskille met betrekking tot voorspelde degradeering opgelewer. Lusern het die hoogste degradeering gehad, gevolg deur sainfoin en dan skaapburnet. 'n Analise vir tanniene het die teenwoordigheid van gekondenseerde tanniene in sainfoin bevestig. Hidroliseerbare tanniene is waarskynlik in skaapburnet teenwoordig. Hierdie tanniene het waarskynlik aanleiding gegee tot die lae degradeering in die rumen van sainfoin en skaapburnet asook die hoër beskikbaarheid en verdwyning van NAN in die dunderm.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Indices of nutritive value and nutritive value of experimental forages

The nutritive value of feeds is conventionally classified under three general components: digestibility, feed consumption (voluntary intake) and the efficiency with which feed energy is utilized (Raymond, 1969). Voluntary intake and the rate and extent of digestion are in turn influenced by the animal consuming the forage, physical characteristics of the forage like the morphological and anatomical characteristics and the chemical properties of the forage. A further component of the nutritive value of feed for ruminants is the degradation of proteins in the rumen. Chalmers and Synge (1954) noted that the nutritive value of feed proteins for ruminants varies inversely with the extent of degradation of the proteins in the rumen.

The extent of dietary protein degradation in the rumen has been related to its solubility (Sherrod & Tillman, 1962; Evans & Biddle, 1971; Peter et al., 1973; Wohlt et al., 1973; Aitchison et al., 1976; Aii & Stobbs, 1980). Protein degradation has led to considerable interest in the concept of by-pass proteins which are thought to leave the rumen intact and escape degradation. By-pass protein fed as a

supplement or forage with by-pass protein characteristics would have the following advantages:

a) it would supply dietary amino acids for absorption.

b) supply glucogenic precursors at tissue level and therefore act as an energy supplement (Lindsay & Armstrong, 1982). This role seems vital when one considers the poor response to energy supplements on high quality pastures due to extensive interaction between energy supplements and pasture digestion (Langlands, 1969).

c) prevent bloat due to reduced plant protein degradation in the rumen (Barry, 1984).

Thus sainfoin or any other forage with by-pass protein characteristics could be used as a sole pasture or in combination with pasture herbs like sheep's burnet which has some feeding potential. Forages with by-pass protein characteristics could also be used in combination with low quality grasses, as by-pass protein has been demonstrated to increase feed intake in sheep (Egan, 1965) and milk production by 20% when formaldehyde treated casein was given to dairy cows feeding on tropical grass (Stobbs et al., 1977). Further evidence was provided by Flores et al. (1979) who showed that a supplement of the tropical legume Leucaena leucocephala increased milk yield in a manner similar to formaldehyde treated casein. They deduced from this observation that L. leucocephala may be more resistant to

deamination in the rumen than the protein in grasses.

The few references to sainfoin in the literature indicate a fodder crop of modest soil requirements and many desirable characteristics, and which has always been prized as a hay for livestock especially brood mares in racing stables (Baker,1952). Work done has shown that the availability to the animal of sainfoin protein appears to be exceptionally high due to low degradation in the rumen (Shedrick & Thomson, 1982). Thomson (1976) noted that sainfoin is a safe food since it does not cause bloat and is more digestible and has a higher voluntary intake than lucerne, red clover or S24 and S22 ryegrass. However there have been contradictory reports on its digestibility (Wilman & Asiedu, 1983; Meissner et al., 1989).

There is virtually no reference to sheep's burnet in the literature. Information gleaned from farmer information leaflets especially in New Zealand, however, indicate a palatable forage that does well even under demanding conditions and provides grazing over a wide range of lower fertility and harsh environmental conditions. Its bloat-free properties have also been noted by farmers in New Zealand Bulletins.

The importance and wide use of lucerne (alfalfa) in many parts of the world, its high digestibility (Wilman et al.,

1977; Wilman & Asiedu, 1983) and nitrogen content (Joyce et al., 1973; Wilman et al., 1983) and its bloat inducing properties makes it an ideal forage for comparison with sainfoin and sheep's burnet.

1.2 Evaluating pastures for yield and quality or nutritive value.

The yield of animal products from pastures depends on a number of associated factors. These factors must be measured, or at least estimated, in order to improve the utilization of forages. Van Soest (1982) summarized these factors or components in the form of a table (Table 1.1). Earlier work in this regard had been done by Raymond (1969), Streeter (1969), Langlands (1975) and Cordova et al. (1978).

Table 1.1 Methods of pasture evaluation.

Aspect	Factor	Method
Forage yield		Cages, clipping
Forage quality	Composition	Chemical analysis
	Digestibility	Oesophageal fistulae, in vitro rumen, faecal bag or grab sampling, markers
	Intake	
Animal yield	Carrying capacity	Stocking rate, put and take, yield/ha
	Efficiency	Portable respiration apparatus

In addition to the factors listed, the rate and extent of degradation of feed protein in the rumen (as discussed before), has been long recognized by several researchers as an important determinant of the nutritive value of feed for ruminants (Chalmers & Synge, 1954; Sherrod & Tillman, 1962; Little et al., 1963; Whitelaw & Preston, 1963; Sharma et al., 1972; Peter et al., 1973; Aitchison et al., 1976). Thus, protein degradation in the rumen is a factor that cannot be ignored in any comprehensive evaluation of pasture for quality or nutritive value.

1.2.1 Dry matter yield as an index of nutritive value and its estimation.

The importance of dry matter yield data in any comprehensive system of pasture evaluation does not lie only in the fact that it ultimately determines the amount of plant material and therefore nutrients available to the grazing animal, but also due to the fact that there is a relationship between forage quality and forage yield. Both experimental and practical evidence suggests that as a forage crop grows to maturity its nutritive value generally tends to decrease (partly due to changes in the ratio of plant parts). Thus, the practical exploitation of any crop involves a compromise between yield and nutritive value. Data on yields at different stages of maturity and digestibilities obtained by in vitro techniques can be used to compile yield-digestibility relationships for different forages and

a comparison in terms of their yields at the given levels effected. Raymond (1969) reported work done by Green at the Grassland Research Institute, Hurley, in the form of graphs for S24 ryegrass during its first growth in spring (Figure 1) and also the yields of different grass varieties at an organic matter digestibility of 65% (Figure 2).

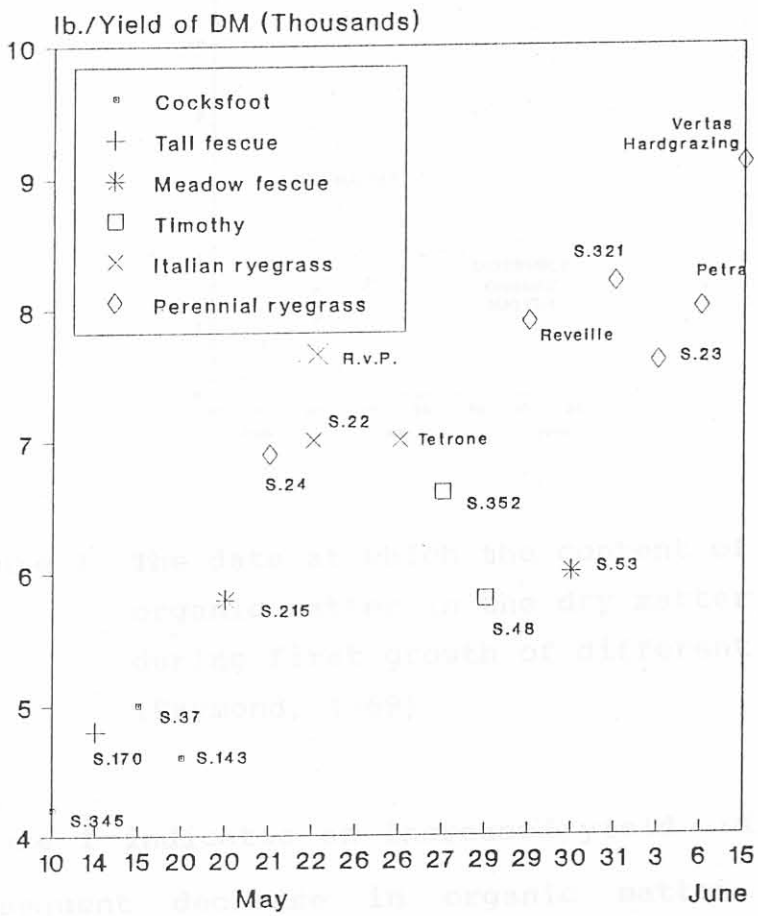

Figure 1. The changes in yield and digestibility of the first growth of S.24 ryegrass during first growth in spring (Raymond, 1969).

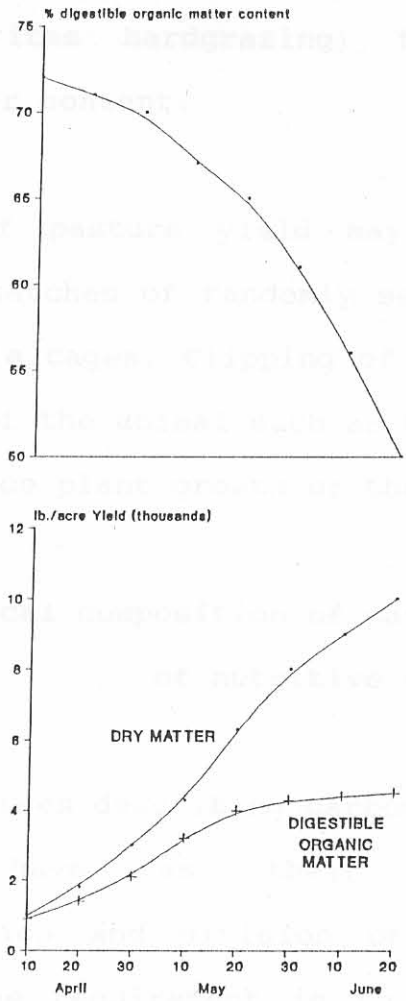

Figure 2. The date at which the content of digestible organic matter in the dry matter falls to 65% during first growth of different grass varieties (Raymond, 1969).

Figure 1 indicates an increased yield with maturity, but a consequent decrease in organic matter digestibility. A compromise therefore has to be sought between digestibility and yield. Figure 2 indicates the higher yields of ryegrass than other grass species harvested at the same digestibility and the interval of 23 days between the dates at which the digestibility of the earliest (S24) and the latest ryegrass

variety (Veritas hardgrazing) falls to 65% digestible organic matter content.

Estimation of pasture yield may be accomplished by the clipping of patches of randomly selected areas protected by use of movable cages. Clipping of protected area eliminates the effects of the animal such as trampling and manure spots which influence plant growth or the plant (Van Soest, 1982).

1.2.2 Chemical composition of pasture as an index of nutritive value.

Chemical analyses describing carbohydrate, protein and fiber fractions, have as their first objective the characterization and division of the dry matter of the feedstuff. The requirement is to establish a relationship between these fractions and a nutritional parameter (eg. digestibility) of forages measured in controlled in vivo experiments from which the nutritive values of other forages can be predicted.

The Weende system of proximate analysis has been generally used for chemical evaluation of feedstuffs in both ruminant and non-ruminant nutrition for more than a century. However criticism of the crude fibre method and the calculation of the nitrogen free extract (NFE) led to serious efforts to find a replacement. Norman (1935) established the inadequacy

of crude fibre as a determinant of nutritive value. Other fibre methods were subsequently proposed including cellulose (Crampton & Maynard, 1938), holocellulose (Ely & Moore, 1955) and eventually the detergent systems (Van Soest, 1963; Van Soest & Wine, 1967) which are in widespread use today. Table 1.2 shows the fractionation of forage organic matter by the system of analysis using detergents.

Table 1.2 Division of forage organic matter by the system of analysis using detergents (Van Soest, 1965).

Fraction	Components
Cell contents (soluble in neutral detergent)	Lipids Sugars, organic acids and water soluble matter Pectin Starch Non-protein nitrogenous compounds Soluble proteins
Cell wall constituents (fiber insoluble in neutral detergent) 1) Soluble in acid detergent 2) Acid-detergent fiber	 Fiber-bound protein Hemicellulose Cellulose Lignin Lignified nitrogenous substances

1.2.2.1 Correlations between chemical composition and nutritive value.

Summarizing work done with a total of 121 forages from different locations in the United States, Van Soest (1965) deduced that in terms of chemical composition and nutritive value the only consistent effect that could be observed for all forages is that of the total fibrous fraction or cell wall constituents. Van Soest (1984) and Allen (1990) reported work done by Mertens with 187 forages (126 grasses and 61 legumes) from 15 experimental sites in the United States and fed at different stages of growth. Table 1.3 and 1.4 indicate correlations of chemical and fermentation parameters with voluntary intake and digestibility.

Table 1.3 Correlations of forage components with voluntary intake and digestibility (Van Soest, 1984).

Component	Intake	Digestibility (in vitro)
Digestibility (in vivo)	+0,61	+0,80
Digestibility (in vitro)	+0,47	
Lignin	-0,08	-0,75
ADF	-0,61	-0,44
Crude protein	+0,56	+0,56
Cellulose	-0,75	-0,45
Cell wall	-0,76	-0,45
Hemicellulose	-0,58	-0,12
Rate of digestion	+0,53	+0,44

Table 1.4 Correlations of ad libitum dry matter intake (kg/$LW^{0.75}$*) with chemical and in vitro fermentation parameters of 126 grasses and 61 legumes (Allen, 1990).

	Grasses	Legumes	Combined
NDF	-0,74	-0,69	-0,76
ADF	-0,69	-0,77	-0,64
Rate of NDF digestibility	+0,66	+0,26	+0,55
Lag	-0,47	-0,45	-0,48
In vitro digestibility			
0h	0,75	0,67	0,75
6h	0,79	0,68	0,79
12h	0,77	0,68	0,74
24h	0,76	0,71	0,64
48h	0,72	0,69	0,46
96h	0,70	0,67	0,36

* LW - livemass

The two tables indicate a high correlation between NDF and intake whilst ADF and lignin are more related to digestibility. It is also evident from the tables the errors that are likely to be made when attempts are made to predict intake from digestibility since the two parameters do not show a consistent relationship.

1.2.3 Voluntary intake and digestibility as indices of nutritive value and their estimation.

Measurements of voluntary intake and total gut tract digestibility are among the most critical estimates required to evaluate the nutritional status of forages for ruminants (Gaylean et al., 1986). Van Soest (1982) noted that intake and efficiency indicate more inter-animal variation (Table

1.5) than digestibility and that it is more difficult to establish feed values for intake and efficiency.

Table 1.5 The approximate relative variation contributed by animal and diet (forage) (Van Soest, 1982).

	Coefficient of variation %	
	Diet	Animal
Digestibility	30	3
Intake	50	30
Efficiency[a]	50	20

a - Use of energy for productive purposes

Thus digestibilities are commonly measured and exclusively used though intake and efficiency are more responsible for total animal responses. Ingalls et al. (1965) concluded that 70% of the variation in production potential between forages can be accounted for in terms of differences in voluntary intake and 30% by differences in digestibility. Gaylean et al. (1986) noted that estimates of nutrient intake in grazing ruminants are typically derived by coupling faecal output data with nutrient indigestibility and that methods that provide accurate and precise estimates of faecal output and digestibility are paramount to a critical evaluation of nutrient intake and digestive physiology in grazing ruminants. Thus, the methods for estimating faecal output, digestibility and intake of grazing ruminants are not mutually exclusive.

1.2.3.1 Methods for estimating faecal output, digestibility and intake of grazing ruminants.

1.2.3.1.1 Total collection of faeces.

This is known as the "standard", "conventional" or direct method. Faecal collection bags have been used in many grazing and confinement trials to determine intake in cattle and sheep. Male animals have generally been used because the separation of urine and faeces is satisfactory. However, when intake and digestibility information particularly relating to nutrition of female physiological processes is required, a female animal must be used and a faeces-urine separator employed (Kartchner & Rittenhouse, 1979).

Problems associated with the method include the fact that it is labour intensive, requires selection of animals with appropriate temperament for harnessing and behavioral modifications due to harnessing and inconsistency of faecal output (Gaylean et al., 1986). However random errors resulting from inconsistency of faecal excretion decrease as the length of the collection period increases. Gaylean et al. (1986) suggested four days as the minimum with seven or more being more appropriate.

Digestibility may be estimated in vitro from samples collected by oesophageally fistulated animals and intake determined from faecal output and digestibility (Langlands,

1975). Digestibility may also be estimated from ratio or index techniques.

1.2.3.1.2 Ratio techniques.

These involve the calculation of digestibility and faecal output through their ratio to an "indigestible" indicator or marker (Cordova et al., 1978). Intake may be calculated once the digestibility and faecal output have been determined from the simple equation:

$$\text{Organic matter intake} = \frac{\text{faecal organic matter output}}{\%\ \text{organic matter indigestibility}}$$

1.2.3.1.2.1 Internal markers or indicators

With internal indicator methods indigestibility is computed by dividing the concentration of a naturally occurring substance in the diet by the concentration of the same substance in the faeces. Digestibility is then calculated by subtracting indigestibility from unity (Streeter, 1969). Internal marker methods that have been used include: chromogen ratio (Reid et al., 1950; Cook & Harris, 1951), lignin ratio (Connor et al., 1963; Wallace & Van Dyne, 1970; Cordova et al., 1978), silica (Jones & Handreck, 1965; McManus et al., 1967), acid insoluble ash (Penning & Johnson, 1983a), potentially indigestible cellulose (Wilkins, 1969; Penning & Johnson, 1983a), indigestible acid detergent fibre (Penning & Johnson, 1983b), indigestible neutral detergent fibre (Lippke et al., 1986), long chain

fatty acids (Body & Hansen, 1978) and long chain n-alkanes (Mayes & Lamb, 1984; Mayes et al., 1986; Dove, Foot & Freer, 1989; Dove, Mayes, Freer, Coombe & Foot, 1989).

1.2.3.1.2.2 External markers or indicators

An external indicator is one which is administered in known amounts. Faichney (1975) summarized the properties of an ideal marker as follows:

1. It must be strictly non-absorbable.
2. It must not affect or be affected by the gastro-intestinal tract or its microbial population.
3. It must be physically similar to or ultimately associated with the material it is to mark.
4. Its method of estimation in digesta samples must be specific and sensitive and it must not interfere with other analyses.

The last point emphasizes the use of markers not only in determining faecal output and feed intake but in the partitioning of digestion within the gastro-intestinal tract, by measuring the quantity and composition of digesta flowing past either re-entrant cannulae (MacRae, 1975) or simple T-shaped cannulae. Digesta flow from the rumen, however, is a discontinuous process, with the fluid and particulate phases flowing at different rates. Therefore the use of a single marker with spot sampling from re-entrant cannulae or simple T-shaped cannulae may give erroneous

estimates of flow due to unrepresentative sampling of the digesta in terms of a preferential selection of the two phases (Siddons et al., 1985). Faichney (1975) demonstrated that this problem could be solved by using two markers in a dual phase system: one to mark the fluid (liquid) phase and the other the particulate phase of digesta.

A comprehensive review of markers and their applications in nutrition has been given by Kotb & Luckey (1972). However, some markers that have been used in recent times in digestibility and intake determinations and the measurement of digesta flow along the alimentary tract include: chromium sesquioxide (Cr_2O_3) (Drennan et al., 1970; Faichney, 1972; Langlands, 1975; Gaylean et al., 1986), the ^{51}Cr complex of ethylenediaminetetraacetic acid (Cr-EDTA) (Downes & McDonald, 1964; Weston & Hogan, 1967; Faichney, 1975), chromium mordant (Uden at al., 1980), the Li or Na salt of Co(III) EDTA (Uden et al., 1980), polyethylene glycol (PEG) (Kay, 1968), Ytterbium (Yb) labelled feedstuffs (Gaylean et al., 1986), Yb acetate (Downes & McDonald, 1964; Drennan et al., 1970; Faichney & Weston, 1971; Hogan & Weston, 1971; Beever et al., 1978; Siddons et al., 1985) and cerium (Uden et al., 1980).

1.2.3.1.3 Index techniques.

These methods generally relate level of intake or digestibility to some component in the faeces through a regression equation. The motivation for the technique arose due to the difficulty in obtaining a representative dietary sample by handclipping of forages (Raymond, 1954). The most common faecal component that has been used is faecal nitrogen. Faecal chromogen has also been used. The faecal nitrogen method entails harvesting herbage for conventional digestion trials to establish a regression between digestibility and percent nitrogen in the faeces. The assumptions behind the faecal index method are:

1) The herbage fed to the animal is similar to that grazed by the animal, and
2) the penned and grazing animals digest herbage to the same degree (Wallace & Van Dyne, 1970).

An advantage of the method is that it does not require sampling of the grazed forage. Weaknesses of the method include:

1) The assumption that the faecal index relationship is the same for forages selected by grazing animals as for forage fed in a conventional feeding trial (Streeter, 1969).
2) The regression varies between first growth and aftermath herbage (Greenhalgh & Corbett, 1960).
3) It estimates digestibility of a group of animals rather

than individuals (Van Dyne & Meyer, 1964).

4) The regression varies from year to year (Topps, 1962).

5) The regression varies for different cultivars of the same species of forage (Minson & Milford, 1967).

1.2.4 Protein degradation in the rumen as an index of nutritive value and its estimation.

Degradation of protein in the rumen is an important value to be used for the prediction of protein passing undegraded to the small intestine for the calculation of protein utilization and protein requirements of ruminants (Raab et al., 1983). Chalmers and Synge (1954) reported an inverse relationship between the nutritive value of feed protein and the extent of its degradation in the rumen.

1.2.4.1 Estimation of protein degradation.

1.2.4.1.1 Protein solubility in mineral buffers.

The extent of dietary protein degradation in the rumen has been related to its solubility by several researchers (Sherrod & Tillman, 1962; Evans & Biddle, 1971; Aitchison, 1976) and attempts have been made to estimate protein degradation from solubility tests (Peter et al., 1973; Wohlt et al., 1973; Crooker et al., 1978; Chamberlain & Thomas, 1979; Krishnamoorthy et al., 1982). Aii and Stobbs

(1980) determined the solubility of some tropical grasses and legumes in Burrough's mineral mixture and found that the solubility varied between grasses with lowest values found for Digitaria decumbens, Panicum maximum and Setaria anceps and the highest protein solubility found in Panicum coloratum, Bracharia mutica and Chloris gayana. With the legumes Desmodium intortum and Desmodium uncinatum had the lowest solubilities and Macroptilum uniflorum, the highest. Abdalla et al. (1988) found no differences in solubility due to forage type or harvest type (Year 1 or 2) when he compared the solubility of Timothy, Brome and Orchard grasses and Fescue and combinations of Trefoil and grass or grass alone. Mineral solvents or buffers that have been suggested for the estimation of protein solubility include Wise Burrough's mineral mixture (Burrough's et al., 1950), McDougalls artificial saliva (Peter et al., 1973), bicarbonate phosphate buffer, autoclaved rumen fluid, NaCl and borate phosphate buffer (Krishnamoorthy et al., 1982). Broderick & Craig (1980) however noted that techniques such as protein-solubility may be inaccurate because they tend to measure the properties of only the rapidly degraded fractions and not those of the protein as a whole.

1.2.4.1.2 Use of commercial proteases.

Poos et al. (1980) reported the use of five commercial proteolytic enzymes (bacterial protein, papain, ficin,

bromelain and fungal protease) in predicting rumen degradation and percent by-pass of various protein sources. They noted that fungal protease gave the highest correlation across all incubation times (1 - 24 hours). Chamberlain & Thomas (1979) used a mixture of artificial saliva and a mixed protease (Streptomyces griseus) and protein breakdown was measured as a proportion of N not precipitated with 1.2 M trichloroacetic acid.

1.2.4.1.3 Use of duodenally cannulated animals and digesta markers (Faichney, 1975; MacRae, 1975).

These techniques have been used to obtain measurements of protein flow into the abomasum. Used in conjunction with microbial markers such as RNA or DAPA (Ling & Buttery, 1978) or isotopes such as ^{35}S, ^{32}P or ^{15}N (Pilgrim et al., 1970; Walker & Nader, 1975; McMeninam et al., 1976; Van Nevel & Demeyer, 1977), determinations of microbial protein synthesis can be obtained. A flow of true protein nitrogen and the proportion of ingested nitrogen degraded in the rumen can then be obtained.

