

Chapter 5 Nitrogen Dynamics in the Rumen of Sheep Determined with ¹⁵N-Labelled Ammonia or ¹⁵N-Labelled Duckweed

5.1 General

The major transactions of N substances in the rumen fluid, *i.e.* the degradation of crude protein in duckweed and cottonseed meal and the subsequent utilisation of the degradation products for microbial protein synthesis, have been studied *in vitro* and reported in Chapter 4. In the rumen, however, the N transactions are more complex than in the test-tube, involving many routes of transaction and also absorption across the gut wall and outflow to the intestines: thus, they are influenced by many factors that do not affect *in vitro* experiments. For example, the degradation of feed N estimated could be over-estimated in longer-term incubations *in vitro* because the outflow of the feed from the rumen, which affects the chance of a feed being ruminally degraded, was not allowed for. Moreover, if the conditions of the *in vitro* system are sub-optimum for the growth and activities of the proteolytic microbes, the rate and extent of feed degradation may be under-estimated.

Quantitative models of N transaction in the rumen (Nolan, 1975) have been built using data obtained from *in vivo* experiments carried out using ¹⁵N dilution techniques. In such experiments, ¹⁵N-labelled ammonia or urea is introduced into the rumen and the appearance of the ¹⁵N label in secondary N pools such as microbial-N

can then be determined. The ^{15}N isotope dilution technique has proved to be a powerful means of understanding the dynamics of N in the rumen. For example, the finding that more than 50 % of microbial-N was derived directly from nitrogenous sources more complex than ammonia (*i.e.* non-protein NAN) when sheep were given lucerne diets was obtained from such experiments (Nolan and Leng, 1972). However, the utilisation of plant NAN materials by microorganisms was determined indirectly after ^{15}N -ammonia utilisation by microbes had been determined, and this method gives lower confidence than a direct measure of plant N incorporation.

This chapter describes experiments that were carried out to investigate the dynamics of N in the rumen using similar ^{15}N dilution techniques. However, ^{15}N was administered into the rumen on different occasions as either ^{15}N -labelled duckweed or ^{15}N -labelled ammonia, and the transfer of the ^{15}N label to various N pools was followed. In this way it was possible to determine, by direct means, the fate of plant-N and the extent of its assimilation in different forms for synthesis of bacterial protein.

5.2 Experiment 4.1: Intra-ruminal ^{15}N Administration

5.2.1 Introduction

In vivo techniques have been used to predict the degradability of dietary protein in the rumen (see Chapter 2). When this method is used, the NAN in digesta passing through the abomasum or duodenum has been separated into microbial and non-microbial-NAN using one of a number of microbial markers. The non-microbial-NAN portion has then been further separated into dietary and endogenous NAN, the former usually being calculated by difference, based on an assumed value for the endogenous NAN flow. When ^{15}N -labelled ammonia has been administered into the rumen and used as the microbial marker, it has also been possible to estimate the N (ammonia-N and NAN) utilisation by microbes for their cellular protein synthesis.

There have been few direct estimates of the rate and extent of dietary protein degradation and of microbial utilisation of the protein digestion products in the rumen. ^{15}N -labelled plant materials offer great possibilities for this purpose. In one of the very few published study of this type, Chapman and Norton (1984) used isotope

dilution techniques with ^{15}N -labelled Pangola grass and Siratro (harvested after 5 weeks of regrowth). They concluded that 95-99 % of the N in these forages was degraded in the rumen.

The following experiment was carried out to determine the feasibility of using ^{15}N -labelled duckweed to estimate the degradability of duckweed protein in the rumen. The extent to which the degradation products of protein in duckweed were used for microbial protein synthesis was also estimated by comparing the patterns of assimilation of the ^{15}N label when it was provided as either a single intra-ruminal injection of ^{15}N -ammonia or ^{15}N -duckweed.

