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