1.2.4.1.4 Use of mathematical models.

In an attempt to recognize the importance of rumen dynamics and associated factors such as rate of passage, rate of degradation and lag time before degradation begins,

mathematical models have been employed to predict dietary protein degradation in the rumen. Prominent are:

1) The model by Ørskov and McDonald (Ørskov & McDonald, 1979) applied basically to the use of polyester bags to incubate feed protein in the rumen of a fistulated animal (In situ technique) (Ørskov & Mehrez, 1977; Nocek, 1985).
2) The model by Broderick and Craig (Broderick & Craig, 1980) to interpret data from in vitro incubations conducted using ratios of protein to ruminal fluid similar to those expected in vivo.

Both models are similar in their assumptions of the heterogeneity of feed protein and first order kinetics for degradation and passage of protein from the rumen.

Thus in the model proposed by Ørskov and McDonald the amount of protein (p) degraded at time (t) is given by the exponential equation

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

where a is interpreted as the rapidly soluble protein fraction, b the amount which in time will degrade and c the fractional-rate constant at which the fraction described by b will be degraded per hour. The equation is normally constrained so that a + b cannot exceed 100%. The constants in the exponential equation are then determined by curve fitting (Ørskov, 1982) or an iterative least square procedure.

1.2.4.1.5 Other techniques for estimating protein degradation in the rumen.

Other methods employed include the prediction of protein degradation from the release of ammonia (NH_3) (Raab et al., 1983; Chamberlain & Thomas, 1979) from the incubation of a feedstuff with rumen fluid in vitro. A colorimetric method (Mahadevan et al., 1979) where the protein is converted into a highly colored diazotized derivative is also employed. The derivative is subsequently incubated in vitro with rumen microorganisms and the amount of the undigested precipitable diazo-protein determined.

1.3 Growth and nutritive value of experimental forages.

1.3.1 Sainfoin.

1.3.1.1 Growth characteristics.

Little research effort has been devoted to the growing of sainfoin. A survey of the few references available reveal the following:

a) It is a deep rooting perennial well adapted to drought conditions and frost tolerant.
b) It seems to prefer calcaerous soils (Baker et al., 1952).
c) It is a crop of modest requirements and does not respond to fertilization with phosphorus (Roath & Graham, 1968).

1.3.1.2 Nutritive value of sainfoin.

1.3.1.2.1 Yield and leaf to stem ratios.

The poor establishment and low yields of sainfoin (Wilman & Asiedu, 1983) in comparison to other forage legumes may account for the low research interest shown in its growing. Varga (1968), however, showed that leafier varieties with improved after growth capacities were attainable through vigorous breeding programs.

Baker (1952) cut sainfoin, grown under dryland conditions, at different physiological stages of growth and reported mean dry matter yields of 9,13 t/ha*. When the first full flowering cut was made into hay, yields of 7,25 t/ha* were obtained.

Leaf:stem ratios ranging from 1,17 to 0,70 were obtained for the first and subsequent re-cuts for the pre-flowering stage; 0,74 to 0,64 for the early flower stage and 1,98 to 0,52 for the full flower stage. Wilman and Asiedu (1983) in a study with sainfoin, red clover, lucerne and ryegrass under dryland conditions noted dry matter yields (t/ha) of 1,017; 1,504 ; 2,030 and 3,899 respectively for three primary growth and four regrowth periods. The percentage of green leaf in dry matter were 79,3; 84,2; 63,2 and 54,8 in the same order. Mean dry matter yields (t/ha*) of 6,73 when

* computed from t/acre by multiplying by 2,5

cut as pasture and 8,68 when sainfoin was cut as hay were obtained in Reno, Nevada (Jensen & Sharp, 1968). Hanna and Smoliak (1968) recorded the following results from several hay tests in Canada: dry matter yields (t/ha*) for different cultivars under dry land conditions ranged from 3,48 to 7,10 (compared with 10,70 for lucerne) in Saskatchewan; 4,95 to 7,55 (8,40 for lucerne) in Lethbridge (irrigated); 4,95 to 7,08 (9,08 for lucerne) also in Lethbridge (dryland conditions); 9,50 to 13,25 (13,38 for lucerne) in Winnipeg and 1,13 to 4,20 (5,60 for lucerne) in Melfort. In these tests Eski and some Russian strains provided the most promise. The authors noted the following results for a dryland grazing test (Table 1.6):

Table 1.6 Relative yields and apparent consumption by sheep in dryland grazing test, Lethbridge, 1968.

	Yield as % of alfalfa	Apparent %	Consumption lbs DM
Alfalfa	100	61	494
Sainfoin	108	83	718
Alfalfa + CWG[a]	100	55	445
Sainfoin + CWG	98	61	476

a - crested wheatgrass

Comparing data on different sainfoin cultivars and other common legume fodder crops, Carleton et al. (1968) drew the following conclusions:

i) Eski sainfoin has yielded less than, as much as, or more than alfalfa (lucerne) depending upon location and year

* computed from t/acre by multiplying by 2,5.

of production.

ii) Eski has been more productive than all other one-cut sainfoin varieties and lines tested and equal in yield to one multi-cut type sainfoin.

iii) Sainfoin yields generally more in the first cutting and less in the second cutting than alfalfa.

iv) Sainfoin recovers more slowly following cutting than alfalfa. Frequent cutting affects sainfoin more adversely than alfalfa due to an increased loss of stand.

v) Yields of sainfoin in the third year after seeding may be reduced because of a decrease in stand.

1.3.1.2.2 Chemical composition, digestibility, voluntary intake and animal performance on Sainfoin.

Baker (1952) recorded the following chemical composition of sainfoin (Table 1.7).

Table 1.7 Average composition of sainfoin at different growth stages.

	Preflowering		Early flower		Full flower	
	1949	1950	1949	1950	1949	1950
Dry matter(%)	24,5	20,2	25,5	20,6	27,3	23,1
	Percentage composition of dry matter					
Crude protein	20,4	21,5	18,7	20,2	17,4	17,5
Ether extract	2,4	2,8	2,5	2,6	2,5	2,4
Crude fibre	15,1	18,2	20,6	21,2	22,4	24,9
N-free extract	56,4	50,2	52,4	49,3	51,8	47,7
Total ash	5,7	7,3	5,8	6,7	5,9	7,1
Silica	0,1	0,2	0,1	0,1	0,2	0,1
Silica-free ash	5,6	7,1	5,7	6,6	5,7	7,0
CaO	1,88	2,13	2,05	2,05	2,13	2,15
P_2O_5	0,66	0,61	0,62	0,62	0,53	0,57
K_2O	1,11	1,76	0,95	1,53	0,87	1,61
MgO	0,49	0,45	0,46	0,44	0,50	0,41
Chlorides as NaCl	0,46	0,71	0,37	0,64	0,32	0,65
True protein	17,6	18,7	15,4	17,6	15,1	15,6
"Amides"	2,8	2,8	3,3	2,6	2,3	1,9

Davis (1968) recorded crude protein content of sainfoin leaf of 11 varieties from the U.S.S.R., ranging from 15,1% to 17,7% (mean 16,3%). Wilman and Asiedu (1983) obtained a nitrogen content (mean of two primary growth and two first regrowth periods of age 8 weeks) of 3,43% compared to red clover (4,83%), lucerne (4,52%) and ryegrass (3,15%).

Terry and Tilley (1964) noted that in contrast to lucerne, the in vitro digestibility of the leaflets and petioles of sainfoin were considerably less than those of lucerne leaves and that sainfoin stems were much more digestible than those of lucerne of a similar whole plant digestibility. Their results showed in vitro dry matter digestibilities of 70%; 71%; 72% and 74% for Cotswold common, Giant, Local Russian and Turkish Anatolian cultivars of sainfoin. Wilman & Asiedu (1983) reported in vitro dry matter digestibility of 63,2% for sainfoin in contrast to 69,1%; 67,2% and 67,7% for red clover, lucerne and ryegrass respectively. The digestibilities for sainfoin 65,2% (leaf) and 64,7% (stem) were in contrast to 75% for lucerne leaf and 60,4% for stem. Meissner et al. (1989) reported an in vitro digestibility of organic matter of material from oesophageal fistulae for sainfoin of 49,9% (November, 1986) and 49,5% (June, 1987) and voluntary organic matter intakes per metabolic livemass of 37,5g and 37,8g for the two periods. Hanna and Smoliak (1968) reported higher apparent consumption of sainfoin and a mixture of sainfoin and crested wheatgrass than lucerne and its mixture with the same grass (Table 1.6). In a study with young sheep grazing pure species plots grown under high soil fertility conditions, Barry (1984) reported that with the exception of Huia white clover, there were higher livemass gains on Fakir sainfoin compared to Maku lotus, Wairau lucerne, Hamua red clover and Ruanui ryegrass.

1.3.1.2.3 Condensed tannins and nutritive value of sainfoin.

Condensed tannins are polymeric flavanols and confer both beneficial and detrimental effects depending upon tannin molecular weight and reactivity with proteins (Barry, 1984). Legumes containing substantial concentrations of condensed tannins in leaf and stem tissue do not cause bloat due to precipitation of soluble plant proteins in the rumen. Another consequence thereof is the higher availability of the protein postruminally. High molecular weight (17000 - 28000) condensed tannins (6% of DM) occur in the leaves and stems of sainfoin (Barry, 1984). Because of the higher molecular weight of the tannins in sainfoin, rumen carbohydrate digestion is not so depressed but the tannins are not so efficient at increasing amino acid supply in the lower digestive tract as the low molecular weight tannins found in Lotus spp. and other plants (Barry, 1984).

1.3.2 Sheep's Burnet.

There appears to be very little scientific information on this promising herb. Information gleaned from New Zealand farmer information leaflets and John Fair's Guide to Profitable Pastures (Fair, 1989) gives the following picture about the growth and nutritive value:

i) Even though its cultivation dates back to the turn of the century in this country, it has only received attention recently when it came into the country as a

contaminant of imported sainfoin seed.

ii) It is a deep-rooting (tap root up to six meters long) herb with a reputation for survival and continued production over a wide range of low soil fertility, cold and dry conditions. It responds vigorously, however, to water and grows best in 500 - 600 mm rainfall regions but can survive where rainfall is as low as 300 mm annually. It tolerates a pH range of 5 - 8. The most productive periods are autumn/winter and early spring but it will continue to give foggage in summer.

iii) It has a long establishment period of about 12 - 18 months.

iv) It is an aggressive competitor for soil moisture and seems to suppress and smother weeds.

v) It can be mixed with hardy grasses such as perennial wheatgrass, Brome or Tall oat grass or planted in conjunction with Lotus corniculatus or sainfoin.

vi) It has a further important role to play in soil conservation (Fisher et al., 1987).

vii) It produces a copious amount of seed and germinates profusely from self sown seeds.

1.3.3 Lucerne.

1.3.3.1 Growth characteristics.

Lucerne is adapted to a wide range of environmental

conditions. Its strong tap root system enables it to draw moisture from great depths resulting in good production on soils with a deep water table and from deep soils during droughts (Fair, 1989).

1.3.3.2 Nutritive value of lucerne.

1.3.3.2.1 Yield and leaf to stem ratios.

A lot of research work has been done on yields of lucerne. A few well chosen examples, however, would give a good overview of the yields of lucerne pasture. Table 1.8 shows yields of lucerne at different physiological stages of growth in a study at Wairakei Experimental station, Taupo, New Zealand.

Table 1.8 Yields of lucerne pasture at different stages of growth (Joyce et al., 1973).

	EV	LV	BF	FL
Yield (kg/ha)				
Dry matter	2498	3469	4159	4650
Digestible organic matter	1631	2184	2392	2607
Crude protein	744	917	1030	1028
Digestible crude protein	600	709	796	771

EV - Early vegetative
LV - Late vegetative
BF - Before flowering
FL - Flowering

Willman and Asiedu (1983) reported dry matter yields (t/ha) of 2,03 for three primary growth and four regrowth periods under dryland conditions and green leaf expressed as a percentage of dry matter of 60,7 for three primary growth and four regrowth periods at Aberystwyth, Wales. Rethman et al. (1986) reported yields (t DM/ha) ranging from 3,75 to 7,01 under dryland conditions at different espacement at the Nooitgedacht Research station, Ermelo, in the Eastern Highveld region of South Africa.

1.3.3.2.2 Chemical composition, digestibility, voluntary intake and animal performance on lucerne.

Table 1.9 shows the chemical composition of lucerne and digestibility of lucerne at different physiological stages of growth.

Table 1.9 Chemical composition and digestibility of lucerne (Joyce et al., 1973).

	EV	LV	BF	FL
% Crude protein	29,8	26,4	24,8	22,1
% Acid detergent fibre	28,2	29,7	33,3	31,4
% Lignin	4,25	5,85	6,5	6,40
% Soluble sugars	5,58	4,85	4,54	5,81
% Ash	14,5	12,3	12,7	10,5
Caloric value (kcal/g)	4,165	4,310	4,110	4,225
% Digestibility				
Organic matter	76,4	71,8	65,9	62,5
Crude protein	80,7	77,4	77,3	74,9
Gross energy	72,1	69,1	61,8	59,9

Terry and Tilley (1964) reported dry matter digestibility of 75% (whole plant), 81% (leaf) and 69% (stem) for young lucerne. The corresponding values for mature lucerne were 66%, 78% and 55% respectively. Wilman and Asiedu (1983) reported a nitrogen content (% of DM) of 4,52 and *in vitro* dry matter digestibility of 67,1% for 8 week old lucerne (mean of two primary and four regrowth periods) and N content of 4,68 (green leaf) and 1,96 (stem) for two primary growth and two first regrowth periods. The corresponding dry matter digestibility for green leaf and stem were 75,0% and 60,4% respectively. Joyce *et al.* (1973) reported the following figures for intake and livemass gain (Table 1.10).

Table 1.10 Growth rate and intake data of hoggets fed lucerne (Joyce *et al.*, 1973).

	Fed *ad libitum*				Fed Maintenance			
	EV	LV	BF	FL	EV	LV	BF	FL
Livemass gain (g/day)	91	85	48	36	16	3	13	16
Wool growth (g/day)	11	12	5	7	5	5	3	3
Dry matter intake (g/day)	724	770	593	571	434	418	441	492
Digestible organic matter intake (g/day)	484	488	375	310	288	265	251	271
Carcass weight (kg)	11,3	11,1	8,8	8,59	8,85	8,13	7,75	8,13
Efficiency of livemass gain (g DM/g LWG)	8,2	9,6	18,4	18,6	-	-	-	-

Meissner *et al.* (1989) working at Hatfield Experimental Farm, Pretoria, recorded organic matter digestibilities (*in*

vitro) of 68,7; 64,3; 63,6; 59,2 (material from oesophageal fistulae) and organic matter intakes (g/kg $W^{0.75}$/day) in mature wethers of 29,8; 33,6; 21,5 and 28,6 in October 1986, November 1987, December 1987 and January 1988.

Cruishank et al. (1985) reported an organic matter intake (g/kg LW*/day) by lambs of 36,5 (DOMI of 31,2). Corbett and Pickering (1979) and Corbett (1979) reported organic matter intakes in lambs of 416 g/day (42g/kg $LW^{0.75}$) and 616 g/day (50 g/kg $LW^{0.75}$/day) respectively. Using cannulated lambs Corbett and Pickering (1979) reported the digestibility of non-ammonia nitrogen postruminally of 70% and 69% for lucerne grazed in February to April and October to December respectively. Corbett (1979) reported a disappearance of non-ammonia nitrogen as a fraction of nitrogen intake of 56,7% and 57,5% for lucerne grazed in February to April and October to December respectively.

* livemass

CHAPTER 2

DRY MATTER YIELD, SELECTION, CHEMICAL COMPOSITION, DIGESTIBILITY AND VOLUNTARY INTAKE OF PASTURES.

2.1 Experimental procedure.

2.1.1 Study objectives in brief.

This study was conducted in two phases

i) the first phase involved the measurement of the above indices on the primary growth of sainfoin and sheep's burnet and the subsequent regrowth (cut in such a way as to yield three areas of each pasture which were grazed at 6 weeks, 12 weeks and 15 weeks of age from midsummer to autumn, 1989).

ii) phase two, which involved the grazing of the second and third regrowths of sheep's burnet and sainfoin and regrowths of lucerne that had been planted a year earlier. The pastures had been cut in a similar way as to yield three areas of each which were grazed at 6 weeks, 8 weeks and 15 weeks of age during the winter of 1989 and after about 9 weeks of regrowth during the spring of 1989.

Dry matter yield, chemical composition and <u>in vitro</u> digestibility of organic material collected by oesophageally

fistulated animals were studied.

An in vivo vs. in vitro study was also undertaken. In vitro digestibility of organic matter values were then corrected to in vivo digestibility values on the basis of the relationships between in vitro and in vivo digestibility for each pasture. Voluntary intake of organic matter was then calculated from in vivo digestibility of organic matter and faecal output of organic matter by the experimental animals. In addition, selection of plant parts (leaf and stem material) was determined by measuring leaf to stem ratios prior to putting the animals on pasture and at the end of the trial periods.

The aim was to obtain an indication of the above indices of nutritive value of sainfoin and sheep's burnet and to compare them to lucerne.

2.1.2 Material

2.1.2.1 Dry matter yield and leaf to stem ratios.

Samples of each pasture were cut using four quadrats each measuring 0,5 m^2 and wool shears.

2.1.2.2 Animals : Preparation and cannulation.

Six Döhne Merino male sheep were fistulated in the oesophagus. The fistulae were similar to the type described by Chapman and Grovum (1984). It consisted essentially of a spatula of a section of a 65 mm diameter pipe made of perspex, a wooden plug, a bolt and a wing nut. These animals were used to obtain pasture samples for quality determination. The fistulae were cleaned regularly. In addition 15 Mutton Merino rams were used during each period as "fillers" for faeces collection and voluntary intake determinations. All animals were vaccinated against enterotoxaemia, regularly dosed to prevent infestation by internal parasites and had their hooves trimmed.

2.1.2.3 Pastures

The sainfoin and sheep's burnet had been established the previous autumn at the Hatfield Experimental Farm of the University of Pretoria. The size of the camps for each

pasture was 0,50ha. The farm is situated in a summer rainfall area at an altitude of 1370m above sea level and has an annual precipitation of 700 mm. The camps were irrigated fortnightly to the equivalent of 15mm rainfall as part of the general irrigation frequency of the small stock section where the trials were undertaken. A lucerne camp of approximately the same size had been established a year earlier and had been used continuously in previous trials. The camps were adjacent to each other. An infestation of ryegrass in the sainfoin and sheep's burnet during the first season of growth was controlled by hoeing and chemical treatment. All subsequent weed infestation was controlled by handweeding.

The camps had been fertilized in the spring of 1988. An analysis of soils in the different camps prior to the autumn/winter of 1989 yielded the results in Table 2.1.

Table 2.1 Results of soil analysis on experimental plots.

Sample	pH (H_2O)	R* ohm	Bray II P mg/kg	Ammonium acetate extractable				Texture
				Ca	Mg	K	Na	
Sainfoin plot	6,51	1300	30	610	203	32	27	ClLm**
S. burnet plot	6,84	1800	45	663	243	48	24	ClLm**
Lucerne plot	6,51	1700	54	633	173	173	16	ClLm**

* Resistance

**ClLm - Clay loam

Subsequently LAN and KCl were applied to the sheep's burnet camp at a rate of 300 kg/ha and KCl at the same rate to the sainfoin camp.

2.1.2.4 Treatments

The experimental animals were allowed to graze the test forages only. Water was provided during the trials. A salt mix (an equal mixture of dicalcium phosphate and common salt) was also provided on an ad lib basis during the winter trials. Intake of the salt lick was not measured.

The treatments during the trials were:

i) Treatment 1 (T1) - Sainfoin
ii) Treatment 2 (T2) - Sheep's burnet
iii) Treatment 3 (T3) - Lucerne

There were five "fillers" (rams) per treatment and two oesophageally fistulated sheep per treatment during each of the eight periods (P1 - P8) [Section 2.1.1] of the study. The "fillers" were adapted for one week and faeces subsequently collected for four days. The sheep with oesophageal fistulae were used to collect pasture material at the beginning of the adaptation period, the beginning of the collection period and at the end of collection. They were allowed to adapt to the pasture for at least three days before collection.

2.1.3 Experimental routine.

Animals were weighed after an overnight fast prior to and at the end of each trial period. The animals were put to pasture at 06h00 and returned to the barn at 18h00 (except the last period in Phase II when animals remained on the pastures for the duration of the trial). The "fillers" were equipped with harnesses and nylon canvas bags for faeces collection. They had access to water at all times.

2.1.4 Parameters.

The following parameters were studied:

2.1.4.1 Plant yield and selection of plant parts.

a) Dry matter yield (t/ha)
b) Leaf:stem ratios prior to adaptation and end of collection periods.

2.1.4.2 Chemical composition of samples collected from oesophageal fistulae and clipped samples[1].

a) Organic matter content (OM).
b) Ash content.

[1] - Clipped samples were used for the determination of mineral contents due to salivary contamination of material from oesophageal fistulae.

c) Crude protein content (CP) of dry matter.

d) Acid detergent fibre content (ADF) of dry matter.

e) Neutral detergent fibre content (NDF) of dry matter.

f) Acid detergent lignin content (ADL) of dry matter.

g) Cellulose content of dry matter (calculated).

h) Hemicellulose content of dry matter (calculated).

i) Acid detergent insoluble nitrogen content (ADIN) of dry matter.

j) Calcium (Ca), Phosphorus (P) and Magnesium (Mg) contents of dry matter.

k) In vitro digestibility of organic matter (IVDOM).

l) In vivo digestibility of organic matter (calculated from IVDOM values using the relationships between IVDOM and in vivo DOM yielded by the in vitro vs. in vivo study (Section 2.1.1)).

m) Voluntary intake of organic matter (OMI).

2.1.5 Methods

2.1.5.1 Trial period

The animals were adapted for one week followed by a collection period of four days. The animals had been put on pasture before the beginning of the trials. Thus the length of each trial period was eleven days.

2.1.5.2 Trial implementation

2.1.5.2.1 Body mass

The mean masses of the experimental animals (Section 2.1.3)were used to determine the metabolic live mass ($LW^{0,75}$) for use in intake calculations.

2.1.5.2.2 Collection of pasture samples.

2.1.5.2.2.1 DM Yield and leaf to stem ratios.

The quadrats employed were placed on the plots using stratified random sampling. The areas with the quadrats were then clipped 5 cm from the ground using an ordinary wool shear. The fresh mass of each sample was weighed after dead material had been removed and subsamples were oven-dried in aluminium foil containers at 100°C for 24 hours and re-weighed. Dry matter content was calculated as follows (AOAC, 1984):

$$\% \text{ Dry matter} = \frac{\text{Dry mass (g)}}{\text{sub sample mass (g)}} \times 100$$

Dry matter yield were obtained by multiplying the mean fresh mass by the mean dry matter percentage. Subsamples were also taken for each pasture and a known mass separated into leaf and stem. The proportions of leaf and stem were noted and expressed as a ratio. This was repeated at the end of the

trial in an area inside and outside the exclusion cages to obtain an indication of the selection of plant parts (leaf and stem) by the experimental animals. Samples of the cut material were also taken and dried at 60°C to be used in determining Ca, P and Mg levels.

2.1.5.2.2.2 Extrusa from oesophageal fistulae.

Pasture samples were collected using the oesophageally fistulated sheep at the beginning of the adaptation period, the beginning of the collection period and the end of the collection period. The animals were starved overnight to prevent contamination of selected pasture material through regurgitation of fermentation products. Care was taken to ensure that no material was eaten on the way to the pastures from the barn. The cannulae were removed and the canvas bags tied around the necks of the animals during the collection process. Sufficient material was usually obtained after about 45 minutes of grazing. The cannulae were reinserted immediately after collection and the animals were allowed to graze with the "fillers" for the rest of the day.