5.2.2 Materials and methods

5.2.2.1 Experimental animals

Two Merino x Border Leicester crossbred sheep, designated as Sheep 1 and 2, with respective live weights of 32.5 and 30.5 kg, were used in this experiment. The animals were each surgically fitted with a rumen cannula. About one month after the surgery, the animals were transferred into individual metabolism crates in a temperature-controlled, continuously lit room. They were allowed about 3 weeks to become accustomed to their new environments and to the experimental diet and 1 week to become accustomed to the hourly feeding regimen before any experiments commenced.

5.2.2.2 Diet and feeding

The animals were given a mixed diet of 400 g oats chaff (1.22 g N/100 g, air-dry basis) and 300 g lucerne chaff (2.97 gN/100 g, air-dry) at hourly intervals by means of an overhead, automatic belt feeder.

5.2.2.3 Experimental procedures

Sheep 1 was given an intra-ruminal single injection of ^{15}N -ammonia while Sheep 2 was dosed intra-uminally with ^{15}N -labelled duckweed. Sixty ml of a solution containing 42.3 mmol N as $(^{15}\text{NH}_4)_2\text{SO}_4$ (enrichment 4.737 mol ^{15}N /100 mol N) and

187.4 mg Co (as the Co-EDTA complex) was drawn into a syringe and injected through the rumen cannula of Sheep 1 over about 0.5 min. The injection was made through a stiff plastic tube that enabled the solution to be directed into different sites in the rumen to facilitate more rapid mixing of the marker throughout the rumen contents.

A similar intra-ruminal administration of ^{15}N -labelled duckweed was given to Sheep 2. Duckweed was labelled by growing it on a medium containing ^{15}N , using a technique described in Appendix 2. The ^{15}N -labelled duckweed had an enrichment of 3.07 mol ^{15}N /100 mol total-N. This duckweed (30 g), dried and finely ground to pass a 1 mm sieve, was first mixed in a beaker with 60 ml of Co-EDTA solution containing 177.5 mg of Co. Tap water was added to the mix to form a slurry that was gently swirled and delivered slowly through the rumen cannula via a funnel and stiff plastic tube. The tube was directed to different locations in the rumen to dissipate the labelled duckweed and assist it to mix more rapidly through the rumen contents. The beaker and tube were washed with a minimum amount of water while the tube was withdrawn carefully from the rumen contents to ensure quantitative delivery of all ^{15}N -labelled material.

Rumen fluid samples were taken from both sheep before and at 0.3, 0.6, 1.1, 1.8, 2.8, 4.4, 6.1, 8.3, 12.4, 22.8 and 32.1 h after ^{15}N -labelled material was administered. These samples were transferred into centrifuge tubes placed in ice and processed immediately as described below.

5.2.2.4 Laboratory analysis

The rumen fluid samples were immediately centrifuged (20,000 x *g*, 15 min, 4°C) to obtain a supernatant fraction, which was acidified with 0.25 ml 18 M H_2SO_4 and stored at -18°C until analysis for ammonia (see Section 3.3.4.2) and NAN (Section 3.3.4.4). Bacteria were isolated from the residue (Section 3.2.7) and stored at -18°C until analysis for N (Section 3.3.4.3). The enrichment of ammonia-N, NAN and bacterial-N was determined as described in Section 3.3.5.

5.2.2.5 Data analysis and calculation

All enrichments of samples from both animals were normalised (linear scaling) to represent 2 mmol ^{15}N (above natural abundance) administered (either as ^{15}N -ammonia or ^{15}N -duckweed) into the rumen. The resulting enrichment data for ammonia-N and NAN in rumen fluid supernatant fractions as well as bacterial-N were fitted by curves with two exponential functions using *GraphPad Prism*, version 3.02 for Windows (Motulsky, 1999). The size of the rumen fluid ammonia compartment, the rates of total flux and net flux of ammonia and the fractions of bacterial-N derived from rumen ammonia were calculated (Nolan and Leng, 1974). The fraction of the ^{15}N in the duckweed administered to Sheep 2 that subsequently entered the ammonia compartment was used as an index of ruminally degradable protein in duckweed, although it was recognised that NAN degradation intermediates (*e.g.* peptides or amino acids) might also be assimilated by microbes. This fraction was determined from the rate of flow of ^{15}N through the ammonia compartment after administration of ^{15}N -labelled duckweed. The latter was taken to be the product of the area under the ammonia curve and the rate of flux of ammonia through the ammonia compartment (which was assumed to be the same as that determined in Sheep 1).

