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.