The material collected from the cannulae was strained through a double layer of cheesecloth to eliminate saliva and then dried at 50° C for 48 hours. It was then milled to pass a one millimeter sieve of a Beaver mill and subsequently stored in glass or plastic bottles for analysis.

2.1.5.2.3 Faeces collection to determine intake.

All the rams (fillers) were equipped with harnesses and nylon canvas bags for faeces collection. The canvas bags were closed at 18h00 on the day prior to the collection of faeces. Faeces were collected twice daily during the last four days of the trial prior to taking the animals to pasture (06h00) and just before driving the animals to the barn (18h00) to reduce loss of faeces. The faeces were also collected at the same times when animals remained on pasture for the whole trial period.

2.2 Experimental design and statistical analysis of data.

There were four trial periods in each phase of the study as described in Section 2.1.1. All experimental animals, which had similar masses and ages, were allocated randomly to treatments in each trial period.

Experimental design

Phase of experiment	Period	Treatments		
		Treatment 1	Treatment 2	Treatment 3
I	P1	Sainfoin	Sheep's burnet	-
	P2	Sainfoin	Sheep's burnet	-
	P3	Sainfoin	Sheep's burnet	-
	P4	Sainfoin	Sheep's burnet	-
II	P5	Sainfoin	Sheep's burnet	Lucerne
	P6	Sainfoin	Sheep's burnet	Lucerne
	P7	Sainfoin	Sheep's burnet	Lucerne
	P8	Sainfoin	Sheep's burnet	Lucerne

The data yielded by the study were analyzed separately for each phase for treatment and period effects and their interaction, using a two-way analyis of variance procedure in the general linear models programme (Freud & Littell, 1981), and utilizing the least square means and a probability level of 5 %.

2.3 Sampling methods.

2.3.1 Pastures.

Clipped samples and extrusa collected from oesophageal fistulae were processed, milled and stored as described in Sections 2.1.5.2.2.1 and 2.1.5.2.2.2.

2.3.2 Faeces.

Ten percent of the daily faeces excretion was pooled over the collection period and stored frozen in plastic bags at -15°C. Pooled samples were subsequently thawed and a

subsample used for the determination of the dry matter content of the faeces. The rest was dried at 60°C in a forced draught oven before grinding to pass a one mm sieve of a Beaver mill and stored in glass and plastic bottles for analysis.

2.4 Analytical methods.

2.4.1 Dry matter content.

Pasture and faecal material were dried at 100°C for 24 hours in a forced draught oven in aluminium foil containers or porcelain crucibles. Where porcelain crucibles were used the samples were cooled in a desiccator containing silica gel and weighed. The aluminium containers used for the determination of dry matter content of cut pastures and wet faeces were weighed directly from the oven, with the scale reading always readjusted to zero before the next reading. The dry matter content (%) was calculated as recommended by the AOAC (AOAC, 1984).

2.4.2 Ash content.

Partially dried samples of pasture and faeces (60°C) were dried overnight at 100°C in porcelain crucibles, weighed and incinerated in a muffle furnace for four hours at 600°C, cooled in a desiccator and weighed.

The ash content of the material used for the determination of in vitro digestibility of organic matter was calculated as follows:

$$\% \text{ Ash} = \frac{\text{Ash mass (g)}}{\text{Sample mass (g)}} \times 100$$

The ash content of faeces was calculated on the basis of the dry matter as recommended in the South African Department of Agriculture Handbook of Laboratory Methods (1989):

$$\% \text{ Ash} = \frac{\text{Ash mass (g)}}{\text{Dried sample mass (g)}} \times 100$$

2.4.3 Organic matter content.

The organic matter (OM) content of pasture material for the in vitro digestibility of OM determinations was calculated as follows:

$$\% \text{ OM} = \frac{\text{Dry matter (g) - Ash mass (g)}}{\text{Sample mass (g)}} \times 100$$

The organic matter content of faeces was calculated as follows:

$$\% \text{ OM} = 100\% - \% \text{ Ash (dry matter basis)}$$

2.4.4 Crude protein content.

The nitrogen (N) content of pasture samples was determined by the macro kjedahl method (AOAC, 1984). A block digester was used for the digestion of the sample and a Tecator Kjeltec System Model 1002 for the distillation.

Percentage crude protein (CP) was calculated as follows:

$$\% \text{ CP} = \% \text{ N} \times 6{,}25$$

2.4.5 The acid detergent fibre.

The acid detergent fibre (ADF) contents of pasture and faeces samples were determined with a Tecator Fibertec System as outlined in the Application Note AN 03/78: A sample milled to pass a one millimeter sieve was weighed (1 g) in a filter crucible and placed in a Hot Extraction Unit of the Fibertec System. The extraction was carried out with 100 ml of acid detergent solution (ADS) (Van Soest, 1963) for one hour (after boiling commenced). This was followed by cold extraction with acetone on the Cold Extraction Unit of the system. The samples were dried at 100°C overnight and ashed at 550°C for three hours.

ADF was calculated as follows:

$$\% \text{ ADF} = \frac{W_1 \text{ (g)} - W_2 \text{ (g)}}{W_0 \text{ (g)}} \times 100$$

where W_1 = dry mass of sample after ADF extraction

W_2 = mass of ash

W_0 = sample mass

2.4.5.1 Nitrogen content of acid detergent fibre (ADIN).

Acid detergent fibre in a sample was extracted using the same procedures as in 2.4.5 except that the sample mass was higher (± 2 g) and 150 ml of ADS was used (to yield a sample large enough for nitrogen determination). The acetone extraction was also omitted and the sample dried at 60° C overnight. Nitrogen content of the residue was determined as in 2.4.4.

2.4.5.2 Lignin content of acid detergent fibre (ADL).

Acid detergent lignin was determined in the manner outlined in Application Note 04/78 of the Tecator Fibertec System. The samples were prepared with the ADF procedure as outlined in 2.4.5 but not ashed. A sequential extraction with 72% sulphuric acid was carried out for three hours. The sample remaining after filtration and washing with hot water was

dried overnight, weighed (W_1) and ashed in a muffle furnace at 550° C for three hours. The residue was then cooled in a desiccator and weighed (W_2).

% ADL was calculated as:

$$\% \text{ ADL} = \frac{W_1 - W_2}{W_0 \text{ (sample mass)}} \times 100$$

2.4.6 Neutral detergent fibre (NDF) content of dry matter.

Neutral detergent fibre contents of pasture and faeces were determined using the same apparatus in 2.4.5 except that Neutral Detergent Solution (NDS) was used (Van Soest & Wine, 1967).

% NDF was calculated as:

$$\% \text{ NDF} = \frac{W_1 \text{ (g)} - W_2 \text{ (g)}}{W_0 \text{ (g)}} \times 100$$

where W_1 = dry mass of sample after NDS extraction

W_2 = mass of ash

W_3 = sample mass

2.4.7 Cellulose.

Cellulose was calculated as:

$$\% \text{ Cellulose} = \text{ADF\%} - \text{ADL\%}$$

2.4.8 Hemicellulose.

Hemicellulose was calculated as:

% Hemicellulose = NDF% - ADF%

2.4.9 Calcium, Magnesium and Phosphorus.

The contents of these minerals were determined on clipped samples due to salivary contamination of samples collected from oesophageal fistulae. A sample milled to pass a one millimeter sieve of a Beaver mill (1 g) was digested in a block digester at 230°C using the wet digestion technique (Manual, Perkin Elmer Atomic Absorption Spectrophotometer pp AY 11). Calcium and magnesium were then determined on a Perkin Elmer 2380 Atomic Absorption Spectrophotometer. Calcium was determined at a wavelength of 422,7 nm and a slit setting of 0,7 nm using a hollow cathode tube.

Magnesium was determined at a wavelength of 285,2 nm and a slit setting of 0,7 nm using a similar lamp. An air-acetylene flame was employed in both the determinations of calcium and magnesium.

Phosphorus was determined on a Technicon Autoanalyser and the concentration determined from a calibration curve.

2.4.10 In vitro digestibility of OM (IVDOM).

In vitro digestibility of OM was determined by the method of Tilley and Terry (1963), as modified by Engels and Van der Merwe (1967). A 0,2 g sample (dried at 50°C and milled to pass a one mm sieve of a Beaver mill) was incubated in a test tube with rumen fluid, urea solution and artificial saliva for 48 hours at 39°C. The samples were shaken three times a day at regular intervals. Hydrochloric acid (1:4) was then used to lower the pH to about 2,0; 3 ml pepsin solution (8g/1000ml) added and incubated for another 48 hours. The contents of the test tubes were then filtered through Gooch crucibles using a vacuum pump and dried for 24 hours at 100°C. The residues were weighed and ashed at 550°C in a muffle furnace for three hours, cooled in a desiccator and weighed. Panicum maximum with an IVDOM of 70 - 75% was used as a standard. This was the standard used in the laboratory and was always kept in a refrigerator. The digestibility of organic matter was calculated as follows:

$$D = 100 - \frac{\text{Undigested residue (g)}}{\text{sample mass (g)}} \times 100$$

where D = digestibility of OM in vitro (IVDOM%)

- The sample mass of the incubated sample is expressed in terms of the OM content.
- Undigested residue is expressed in terms of the OM content.

As a result of the in vivo vs. in vitro DOM study (Table 2.14) the IVDOM figures were converted into in vivo DOM

values as follows:

(i) Lucerne and sheep's burnet:

% *in vivo* DOM = 0,746 IVDOM + 18,16[a]

(ii) Sainfoin:

% *in vivo* DOM = % IVDOM x 1,17[b]

The voluntary intake of OM was calculated from the *in vivo* DOM of the respective pastures and the organic matter content of the faeces (Langlands, 1975).

$$OMI = FO \times 100/(100 - DIG)$$

where OMI = organic matter intake (g/day)

FO = faecal output (g OM/day)

DIG = digestibility of the forage (%)

2.5 Results.

The results were divided into treatment effects and time period (P1 - P8) effects. The results for treatment effects are summarized with the standard error of means whilst period effects show the standard deviations and coefficients of variation of the parameters measured. The standard

[a] - Relationship (unpublished) between *in vivo* DOM and IVDOM obtained in this laboratory from work done on several planted pastures

[b] - the ratio of *in vivo* DOM to *in vitro* DOM obtained in the study (Table 2.14).

deviations, standard errors of means and coefficients of variation were calculated using the method of Snedecor (Snedecor, 1956). Unless otherwise stated values with at least one common letter on the same horizontal line do not differ significantly.

2.5.1 Dry matter yield and leaf:stem ratios[1].

Tables 2.2a and 2.2b show the dry matter yields (t/ha) and leaf:stem ratios of the pastures for phases I and II respectively. There were no significant differences between the yields of sainfoin and sheep's burnet in Phase I (first regrowths). However in Phase II (subsequent regrowths) the yields of sheep' burnet were significantly higher than sainfoin and the yields of both were significantly higher than those of lucerne. There were significant differences in leaf:stem ratios of the three pastures in both phases.

Table 2.2a. Dry matter yield (t/ha) and leaf:stem ratios of pastures. Means of three regrowth periods. Phase I.

Parameter	Treatment		
	Sainfoin	Sheeps' burnet	SE_m
DM yield (t/ha)	$5,1^{a}$	$6,0^{a}$	0,45
Leaf:stem ratio	$1,6^{b}$	$1,1^{a}$	0,25

[1] - No yield and leaf:stem ratios are given for the primary growth (P1) because of extensive weed infestation and shattering of material during cutting and sorting.

Table 2.2b. Dry matter yield (t/ha) and leaf:stem ratios of pastures. Means of three second regrowths[2] and one third regrowth period.

Parameter	Treatment			
	Sainfoin	Sheeps' burnet	Lucerne	SE_m
DM yield (t/ha)	$3,3^{b}$	$3,9^{c}$	$2,8^{a}$	0,32
Leaf:stem ratio	$3,3^{c}$	$0,9^{a}$	$1,6^{b}$	0,62

Tables 2.3a to 2.5 indicate the yields and leaf:stem ratios of the three pastures during the different periods of the study.

The results for sainfoin (Tables 2.3a and 2.3b) show that, the 12 week regrowth in Phase I (Table 2.3a) had a significantly higher yield whilst the 15 week regrowth had a significantly higher leaf:stem ratio. In Phase II (Table 2.3b) the 15 week autumn regrowth had a significantly higher yield. The spring regrowth (P8) had a significantly lower leaf:stem ratio compared to the 6 week and 8 week autumn/winter regrowths.

[2] - Sainfoin and sheeps' burnet only. Lucerne has been cut several times previously.

Table 2.3a. The influence of period on the DM yield and leaf:stem ratios of sainfoin. Phase I.

Parameters	Trial period and chronological age of pastures.		
	P2 (21/2/89-2/3/89) 6 weeks	P3 (4/4/89-14/4/89) 12 weeks	P4 (25/4/89-5/5/89) 15 weeks
DM yield (t/ha)	4,2[a]	6,7[b]	4,4[a]
S.D.	0,24	1,58	0,84
Leaf:stem ratio	1,4[a]	1,3[a]	2,0[b]
S.D.	0,29	0,38	0,61

Table 2.3b. The influence of period on the DM yield and leaf:stem ratios of sainfoin. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (18/6/89-28/6/89) 8 weeks	P7 (28/6/89-8/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
DM yield (t/ha)	4,7[b]	2,9[a]	2,4[a]	3,2[a]
S.D.	1,37	0,24	0,22	0,6
Leaf:stem ratio	3,2[a]	3,4[b]	3,5[b]	3,0[a]
S.D.	0,03	0,36	0,18	0,37

The results for sheeps' burnet indicate a significantly higher yield for the 12 week regrowth in Phase I whilst there were no significant differences in leaf:stem ratio between periods (Table 2.4a). In Phase II (Table 2.4b), the 15 week regrowth had a significantly higher yield compared to the other regrowths. However there were no significant differences in yield between the 8 week autumn/winter regrowth and 8-9 week spring regrowth. The spring regrowth had a significantly lower leaf:stem ratio.

Table 2.4a. The influence of period on DM yield and leaf:stem ratios of sheep's burnet. Phase I.

Parameters	Trial period and chronological age of pastures.		
	P2 (21/2/89-2/3/89) 6 weeks	P3 (4/4/89-14/4/89) 12 weeks	P4 (25/4/89-5/5/89) 15 weeks
DM yield (t/ha)	5,2[a]	7,1[b]	5,6[ab]
S.D.	1,20	1,88	0,59
Leaf:stem ratio	1,0[a]	1,0[a]	1,4[a]
S.D.	0,08	0,21	0,08

Table 2.4b. The influence of period on DM yield and leaf:stem ratios of sheeps' burnet. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5(8/6/89-18/6/89) 15 weeks	P6(18/6/89-28/6/89) 8 weeks	P7(28/6/89-8/7/89) 6 weeks	P8(7/11/89-18/11/89) 8-9 weeks
DM yield (t/ha	5,1[c]	4,1[b]	2,8[a]	3,8[b]
S.D.	1,10	0,80	0,48	1,36
Leaf:stem ratio	1,0[b]	1,0[b]	1,0[b]	0,4[a]
S.D.	0,18	0,08	0,36	0,26

Table 2.5 shows the results obtained for different cuts of lucerne. There were no significant differences in yield between cuts. The 15 week regrowth had a significantly lower leaf:stem ratio compared to the others whereas the 6 week regrowth had a significantly higher leaf:stem ratio. There were no significant differences in leaf:stem ratios between the 8 week winter and 8-9 week spring regrowths of lucerne.

Table 2.5. The influence of period on DM yield and leaf:stem ratio of lucerne. Phase II only.

Parameters	Trial period and chronological age of pastures.			
	P5(8/6/89 -18/6/89) 15 weeks	P6(18/6/89 - 28/6/89) 8 weeks	P7(28/6/89 -8/7/89) 6 weeks	P8(7/11/89-18/11/89) 8-9 weeks
DM yield (t/ha)	2,7[a]	2,5[a]	2,9[a]	3,1[a]
S.D.	0,46	0,26	0,12	0,24
Leaf:stem ratio	0,7[a]	1,4[b]	3,0[c]	1,3[b]
S.D.	0,18	0,28	0,36	0,18

Tables 2.6 to 2.9 provide an indication of the selection of plant parts by sheep grazing on the three pastures.

Table 2.6. Selection of plant parts by sheep. Leaf:stem ratios before grazing and at the end of grazing.

Phase of study	Treatment	Leaf:stem ratio		SE_m
		Before grazing	End of grazing	
I	Sainfoin	1,6[b]	1,2[a]	0,20
	Sheeps' burnet	1,1[a]	1,0[a]	0,05
II	Sainfoin	3,3[b]	2,9[a]	0,20
	Sheeps' burnet	0,9[a]	0,7[a]	0,10
	Lucerne	1,6[b]	1,0[a]	0,30

There were significant changes in leaf:stem ratios (before grazing and at the end of grazing) for sainfoin and lucerne (Table 2.6) but the change in leaf:stem ratio was not significant in the case of sheeps' burnet.

Table 2.7. Selection of plant parts by sheep during different stages of growth of sainfoin.

Phase of study	Trial Period	Chronological age of pastures	Leaf:stem ratio	
			Before grazing	End of grazing
I	P2 (21/2/89-2/3/89)	6 weeks	$1,4^{a}$	$1,0^{a}$
	P3 (4/4/89-14/4/89)	12 weeks	$1,3^{a}$	$1,3^{a}$
	P4(25/4/89-5/5/89)	15 weeks	$2,0^{b}$	$1,3^{a}$
II	P5 (8/6/89-18/6/89)	15 weeks	$3,2^{b}$	$2,5^{a}$
	P6 (17/6/89-27/6/89)	8 weeks	$3,4^{a}$	$3,2^{a}$
	P7 (25/6/89-5/7/89)	6 weeks	$3,5^{a}$	$3,5^{a}$
	P8 (7/11/89-18/11/89)	8-9 weeks	$3,0^{b}$	$2,5^{a}$

There were significant changes in leaf:stem ratio of sainfoin for the 15 week regrowth in both phases and 8 - 9 week regrowth in spring. There were no significant changes in leaf:stem ratios for the 6 week and 12 week regrowths in Phase I and 6 and 8 week regrowths in Phase II (Table 2.7).

Table 2.8. Selection of plant parts by sheep during different stages of growth of sheeps' burnet.

Phase of study	Trial Period	Chronological age of pastures	Leaf:stem ratio	
			Before grazing	End of grazing
I	P2 (21/2/89-2/3/89)	6 weeks	$1,0^{a}$	$1,1^{a}$
	P3 (4/4/89-14/4/89)	12 weeks	$1,0^{a}$	$0,9^{a}$
	P4(25/4/89-5/5/89)	15 weeks	$1,4^{a}$	$1,1^{a}$
II	P5 (8/6/89-18/6/89)	15 weeks	$1,0^{a}$	$0,9^{a}$
	P6 (17/6/89-28/6/89)	8 weeks	$1,0^{a}$	$0,9^{a}$
	P7 (28/6/89-8/7/89)	6 weeks	$1,0^{a}$	$0,8^{a}$
	P8 (7/11/89-18/11/89)	8-9 weeks	$0,4^{a}$	$0,3^{a}$

There were no significant changes in leaf:stem ratios before and after grazing by sheep of sheeps' burnet in both phases (Table 2.8).

Table 2.9. Selection of plant parts by sheep during different stages of growth of lucerne.

Phase of study	Trial Period	Chronological age of pastures	Leaf:stem ratio	
			Before grazing	End of grazing
II	P5 (8/6/89-18/6/89)	15 weeks	$0,7^{a}$	$0,5^{a}$
	P6 (17/6/89-27/6/89)	8 weeks	$1,4^{a}$	$1,1^{a}$
	P7 (25/6/89-5/7/89)	6 weeks	$3,0^{b}$	$1,4^{a}$
	P8 (7/11/89-18/11/89)	8-9 weeks	$1,3^{a}$	$1,0^{a}$

There was a significant change in leaf:stem ratio only in the 6 week regrowth of lucerne.

2.5.2 Chemical components of pastures.

Pooled samples were used for the Ca, P, Mg as well as ADL determinations. These values were therefore not suited for an analysis of variance procedure and therefore only means and standard errors of the means are shown in the tables.

Tables 2.10a and 2.10b show the mean chemical composition of the three pastures for Phases I and II respectively. There were significant differences between pastures with respect to CP, ADF, NDF and ADIN in both phases. In Phase I (Table 2.10a) there were also significant differences between sainfoin and sheeps' burnet with respect to OM, cellulose,

hemicellulose and ash contents. In Phase II, sainfoin and lucerne contained significantly higher OM and cellulose contents compared to sheeps' burnet (Table 2.10b) but the three pastures did not differ significantly with respect to hemicellulose content. Although no tests of significance were done on ADL, Ca, P and Mg contents, sainfoin had considerably higher ADL levels compared to the other two pastures. Sheeps' burnet had significantly higher ash contents in both phases. The results indicate a higher Ca content in lucerne and a higher Mg content in sheeps' burnet. The Ca:P ratio was highest (widest) in lucerne, followed by sheeps' burnet and sainfoin in that order.

Table 2.10 a. Chemical composition of forages (% of DM). Mean of the four periods in Phase I.

Parameter	Pasture		SE_m
	Sainfoin	Sheeps' burnet	
OM	$90,0^{b}$	$89,1^{a}$	0,45
CP	$23,1^{b}$	$18,1^{a}$	2,50
ADF	$41,1^{b}$	$21,6^{a}$	9,75
NDF	$51,5^{b}$	$36,0^{a}$	7,75
ADL	$14,8^{b}$	$5,6^{a}$	4,60
Cellulose	$26,7^{b}$	$16,0^{a}$	5,35
Hemicellulose	$10,4^{a}$	$14,3^{b}$	1,95
ADIN	$2,6^{b}$	$1,2^{a}$	0,70
Ash	$10,0^{a}$	$10,9^{b}$	0,45
Ca	1,00	0,90	0,05
P	0,29	0,25	0,02
Mg	0,57	0,70	0,07
Ca:P	3,45	3,60	

Table 2.10 b. Chemical composition of forages (% of DM). Means of the four periods in Phase II.

Parameter	Pasture			SE_m
	Sainfoin	Sheeps' burnet	Lucerne	
OM	$89,5^{b}$	$88,7^{a}$	$89,3^{b}$	0,24
CP	$23,4^{b}$	$17,2^{a}$	$26,1^{c}$	2,63
ADF	$39,1^{c}$	$21,3^{a}$	$25,3^{b}$	5,39
NDF	$48,0^{c}$	$31,3^{a}$	$35,5^{b}$	5,02
ADL	$18,1_{b}$	$6,1_{a}$	$4,8_{b}$	4,23
Cellulose	$21,0^{b}$	$15,2^{a}$	$20,7^{a}$	1,89
Hemicellulose	$8,9^{a}$	$10,0^{a}$	$10,2^{a}$	0,40
ADIN	$2,5^{b}$	$1,0^{a}$	$1,0^{a}$	0,50
Ash	$10,5^{a}$	$11,2^{b}$	$10,7^{a}$	0,21
Ca	0,84	0,99	1,43	0,18
P	0,41	0,39	0,33	0,02
Mg	0,45	0,97	0,49	0,17
Ca:P	2,05	2,54	4,33	

Tables 2.11a to 2.13 show the chemical composition of the pastures during the different trial periods and stages of growth.

Table 2.11 a. The influence of period on chemical components (% of DM) of sainfoin. Phase I.