5.2.3 Results

The dietary N intake was 14.2 g N/d. An additional 0.59 g N was provided as $(^{15}\text{NH}_4)_2\text{SO}_4$ for Sheep 1 and 0.84 g N as ^{15}N -duckweed for Sheep 2 on the experimental days. The actual (*i.e.* unscaled) amount of ^{15}N administered was 2.02 and 1.71 mmol for Sheep 1 and 2, respectively. About 8 % of N in the dried ^{15}N -duckweed that was administered to Sheep 2 was present in the form of ammonia.

The curve for Co concentration (ln mg/l) *v.* time (h) in the rumen fluid following the intra-ruminal injection of Co-EDTA is shown in Figure 23. The rumen concentration of ammonia (mmol N/l) is given in Figure 24. The enrichment *v.* time results in the period after ^{15}N administration and the fitted curves with two-exponential components are given in Figure 25.

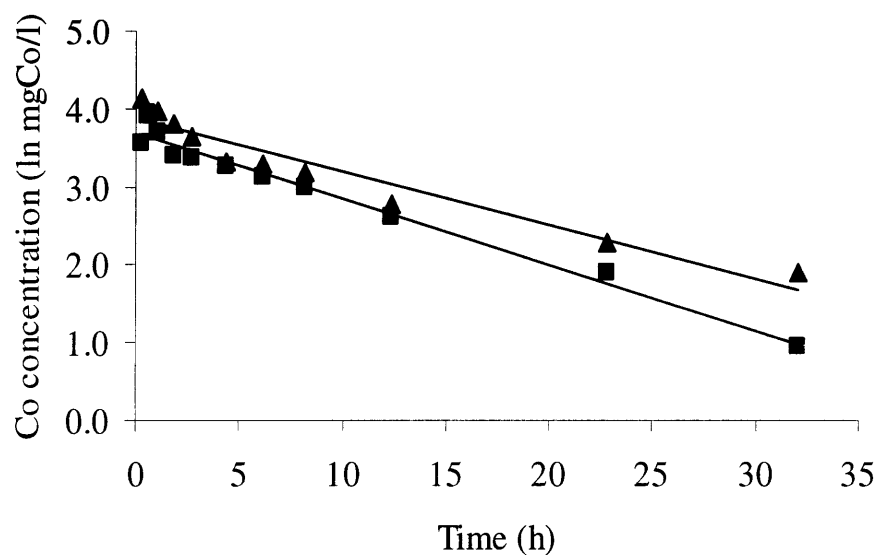


Figure 23. The rumen concentration of Co (ln mg/l) with time (h) for Sheep 1 (▲) and Sheep 2 (■) following an intra-ruminal injection of Co-EDTA.