Parameters	Trial period and chronological age of pastures.			
	P1 (26/12/88 -6/1/89) Sown previous spring	P2 (21/2/89 - 2/3/89) 6 weeks	P3 (4/4/89- 14/4/89) 12 weeks	P4 (25/4/89- 5/5/89) 15 weeks
OM	90,7^{b}	90,0^{b}	90,4^{b}	88,8^{a}
S.D.	0,14	1,21	1,32	1,29
CP	29,6^{b}	29,2^{b}	16,9^{a}	16,8^{a}
S.D.	0,85	1,79	0,69	3,12
ADF	34,7^{a}	45,4^{b}	41,1^{ab}	43,2^{b}
S.D.	2,62	7,04	7,19	6,73
NDF	50,9^{a}	53,5^{a}	51,1^{a}	50,6^{a}
S.D.	0,99	3,43	3,54	4,09
ADL	11,1	16,7	15,0	16,3
Cellulose	23,6^{a}	28,7^{a}	26,1^{a}	28,4^{a}
S.D.	2,97	7,04	7,19	5,35
Hemicellulose	16,2^{b}	8,0^{a}	10,0^{a}	7,5^{a}
S.D	3,61	3,91	4,52	3,10
ADIN	3,5^{c}	3,0^{b}	2,0^{a}	2,0^{a}
S.D.	0,78	0,48	0,38	0,48
Ash	9,3^{a}	10,0^{a}	9,6^{a}	11,3^{b}
S.D	0,14	1,21	0,40	1,29
Ca	0,80	0,62	1,36	1,23
P	0,27	0,43	0,27	0,19
Mg	0,31	0,48	0,55	0,93

Crude protein content was significantly higher in the primary growth compared to the regrowths in Phase I. There was a significant decrease in CP from the 6 week old pasture (P2) to the 12 week old pasture (P3). There were no significant differences however between the 12 week (P3) and 15 week (P4) old pastures.

In Phase I (Table 2.11a) there were no significant differences between the summer regrowths (P2, P3, P4) of

sainfoin with respect to ADF, NDF, cellulose and hemicellulose despite the different periods (age) of the pastures. However, there were significant differences in these parameters when the regrowths were compared with the primary growth (P1) which had significantly lower values for the fibre indices.

Table 2.11 b. The influence of period on the chemical components (% of DM) of sainfoin. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (17/6/89-28/6/89) 8 weeks	P7 (25/6/89-8/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
OM	$90,0^{bc}$	$89,4^{b}$	$88,3^{a}$	$90,3^{c}$
S.D.	$0,47_{a}$	$0,83_{a}$	$0,90_{a}$	$0,56_{b}$
CP	$21,9^{a}$	$22,1^{a}$	$21,6^{a}$	$28,1^{b}$
S.D.	$3,36_{b}$	$0,75_{b}$	$2,35_{b}$	$1,55_{a}$
ADF	$41,4^{b}$	$41,0^{b}$	$41,9^{b}$	$32,3^{a}$
S.D.	$2,41_{b}$	$3,42_{b}$	$7,61_{b}$	$3,38_{a}$
NDF	$50,4^{b}$	$50,4^{b}$	$49,0^{b}$	$42,4^{a}$
S.D.	1,26	3,38	5,51	4,09
ADL	$22,4_{a}$	$18,1_{a}$	$18,7_{a}$	$13,2_{a}$
Cellulose	$19,0^{a}$	$22,9^{a}$	$23,1^{a}$	$19,1^{a}$
S.D.	$2,41_{a}$	$3,42_{a}$	$7,61_{a}$	$3,38_{a}$
Hemicellulose	$9,0^{a}$	$9,4^{a}$	$7,1^{a}$	$10,0^{a}$
S.D	$2,54_{c}$	$2,91_{a}$	$2,30_{c}$	$1,37_{b}$
ADIN	$3,0^{c}$	$2,0^{a}$	$3,0^{c}$	$2,3^{b}$
S.D.	$0,28_{ab}$	$0,32_{b}$	$0,42_{c}$	$0,38_{a}$
Ash	$10,0^{ab}$	$10,7^{b}$	$11,7^{c}$	$9,7^{a}$
S.D	0,47	0,83	0,90	0,56
Ca	0,92	0,76	-	0,83
P	0,35	0,39	-	0,49
Mg	0,49	0,44	-	0,42

A similar trend is noticed in Phase II (Table 2.11b) where there were no significant differences in the fibre indices for the late autumn (P5) and winter (P6 & P7) regrowths despite chronological age but significantly lower values

were obtained in the spring regrowth (P8).

Table 2.12a. The influence of period on the chemical components (% of DM) of sheeps' burnet. Phase I.

Parameters	Trial period and chronological age of pastures.			
	P1 (26/12/88 -6/1/89) Sown previous spring	P2 (21/2/89 - 2/3/89) 6 weeks	P3 (4/4/89- 14/4/89) 12 weeks	P4 (25/4/89- 5/5/89) 15 weeks
OM	89,1^{a}	89,1^{a}	89,6^{a}	88,7^{a}
S.D.	0,35$_{b}$	0,60$_{b}$	0,39$_{a}$	1,08$_{a}$
CP	20,5^{b}	23,6^{b}	14,2^{a}	14,0^{a}
S.D.	1,98$_{a}$	2,49$_{a}$	1,91$_{a}$	1,12$_{a}$
ADF	24,0^{a}	20,8^{a}	21,1^{a}	20,3^{a}
S.D.	1,27$_{ab}$	3,55$_{b}$	4,30$_{ab}$	2,80$_{a}$
NDF	37,9^{ab}	38,7^{b}	35,0^{ab}	32,3^{a}
S.D.	0,78	5,56	3,81	0,98
ADL	4,8^{a}	4,5^{a}	7,2^{a}	5,9^{a}
Cellulose	19,3^{a}	16,3^{a}	13,8^{a}	14,4^{a}
S.D.	1,63$_{ab}$	3,55$_{b}$	4,30$_{a}$	2,80$_{a}$
Hemicellulose	13,9^{ab}	17,9^{b}	13,6^{a}	12,0^{a}
S.D.	2,05	3,10	2,32	2,65
ADIN	1,0^{a}	2,0^{b}	1,0^{a}	1,0^{a}
S.D.	0,42$_{a}$	0,58$_{a}$	0,38$_{a}$	0,26$_{a}$
Ash	11,0^{a}	10,9^{a}	10,4^{a}	11,3^{a}
S.D	0,35	0,60	0,39	1,08
Ca	0,82	0,70	0,97	1,10
P	0,22	0,31	0,25	0,22
Mg	0,74	0,55	0,78	0,74

Whereas there was no significant difference between the primary growth and the 6 week regrowth of sheeps' burnet as far as CP was concerned (Table 2.12a, Phase I), there was a significant decrease in CP in the 12 week and 15 week regrowth - a similar trend as in sainfoin. The ADF contents were similar for all growth stages. The same trend

occurred for cellulose and ash. There was a significant difference in hemicellulose and NDF contents between the primary growth and the 6 week regrowth. Calcium tended to increase with age whilst P showed the opposite trend.

Table 2.12b. The influence of period on chemical components (% of DM) of sheeps' burnet. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (17/6/89-27/6/89) 8 weeks	P7 (25/6/89-5/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
OM	88,6^{a}	88,6^{a}	88,8^{a}	89,0^{a}
S.D.	0,91	0,24	0,50	0,64
CP	16,4^{a}	14,7^{a}	14,8^{a}	22,9^{b}
S.D.	0,61	0,75	2,17	4,32
ADF	22,2^{a}	20,3^{a}	19,8^{a}	23,0^{a}
S.D.	0,58	3,85	2,36	1,09
NDF	31,0^{a}	29,5^{a}	27,3^{a}	37,4^{b}
S.D.	1,34	1,34	3,24	1,72
ADL	6,7	7,3	5,9	4,5
Cellulose	15,5^{ab}	13,0^{a}	13,9^{ab}	18,5^{b}
S.D.	0,58	3,85	2,31	1,09
Hemicellulose	8,8^{a}	9,2^{a}	7,6^{a}	14,4^{b}
S.D.	1,92	4,48	0,93	2,01
ADIN	1,0^{a}	1,0^{a}	1,0^{a}	0,8^{a}
S.D.	0,34	0,42	0,26	0,37
Ash	11,4^{a}	11,1^{a}	11,7^{a}	11,0^{a}
S.D	0,91	0,29	0,50	0,64
Ca	1,02	1,11	-	0,83
P	0,36	0,33	-	0,49
Mg	1,10	1,11	-	0,70

There were no significant differences between the late autumn (P5) and winter (P6 & P7) regrowths with respect to CP (Table 2.12b, Phase II). However, they differed significantly from the spring regrowth. Acid detergent fibre remained similar for all growth stages as in Phase I. The same trend was noticed with hemicellulose except with the spring regrowth. Neutral detergent fibre was similar for the winter regrowths but these differed significantly with the spring regrowth. Ash content followed the same trend as in Phase I.

Table 2.13. The influence of period on chemical components (% of DM) of lucerne. Phase II only.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (17/6/89-27/6/89) 8 weeks	P7 (25/6/89-5/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
OM	87,9^{a}	90,3^{b}	89,1^{c}	90,1^{b}
S.D.	0,64$_{a}$	0,50$_{bc}$	0,45$_{b}$	4,25$_{c}$
CP	17,9^{a}	29,2^{bc}	26,4^{b}	30,7^{c}
S.D.	0,28$_{b}$	1,45$_{a}$	2,01$_{a}$	3,03$_{a}$
ADF	36,0^{b}	19,9^{a}	21,5^{a}	24,0^{a}
S.D.	0,57$_{b}$	2,78$_{a}$	4,16$_{ac}$	3,07$_{c}$
NDF	50,8^{b}	27,2^{a}	30,3^{ac}	33,8^{c}
S.D.	0,78	3,86	2,86	3,79
ADL	6,2	4,7	4,3	4,0
Cellulose	29,8^{b}	15,2^{a}	18,1^{a}	19,5^{a}
S.D.	0,57$_{b}$	2,54$_{a}$	4,84$_{a}$	3,33$_{a}$
Hemicellulose	14,8^{b}	7,3^{a}	8,8^{a}	9,8^{a}
S.D.	0,21$_{a}$	1,44$_{a}$	5,33$_{a}$	1,76$_{a}$
ADIN	1,0^{a}	1,0^{a}	1,0^{a}	1,0^{a}
S.D.	0,07$_{c}$	0,28$_{a}$	0,36$_{b}$	0,26$_{a}$
Ash	12,2^{c}	9,7^{a}	11,0^{b}	9,9^{a}
S.D	0,64	0,50	0,45	0,76
Ca	1,47	1,44	-	1,37
P	0,28	0,43	-	0,30
Mg	0,54	0,45	-	0,49

There were significant differences between the 15 week regrowth (P5) and 6 and 8 weeks winter regrowths of lucerne (P6 & P7) with respect to CP (Table 2.13). There were also significant differences in CP content between the spring regrowth and the winter regrowths except the 8 week regrowth. There were significantly higher ADF and NDF contents in the 15 week regrowth compared with the shorter regrowth periods. The same trend occurred for cellulose and hemicellulose. High Ca levels occurred in lucerne of all growth stages.

2.5.3 Digestibility and voluntary intake of organic matter.

As stated in Section 2.4.10 an *in vivo* vs. *in vitro* study was carried out indoors in metabolism cages. The results of the study are shown in Table 2.14.

Table 2.14. Differences between *in vitro* and *in vivo* digestibilities as affected by pasture species.

	Digestibility of OM (%)		
Pasture	*In vitro*	*In vivo*	SE_m
Sainfoin	55.7	65.0	4.65
Sheeps' burnet	54.4	57.6	1.60
Lucerne	52.6	55.2	1.30

Lucerne and sheeps' burnet were deemed to fit in the prediction equation (Section 2.4.10):

$$\%\ \textit{in vivo}\ \text{DOM} = 0{,}746\%\ \text{IVDOM} + 18{,}16$$

In vivo digestibilities of organic matter for the two pastures for all the trial periods were then calculated from the in vitro digestibilities, using the equation. The calculated in vivo digestibilities of organic matter were used in all voluntary intake of organic matter determinations.

Based on the results of the digestibility study (Table 2.14), however, in vivo digestibilities of organic matter of sainfoin were estimated from the in vitro digestibilities by simple proportion, i.e.

$$\% \text{ in vivo DOM} = \% \text{ IVDOM} \times \frac{\text{in vivo DOM}}{\text{in vitro DOM}}$$

$$= \% \text{ IVDOM} \times \frac{65,0}{55,7}$$

$$= \% \text{ IVDOM} \times 1,17$$

The calculated in vivo digestibilities were then used for the voluntary intake of organic matter determinations.

Tables 2.15a and 2.15b show the differences between the pastures with respect to IVDOM and in vivo DOM. There were significant differences between the pastures in both IVDOM and in vivo DOM in both phases.

Table 2.15 a. IVDOM and in vivo DOM of pastures (Mean of the four periods in Phase I).

	Pasture		
Parameter	Sainfoin	Sheeps'burnet	SE_m
IVDOM (%)	$52,4^{a}$	$61,5^{b}$	4,55
In vivo DOM (%)	$61,1^{a}$	$64,0^{b}$	1,45

Table 2.15 b. IVDOM and in vivo DOM of pastures (Mean of the four periods in Phase II).

	Pasture			
Parameter	Sainfoin	Sheeps'burnet	Lucerne	SE_m
IVDOM (%)	$49,5^{a}$	$55,1^{b}$	$64,4^{c}$	4,35
In vivo DOM (%)	$57,7^{a}$	$59,2^{b}$	$66,2^{c}$	3,62

Table 2.16a portrays in vitro and in vivo digestibilities of OM of sainfoin for the different growth stages (trial periods) in Phase I. There were significant differences between the 6 week regrowth (P2) and 15 week regrowth (P4) in both parameters.

In phase II (Table 2.16b) there was a significant difference between the spring regrowth of sainfoin (P8) and the three preceding periods in both IVDOM and in vivo DOM. In addition there was a significantly lower in vivo DOM on the 6 week regrowth (P7) compared with the 8 week regrowth (P6).

Table 2.16 a. The influence of period on IVDOM and in vivo DOM of sainfoin. Phase I.

Parameters	Trial period and chronological age of pastures.			
	P1 (26/12/88 -6/1/89) Sown previous spring	P2 (21/2/89 - 2/3/89) 6 weeks	P3 (4/4/89- 14/4/89) 12 weeks	P4 (25/4/89- 5/5/89) 15 weeks
IVDOM (%)	$53,8^{ab}$	$49,6^{a}$	$52,2^{ab}$	$54,1^{b}$
S.D.	2,12	4,58	2,79	1,63
In vivo DOM(%)	$62,8^{ab}$	$57,8^{a}$	$60,8^{ab}$	$63,0^{b}$
S.D.	2,47	5,33	3,26	1,89

Table 2.16 b. The influence of period on IVDOM and in vivo DOM of sainfoin. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89- 18/6/89) 15 weeks	P6 (17/6/89- 28/6/89) 8 weeks	P7 (28/6/89- 8/7/89) 6 weeks	P8 (7/11/89- 18/11/89) 8-9 weeks
IVDOM (%)	$48,9^{ab}$	$49,3^{b}$	$46,9^{a}$	$53,1^{c}$
S.D.	1,16	0,91	0,16	2,16
In vivo DOM(%)	$56,9^{ab}$	$57,5^{b}$	$54,7^{a}$	$61,9^{c}$
S.D.	1,11	1,05	0,16	2,54

Tables 2.17a and 2.17b show the in vitro and in vivo digestibilities of OM of sheeps' burnet for the different growth stages (trial periods) in Phases I and II respectively.

In Phase I, there were no significant differences in in vivo DOM. However, there was a significant difference in IVDOM between the 6 week (P2) and 12 week (P3) regrowths and also between the 6 week regrowth (P2) and primary growth (P1).

In Phase II, there were significant differences between the spring regrowth (P8) and the 15 week (P5) and 6 week (P7) regrowths in both parameters. There was also a significant difference between the 6 week regrowth and 8 week regrowth (P6) with respect to IVDOM.

Table 2.17 a. The influence of period on IVDOM and in vivo DOM of sheeps' burnet. Phase I.

Parameters	Trial period and chronological age of pastures.			
	P1 (26/12/88 -6/1/89) Sown previous autumn	P2 (21/2/89 - 2/3/89) 6 weeks	P3 (4/4/89- 14/4/89) 12 weeks	P4 (25/4/89- 5/5/89) 15 weeks
IVDOM (%)	$59,2^{a}$	$64,1^{b}$	$59,4^{a}$	$63,4^{ab}$
S.D.	0,14	4,24	5,09	3,19
In vivo DOM(%)	$62,3^{a}$	$66,0^{a}$	$62,5^{a}$	$65,5^{a}$
S.D.	0,14	3,15	3,80	2,37

Table 2.17 b. The influence of period on IVDOM and in vivo DOM of sheeps' burnet. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (18/6/89-28/6/89) 8 weeks	P7 (28/6/89-8/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
IVDOM (%)	$53,9^{ab}$	$56,0^{bc}$	$53,0^{a}$	$57,3^{c}$
S.D.	1,73	1,88	2,05	2,61
In vivo DOM(%)	$58,4^{ab}$	$59,9^{c}$	$57,7^{a}$	$60,9^{c}$
S.D.	1,27	1,40	1,54	1,93

Table 2.18 shows the in vitro and in vivo digestibilities of OM of lucerne at different growth stages (trial periods) in Phase II. The spring regrowth of lucerne had a significantly higher IVDOM and in vivo DOM. There were no significant differences between the 8 week and 6 week regrowths. The 15 week regrowth had a significantly lower IVDOM and in vivo DOM than the other 6 and 8 week regrowths.

Table 2.18. The influence of period on IVDOM and <u>in vivo</u> DOM of lucerne. Phase II only.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (18/6/89-28/6/89) 8 weeks	P7 (25/6/89-8/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
IVDOM (%)	$57,2^{a}$	$66,3^{b}$	$64,0^{b}$	$70,2^{c}$
S.D.	0,49	1,47	1,54	2,43
<u>In vivo</u> DOM(%)	$60,9^{a}$	$67,6^{b}$	$65,9^{b}$	$70,5^{c}$
S.D.	0,35	1,32	1,40	2,32

2.5.3.1 Voluntary intake of organic matter (OMI) of sheep on pastures.

Tables 2.19a and 2.19b portray the intake of organic matter by intact rams on the pastures in Phases I and II respectively.

There were no significant differences between sainfoin and sheeps' burnet with respect to both intake measures in both phases. However, sainfoin had a significantly higher intake per metabolic livemass than lucerne. There were no significant differences between sheeps' burnet and lucerne with respect to both intake parameters (Phase II).

Table 2.19 a. The intake of organic matter as influenced by pasture type (Mean of the four periods in Phase I).

	Pasture		
Parameter	Sainfoin	Sheeps'burnet	SE_m
OMI (g/day)	$995,4^a$	$1030,5^a$	17,55
OMI ($g/kgLW^{0,75}$/day)	$61,3^a$	$63,4^a$	1,05

Table 2.19 b. The intake of organic matter as influenced by pasture type (Mean of the four periods in Phase II).

	Pasture			
Parameter	Sainfoin	Sheeps'burnet	Lucerne	SE_m
OMI (g/day)	$1243,9^a$	$1201,3^a$	$1099,9^a$	42,7
OMI ($g/kgLW^{0,75}$/day)	$66,4^a$	$64,0^{ab}$	$57,8^b$	2,56

Tables 2.20a to 2.22 indicate the intake of organic matter by the rams during the different growth stages (trial periods) of the study. The rams were kept on the pastures for 12 hours/day from Period I (P1) to Period 7 (P7). During the trial in Period 8 (P8) however they remained on the pastures for 24 hours/day.

Tables 2.20a and 2.20b show the intake of OM by rams on sainfoin for the different periods of Phases I and II respectively.

Table 2.20 a. The influence of period on OMI of sheep grazing sainfoin. Phase I.

Parameters	Trial period and chronological age of pastures.			
	P1 (26/12/88 -6/1/89) Sown previous spring	P2 (21/2/89 - 2/3/89) 6 weeks	P3 (4/4/89- 14/4/89) 12 weeks	P4 (25/4/89- 5/5/89) 15 weeks
OMI (g/day)	1073,0[a]	936,5[a]	923,5[a]	1048,6[a]
C.V.(%)	11,6	6,6	7,6	17,9
OMI (g/kg $LW^{0,75}$/day)	68,7[a]	58,2[a]	56,2[a]	62,2[a]
C.V.(%)	9,8	7,3	9,3	16,7

Table 2.20 b. The influence of period on OMI of sheep grazing sainfoin. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89- 18/6/89) 15 weeks	P6 (17/6/89- 28/6/89) 8 weeks	P7 (28/6/89- 8/7/89) 6 weeks	P8 (7/11/89- 18/11/89) 8-9 weeks
OMI (g/day)	943,4[a]	960,4[a]	970,0[a]	2101,9[b]
C.V.(%)	11,6	12,5	5,3	29,7
OMI (g/kg $LW^{0,75}$/day)	54,2[a]	53,6[a]	52,8[a]	105,9[b]
C.V. (%)	15,7	13,6	2,8	24,9

There were no significant differences between periods with respect to the two intake measures in Phase I (Table 2.20a). In Phase II (Table 2.20b) there were no significant differences with respect to both measures between the late autumn and winter trials (P5-P7). However there were

significantly higher intakes on the trial with the spring regrowth (P8: during which the animals remained on the pasture for the duration of the trial) than each of the preceding periods.

Tables 2.21a and 2.21b show the intake of OM by rams on sheeps' burnet for the different periods of Phases I and II respectively.

In Phase I there were no significant differences in OMI (g/day) of sheeps' burnet between all periods. However, there was a significantly higher OMI (g/kg $LW^{0,75}$/day) on the primary growth (P1) compared to the 12 week regrowth (P2). In Phase II there were no significant differences with respect to both parameters between the late autumn and winter regrowths (P5, P6, P7). The trial on the spring regrowth (P8) yielded significantly higher intakes compared to each of the preceding three periods as noticed in the case of sainfoin.

Table 2.21 a. The influence of period on OMI of sheep grazing sheeps' burnet. Phase I.

Parameters	Trial period and chronological age of pastures.			
	P1 (26/12/88 -6/1/89) Sown previous spring	P2 (21/2/89 - 2/3/89) 6 weeks	P3 (4/4/89- 14/4/89) 12 weeks	P4 (25/4/89- 5/5/89) 15 weeks
OMI (g/day)	1099,6[a]	996,9[a]	934,9[a]	1090,5[a]
C.V.(%)	13,2	8,6	12,9	16,1
OMI (g/kg $LW^{0,75}$/day)	69,9[a]	62,2[ab]	56,9[b]	64,7[ab]
C.V.(%)	10,9	9,5	13,9	15,4

Table 2.21 b. The influence of period on OMI of sheep grazing sheeps' burnet. Phase II.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89- 18/6/89) 15 weeks	P6 (18/6/89- 28/6/89) 8 weeks	P7 (28/6/89- 8/7/89) 6 weeks	P8 (7/11/89- 18/11/89) 8-9 weeks
OMI (g/day)	1018,3[a]	1046,9[a]	1123,3[a]	1616,8[b]
C.V.(%)	19,4	10,6	19,4	14,8
OMI (g/kg $LW^{0,75}$/day)	57,9[a]	58,1[a]	60,5[a]	79,6[b]
C.V. (%)	24,6	12,7	17,1	18,0

Table 2.22 shows the intake of OM by rams on lucerne in Phase II of the study. There were significantly higher intakes on the 15 week regrowth compared with the 6 week regrowth, with the 8 week regrowth in between.

There were significantly higher intakes on the trial with the spring regrowth (P8) than each of the preceding periods as in the case of sainfoin and sheeps' burnet.

Table 2.22. The influence of period on OMI of sheep grazing lucerne. Phase II only.

Parameters	Trial period and chronological age of pastures.			
	P5 (8/6/89-18/6/89) 15 weeks	P6 (18/6/89-28/6/89) 8 weeks	P7 (25/6/89-8/7/89) 6 weeks	P8 (7/11/89-18/11/89) 8-9 weeks
OMI (g/day)	$1090,0^{b}$	$827,7^{ab}$	$658,8^{a}$	$1823,0^{c}$
C.V. (%)	18,2	22,9	35,1	23,0
OMI(g/kg $LW^{0.75}$/day)	$60,5^{a}$	$47,3^{ab}$	$36,1^{a}$	$87,6^{c}$
C.V. (%)	10,5	16,2	28,2	23,7

CHAPTER 3

NITROGEN INTAKE AND UTILIZATION

3.1 Experimental procedures.

3.1.1 Study objectives in brief.

The objective of this experiment was to obtain an indication of the utilization of feed nitrogen by sheep of the three test forages: sainfoin, sheeps' burnet and lucerne. Döhne Merino wethers with cannulae in the rumen, abomasum and ileum were used to determine the intake and flow of nutrients along different sections of the digestive tract. Ruminal ammonia levels, total nitrogen, ammonia nitrogen and non-ammonia nitrogen flows at the abomasum and ileum were determined. Volatile fatty acid production in the rumen was also measured as an index of energy production on the pastures.

The experiment was conducted in two phases:

i) the first phase involved the measurement of the above parameters on the first regrowth of sainfoin and sheeps' burnet. The pastures had been mowed at different dates in such a way as to yield three areas of each pasture which were grazed by the experimental animals at 6 weeks, 12 weeks and 15 weeks of age from midsummer to

autumn 1989. During this phase the animals were allowed to graze on the pastures from 06h00 to 18h00 during the trials.

ii) phase two involved the feeding of harvested spring regrowths (1989 and 1990) of sainfoin, sheeps' burnet and lucerne to the experimental animals indoors in metabolism cages[a].

In each phase the trials were run simultaneously as the intake studies (Chapter 2) on the same pastures. The idea was to determine whether the experimental animals were having a sufficient level of intake to justify a quantification of the data[b].

3.1.2 Material.

3.1.2.1 Animal: Preparation and cannulation.

Nine Döhne Merino wethers equipped with multiple cannulae in the rumen, abomasum and ileum were used. The rumen cannulae were made of rubber and each had an internal diameter of

[a] - It was decided to feed animals indoors to eliminate errors due to faecal losses (causing problems in Phase I with accurate intake determinations and infusion of external markers).

[b] - As a result samples taken during the trial on the 6 week regrowths in Phase I were deemed not suitable for analysis.

25mm. The abomasal and ileal cannulae were T-type simple cannulae manufactured from silicone rubber. All cannulae were closed with cork plugs which were opened only at the time of sampling.

The cannulae were cleaned and regularly disinfected to avoid infestation by maggots. The wool around them was also clipped regularly. Like the animals used in the intake studies the experimental animals were vaccinated against enterotoxaemia, regularly dosed to prevent infestation of internal parasites, treated against external parasites and had their hooves trimmed.

3.1.2.2 Pastures.

Pasture material was grazed or harvested with wool shears, to a height of 2cm (See Section 2.1.2.3).

3.1.2.3 Treatments.

The treatments in Phase I were

i) Treatment I - Sainfoin

ii) Treatment 2 - Sheeps' burnet.

There were three multiple cannulated sheep and two oesophageal fistulated sheep per treatment.

In Phase 2 harvested pasture material was fed indoors in metabolism cages. Fresh water was always available.

The treatments were:

i) Treatment 1 - Sainfoin

ii) Treatment 2 - Sheeps' burnet

iii) Treatment 3 - Lucerne.

There were two and four multiple cannulated animals per treatment in 1989 and 1990 respectively.

3.1.3 Experimental routine.

In Phase 1 of the experiment the animals grazed from 06h00 to 18h00. They were returned to the barn at 18h00 where they had no access to feed. Fresh water was always available. In Phase 2 the animals were kept in metabolism cages for the duration of the trials and fed fresh material twice daily at 06h00 and 18h00. All the multiple cannulated animals were also equipped with harnesses and nylon canvas bags for faeces collection. The oesophageally fistulated animals were used for collection of pasture at the beginning of the adaptation period, at the beginning of the collection period and again at the end of the collection period of each trial.

3.1.4 Parameters.

The following variables were studied:

3.1.4.1 Dry matter content of pastures.

3.1.4.2 Organic matter content of pastures.

3.1.4.3 Nitrogen content of pastures.

3.1.4.4 Organic matter intake.

3.1.4.5 Nitrogen intake.

3.1.4.6 Rumen parameters.

3.1.4.6.1 Rumen ammonia nitrogen concentration.

3.1.4.6.2 Volatile fatty acid concentration.

3.1.4.6.3 Molar proportions of volatile fatty acids.

3.1.4.7 Flow study.

3.1.4.7.1 Abomasum.

3.1.4.7.1.1 Total nitrogen flow.

3.1.4.7.1.2 Ammonia nitrogen flow.

3.1.4.7.2 Ileum.

3.1.4.7.2.1 Total nitrogen Flow.

3.1.4.7.2.2 Ammonia nitrogen flow.

3.1.4.8 Non-ammonia nitrogen disappearance.

3.1.4.9 Non-ammonia nitrogen disappearance as a proportion of N intake.

3.1.4.10 Digestibility of non-ammonia nitrogen in the small intestine.

3.1.5 Methods.

3.1.5.1 Trial period.

An adaptation period of one week, of which the last four days were used for the infusion of the external markers chromium (Cr) EDTA and Ytterbium (Yb) acetate to achieve steady state conditions (Faichney & White, 1977) was used. The animals had been put on pasture before the beginning of the trials. This was followed by a collection period of four days for rumen, abomasal, ileal and faecal samples.

3.1.5.2 Trial implementation.

3.1.5.2.1 Feeding.

In Phase I the animals were given access to pasture and fresh water in the manner described in Section 3.1.3.

In Phase 2 the animals were fed freshly harvested material to supply them with at least 1000 g OM per day (1989). This was adjusted to 1500 g OM per day in 1990. Fresh water was available at all times in the metabolism cages.

3.1.5.2.2 Digesta flow markers.

The external markers Cr-EDTA and Yb-acetate were used to

determine the flow of digesta in the different sections of the gastrointestinal tract. Chromium-EDTA (Downes & McDonald, 1964; Faichney, 1975) was used to mark the liquid phase and Yb-acetate (Siddons et al., 1985) the particulate phase.

3.1.5.2.2.1 Preparation and infusion of markers.

Chromium-EDTA was prepared according to the method of Morgan et al. (1976):

Exactly 71,6 g of $CrCl_3.6H_2O$ was added to 100 g $Na_2EDTA.2H_2O$. The mixture was boiled for an hour, cooled and then adjusted to a pH of 7,0 by addition of NH_4OH. It was then made up to a liter with de-ionized water. This solution would contain 13,976 Cr/l. Twenty ml of the solution was added to 1 liter of de-ionized water each day and infused into the rumen with the aid of an electric peristaltic proportioning pump mounted at the back of each animal in Phase I. In Phase II the same quantity was continuously infused into the rumen with the aid of an autoanalyser proportioning pump adapted for the purpose. This ensured that about 300 mg/day of chromium was infused per sheep. The pumps were calibrated to infuse 1 liter in 24 hours (about 0,7 ml/minute). The actual amount of Cr in the solution was determined by atomic absorption spectrophotometry.

Ytterbium-acetate was dried at 100° C overnight. Due to the

high importation costs the total number of sheep and days to be infused plus a small margin was determined and a calculated amount of dried Yb-acetate dissolved in a predetermined volume of de-ionized water so that 10 ml of the solution would contain 100mg Yb. One hundred mg Yb per sheep per day (Siddons et al., 1985) was supposed to be infused. Ten ml of the solution was added to 1 liter of de-ionized water per sheep and infused continuously into the rumen via separate infusion lines using the same proportioning pumps. The actual amount of Yb in the prepared solution was measured by Atomic Absorption Spectrophotometry. The infusion of markers started from the fourth day of adaptation till the end of each trial.

3.2 Experimental design and statistical analysis of data.

The experiment was undertaken in two phases: phase I with two treatments (sainfoin and sheeps' burnet) and phase II with three treatments (lucerne included with sainfoin and sheeps' burnet).

There were two trial periods in each phase. Experimental animals were allocated randomly to each treatment in each trial period.

Experimental design

Phase of experiment	Period[1]	Treatments		
		Treatment 1	Treatment 2	Treatment 3
I	P3	Sainfoin	Sheeps' burnet	-
	P4	Sainfoin	Sheeps' burnet	-
II	P8	Sainfoin	Sheeps' burnet	Lucerne
	P9	Sainfoin	Sheeps' burnet	Lucerne

The data yielded by the study were analyzed separately for each phase for treatment and period effects and their interaction using the two-way analysis of the general linear models programme. The least square means and a probability level of 5% was utilized.

3.3 Sample collection and preservation.

3.3.1 Intake of organic matter and nitrogen.

3.3.1.1 Pasture samples.

Samples were collected from the oesophageally fistulated sheep and hand clipped samples were taken at the beginning of the adaptation period, at the beginning of the collection period and again at the end of the collection period.

[1] - Periods coincide with those of the intake trials (Chapter 2) except P9 which was spring 1990.

Dry matter content of the cut samples was determined from the subsample at 100° C, the remainder being dried at 50° C immediately after cutting for N determination. Samples from the oesophageal fistulae were treated and dried in the same manner as 2.1.5.2.2. All dried samples were milled to pass a 1 mm sieve of a Beaver mill and stored in glass and plastic bottles for analysis.

3.3.1.2 Faecal samples.

Faeces were collected twice daily at 06h00 and 18h00 during the collection period. The total daily excretion was weighed, 10% taken and pooled and stored frozen in polythene bags at -15° C. At the end of each trial the faeces were thawed and a subsample taken immediately for the determination of dry matter at 100° C. Thus H_2O loss during freezing and thawing was averted. This was necessary if DM content of the faeces was to be related to wet faeces excretion. The remainder of the sample was dried at 60° C, ground to pass a 1 mm sieve of a Beaver mill and stored in glass bottles for analysis.

3.3.2 Flow study: Ruminal, abomasal and ileal samples.

3.3.2.1 Sampling times.

Rumen fluid samples, abomasal and ileal digesta samples were taken three times daily over the last four days in such a

manner that each 2 h period of the 24 h feeding cycle was represented (Faichney, 1975).

Day 1	07h00	15h00	23h00
Day 2	09h00	17h00	01h00
Day 3	11h00	19h00	03h00
Day 4	13h00	21h00	05h00

3.3.2.2 Rumen fluid samples.

At each sampling about 50 ml of rumen fluid was drawn with a perspex pipe using suction provided by a 60 ml syringe. The rumen fluid was then filtered through cheese cloth and subsequently preserved for ammonia and volatile fatty acid determinations.

3.3.2.2.1 Preservation of sample for rumen ammonia determinations.

Ten ml of the filtered rumen fluid sample drawn at each sampling was preserved with 2 ml of 0,5M H_2SO_4, pooled and frozen pending subsequent NH_3 determination.

3.3.2.2.2 Preservation of samples for volatile fatty acid determination.

Ten ml of the filtered rumen fluid sample drawn at each sampling was preserved with 0,5ml 10% NaOH, pooled and frozen for subsequent determinations of volatile fatty acids.

3.3.2.3 Abomasal and ileal samples.

Fifty ml each of abomasal and ileal digesta was collected at each sampling, pooled and stored frozen for subsequent analysis. The cork plugs were replaced immediately after sampling. Before chemical analyses the samples were thawed, a subsample taken immediately for DM determination and the rest dried at 60° C in a force draught oven. It was then ground in a mortar with a pestle and stored in glass bottles.

3.4 Analytical methods.

3.4.1 Nitrogen in pasture.

The samples of pasture that had been taken (clipped material or material from oesophageal fistulae) were dried at 50° C, milled to pass a 1 mm screen of a Beaver mill and then analyzed by macro kjedahl (AOAC, 1984) using the apparatus described in 2.4.4.

3.4.2 Dry matter.

The DM content of clipped pasture, faecal, abomasal and ileal digesta were determined by drying samples at 100° C for 24 hours in a forced draught oven and calculating the DM content as stated in 2.4.1.

3.4.3 Organic matter content.

The OM content of clipped samples and faeces were expressed on the basis of the dry matter in the South African Department Of Agriculture Handbook of Laboratory Methods (1989). See Section 2.4.3.

3.4.4 Organic matter intake.

The intake of OM by each animal that grazed the pastures in Phase I was calculated from the mean calculated in vivo indigestibility of OM of material collected from the oesophageal fistulae and the OM content of faeces excreted as stated in 2.4.10. The intake of OM by each animal fed clipped material in Phase II was determined by multiplying the mean OM content of grab samples collected by the mean amount of DM eaten daily.

3.4.5 Nitrogen intake.

The nitrogen intake of each animal in Phase I was determined by multiplying the N content of the material collected from the oesophageal fistulae (corrected for OM content) by the OM intake of that animal. The N intake of each animal in Phase II was determined by multiplying the mean N content of the grab samples of pasture (corrected for OM content) by the OM intake of each animal.

3.4.6 Rumen parameters.

3.4.6.1 Rumen NH_3-N concentration.

The concentration of NH_3-N in the rumen was determined from the filtered sample on a Technicon Autoanalyser. The reading obtained was multiplied by the dilution factor. The concentration (mg/100ml) was calculated as follows:

$$\frac{\text{Reading} \times \text{Dilution factor} \times 1{,}2175}{10}$$

(Manual Technicon Autoanalyser).

3.4.6.2 Volatile fatty acid concentration.

Ten ml of the filtered and preserved sample was taken for preparation of sample for analysis. One ml 50% orthophosphoric acid was added. The sample was centrifuged at 4500rpm for 20 minutes. Nine ml of the clear supernatant was pipetted into a clean bottle and to this exactly 1 ml of an internal standard, Pivalic acid (2000mg/1000ml) was added.

A Varian 3300 Gas Chromatograph with a flame ionization detector and a Varian 4290 integrator were used for the determination of the concentrations of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids.

The apparatus had a glass column with an internal diameter of 3mm and packed with 60/80 Carbopack c/o, 3% Carbowax

20m/o, 1% H_3PO_4. The column was conditioned overnight at 150° C and a flow of 15 ml N (carrier gas) per minute. The standard solution for the determination of volatile fatty acids (VFA) was made up at 15° C by adding to 50 ml de-ionized water and 2 ml orthophosphoric acid, the VFA in roughly the quantities that are normally present in rumen fluid:
450 mg acetic acid, 200 mg propionic acid, 70 mg n-butyric acid, 25 mg iso-butyric acid and 25 mg isovaleric acid. Ten ml of the internal standard was then added and made up to 100 ml with de-ionized water.

Standards were injected repeatedly until consecutive results were comparable. A 1 ml sample of each of the prepared sample was then injected. The readings on the integrator for each of the VFA was multiplied by the dilution factor to obtain their concentrations (mmol/100ml). The molar proportions were calculated by dividing the concentration of each VFA by the total concentration.

3.4.7 Flow study.

3.4.7.1 Determination of concentrations of Cr and Yb in abomasal and ileal digesta.

Samples of the wet abomasal and ileal digesta were centrifuged at 4500 rpm for 15 minutes. The supernatant was separated and kept in small glass bottles. The remainder of the wet abomasal and ileal digesta were dried at 60° C. Since the markers, especially Cr, were not expected to behave as ideal markers (Faichney, 1975) the concentrations of both Yb and Cr were measured in the supernatant and the solid phase. Chromium and Yb concentrations in the supernatant were measured directly on a Perkin-Elmer 2380 atomic absorption spectrophotometer after an appropriate dilution.

The solid samples were prepared by a wet digestion technique as in 2.4.7 and diluted to an appropriate concentration. The dry ashing method proposed by Siddons et al. (1985) was not used because the recovery of Yb in a spiked sample was lower than in the wet digestion technique. Chromium was determined at a wavelength of 357,9 nm and a slit setting of 0,7 nm. A hollow cathode lamp and an air-acetylene flame were used. Ytterbium was determined at a wavelength of 398,8 nm and a slit setting of 0,2 nm. A hollow cathode tube and a nitrous-oxide acetylene flame were used. The readings obtained were multiplied by the dilution factor to obtain the concentrations in mg/l.

3.4.7.2 Digesta flow (g/d) at the abomasum and ileum.

The flow of digesta into the abomasum and ileum were calculated by employing the double marker method described by Faichney (1975).

3.4.7.2.1 Nitrogen flow (g/d) at the abomasum and ileum.

Nitrogen flow was determined by multiplying the digesta flow rate at the abomasum and ileum by the respective N content (2.4.4) of the dried digesta (60° C).

3.4.7.2.2 Ammonia nitrogen flow (g/d) at the abomasum and ileum.

Ammonia-N in the abomasum and ileum was determined on the supernatant of the centrifuged samples (at 4500 rpm for 10 minutes) with a Technicon auto-analyser. The concentration of NH_3-N was calculated as in 3.4.6.1.

Ammonia - N flow was calculated by multiplying the digesta flow rate by the respective concentrations of NH_3-N (mg/l).

3.4.7.3 Non-ammonia nitrogen (NAN) flow (g/d) at the abomasum and ileum.

The NAN flow at the abomasum and ileum (g/d) was determined by subtracting the NH_3-N flow from the total N flow at the abomasum and ileum respectively.

3.4.7.4 Non-ammonia nitrogen disappearance in small intestine.

The disappearance of non-ammonia nitrogen (NAN) in the small intestine was calculated as follows:

NAN disappearance (g/d) = NAN flow at abomasum (g/d) - NAN flow at ileum (g/d).

3.4.7.5 Non-ammonia nitrogen disappearance as a proportion of N intake.

This was calculated as follows:

$$\text{NAN disappearance (proportion of intake)} = \frac{\text{NAN disappearance (g/d)}}{\text{N intake (g/d)}} \times 100$$

3.4.7.6 Digestibility of NAN.

Digestibility of NAN was calculated as follows:

$$\% \text{ NAN digestibility} = \frac{\text{NAN disappearance (g/d)}}{\text{NAN Flow in Abomasum (g/d)}} \times 100$$

3.5 Results.

The results were subdivided into treatment effects and time period effects. The results for treatment effects are summarized with the standard error of means whilst period effects show the standard deviations of the parameters measured.

The standard deviations and standard error of means and coefficients of variation were calculated using the method of Snedecor (1956). Unless otherwise stated values with at least one common letter on the same horizontal line do not differ significantly. Samples were pooled for plant N content determination and therefore N figures do not show letters denoting differences of significance.

3.5.1 pH and Volatile Fatty acid (VFA) production in rumen and N utilization.

3.5.1.1 pH, VFA production and molar proportions of VFA in the rumen.

Tables 3.1a and 3.1b portray differences in pH, total VFA levels and molar proportions of VFA in the rumen of sheep fed the pastures. In Phase I (Table 3.1a) there were no significant differences between treatments with all the parameters except the molar proportions of butyric and valeric acids. In Phase II (Table 3.2a) there were no significant differences between all three pastures with respect to VFA production, molar proportions of propionic acid and the ratio of acetic acid to propionic acid. As in Phase I there were no significant differences between sainfoin and sheeps' burnet with respect to pH and the molar proportion of acetic acid. However there were significant differences between the two pastures as far as the molar proportions of butyric and valeric acids were concerned. Lucerne differed significantly from sheeps' burnet with respect to pH and the molar proportions of butyric acid and valeric acid and from sainfoin with respect to the molar proportion of valeric acid.

Table 3.1 a pH, VFA production and molar proportions of VFA as influenced by pasture type (Mean of the two periods in Phase I).

Parameters	Pasture		SE_m
	Sainfoin	Sheeps' burnet	
pH	$6,2^{a}$	$6,3^{a}$	0,05
VFA conc.(mmol/100ml)	$14,4^{a}$	$14,3^{a}$	0,05
Molar proportions:			
Acetic	$0,68^{a}$	$0,64^{a}$	0,02
Propionic	$0,21^{a}$	$0,20^{a}$	0,01
Butyric[1]	$0,10^{a}$	$0,15^{b}$	0,03
Valeric[1]	$0,02^{b}$	$0,01^{a}$	0,01
Ratio acetic to propionic acid	$3,3^{a}$	$3,2^{a}$	0,05

Table 3.1 b pH, VFA production and molar proportions of VFA as influenced by pasture type (Mean of the two periods in Phase II).

Parameters	Pasture			SE_m
	Sainfoin	Sheeps' burnet	Lucerne	
pH	$6,0^{ab}$	$5,9^{a}$	$6,2^{b}$	0,09
VFA concentration (mmol/100ml)	$30,4^{a}$	$31,5^{a}$	$39,7^{a}$	2,93
Molar proportions:				
Acetic	$0,66^{b}$	$0,64^{ab}$	$0,63^{a}$	0,01
Propionic	$0,22^{a}$	$0,21^{a}$	$0,23^{a}$	0,01
Butyric	$0,10^{a}$	$0,14^{b}$	$0,11^{a}$	0,01
Valeric	$0,02^{b}$	$0,01^{a}$	$0,03^{c}$	0,01
Ratio acetic to propionic acid.	$3,0^{a}$	$3,1^{a}$	$2,8^{a}$	0,09

[1] - In all instances butyric and valeric acids are expressed as the total of the normal and iso-acids.

Tables 3.2a to 3.4 show the pH, VFA levels and molar proportions of VFA in the rumen of sheep during the two trial periods within each phase.

Tables 3.2a and 3.2b indicate the parameters as measured for sheep fed sainfoin for Phases I and II respectively. There were significant differences between periods only with respect to pH and VFA production in both phases.

Table 3.2 a The influence of period on pH, VFA and molar proportions of VFA in sheep on sainfoin pasture (Phase I).

Parameters	Periods and chronological age of pastures	
	P3 (4/4/89-14/4/89) 12 weeks	P4 (25/4/89-5/5/89) 15 weeks
pH	5,9[a]	6,4[b]
S.D	0,12	0,12
VFA conc. (mmol/100ml)	16,5[b]	12,2[a]
S.D.	2,3	0,45
Molar proportions:		
Acetic acid	0,67[a]	0,69[a]
S.D.	0,04	0,03
Propionic acid	0,22[a]	0,20[a]
S.D.	0,02	0,03
Butyric acid	0,11[a]	0,09[a]
S.D	0,02	0,01
Valeric acid	0,01[a]	0,02[a]
S.D.	0	0
Ratio acetic to propionic acid	3,1[a]	3,5[a]
S.D.	0,55	0,66

Table 3.2 b The influence of period on pH, VFA and molar proportions of VFA in sheep fed sainfoin (Phase II).

Parameters	Periods and chronological age of pastures	
	P8 (7/11/89 -18/11/89) 8-9 weeks	P9 (17/10/90 -28/10/90) Regrowth from winter
pH	5,7[a]	6,4[b]
S.D	0,42	0,13
VFA conc. (mmol/100ml)	16,7[a]	44,2[b]
S.D.	1,34	5,16
Molar proportions:		
Acetic acid	0,66[a]	0,66a
S.D.	0	0,03
Propionic acid	0,23[a]	0,22[a]
S.D.	0,01	0,02
Butyric acid	0,10[a]	0,09[a]
S.D	0	0,01
Valeric acid	0,02[a]	0,03[a]
S.D.	0	0,01
Ratio acetic to propionic acid	3,0[a]	3,1[a]
S.D.	0,14	0,39

Table 3.3a and 3.3b show the parameters as measured for sheep fed sheeps' burnet. There was a significant difference between periods only with respect to VFA production in both phases.

Table 3.3 a The influence of period on pH, VFA and molar proportions of VFA in sheep on sheeps' burnet pasture (Phase I).

Parameters	Periods and chronological age of pastures	
	P3 (4/4/89-14/4/89) 12 weeks	P4 (28/4/89-5/5/89) 15 weeks
pH	6,3[a]	6,3[a]
S.D	0,21	0,21
VFA conc. (mmol/100ml)	16,0[a]	12,7[b]
S.D.	2,17	0,85
Molar proportions:		
Acetic acid	0,63[a]	0,66[a]
S.D.	0,06	0,03
Propionic acid	0,19[a]	0,21[a]
S.D.	0,04	0,02
Butyric acid	0,18[a]	0,13[a]
S.D	0,06	0,01
Valeric acid	0,01[a]	0,01[a]
S.D.	0	0
Ratio acetic to propionic acid	3,4[a]	3,1[a]
S.D.	0,76	0,36

Table 3.3 b The influence of period on pH, VFA and molar proportions of VFA in sheep fed sheeps' burnet (Phase II).