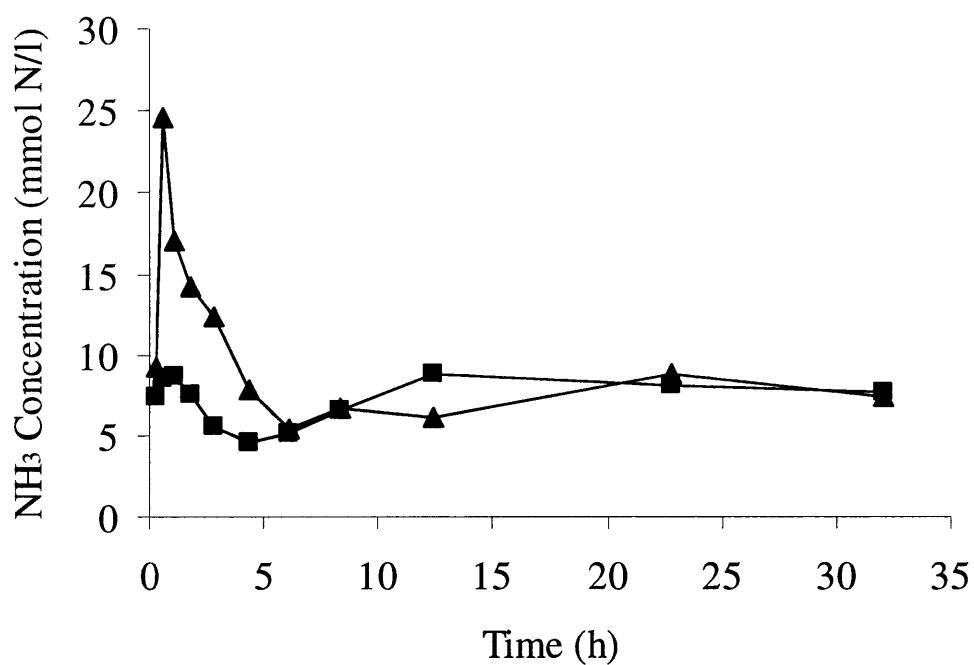


Figure 24. The rumen ammonia concentration (mmol N/l) with time (h) for Sheep 1 (▲) and Sheep 2 (■) after administration of ^{15}N -labelled materials.

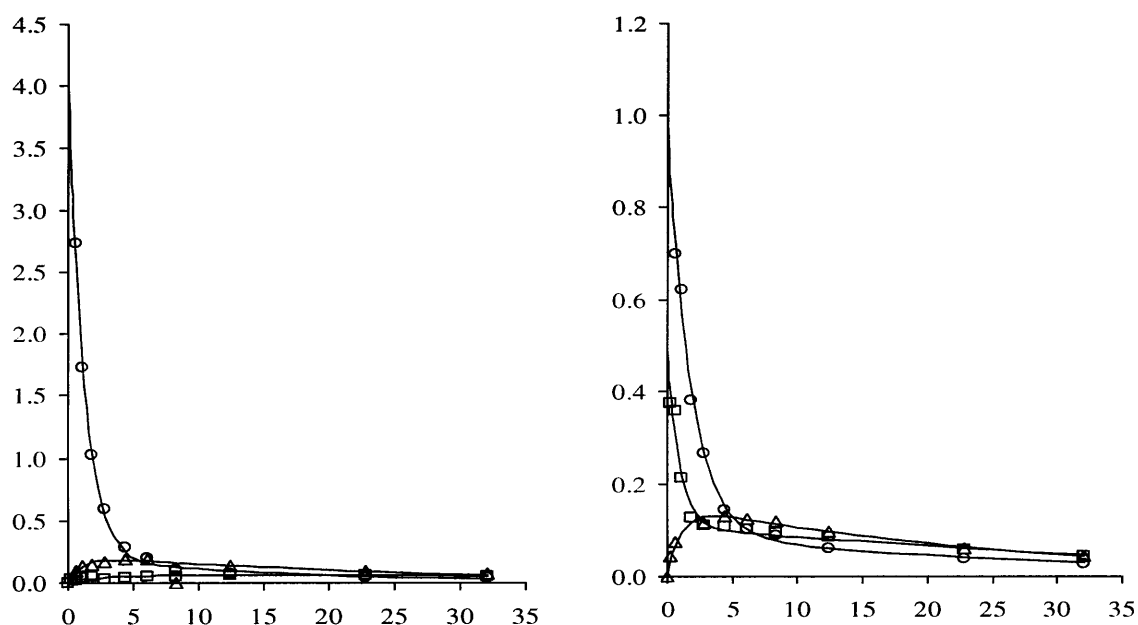


Figure 25. Enrichments (y axis; mol $^{15}\text{N}/100$ mol total-N) in rumen fluid ammonia ($\circ$), rumen fluid NAN ($\square$) and bacterial-N ($\triangle$) versus time (x axis; h) after intra-ruminal administration of 2 mmol ^{15}N as ^{15}N -labelled ammonia (Sheep 1, left) or ^{15}N -labelled dried duckweed (Sheep 2, right). The lines represent fitted curves with two exponential functions.