Parameters	Periods and chronological age of pastures	
	P8 (7/11/89 -18/11/89) 8-9 weeks	P9 (17/10/90 -28/10/90) Regrowth from winter
pH	5,8[a]	6,1[a]
S.D	0,28	0,10
VFA conc. (mmol/100ml)	16,6[a]	46,4[b]
S.D.	0,84	12,16
Molar proportions:		
Acetic acid	0,64[a]	0,64[a]
S.D.	0,01	0,01
Propionic acid	0,21[a]	0,21[a]
S.D.	0,01	0,02
Butyric acid	0,14[a]	0,13[a]
S.D	0,01	0,02
Valeric acid	0,02[a]	0,01[a]
S.D.	0,01	0,01
Ratio acetic to propionic acid	3,1[a]	3,0[a]
S.D.	0,14	0,25

Table 3.4 indicates the parameters as measured for sheep fed lucerne during its inclusion in Phase II. There were significant differences between periods only with respect to VFA production and molar proportion of butyric acid.

Table 3.4 The influence of period on VFA production and molar proportions of VFA in sheep fed lucerne (Phase II only).

Parameters	Periods and chronological age of pastures	
	P8 (7/11/89 -18/11/89) 8-9 weeks	P9 (17/10/90 -28/10/90) Regrowth from winter
pH	6,1[a]	6,3[a]
S.D	0,28	0,15
VFA conc. (mmol/100ml)	20,1[a]	59,3[b]
S.D.	1,56	5,66
Molar proportions:		
Acetic acid	0,63[a]	0,64[a]
S.D.	0,03	0,02
Propionic acid	0,22[a]	0,24[a]
S.D.	0,04	0,02
Butyric acid	0,13[b]	0,09[a]
S.D	0,02	0,01
Valeric acid	0,03[a]	0,03[a]
S.D.	0	0,01
Ratio acetic to propionic acid	2,9[a]	2,7[a]
S.D.	0,71	0,29

3.5.1.2 Nitrogen flow and utilization.

Tables 3.5a and 3.5b show differences in nitrogen intake and utilization in sheep fed the pastures in Phases I and II respectively. In phase I there were significant differences between pastures with respect to rumen NH_3 production and the disappearance of NAN (both in absolute amounts and relative to intake) as well as the digestibility of NAN.

In Phase II there were significant differences between pastures with respect to rumen NH_3 production and the disappearance of NAN (% of intake). There was significantly higher disappearance of NAN (g/day) in sheep on sainfoin compared to sheeps' burnet and lucerne which did not differ significantly from each other.

The digestibility of NAN did not differ significantly between lucerne and sainfoin but was significantly higher in both pastures compared to sheeps' burnet.

Table 3.5 a Nitrogen intake and utilization as influenced by pasture type (Mean of the two periods in Phase I).

Parameters	Pasture		SE_m
	Sainfoin	Sheeps' burnet	
Rumen NH_3 (mg/100ml)	$9,3^b$	$4,2^a$	2,55
OM intake (g/day)	$986,2^a$	$1015,1^a$	14,45
N (% of DM)	3,0	2,5	0,25
N intake (g/day)	$29,6^a$	$25,4^a$	2,10
ABOMASUM			
Digesta flow (l/day)	$21,9^a$	$19,8^a$	1,05
Total N flow (g/day)	$37,7^b$	$27,3^a$	5,20
NH_3-N flow (g/day)	$2,0^b$	$0,9^a$	0,55
NAN flow (g/day)	$35,7^a$	$26,4^a$	4,65
ILEUM			
Digesta flow (l/day)	$5,6^a$	$6,3^a$	0,35
Total N flow (g/day)	$13,8^a$	$13,8^a$	0,02
NH_3-N flow (g/day)	$1,3^a$	$1,4^a$	0,05
NAN flow (g/day)	$12,5^a$	$12,4^a$	0,05
NAN disappearance (g/day)	$23,2^b$	$14,0^a$	4,60
NAN disappearance (% of intake)	$78,4^b$	$55,1^a$	11,65
NAN digestibility(%)	$65,0^b$	$53,0^a$	6,00

Table 3.5 b Nitrogen intake and utilization as influenced by pasture type (Mean of the two periods in Phase II).

Parameters	Pasture			SE_m
	Sainfoin	Sheeps' burnet	Lucerne	
Rumen NH_3 (mg/100ml)	$25,3^{b}$	$6,6^{a}$	$65,3^{c}$	17,3
OM intake (g/day)	1139^{b}	888^{a}	1044^{ab}	73,2
N (% of DM)	$3,8_{b}$	$3,3_{a}$	$4,2_{b}$	0,26
N intake (g/day)	$43,3^{b}$	$29,3^{a}$	$43,9^{b}$	4,77
ABOMASUM				
Digesta flow (l/day)	$21,3^{b}$	$18,7^{ab}$	$15,9^{a}$	1,56
Total N flow (g/day)	$50,7^{b}$	$32,9^{a}$	$40,0^{a}$	5,17
NH_3-N flow (g/day)	$4,1^{a}$	$1,4^{a}$	$10,1^{b}$	2,57
NAN flow (g/day)	$46,6^{b}$	$31,5^{a}$	$29,9^{a}$	5,32
ILEUM				
Digesta flow (l/day)	$5,8^{b}$	$5,1^{ab}$	$4,8^{a}$	0,30
Total N flow (g/day)	$14,8^{a}$	$13,2^{ab}$	$10,8^{b}$	1,16
NH_3-N flow (g/day)	$1,8^{a}$	$1,0^{b}$	$2,6^{c}$	0,46
NAN flow (g/day)	$13,0^{b}$	$12,2^{b}$	$8,2^{a}$	1,48
NAN disappearance (g/day)	$33,6^{b}$	$19,3^{a}$	$21,7^{a}$	4,42
NAN disappearance (% of intake)	$77,6^{c}$	$65,9^{b}$	$49,4^{a}$	8,18
NAN digestibility(%)	$72,1^{b}$	$61,3^{a}$	$72,6^{b}$	3,65

Tables 3.6a to 3.8 portray nitrogen intake and utilization indices measured in sheep fed on pastures during the two trial periods within each phase.

Tables 3.6a and 3.6b show the indices as measured for sheep fed sainfoin for Phases I and II respectively. In Phase I, there were no significant differences between periods with respect to rumen NH_3 production, NAN disappearance (both absolute amounts and relative to intake) and NAN digestibility.

In Phase II, there were significant differences between periods with respect to rumen NH_3 production and NAN disappearance (g/day).

However, there were no significant differences between periods with respect to NAN disappearance (% of intake) and NAN digestibility (%).

Table 3.6 a. The influence of period on nitrogen intake and utilization in sheep on sainfoin pasture (Phase I).

Parameters	Periods and chronological age of pastures	
	P3 (4/4/89-14/4/89) 12 weeks	P4 (25/4/89-5/5/89) 15 weeks
Rumen NH_3 (mg/100ml)	7,7[a]	11,0[a]
S.D	3,71	3,62
OM intake (g/day)	924[a]	1049[a]
S.D.	143,2	59,2
N (% of DM)	3,0	3,0
N intake (g/day)	27,7[a]	31,5[a]
S.D.	4,3	1,76
ABOMASUM		
Digesta flow (l/day)	21,6[a]	22,3[a]
S.D.	2,71	3,52
Total N flow (g/day)	33,9[a]	41,6[a]
S.D	5,69	8,91
NH_3-N flow (g/day)	1,7[a]	2,3[a]
S.D.	0,23	0,50
NAN flow (g/day)	32,2[a]	39,3[a]
S.D.	5,58	8,60
ILEUM		
Digesta flow (l/day)	4,3[a]	6,8[a]
S.D.	0,45	1,51
Total N flow (g/day)	10,3[a]	17,4[a]
S.D	1,97	5,06
NH_3-N flow (g/day)	1,0[a]	1,6[a]
S.D.	0,21	0,62
NAN flow (g/day)	9,3[a]	15,8[a]
S.D.	2,41	4,45
NAN disappearance (g/day)	22,9[a]	23,5[a]
C.V. (%)	16,3	21,6
NAN disappearance (% of intake)	82,3[a]	74,6[a]
C.V. (%)	16,3	21,6
NAN digestibility(%)	71,1[a]	59,8[a]
C.V. (%)	3,3	8,6

Table 3.6 b. The influence of period on nitrogen intake and utilization in sheep fed sainfoin (Phase II).

Parameters	Periods and chronological age of pastures	
	P8 (7/11/89 -18/11/89) 12 weeks	P9 (17/10/90 -28/10/90) 15 weeks
Rumen NH_3 (mg/100ml)	14,5[a]	36,1[b]
S.D	4,81	10,34
OM intake (g/day)	882[a]	1396[b]
S.D.	102,53	16,09
N (% of DM)	3,8	3,7
N intake (g/day)	33,5[a]	51,6[b]
S.D.	3,96	0,61
ABOMASUM		
Digesta flow (l/day)	20,1[a]	22,5[a]
S.D.	5,94	2,10
Total N flow (g/day)	39,0[a]	62,4[b]
S.D	8,34	4,41
NH_3-N flow (g/day)	2,2[a]	6,1[a]
S.D.	0,92	2,71
NAN flow (g/day)	36,8[a]	56,3[b]
S.D.	7,42	1,85
ILEUM		
Digesta flow (l/day)	4,0[a]	7,7[b]
S.D.	0,99	0,17
Total N flow (g/day)	12,6[a]	17,1[b]
S.D	1,34	2,42
NH_3-N flow (g/day)	1,4[a]	2,2[a]
S.D.	0,35	0,51
NAN flow (g/day)	11,2[a]	14,9[b]
S.D.	1,70	2,05
NAN disappearance (g/day)	25,6[a]	41,4[b]
C.V. (%)	22,3	3,3
NAN disappearance (% of intake)	76,4[a]	80,2[a]
C.V. (%)	10,6	4,0
NAN digestibility(%)	69,6[a]	73,6[a]
C.V. (%)	2,1	4,1

Tables 3.7a and 3.7b show the indices as measured for sheep fed sheeps' burnet for Phases I and II respectively. In Phase I, there were no significant differences between periods with respect to rumen NH_3 production, NAN disappearance (both absolute and relative to intake) and NAN digestibility (%).

In Phase II, there were no significant differences between periods with respect to rumen NH_3 production, NAN disappearance (both absolute and relative to intake) and NAN digestibility (%).

Table 3.7 a The influence of period on nitrogen intake and utilization in sheep on sheeps' burnet pasture (Phase I).

Parameters	Periods and chronological age of pastures	
	P3 (4/4/89-14/4/89) 8-9 weeks	P4 (25/4/89-5/5/89) Regrowth from winter
Rumen NH_3 (mg/100ml)	3,8[a]	4,5[a]
S.D	1,72	1,92
OM intake (g/day)	940[a]	1091[a]
S.D.	58,8	98,7
N (% of DM)	2,5	2,5
N intake (g/day)	23,5[a]	27,3[a]
S.D.	1,45	2,48
ABOMASUM		
Digesta flow (l/day)	20,6[a]	19,1[a]
S.D.	5,83	4,48
Total N flow (g/day)	28,7[a]	25,9[a]
S.D	8,83	5,96
NH_3-N flow (g/day)	1,0[a]	0,9[a]
S.D.	0,61	0,31
NAN flow (g/day)	27,7[a]	25,0[a]
S.D.	8,25	5,65
ILEUM		
Digesta flow (l/day)	7,4[a]	5,2[a]
S.D.	3,06	1,40
Total N flow (g/day)	16,2[a]	11,4[a]
S.D	7,66	3,66
NH_3-N flow (g/day)	1,6[a]	1,1[a]
S.D.	0,78	0,65
NAN flow (g/day)	14,6[a]	10,3[a]
S.D.	7,11	2,80
NAN disappearance (g/day)	13,1[a]	14,7[a]
C.V. (%)	11,7	19,0
NAN disappearance (% of intake)	55,7[a]	53,8[a]
C.V. (%)	11,7	19,0
NAN digestibility(%)	47,3[a]	58,8[a]
C.V. (%)	20,4	7,3

Table 3.7 b The influence of period on nitrogen intake and utilization in sheep fed sheeps' burnet (Phase II).

Parameters	Periods and chronological age of pastures	
	P8 (7/11/89 -18/11/89) 8-9 weeks	P9 (17/10/90 -28/10/90) Regrowth from winter
Rumen NH_3 (mg/100ml)	4,8[a]	8,4[a]
S.D	0,99	1,33
OM intake (g/day)	733[a]	1043[b]
S.D.	0,42	104,7
N (% of DM)	3,5	3,1
N intake (g/day)	25,7[a]	32,3[a]
S.D.	0,07	3,25
ABOMASUM		
Digesta flow (l/day)	19,8[a]	17,7[a]
S.D.	0,64	0,71
Total N flow (g/day)	28,7[a]	37,1[b]
S.D	1,27	1,70
NH_3-N flow (g/day)	1,2[a]	1,5[a]
S.D.	0,28	0,14
NAN flow (g/day)	27,5[a]	35,6[b]
S.D.	1,56	1,84
ILEUM		
Digesta flow (l/day)	4,6[a]	5,5[a]
S.D.	0,42	0,71
Total N flow (g/day)	10,8[a]	15,8[b]
S.D	0,57	1,63
NH_3-N flow (g/day)	1,0[a]	1,1[a]
S.D.	0,07	0,07
NAN flow (g/day)	9,8[a]	1,70[b]
S.D.		
NAN disappearance (g/day)	17,7[a]	20,9[a]
C.V. (%)	11,6	0,68
NAN disappearance (% of intake)	68,9[a]	64,7[a]
C.V. (%)	11,6	9,36
NAN digestibility(%)	64,4[a]	58,7[a]
C.V. (%)	5,93	4,46

Table 3.8 indicates the indices as measured for sheep fed lucerne during its inclusion in Phase II. There were significant differences between periods with respect to rumen NH_3 production and NAN disappearance (g/day). There were no significant differences, however, between periods with respect to NAN disappearance (% of intake) and NAN digestibility (%).

Table 3.8 The influence of period on nitrogen intake and utilization in sheep fed lucerne (Phase II only).

Parameters	Periods and chronological age of pastures	
	P8 (7/11/89 -18/11/89) 8-9 weeks	P9 (17/10/90 -28/10/90) Regrowth from winter
Rumen NH_3 (mg/100ml)	72,3[b]	58,3[a]
S.D	2,05	7,88
OM intake (g/day)	782[a]	1307[b]
S.D.	43,1	203,5
N (% of DM)	3,8	4,5
N intake (g/day)	29,7[a]	58,8[b]
S.D.	1,70	9,13
ABOMASUM		
Digesta flow (l/day)	10,5[a]	21,3[b]
S.D.	2,40	3,58
Total N flow (g/day)	23,0[a]	56,9[b]
S.D	3,54	5,00
NH_3-N flow (g/day)	3,3[a]	16,9[b]
S.D.	1,13	4,01
NAN flow (g/day)	19,7[a]	40,0[b]
S.D.	2,55	1,95
ILEUM		
Digesta flow (l/day)	2,2[a]	7,4[b]
S.D.	0,35	0,76
Total N flow (g/day)	4,9[a]	16,7[b]
S.D	0,03	2,38
NH_3-N flow (g/day)	0,6[a]	4,6[b]
S.D.	0,14	0,66
NAN flow (g/day)	4,3[a]	12,1[b]
S.D.	0,14	1,94
NAN disappearance (g/day)	15,4[a]	27,9[b]
C.V. (%)	17,5	11,0
NAN disappearance (% of intake)	51,9[a]	47,4[a]
C.V. (%)	11,9	5,38
NAN digestibility(%)	78,2[a]	69,8[a]
C.V. (%)	4,6	8,92

CHAPTER 4

DEGRADATION OF FORAGE PROTEINS IN THE RUMEN AND TANNIN ANALYSIS.

4.1 Experimental procedure.

4.1.1 Study objectives in brief.

This experiment was conducted to determine the rate and extent of degradation of the proteins in sainfoin, sheeps' burnet and lucerne in the rumen. As an adjunct to the experiment, analyses for tannins were also carried out.

The nylon bag technique, alternatively called the in situ or in sacco method (Ørskov & Mehrez, 1977; Ørskov & McDonald, 1979), was employed in the degradation studies.

The radial diffusion technique (Hagerman, 1987) was used for the detection of total tannins and a modified acidified vanillin technique (Terrill et al., 1990) for condensed tannins.

4.2 Degradation of proteins in rumen.

4.2.1 Material.

4.2.1.1 Animals.

Nine Döhne Merino wethers with multiple cannulae in the rumen, abomasum and ileum were used. The rumen cannulae were of the type described in 3.1.2.1.

4.2.1.2 Nylon bags.

The nylon bags were of the same type (manufacturer of material; Rhologan Engineering Box 84158, Greenside 2034, RSA) used by Erasmus et al. (1988). They had an average pore size of 53μm and dimensions 14 cm x 9 cm. The bags were sewn with a double row of stitching with rounded corners to allow easy removal of particulate material. The seams were also sealed with a contact adhesive.

4.2.1.3 Sample preparation.

Cut samples of the forages which were collected during the partial digestion trial of the spring of 1990 were freeze dried for 48 h and milled to pass a 1 mm screen of a Beaver mill.

4.2.1.4 Treatments

The treatments were;

Treatment 1 - Sainfoin

Treatment 2 - Sheeps' burnet

Treatment 3 - Lucerne.

4.2.2 Parameters.

The following variables were under investigation:

4.2.2.1 Nitrogen content (%) of the samples prior to and after the incubation periods.

4.2.2.2 The percentage disappearance of N at each incubation time.

4.2.2.3 pH of the rumen during incubation.

4.2.2.4 The constants a,b and c in the equation $p = a + b(1 - e^{-ct})$ (Ørskov, 1982). (See Section 1.2.4.1.4)

4.2.2.5 The predicted degradation (Ørskov, 1982) of proteins

$$h = a + \frac{bc}{c + k}$$

a,b,c correspond with the definitions above and k is the fractional outflow rate (0,02 and 0,05/hr in this study).

4.2.3 Methods.

4.2.3.1 Trial period.

The cannulated animals employed for the partial digestion trials in 1990 (Chapter 3) were used immediately after the said trials. Thus, the sheep were adapted for at least eleven days on the same pasture material under study. Two successive incubation periods of 24 h were used giving a total trial period of 13 days.

4.2.3.2 Trial implementation.

4.2.3.2.1 Feeding, housing and management of animals.

The sheep were fed with fresh, daily harvested material of the forage being incubated. They were fed enough forage to provide at least 1500 g organic matter/day. They were housed in metabolism cages and supplied a constant and fresh supply of water.

4.2.3.2.2 In situ procedure.

Seven bags containing 5 g each of the dried test forage were incubated in the rumen of each sheep for 1, 2, 4, 6, 8, 12 and 24 h. There were three sheep per treatment and the test was repeated once. The bags were securely tied with nylon twine and for each sheep the seven bags were attached to a further nylon cord (Orskov & Mehrez, 1977) 25cm long and suspended in the rumen. The end of the main line was secured in the rumen and weighed down with a 250 ml plastic bottle filled with an appropriate amount of pebbles (Murphy & Kennely, 1987).

Incubations commenced prior to feeding in the morning (06h00). Bags were sequentially removed after 1, 2, 4, 6, 8, 12 and 24 h and gently washed and squeezed clear under running water until the rinsing water was clear.

They were then put through a cold wash, rinse and spin cycle of an automatic washing machine for 10 minutes. Zero time disappearance values were obtained by washing unincubated bags filled with the same mass of material in a similar fashion. The bags were then dried at 60° C for 48 h. The contents of the bag were analyzed for N by macro kjedahl (AOAC, 1984).

The procedure was repeated immediately after the last bag was removed to give seven values for each incubation period per treatment.

4.2.3.2.3 Monitoring of pH.

Samples of rumen fluid were taken three times daily during the duration of the trials. They were filtered and the pH read immediately with a pH meter.

4.3 Analyses for tannins.

4.3.1 Methods.

Clipped samples of pasture material collected in the course of the study were used. They had been dried at 60° C in a forced draught oven or freeze dried and milled to pass a 1 mm screen of a Beaver mill. They were then subjected to the undermentioned tests.

4.3.1.1 Radial diffusion technique (Hagerman, 1987).

This technique is used for the detection and measurement of total tannin content (both hydrolysable and condensed). The tannin content is essentially determined by reacting the tannin with a protein and quantitating the precipitated complex. Non-tannin phenolics do not interfere with the method nor do water-soluble compounds. The detection limit of the method is 0,025 mg tannic acid or condensed tannin and precision is 6% (relative standard deviation).

4.3.1.2 Modified vanillin HCl technique (Terrill et al., 1990).

This technique is used for the detection and measurement of condensed tannins only. A 7:3 acetone/water solution for tannin extraction and a purified tannin standard was employed for the detection of condensed tannin.

4.4 Results.

The data yielded by the experiment were analysed for replicate and treatment effects using the one-way analysis of variance procedure of the general linear models procedure (Freud & Littel, 1984) and utilising the least square means and a probability level of 5%.

The results for replicates are summarized with the standard deviations (S.D.) for the parameters for the three sheep in each treatment (Table 4.1), whilst the summary for the treatments show the respective standard error of means (SE_m) in Table 4.2. The standard deviations and standard error of means were calculated using the method of Snedecor (1956).

Table 4.1 The influence of replication on protein degradation parameters for the three pastures.

Parameters	Treatments and Replicates					
	Sainfoin		Sheeps' burnet		Lucerne	
	Repl. 1	Repl.2	Repl. 1	Repl.2	Repl. 1	Repl.2
a	1,7[a]	2,3[a]	-8,5[a]	-10,3[a]	31,7[a]	29,7[a]
S.D.	1,15	0,29	2,29	1,15	1,44	1,26
b	82,0[a]	79,0[a]	62,8[a]	58,7[a]	64,3[a]	66,5[a]
S.D.	2,65	4,36	4,04	5,84	1,76	0,87
c	0,086[a]	0,092[a]	0,062[a]	0,092[b]	0,320[b]	0,234[a]
S.D.	0,010	0,010	0,005	0,013	0,002	0,022
h_1*	68,0[a]	67,2[a]	39,0[a]	37,8[a]	92,2[a]	90,9[a]
S.D.	0,91	4,88	4,91	4,41	0,95	1,06
h_2*	53,3[a]	53,5[a]	26,4[a]	27,6[a]	87,3[a]	84,4[a]
S.D.	2,32	4,65	4,58	3,33	0,92	0,70
pH	6,7[b]	6,4[a]	6,1[a]	6,1[a]	6,4[a]	6,6[b]
S.D.	0,10	0,10	0,10	0,06	0,15	0,15

N.B. Values in the same line with different letters within each treatment only, differ significantly ($p \leq 0,05$).

* h_1 and h_2 are effective degradations at fractional outflow rates of 0,02/h and 0,05/h respectively.

Table 4.2 Influence of treatment on protein degradation parameters. Means of two replicates.

Parameters	Treatments			
	Sainfoin	Sheeps' burnet	Lucerne	SE_m
a	$2,0^{b}$	$-9,4^{a}$	$30,7^{c}$	11,9
b	$80,5^{c}$	$60,8^{a}$	$65,4^{b}$	5,95
c	$0,089^{a}$	$0,077^{a}$	$0,277^{b}$	0,06
h_1	$67,6^{b}$	$38,4^{a}$	$91,6^{c}$	15,4
h_2	$53,4^{b}$	$27,0^{a}$	$85,9^{c}$	17,0
pH	$6,6^{b}$	$6,1^{a}$	$6,7^{b}$	0,19

Values in the same line with different superscripts differ significantly ($p \leq 0,05$).

No significant differences between replicates could be detected for a and b on all three pastures (Table 4.1). Though there were significant differences between replicates for the fractional rate constant c for sheeps' burnet and lucerne, they did not have any significant effect on b and the effective degradations h_1 and h_2. There were significant pH differences between replicates for sainfoin and sheeps' burnet. This also, did not have any significant effect on the effective degradations between replicates. Effective degradation was significantly highest in lucerne, followed by sainfoin and sheeps' burnet in that order.

Tables 4.3 and 4.4 portray the results of analyses for total tannins and condensed tannins, respectively.

Table 4.3 Analysis for tannins by the radial diffusion technique.

Period	Sample	Tannin conc. (mg/g)
Late summer to autumn, 1989	Sainfoin : Leaf	11,2
	Stem	ND[1]
	Sheeps' burnet: Leaf	30,9
	Stem	24,3
Winter, 1989	Sainfoin: Whole	10,4
	Sheeps' burnet: Whole	27,7
	Lucerne: Whole	ND[1]
Spring, 1989	Sainfoin : Leaf	10,4
	Stem	ND[1]
	Sheeps' burnet: Leaf	20,0
	Stem	10,4
	Lucerne: Leaf	ND[1]
	Stem	ND[1]
Spring, 1990[2]	Sainfoin: Leaf	16,6
	Stem	7,9
	Inflorescence	18,0
	Sheeps' burnet: Leaf	24,3
	Stem (plus seed head)	52,5
	Lucerne : Leaf	ND[1]
	Stem	ND[1]

[1] - Not detected (ND) in measurable quantities.

[2] - Freeze dried. All other samples oven dried.

Table 4.4 Analysis for condensed tannins by the acidified vanillin technique.

Period	Sample	Tannin conc. (mg/g)
Spring, 1990	Sainfoin: Leaf	5,3
	Stem	3,6
	Inflorescence	13,0
	Sheeps' burnet: Leaf	ND
	Stem	ND

ND - not detected in measurable quantities

The results indicate the presence of condensed tannins in

the leaf, stem and inflorescence of sainfoin. In contrast, the tannins in the leaf and stem of sheeps' burnet were not of the condensed type, but probably hydrolysable. No tannins were detected in lucerne with the methods employed in this study.

CHAPTER 5

DISCUSSION

A common weakness of the experimental design in the yield and plant characteristics as well as the chemical composition, digestibility, voluntary intake and nitrogen utilization trials was the unequal number of treatments in the two phases. This was due to structural and other constraints on the experimental farm.

This fact necessitated the limiting of statistical comparisons of parameters to those within each phase and not over the whole study period. Within each phase of the study however, very useful information on treatment and period effects were obtained.

5.1 Dry matter yield and leaf to stem ratios.

Dry matter yield and leaf to stem ratios for the primary growth (P1) were not reported due to extensive loss of material during hoeing to get rid of weed infestation. Extensive shattering of leaf material during clipping and sorting also occurred due to the state of the pastures at the time.

The yields and leaf to stem ratios in Phase I were therefore

reported for the first aftermath and represented chronological ages of the pastures from 6 - 15 weeks, whereas Phase II represented yields and leaf to stem ratios for the second and third aftermaths with chronological ages of 6 - 15 weeks.

Leaf to stem ratios were expressed as decimal fractions with a ratio of unity representing equal proportions of leaf and stem material. An increase or decrease of the ratio from unity therefore corresponded to increases or decreases of leaf and stem material respectively (for purposes of comparison to references in the literature the proportion of leaf (%) is given in parenthesis).

5.1.1 Dry matter yield.

Even though sheeps' burnet apparently had a higher mean dry matter yield (6,0 t/ha) than sainfoin (5,1 t/ha) in Phase I, the difference was not significant (Table 2.2a). However, in Phase II sheeps' burnet had a significantly higher mean yield (3,9 t/ha) whilst both sainfoin (3,3 t/ha) and sheeps' burnet had significantly higher yields than lucerne (2,8 t/ha) as indicated in Table 2.2 b. There is little published scientific results on sheeps' burnet which makes a comparison with previous work impossible. However, the higher yields on sainfoin compared to lucerne seems to be in agreement with results at Lethbridge, Canada under dryland

conditions reported by Hanna and Smoliak (1968). They reported relative yields of 108% and 100% for sainfoin and lucerne respectively and Carleton et al. (1968) also reported that Eski sainfoin had yielded less, as much or more than lucerne depending upon location and year of production. However, the results also contradict those obtained by the same authors in most other locations and conditions (dryland or irrigation) in Canada where yields of sainfoin tended to be generally lower than those of lucerne. The results also contradict those obtained by Wilman and Asiedu in Aberytswyth, Wales (Wilman & Asiedu, 1983).

The comparative yields of the pastures in this study must however be interpreted with caution as the lucerne plot had been planted earlier and used more intensively in previous experiments. Also it was evaluated during a period when it was probably more sensitive to cold. Nevertheless, the range of DM yields in the study for lucerne (2,7 - 3,1 t/ha) are partially in agreement with results obtained by Wilman and Asiedu (1983) who obtained a mean DM yield of 2,03 t/ha under dryland conditions for three primary growth and four regrowth periods and Joyce et al. (1973) who reported DM yields of 2,5 - 4,6 t/ha for different physiological ages. These yields seem lower compared with those obtained under dryland conditions by Rethman et al. (1986) at Ermelo in the Eastern Highveld region (3,75 - 7,01 t/ha). It must be borne in mind, however, that the yields in this study were reported for only autumn and winter regrowths under irrigation, wheras those of Rethman et al. (1986)

represented total seasonal production under dryland conditions.

In Phase I, the yields of both sainfoin and sheeps' burnet were significantly higher at 12 weeks of age than the other regrowths (Tables 2.3a and 2.4a). The decline in yield of the 15 week regrowths could be due to the fact that necromass was removed from clipped material before weighing.

Whilst there was not much difference in the yields of lucerne during the different stages of growth in Phase II (Table 2.5), the DM yields of both sainfoin and sheeps' burnet increased with age, with the 15 week old pastures being significantly higher in DM yields compared to the 8 week and 6 week winter regrowth and the 8 - 9 week spring regrowth (Tables 2.3b and 2.4b).

Whereas age or maturity might have been a further factor in the different yields the generally lower yields in winter might also encompass a seasonal effect due to low temperatures.

5.1.2 Leaf to stem ratios.

There were significant differences in leaf to stem ratios between the pastures in both phases with the mean leaf to stem ratios for sainfoin and sheeps' burnet in Phase I being 1,6 (61,5%*) and 1.1 (52,4%*) respectively (Table 2.2a), and

* corresponding proportion of leaf(%).

the ratios in Phase II for sainfoin, sheeps' burnet and lucerne being 3,3 (76,7%*), 0,9 (47,4%*) and 1,6 (61,5%*) respectively (Table 2.2b).

The findings for sainfoin and lucerne are consistent with results of Wilman and Asiedu (1983) who reported a consistently higher proportion of green leaf in sainfoin compared to lucerne (a higher proportion of stem in lucerne). They reported values of green leaf as a percentage of dry matter (mean of four growth periods) of 63,2% for lucerne and 79,3% for sainfoin which are close to those obtained in Phase II of this study (Table 2.2b). The mean leaf to stem ratio of sainfoin of 1,6 also falls within the range of 0,52 - 1,98 reported by Baker et al. (1952) for different growth stages of sainfoin. It does seem from this study that with repeated cutting sainfoin acquires a spreading habit with higher proportions of leaf material as noticed in Phase II of this study (Table 2.3b).

Lucerne seems to have a high proportion of green leaf at a young age with the proportion of stem increasing with maturity as evident by the leaf to stem ratios of 3,0 (75,0%*), 1,4 (58,3%*) and 0,7 (41,2%*) at 6 weeks, 8 weeks and 15 weeks respectively (Table 2.5).

* corresponding proportion of leaf (%).

The leaf to stem ratio of sheeps' burnet remained essentially unchanged for both phases (Tables 2.2a and 2.2b) during all periods (Tables 2.4a and 2.4b) except in spring when the plant changes its tuft-like spreading habit into an erect form with a higher proportion of stem which quickly proceeds to the seed head stage. Thus, with the exception of spring, there is about as much leaf material as there is of stem.

5.1.3 Selection of plant parts by sheep.

There was a significant decrease in the mean leaf to stem ratio at the beginning and end of the trial periods in the case of sainfoin and lucerne (Tables 2.7 and 2.9). This suggests a consumption of the sheep of relatively more leaf material compared to stem whereas there were no significant changes in leaf to stem ratios in the case of sheeps' burnet (Table 2.8), implying that the sheep consumed about equal proportions of leaf and stem of this pasture.

The explanation for the observation in sheeps' burnet could lie in its tuft-like growth habit during most periods making selection by sheep of the plant parts difficult. The higher proportion of stem in lucerne compared to sainfoin and the lower digestibility of the stem especially with increasing maturity (Terry & Tilley, 1964) could have resulted in

selective grazing by sheep of the leaf. Wilman and Adiedu (1983) also reported the low attractiveness of the stem of lucerne to sheep.

Although sainfoin stems are much more digestible than those of lucerne of a similar whole plant digestibility (Terry & Tilley, 1964) and is readily eaten as well as the leaf (Wilman & Asiedu, 1983), the sheep in this study tended to consume significantly more leaf material. This observation seems to contradict assertions (Reynolds et al., 1967; Burns et al., 1972) that the presence of condensed tannins (occurring to a greater extent in the leaves of sainfoin than the stem as indicated by analysis of tannins, Chapter 4) reduces palatability. It is possible, however, that the level of tannins in the present study was not high enough to reduce palatability. The high OM intakes obtained on sainfoin apparently confirms this fact. This seems plausible considering the relatively warm conditions and mild winters in the experimental area and the fact that the soils had been well fertilized. Barry and Forss (1983) reported a condensed tannin concentration of 2 - 4% of DM when Lotus pedunculatus was grown in high fertility soils under warm conditions, whilst the concentration increased to 8 - 10% of DM when grown in lower fertility acid soils under cold conditions. The tannin content was, furthermore, reduced by fertilizer addition and was negatively correlated with DM

yield. The results on tannin analysis (Table 4.3 and 4.4) indicate generally low levels of tannin albeit the fact that the leaf generally contained more tannin than the stems.

The figures of the oven dried samples, however must be treated with circumspection as heat treatment (oven drying) has been shown to reduce extractable tannin levels caused probably by polymerization (Terrill et al., 1990). This could also have affected the tannins in sainfoin (condensed) and sheeps' burnet (hydrolysable) differentially due to their different chemical structures.

5.2 Chemical composition of pastures.

Samples were pooled for Ca, P, Mg as well as ADL determinations partly to reduce costs or in the case of ADL due to difficult filtration after 72% sulphuric acid extraction. As a result, no statistical comparisons were done for these parameters.

There were significant differences between the three pastures with respect to CP, ADF and NDF contents in both phases (Tables 2.10a and 2.10b).

Crude protein content was significantly higher in lucerne, followed by sainfoin and sheeps' burnet respectively. Of

interest is the fact that mean CP contents of sainfoin and sheeps' burnet for the two phases coincided i.e. 23,1% and 23,4% for Phases I and II respectively and 18,1% and 17,2% for Phases I and II respectively even though the two phases represented different seasons of the year. However, since the parameters were measured over the same age range it could be argued that CP was not likely to be a limiting factor on animal performance on these pastures during any season of the year. The limited scientific information on sheeps' burnet again makes comparison with other studies impossible.

The mean CP content of sainfoin for the two phases are in close agreement with that reported by Meissner et al. (1989) (24,9%*) in November 1986 at the same experimental site. The mean CP value (25,0%*) was also similar to that reported by the same authors in October 1986. No information however was given in that study on the stage of maturity of the pastures. Wilman and Asiedu (1983) reported mean CP contents of 21,4% and 28,3% for sainfoin aged 1 - 8 weeks which did not differ much from the values obtained in this study. They also determined the CP content of the green leaf and stems and reported a higher content of CP in both the leaf and stem of lucerne than those of sainfoin.

* computed from %N by multiplying by 6,25.

Baker et al. (1952) recorded a range of CP values of 21,5 - 17,4 for different growth stages of sainfoin. The slightly lower values are probably due to the fact that the whole plant was used and not the material selected by fistulated sheep as in this study. The range of CP values (15,1 - 17,7%) with a mean of 16,3% reported by Davis (1968) for 11 varieties of sainfoin leaf from the USSR are much lower than would be expected from this study, as the sheep selected much more leaf material.

Joyce et al. (1973) reported a mean CP value of 25,8% for different growth stages of lucerne which is in close agreement with the results (26,1 %) obtained in this study.

Both sainfoin and sheeps' burnet retained a high CP content in the primary growth despite the advanced stage of maturity. Crude protein contents, however, declined rapidly with maturity in both pastures in Phase I (Tables 2.11a and 2.12a) as was reported by Wilman and Asiedu (1983) for sainfoin. Lucerne maintained a high CP content even in winter for the 6 - 8 week regrowths but the 15 week regrowth had a markedly lower CP content (Table 2.13) as would be expected (Wilman & Asiedu, 1983). In all pastures there was a markedly higher CP content in the spring regrowths (Table 2.11b, 2.12b and 2.13) which in all probability was due to a seasonal effect.

As noted there were significant differences between the three pastures in NDF and ADF contents in both phases (Tables 2.10a and 2.10b) with sainfoin having the highest mean ADF and NDF contents, followed by lucerne and sheeps' burnet, in that order.

Sainfoin had a significantly higher cellulose content than sheeps' burnet in both phases but did not differ significantly from lucerne. Sheeps' burnet had a significantly higher hemicellulose content than sainfoin in Phase I but did not differ significantly from the other pastures in Phase II. Even though no statistical comparisons were done on the ADL fraction, sainfoin obviously had a much higher ADL content than sheeps' burnet and lucerne.

The higher ADF and NDF contents of sainfoin therefore seems to result mainly from the lignin fraction. The results seem to agree with the report by Woodman in 1948, cited by Baker et al. (1952), that sainfoin is a crop that readily becomes fibrous.

Wilman and Asiedu (1983), in contrast, reported lignin contents at 8 weeks of age of 12,1% and 11,1% and cell wall contents of 29,8% and 37,6% for sainfoin and lucerne respectively. Though the method of lignin determination was not stated the lignin and higher cell wall value of lucerne compared to sainfoin seem to contradict the results of this

study. However, it could also be a pointer that there might be notable variations in cultivar and type differences or even in the environment in which the pastures are grown.

The range of lignin values (4,25% - 6,50%) reported by Joyce et al. (1973) for lucerne at different physiological growth stages however, are in agreement with the results obtained in this study.

As shown in Tables 2.11a and 2.11b the spring growths (primary growth and third regrowth) of sainfoin had significantly lower ADF contents in both phases whilst NDF was also significantly lower in the spring regrowth in Phase II. Hemicellulose was significantly higher in the primary growth. Otherwise ADF, NDF, cellulose and hemicellulose remained essentially the same in all periods. The lower ADF and NDF values in the spring regrowths are most likely the consequence of lower lignification.

The NDF values of sainfoin were in close agreement with the value of 50,9% obtained by Meissner et al. (1989) in November 1986 and June 1987 at the same experimental station. The authors also suggested that the constancy of the cell wall during seasons could be due to the fact that tannins or other chemicals stabilize the cell wall partially, since the cell wall would normally increase as the season progresses.

A similar trend occurred in sheeps' burnet (Tables 2.12a and 2.12b), except that NDF content was significantly higher in the spring regrowth. This could probably be related to the proportion of stem material during spring (Table 2.4b).

The constancy of the cell wall could also be due to the stabilization of the cell wall by tannins or other chemical constituents as suggested by Meissner et al. (1989) as sheeps' burnet has been shown in this study (Table 4.3 and 4.4) to contain tannins, albeit probably hydrolysable.

The cell wall components of lucerne remained reasonably constant for 6 and 8 week winter and 8 - 9 week spring regrowths (Table 2.13). The significant increase in cell wall components for the 15 week regrowth was as would be generally expected due to its higher maturity, with consequent increase in stem fraction.

The percentage of ADIN in the OM was significantly higher in sainfoin than in sheeps' burnet and lucerne which did not differ significantly (Tables 2.10a and 2.10b). Taking the percentage of total N (CP) of the pasture into account however, the results indicate a higher proportion of insoluble N in sheeps' burnet compared to lucerne.

Sheeps' burnet had significantly higher total ash contents than both sainfoin and lucerne, which did not differ significantly from each other. This seems to confirm reports

in New Zealand farmer manuals on observations by farmers of the mineral status of the herb. Fair (1989) noted claims by European literature as to the richness of sheeps' burnet in minerals especially iron and its health promoting properties.

Even though there were no statistical comparisons on Ca, P and Mg contents due to reasons already given, the following trends were discernible (Tables 2.10a and 2.10b);

(i) Ca content was considerably higher in lucerne followed by sainfoin and sheeps' burnet in that order.

(ii) P was highest in sainfoin followed by sheeps' burnet and lucerne in that order. Ca:P ratio was more favourable in sainfoin, followed by sheeps' burnet and lucerne in that order.

(iii) Mg was considerably higher in sheeps' burnet followed by lucerne and sainfoin in that order.

Ca and Mg contents appeared to increase with maturity in sainfoin and sheeps' burnet but P seemed to decrease with maturity (Tables 2.11a and 2.12b). McDowell et al. (1983) reported the following values (converted from g.kg to %) as the requirement of Ca, P and Mg in feeds for grazing animals:

Ca (%)	0,18 - 0,60
P (%)	0,18 - 0,43
Mg (%)	0,04 - 0,18

Thus the contents of Ca, P and Mg in all three pastures, even in winter, fulfil or surpass the requirements and unless there are problems of metabolism of the minerals by animals, deficiencies of these essential nutrients are not likely to occur. As shown in Tables 2.10a and 2.10b there was a marked difference between the Ca:P ratio of lucerne on one hand and sainfoin and sheeps' burnet on the other. There is, however, evidence that strongly indicates that ruminants can tolerate wide Ca:P ratios (Wise et al., 1963). The Ca:P ratio may therefore not be an important factor in the relative nutritive values of the three forages.

Baker et al. (1952) reported a mean ash content of 6,4%* for sainfoin at different growth stages which appears low compared to the results of this study (10,0% and 10,5% respectively for Phases I and II). However, they used clipped samples with a lower risk of soil contamination, and no salivary contamination as with the oesophageal fistulate used in this study. They also reported mean Ca and P contents of 2,1% and 0,6%* which were much closer to that obtained in this study. Their observation that the level of Ca in sainfoin is not as high as lucerne was confirmed in this study. The mean ash content of lucerne of 12,5% reported by Joyce et al. (1973) is also close to the value of 10,7% obtained in this study.

* Computed from published results.

5.3 Digestibility and calculations of voluntary intake of organic matter.

An in vitro vs. in vivo study conducted in the autumn of 1989 in metabolism cages with freshly cut pasture, yielded the results in Table 2.14. Since the in vitro and in vivo digestibilities fitted in the prediction equation

% in vivo DOM = 0,746 IVDOM + 18,16

all in vitro DOM figures obtained for the two pastures in all periods were adjusted to in vivo DOM and used in the voluntary intake determinations.

The IVDOM of sainfoin was, however, lower than the in vivo digestibility by about 10 percentage units and therefore the adjustment of IVDOM to in vivo DOM was done by simple proportion ie.

% in vivo DOM = % IVDOM x 1,17*

and the in vivo DOM values used for the voluntary intake determination.

There is evidence to suggest that tannins (as found in sainfoin) have inhibitory effects on various enzymes in vitro, including some rumen enzymes (McLeod, 1974; Kumar & Singh, 1984) and microbes. McLeod (1974) cited Gustavson 1956), Pridham (1960) and Feeny (1969) about the fact that

* Ratio of in vivo to in vitro digestibility of OM (Table 2.14).

condensed tannins have a greater inhibitory effect on the activity of both enzymes and microorganisms than hydrolysable tannins and phenols of low molecular weight. This fact could explain the different relationships observed between the in vitro and in vivo DOM with respect to sainfoin and sheeps' burnet. The presence of condensed tannins in sainfoin is widely quoted in the literature and was confirmed in the tannin analysis in this study, whilst sheeps' burnet has been shown to probably contain hydrolysable tannins (Tables 4.3 and 4.4).

A further justification for the manner of conversion of IVDOM to in vivo DOM, particularly for sainfoin, lies in work done by Cope and Burns (1971), Donelly and Anthony (1969) and Donelly and Anthony (1970). Cope and Burns (1971) reported that mean in vitro digestibilities of dry matter for Lespedeza cuneata (sericea) containing condensed tannins were about half as high as those found by Donelly and Anthony (1970) with the intra-ruminal nylon bag method (in vivo). They suggested that the different mean values for the two studies possibly indicated a more complete in vivo digestion and that the rumen fermentation process may be capable of overcoming some of the initial effects of the tannin. This seems plausible when one considers a static batch in vitro system as the Tilley and Terry method (Tilley & Terry, 1963) used in this study and a continuous culture system like the rumen with the possibility of renewal and adaptation. Barry (1984) reported that sheep adapt to high tannin concentrations to some degree.

However, the use of a single proportion and a single prediction equation might still lead to some error since Donelly and Anthony (1970) suggested that there might be tannin threshold levels above which digestibility does not vary in proportion to tannin. Nevertheless, it was anticipated in this study, that the errors that would probably arise from such an adjustment would be relatively small compared to those that would result in determining voluntary intake from IVDOM for sainfoin.

5.3.1 Digestibility and voluntary intake on experimental pastures.

Sheeps' burnet had significantly higher IVDOM and in vivo DOM than sainfoin in Phase I (Table 2.15a) whereas lucerne had the highest IVDOM and in vivo DOM, followed by sheeps' burnet and sainfoin in that order in Phase II, with the differences between the three pastures being significant (Table 2.15b).

The difference between the 6 week regrowth and the 15 week regrowth of sainfoin in Phase I was almost insignificant (P value of 0,0424) and could simply have been due to selection of material by the different oesophageally fistulated sheep used in the two periods (Table 2.16a). Even though the spring regrowth of sainfoin in Phase II had a significantly higher IVDOM and in vivo DOM (Table 2.16b), there was no evidence of any dramatic change in OM digestibility with

maturity or season. The results seem to reinforce the observation by Meissner et al. (1989) of the little variation in the digestibility of sainfoin as the season progressed, and their speculation of probable stabilization of the cell wall by tannins or other chemical constituents.

A similar trend was noticed with sheeps' burnet, probably due to a similar reason as suggested by the authors, since sheeps' burnet has been shown to contain tannins in this study.

Since lucerne was studied only in Phase II its behavior over a relatively brief time span was noted. There were no significant differences between the 6 week and 8 week winter regrowth of lucerne. However, both had significantly higher IVDOM and in vivo DOM than the 15 week regrowth which could be partially ascribed to the high proportion of stem material, high total cell wall and lignin content and consequent lower digestibility. The significantly higher digestibility in the 8 - 9 week spring regrowth is with all probability a seasonal effect.

There were no significant differences in OM intake (OMI) (absolute or relative to metabolic livemass) between sheep on sainfoin and sheeps' burnet in Phase I (Table 2.19a). In Phase II there were no significant differences between the three pastures in OMI (g/day) (Table 2.19b). However, there was a significantly higher intake of OM per metabolic

livemass of sheep on sainfoin compared to lucerne. The differences in intake between sheep on sainfoin and sheeps' burnet and sheeps' burnet and lucerne were not significant.

There was a highly significant interaction (P value 0,005) between period and treatment in Phase II. This was due to the fact that during the trial in spring (P8) the animals were kept on the pastures (24 hours a day) during the duration of the trial, unlike in the previous trials when they were taken to the pastures at 06h00 and returned at 18h00 to the barn. There was, therefore, a dramatic increase in intake during P8 which resulted in the interaction.

There were no significant differences in intake on sainfoin during different periods in Phase I (Table 2.20a) whereas in Phase II (Table 2.20b) there was a highly significant difference in OMI on the spring regrowth (P8) compared to the preceding periods. The extent to which the higher digestibility in spring (a seasonal effect) contributed to the higher intake or the prolonged grazing time is a factor, cannot be assessed as a result of the interaction between period and treatment. However, the very high intake obtained during this period for sainfoin as well as the other two pastures is a reflection of the potential of the pastures in livestock production. This observation is very important for the practical management of pastures, as many farmers tend to pen animals at night. This practice would therefore not assure optimum intake.