For Sheep 1, the mean ammonia concentration in the rumen fluid was 152 mg N/l and the rumen volume estimated from the decline in the ruminal concentration of Co was 3.9 l. Therefore, the size of the rumen ammonia compartment was 593 mg N. The total ammonia flux was 12.3 g N/d and the net flux rate was 8.17 g N/d. About 4.07 g ammonia-N therefore left the ammonia compartment and returned to it each day, *i.e.* recycled. The ratio of the area under the rumen bacterial-N curve to that under the rumen fluid ammonia curve for Sheep 1 was 0.70, indicating that 70 % of the bacterial-N was derived from ammonia, and the remaining 30 % was therefore derived from NAN. The corresponding ratio for Sheep 2 that was ingesting ^{15}N -labelled duckweed was considerably higher (0.92) indicating that the rumen bacteria did not assimilate only ^{15}N -ammonia, but also used ^{15}N -labelled NAN intermediates from the labelled duckweed as a source of N for their protein synthesis.

Sheep 2 had a lower mean rumen ammonia concentration than Sheep 1, *i.e.* 100 v. 152 mg N/l. The estimated rumen volume was 4.5 l and the rumen ammonia compartment size was therefore about 450 mg N. After receiving the same dose of

^{15}N in duckweed as Sheep 1 (2 mmol), the ammonia compartment of Sheep 2 became labelled with ^{15}N , but to a far lower extent than occurred in Sheep 1 (see Figure 25), indicating that only a small fraction of the duckweed N was degraded to ammonia. The rumen NAN pool of Sheep 2 also became labelled with ^{15}N , but the rate of appearance of ^{15}N and the shape of the NAN curve were different from that for Sheep 1 and the enrichments of rumen NAN and bacterial-N were considerably higher relative to ammonia enrichment than for Sheep 1. These results indicate that duckweed N contributed directly to these pools, with only a small fraction of the NAN being derived from rumen ammonia. About 50 % of the ^{15}N administered as duckweed to Sheep 2 subsequently passed through the rumen ammonia compartment whereas, because ^{15}N was administered as ^{15}N -ammonia to Sheep 1, 100% of the ^{15}N administered to Sheep 1 passed through the ammonia compartment.

5.3 Experiment 4.2: Continuous ^{15}N Administration (1)

5.3.1 Introduction

There were clear indications from the results of Experiment 4.1 that duckweed was degraded in the rumen to NAN compounds that were important precursors for microbial protein synthesis. The results were obtained from 2 animals, each given both ^{15}N -ammonia and ^{15}N -labelled duckweed at different times in a cross-over design. The ^{15}N -labelled duckweed used in Experiment 4.1 was directly introduced into the rumen through a cannula after being finely ground to mimic a well-masticated feed.

It seemed likely, however, that prediction of duckweed degradability *in vivo* would be more realistic if the experimental animals were allowed to masticate and ingest the ^{15}N -labelled duckweed themselves. In the next and subsequent experiments, it was therefore decided to allow the sheep to ingest ^{15}N -labelled duckweed with the rest of their diet.

5.3.2 Materials and methods

5.3.2.1 Experimental animals

Four Merino x Border Leicester sheep, designated as Sheep A, B, C and D (mean live weight 32.4 ± 0.66) were used in this experiment. Each animal was equipped with a rumen and an abomasal cannula. The sheep were kept in individual metabolism crates in a temperature-controlled, continuously lit room during the experiment.

5.3.2.2 Diets and feeding

A daily ration of 400 g oat chaff (91.6 % DM, 1.23 g N/100 g DM) and 300 g lucerne chaff (91.1 % DM, 2.99 g N/100 g DM) was delivered in equal portions at hourly intervals to each sheep from an automatic, overhead, moving-belt feeder.

5.3.2.3 Experimental procedures

The experiment was carried out in two periods using a cross-over design. In each period, one animal received $(^{15}\text{NH}_4)_2\text{SO}_4$ and the other ^{15}N -labelled duckweed. A solution containing $(^{15}\text{NH}_4)_2\text{SO}_4$ (approximately 400 μg N/ml and 4.683 mol ^{15}N /100 mol N above natural abundance) and Co-EDTA complex (72.6 μg Co/ml) was continuously infused (0.84 g/min) for 48 h into the rumen via the cannula. A solution of Co-EDTA (82.8 μg Co/ml) was also infused for 48 h into the rumen of the sheep given the diet with ^{15}N -duckweed. ^{15}N -labelled duckweed (30 g) containing 2.56 mg N/100 mg DM and 2.972 mol ^{15}N /100 mol N was dried but not milled. This ^{15}N -labelled duckweed was evenly distributed on top of the oat-lucerne chaff mixture on the belt of the automatic feeder and about 1/24th of the daily ration was delivered, at hourly intervals, during the last 24 h of the 48-h intra-ruminal infusion.

During the 48-h period, rumen fluid and abomasal digesta samples were collected as described in Sections 3.2.4 and 3.2.5, respectively.

5.3.2.4 Laboratory analysis

Treatment of rumen fluid samples, procedures for isolating bacterial samples and for storage and analysis of N and ^{15}N were the same as those applied in Experiment 4.1. Stored abomasal digesta samples were thawed and separated into liquid- and particle-phase digesta as described in Section 3.3.1.1 and they were subjected analyses for Co (Section 3.3.1.1) and AIA (Section 3.3.2).

5.3.2.5 Data analysis and calculation

All enrichment values were normalised to represent administration into the rumen of $2\ \mu\text{mol } ^{15}\text{N}/\text{min}$ in excess of natural abundance. The concentrations of AIA and Co were determined on particle-rich and fluid-rich portions of abomasum digesta: 'true' abomasum digesta were then physically reconstituted (Faichney, 1975). The concentrations of DM, NAN and ammonia-N and the enrichments of the N fractions were then determined on the reconstituted abomasum digesta and the flows of these constituents were calculated.

5.3.3 Results

During the intra-ruminal infusion of ^{15}N -ammonia, the enrichment in rumen ammonia-N appeared to have reached a plateau value after about 10 h, although there was considerable variation about the mean enrichment from 10–48 h after the start of the infusion (Figure 26). The enrichments of rumen NAN and bacterial-N were much less variable and appeared to increase exponentially but generally did not reach plateau values during the 48 h of the tracer infusion. Extrapolation of the fitted exponential curves for bacterial N indicated that enrichments would have reached 95 % of their eventual plateau value at about 70 h, when the transfer coefficient (fraction of bacterial-N derived from ammonia-N) would have been 35–66 %. The ^{15}N label appeared in abomasum NAN where the enrichment was lower than the concurrent enrichment in rumen bacterial NAN (due to the dilution of the ^{15}N tracer by the unlabelled non-microbial-NAN, *i.e.* undegraded feed and endogenous NAN).

In contrast, when the 4 sheep ingested their diets with ^{15}N -labelled duckweed over a 24-h period, the enrichments of rumen ammonia-N, NAN and bacterial N followed an almost linear increase, and certainly did not approach plateau values (Figure 27). The enrichment of rumen bacterial-N and NAN was closer to that of rumen ammonia-N than when ^{15}N -ammonia was administered. Abomasum NAN, which was presumably enriched due to the presence of both labelled bacteria and undegraded labelled duckweed, appeared to have a higher enrichment at any particular time than rumen bacterial-N.