A similar trend in intake occurred in sheep on sheeps' burnet (Tables 2.21a and 2.21b).

An interesting fact noticed on lucerne was the progressive increase in OMI as the crop matures and the significantly higher intake on the 15 week regrowth compared to the 6 week regrowth (Table 2.22), despite the higher quality (higher CP, significantly lower cell wall components and digestibility) of the younger pasture. It is possible that differences in solubility in the rumen and its contribution to gas production (bloat) in the rumen or availability of amino acids postruminally (Egan, 1965) could have been a contributory factor due to the higher intake recorded on the mature (15 week) crop.

Table 5.1 indicates digestibility and OMI figures obtained by several authors on sainfoin and lucerne. As with other nutritive value indices there are virtually no references to sheeps' burnet in the literature.

Table 5.1 Digestibility and voluntary intake of OM on sainfoin and lucerne reported in the literature.

Pasture	In Vitro		OMI	Author
	DDM[a]	DOM	g/LW^{0,75}/d	
O. viciifolia				
November		49,9	45,2	Meissner et al. (1989)
		58,4*		
June		49,5	45,3	"
		57,9*		
O. viciifolia				
var. Cotswold common	70			Terry & Tilley (1964)
Giant	71			"
Local Russian	72			"
Turkish Anatolian	74			"
O. viciifolia	63,2			Wilman & Asiedu (1983)
M. sativa				
October		68,7	29.8	Meissner et al. (1989)
November		64,3	33,6	"
December		63,6	21,5	"
January		59,2	28,6	"
M. sativa	75,0			Terry & Tilley (1964)
M. sativa	67,1			Wilman & Asiedu (1983)
M. sativa		69,2*		Joyce et al. (1973)
M. sativa			42,0	Corbett & Pickering (1979)
M. sativa			50,0	Corbett (1979)
M. sativa			70,0***	Cruikshank (1985)

* Values that would be obtained if IVDOM figures are adjusted by the in vitro vs. in vivo relationship used in this study.
** Mean intake for different maturity stages computed from published results.
*** Estimated from OMI (g/LM/d) by Meissner et al. (1989).

a - DDM - Digestibility of dry matter

Whereas the IVDOM figures reported by Meissner et al. (1989) at the same experimental station are close to those obtained in this study (52,4% and 49,5% respectively for Phases I and II) the OMI figures are considerably lower than those in this study (61,3 g/kg $W^{0,75}$/day and 66,4 g/kg $W^{0,75}$/day for Phases I and II respectively).

This is due to the fact that they computed intake from IVDOM instead of in vivo DOM. As a result they did not consider the depression of digestibility in vitro by condensed tannins as reported in the literature (Cope & Burns, 1971; Donelly & Anthony, 1969; Donelly & Anthony, 1970) and confirmed in this study. An adjustment of their IVDOM figures would give results that are closer to those obtained in this study.

Though the digestibility figures by Terry and Tilley (1964) and Wilman and Asiedu (1983) are expressed on dry matter basis, it serves to confirm the fact that whole lucerne has a higher digestibility than sainfoin of the same maturity.

The mean digestibility of 69,2% for lucerne reported by Joyce et al. (1973) agrees fairly well with the results of this study. The IVDOM figures reported by Meissner et al. (1989) (59,2% - 68,7%) for lucerne are also close to those obtained in this study. Their mean intake figures(21,5 - 33,6 g/$LM^{0,75}$/day) are closer to those obtained in this study when sheep were grazed for 12 hours on immature

lucerne (as was the grazing time in their study).

The high intakes on the 8 - 9 week spring regrowth of lucerne of 87,6 g/$LM^{0,75}$/day (Table 2.22), 105,0 g/$LM^{0,75}$/day on sainfoin (Table 2.20b) and 79,6 g/$LM^{0,75}$/day on sheeps' burnet (Table 2.21b) reflect the potential of the pastures and the inadequacy of truncated grazing periods in intake trials and in general management of sheep flocks on intensive pastures.

The intake figures reported by Corbett and Pickering (1979) and Corbett (1979) are much closer to the intake figures obtained in this study for the 8 week winter regrowth (12 hours grazing time) (Table 2.22) whilst the estimated OMI of 70g/$LM^{0,75}$/day of Cruikshank (1985) confirms the potential intake possible on lucerne.

The results of this study also confirms the report of Hanna and Smoliak (1968) on relative intakes of 108% and 100% for sainfoin and lucerne respectively. The results also are in agreement with those reported by Meissner et al. (1989) on the higher intakes of sainfoin compared to lucerne (Table 2.23).

5.4 pH and VFA production in the rumen, N flow and utilization.

The results of the above experiment are shown in Table 3.1a through to 3.8. Very minor and negligible discrepancies may exist between the treatment means when calculated from the tables for the period means due to the rounding off of figures to 1 or 2 decimal places.

The variation especially in N utilization by individual sheep (as indicated by the coefficients of variation of NAN disappearance), especially with the animals grazing on pasture (P3 and P4), suggests that it would have been more appropriate to use more experimental animals in order to reduce the errors caused by variation between animals. Within this limitation, however, useful information on differences in the utilization of N for these forages was obtained.

5.4.1 pH and VFA production.

There were no significant differences in rumen pH between sheep on sainfoin and sheeps' burnet in Phase I (Table 3.1a). Even though there were differences between the rumen pH of sheep on sainfoin and lucerne in Phase II as indicated in Table 3.1b, the pH differences were not so wide as to probably influence rumen microbial composition and

subsequent fermentation. There were also generally no significant period differences in rumen pH on the pastures with the pH values falling between 6 - 7, which is the considered range for a normal forage fed rumen (Van Soest, 1982). Sheep fed on sainfoin, however, had significantly lower pH values of 5,9 and 5,7 on the relatively younger pastures (Tables 3.2a and 3.2b).

It has long been recognized that VFA production in the digestive tract of the ruminant represents an important source of energy and among other factors, is diet dependent (Stewart et al., 1958). In a forage fed animal particularly, VFA production is a major function of energy and has been related to the intake of digestible OM (Langlands, 1975). There were no significant differences in total VFA production between sheep on the pastures in both phases as indicated in Tables 3.1a and 3.1b. Sheep on lucerne tended to have a higher VFA production compared to those on the other pastures, due probably to the higher digestibility of its OM. The significantly higher total VFA production during P9 in Phase II on all pastures (Tables 3.2b, 3.3b and 3.4) was due to the dramatic increase in intake of OM.

The principal VFA's occurred in their normal order of abundance: acetic, propionic, butyric and valeric (Van Soest, 1982). There were no significant treatment differences with respect to the proportions of the volatile

fatty acids and acetate to propionate ratio in the rumen.

The proportions also stayed reasonably constant in all periods of the study despite the different intake levels and total VFA concentrations. This coupled with the high total VFA production (Bath & Rook, 1965) obtained in the rumen, especially at the high intake level in Phase II, is an indication that energy production is not likely to be a limiting factor to animal production on these forages, since a significant linear relationship has been found to exist between total VFA production and metabolizable energy intake (Griffiths & Bath, 1973).

5.4.2 Nitrogen flow and utilization in the small intestine.

There were significant differences in rumen NH_3 production by sheep on the three pastures (Tables 3.5a and 3.5b). Sheeps' burnet had the lowest (4,2 and 6,6 mg/100 ml in Phases I and II respectively), sainfoin 9,3 and 25,3 mg/100 ml in Phases I and II respectively and lucerne 65,3 mg/100 ml (Phase II only). Whereas suggestions have been made that mean rumen NH_3 concentrations below 5 mg/100 ml (Satter & Roffler, 1977) (as found in sheep on sheeps' burnet in Phase I) could be limiting for optimal microbial protein production with consequent effects on forage fiber digestibility, the comparatively high digestibility of OM and similar VFA production levels on sheeps' burnet appear

to negate this view. The similar flows of N into the abomasum in sheep on sainfoin and sheeps' burnet at similar intakes in Phase I and significantly different intakes in Phase II seem to be in agreement with the scenario painted by Satter and Roffler (1977). They postulated that it does not make much difference whether true dietary protein is degraded in the rumen (provided NH_3 production does not exceed the ability of rumen bacteria to convert the NH_3 to microbial protein) as long as the rumen bacteria are able to utilize all the ammonia produced, since in either way, dietary or recycled nitrogen ultimately ends up as protein presented to the intestine for absorption. This would imply that the protein in sheeps' burnet is more effectively by-passed in the rumen compared to that of sainfoin. This conclusion would agree with the report by Losada et al., as quoted by Paulsmeier (1987), that the apparently efficient conversion of dietary N to duodenal N is associated with relatively low rumen NH_3 concentrations (4 - 10 mg NH_3-N/100 ml rumen liquor). The scenario would also explain the low availability of N in the abomasum of sheep on lucerne and subsequently low disappearance of NAN. The high concentrations of NH_3 in the rumen resulting from degradation of dietary protein, results in the flow into the abomasum of only the dietary true protein that escapes ruminal degradation and the portion of degraded protein or NPN that is utilized by rumen microbes. The excess NH_3 is absorbed from the reticulorumen or passes to the lower gut

where it is absorbed and is eventually converted to urea and the greater part excreted (Satter & Roffler, 1977). This would constitute a serious tax on the energy household of the animal. The phenomenon seems to explain the high NH_3-N flows in the abomasum especially at the high intake level (P9) of sheep on lucerne (Table 3.8).

The significantly higher levels of rumen NH_3 in the sheep on sainfoin in P9 (Table 3.6b) and higher though insignificant level in sheep on sheeps' burnet (Table 3.7b) are most likely due to the higher nitrogen intakes (Adams & Kartchner, 1984). The significantly lower rumen NH_3 levels in sheep on lucerne in P9 compared to P8 despite the higher N intake by sheep (Table 3.8) is explained by the very high flows of NH_3-N in the abomasum. The phenomenon responsible for this occurrence needs to be investigated.

The disappearance of NAN in the small intestine was significantly different on all pastures for both phases in the order sainfoin, sheeps' burnet and lucerne (Tables 3.5a and 3.5b). Whereas it appears that the protein in sheeps' burnet is more efficiently passed undegraded into the abomasum than sainfoin, there was a significantly lower digestibility of its NAN due to the flow of a higher proportion of NAN through the ileum into the faeces. This could be due to the recomplexing of protein by undegraded tannin in the intestine as speculated by McLeod (1974). He also advocated the examination of a wider range of

hydrolysable tannins before any definite conclusions could be reached regarding the advantages of tannin protected proteins.

There were no significant differences in NAN digestibility of sainfoin and lucerne. Lucerne also had a significantly higher digestibility compared to sheeps' burnet.

There were no significant period differences in NAN disappearance, in all pastures in both phases (Tables 3.6a to 3.8). Even though the values of NAN digestibility differed, especially for the animals on pasture (P3 and P4), the differences were not statistically significant as shown in Table 3.6a through to Table 3.8. Although little work has been done on sainfoin and sheeps' burnet the results are in general agreement with the high availability of N postruminally of sainfoin (Shedrick & Thomson, 1982) and the consequent higher livemass gains and animal production associated with it (Barry, 1984).

The results are also in agreement with the work of Corbett and Pickering (1979) who reported post-ruminal NAN digestibilities of 70% and 69% (72,6% in this study) in sheep grazing lucerne in February - April and October - December respectively, and fairly in agreement with Corbett (1979) who reported NAN disappearance as a fraction of N

intake of 56,7% and 57,5% for lucerne grazed in the same periods. A mean value of 49,4% was obtained in this study with a range of 45% to 55,7% in individual sheep in the two periods studied.

5.5 Protein degradation in the rumen and analysis of tannins.

The results of the protein degradation trial using the in sacco method are shown in Tables 4.1 and 4.2. Freeze dried samples had been used for the trial as well as the subsequent condensed tannin analysis, because of the effect of heat treatment on the solubility of feed proteins (Sherrod & Tillman, 1962) and heat preservation on tannin extraction (Ørskov, 1982). The sheep were also fed fresh pasture material in line with the recommendation by Ørskov (1982) that the diet given to the animals fitted with nylon bags, must be similar to the diets for which the results are to be applied.

As shown in Table 4.1 there were significant differences between replicates for the fractional rate constant c for sheeps' burnet and lucerne. However this did not have any significant effects on b and the effective degradation calculated at fractional outflow rates of 0,02/h (h_1) and 0,05/h (h_2). There were also significant rumen pH differences between replicates for sheep on sainfoin and

sheeps' burnet. Table 4.2 shows the means of the two replicates for each treatment and the treatment differences with respect to the degradation parameters.

There were significant differences between all pastures with respect to a, b, c and the effective degradation whilst the rumen pH did not differ between sheep on sainfoin and lucerne. However, the rumen pH for sheep on both pastures were significantly higher than those on sheeps' burnet. Nevertheless, the rumen pH differences between replicates or treatments were not marked enough to probably produce different rumen environments which would support different rates of cellulose degradation; a factor that has been shown to influence rates at which proteins, especially of vegetable origin, degrade (Ørskov, 1982).

The positive a (soluble fraction) values for lucerne and sainfoin implies that there is a soluble fraction which rapidly disappears and an insoluble fraction which starts to disappear almost immediately. There is also a fraction which does not degrade. The negative value for sheeps' burnet implies that there is no fraction which disappears immediately and there is a lag phase for the insoluble fraction. There is also a fraction which does not disappear (Ørskov, 1982). The relative magnitudes of a for sainfoin (2%) and lucerne (30,7%) however indicate a much higher soluble fraction in lucerne, which is assumed to be

instantly degradable. Lucerne also exhibits a faster rate (0,277) at which the fraction b (60,8%) will degrade and the consequent higher effective degradations of 91,6% and 85,9% at the fractional outflow rates of 0,02/h (h_1) and 0,05/h (h_2), respectively. Whereas sainfoin and sheeps' burnet will degrade at almost similar rates, effective degradation in sainfoin (67,6% and 53,4% respectively) at the two outflow rates is higher, because of the negative value of a in sheeps' burnet.

Although no comparative work was found in the literature, degradations of 93% and 82% respectively, were detected for the leaf and stem of the legume clover (Nordkvist et al., 1987), which is quite similar to lucerne. The results of the present trail do offer a valid explanation for the significant differences in rumen NH_3 levels and the consequent postruminal flows and disappearance of NAN. The high rumen NH_3 levels in lucerne are apparently due to a higher rate and extent of protein degradation. Because of loss of NH_3 through the reticulo-ruminal wall or lower digestive tract and the subsequent excretion thereof, a serious tax on protein metabolism occurs on lucerne resulting in a lower flow and disappearance of true protein in the small intestine. Whereas sheeps' burnet appears more efficient at providing undegraded protein in the abomasum due to its significantly lower degradation into NH_3 in the rumen compared to sainfoin, there is nevertheless a lower

disappearance of NAN due to passage of a higher proportion of N into the faeces.

The analyses for tannins were undertaken only to provide an indication of the presence or absence of tannins. No statistical analyses were done since the samples had undergone different preservation regimes and were mostly single samples. However, it can be deducted from Table 4.3 that the total tannin content is much higher in sheeps' burnet than in sainfoin. The total tannin content was measured by the radial diffusion technique (Hagerman, 1987). Tannins were not detected for lucerne samples and therefore they were not included in the subsequent analysis for condensed tannins.

The results of the two procedures indicate the presence of condensed tannins in both the leaf and stem of sainfoin (Osbourn et al, 1966; Barry, 1984) with higher levels in leaf material and probably hydrolysable tannins in both the leaf and stem of sheeps' burnet with probably higher levels in the leaf and seed head.

The presence of tannins confirms the higher postruminal flows and availability of NAN in sainfoin and sheeps' burnet due to protection of protein from degradation in the rumen (McLeod, 1974; Barry, 1984). The different rates and extent of degradation and availability of NAN postruminally may be

due to differences in tannin levels in the two forages, different effects of the two types of tannins on proteolytic enzymes or different mechanisms for tannin binding and release in the digestive tract.

Tannins furthermore have been shown to inhibit foam production (Kendall, 1966), which has been associated with the incidence of bloat in ruminants. This may account for the bloat free properties of sainfoin reported in the literature (Barry, 1984) and the reputation of sheeps' burnet as a bloat free pasture among New Zealand farmers, as reported in farmer bulletins.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS.

The aim of this study was to determine the nutritive value of sainfoin (Onobrychis viciifolia) and sheeps' burnet (Sanguisorba minor). Lucerne (Medicago sativa) was used as a control. The nutritive value indices employed were as follows: DM yield, leaf to stem ratios and selection of plant parts by sheep, chemical composition of pasture material, digestibility and voluntary intake of OM of the forages, post-ruminal disappearance of NAN and degradation of the forage proteins in the rumen of sheep.

The study was conducted in two phases with Phase I covering mid-summer to mid-autumn and with only sainfoin and sheeps' burnet as treatments. Phase II covered late autumn to early summer with lucerne included as a treatment.

Each phase comprised four periods with the pastures cut in a way as to yield portions with different chronological ages.

Dry matter yields were obtained by clipping samples of pasture during each period of the study using quadrats and wool shears. Leaf:stem ratios were measured by separating a weighed sample of clipped material into leaf and stem and expressing their masses as a ratio. An indication of the selection of plant parts by sheep was obtained by measuring leaf:stem ratios inside and outside exclusion cages.

Extrusa from oesophageal fistulae of sheep were collected during the different periods and used for the determination of OM, CP, ADF, NDF, Cellulose, Hemicellulose, Lignin, ADIN and Ash. Clipped samples were used for the determination of Ca, P and Mg because of salivary contamination of material from oesophageal fistulae.

The dried extrusa from the oesophageal fistulae were also used for the determination of IVDOM of the pastures during the different periods of the study. The IVDOM values were corrected to in vivo DOM values using the relationships obtained in an in vitro vs. in vivo study. Organic matter intake by sheep on the pastures was calculated using the calculated in vivo values and faecal output of intact rams.

Multiple cannulated sheep were used to measure the flow and disappearance of NAN in the small intestine. Chromium-EDTA and Yb-acetate were infused as liquid phase and particulate phase marker respectively to determine the flow of digesta in the digestive tract. Rumen NH_3 and VFA production were also measured.

An explanation for the relative flows and disappearance of NAN was then sought in terms of the degradation of forage proteins in the rumen (nylon bag technique employed) and the occurrence of tannins.

Sheep used in the study were randomly allocated to treatments. Data were analysed using the analysis of variance procedure in the General Linear Models programme, using the least square means and a probability level of 5%.

From the results of the study the following conclusions and recommendations were reached:

1) Dry matter yields were generally higher on sheeps' burnet than on sainfoin whilst both sainfoin and sheeps' burnet produced higher yields than lucerne. Although there is evidence in the literature (Hanna & Smoliak, 1968; Carleton et al., 1968) to support the higher yields of sainfoin compared to lucerne, the results in this study must be treated with circumspection since the lucerne pasture had been more intensively utilized prior to the study. Lucerne was also evaluated during the period where it was probably more sensitive to cold. Furthermore, the problem of the persistence of sainfoin mentioned in the literature (Sheehy & Popple, 1981; Wilman & Asiedu, 1983) needs to be investigated under local conditions and if confirmed, a selection and breeding program to obtain cultivars with more vigorous regrowth (Varga, 1968) initiated.

   Both sainfoin and lucerne had higher proportions of leaf material compared to sheeps' burnet which had about equal proportions of leaf and stem. The proportion of leaf

in sainfoin increased with frequent cutting. The proportion of leaf in lucerne declined with maturity.

Sheep consumed considerably more leaf than stem material in sainfoin and lucerne and about equal proportions of leaf and stem in sheeps' burnet. The selection of more leaf material by sheep on sainfoin also diminishes the notion of unpalatability (at least at the levels present in sainfoin in this study) attributed to condensed tannins which has been shown to be higher in leaf material.

2) Chemical composition.

All three forages contained adequate amounts of CP even at mature stages of growth or in winter. Crude protein content was highest in lucerne, followed by sainfoin and sheeps' burnet in that order and declined with maturity.

Mineral (Ca, P, Mg) contents in all three forages exceeded normal requirements by grazing animals. The differences in the Ca:P ratio may not be an important factor influencing the nutritive value since there is evidence that strongly suggests that wide Ca:P ratios are tolerated by the ruminant animal.

Higher fibre contents occurred in sainfoin compared to the others and was typified by high ADF and NDF contents.

This was due largely to the high lignin contents and did not vary much with season as reported by Meissner et al. (1989) or maturity. However this apparently did not hamper intake. Evidence from the literature (Wilman & Asiedu, 1983) suggests the availability of cultivars/ecotypes with lower fibre and lignin contents; a fact that must be borne in mind in any improvement program.

3) The digestibility of OM was highest in lucerne, followed by sheeps' burnet and sainfoin, in that order. The digestibility of sainfoin did not vary much as the season progressed as previously reported (Meissner et al., 1989). Sheeps' burnet behaved similarly to a fair degree. The digestibility of lucerne, however, declined markedly at very mature stages of growth. The results of the in vitro vs. in vivo study revealed that in future studies with sainfoin or other condensed tannin containing legumes, a relationship should be established between in vitro and in vivo digestibilities in order to accurately determine intake. This should be related to tannin content and if possible tannin threshold levels established.

Organic matter intake by sheep on sainfoin was markedly higher than on lucerne and could be similar to or higher than on sheeps' burnet. Organic matter intake in sheeps' burnet was generally higher than on lucerne. Like

digestibility, OM intake on sainfoin and sheeps' burnet did not vary much as the season progressed. Organic matter intake was considerably lower in young lucerne and increased with maturity.

The results of this study make it pertinent to graze animals for 24 hours/day (instead of the 12 hours/day used in part of this study and previous studies at the same research facility) due to the highly significant changes in intake for all three forages noted after changing the grazing time. Furthermore, the extended grazing time is what one would expect under practical livestock management regimes.

4) The different rates and extent of degradation of proteins in the rumen and the consequent differences in NAN availability, clearly portrayed the inadequacy of the use of CP content as the sole index of N availability and pasture quality. Non-ammonia nitrogen availability was markedly different with sainfoin having the highest availability followed by sheeps' burnet and lucerne, in that order. The availability of amino acids postruminally could also account partially for the intake differences on these pastures (Egan, 1965). Though degradation was lowest in sheeps' burnet (lucerne had the highest), there was a lower availability of its NAN compared to sainfoin due to a lower digestibility of NAN. The results also confirmed the presence of condensed tannins and revealed

the occurrence of maybe hydrolysable tannins in sheeps' burnet which accounted for the relatively lower degradation in the rumen.

It is recommended that in future studies with very slowly degradable feedstuffs or forages like sheeps' burnet the period of incubation in the nylon bag studies must be extended to at least 48 hours. Although crude protein degradation in this study appeared to have peaked after 24 hours incubation and no problems were therefore encountered in the derivation of the asymptotes for the calculation of proportion of N disappearing (Ørskov, 1982), a better picture might be obtained using longer incubation periods.

5) The common practice of growing sainfoin in conjunction with sheeps' burnet (Fair, 1989) might be justifiable when dry matter yields are used as a criterion as noticed in this study. However, the results of this study also questions the rationale behind the mixing since the two forages are similar in the manner in which they are utilized by the grazing animal, especially with respect to N intake and utilization.

It is recommended therefore that either sainfoin or sheeps' burnet be mixed with other forages with highly soluble protein fractions. Palatability evaluations, however, must form an important part of investigations into such mixtures.

6) The generally low intake, very high degradation of protein in the rumen and consequent low proportion of ingested N absorbed postruminally found in sheep fed lucerne, are serious problems that need to be addressed. Research efforts should be geared towards:

   (i) Grazing different cultivars at different maturity stages to find out the effect on these indices of nutritive value.

   (ii) The possibility of incorporating into lucerne the gene responsible for condensed tannin production in sainfoin by genetic engineering. This would increase amino acid supply to the lower digestive tract without adversely affecting carbohydrate digestion in the rumen (Barry, 1984).

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