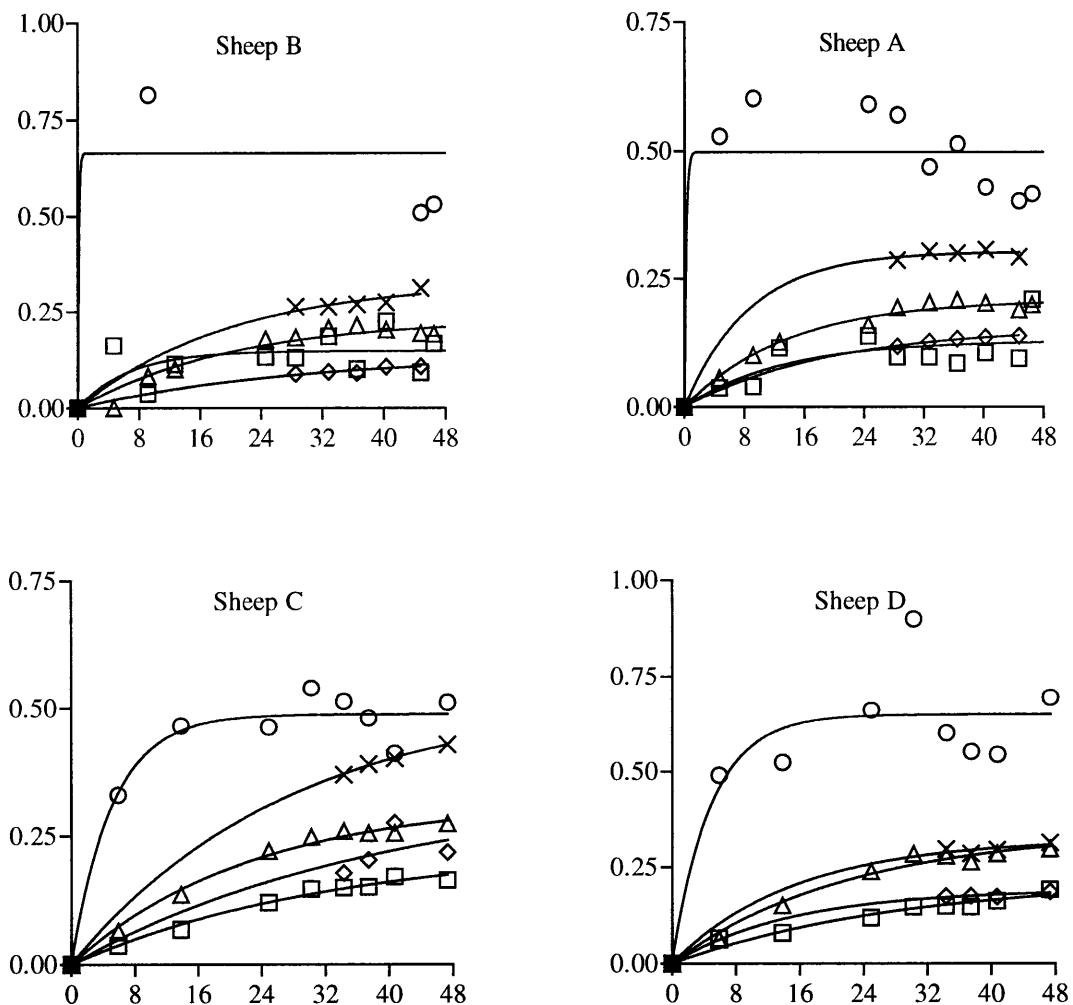


Figure 26. Enrichments (y axis; mol $^{15}\text{N}/100$ mol total N) versus time (x axis; h) in rumen fluid ammonia ($\circ$), rumen fluid NAN ($\square$) rumen bacterial-N ($\triangle$), reconstituted abomasum ammonia ($\times$) and abomasum NAN ($\diamond$) of sheep during a 48 h continuous infusion of $2 \mu\text{mol } ^{15}\text{N}/\text{min}$ as ^{15}N -labelled ammonia.

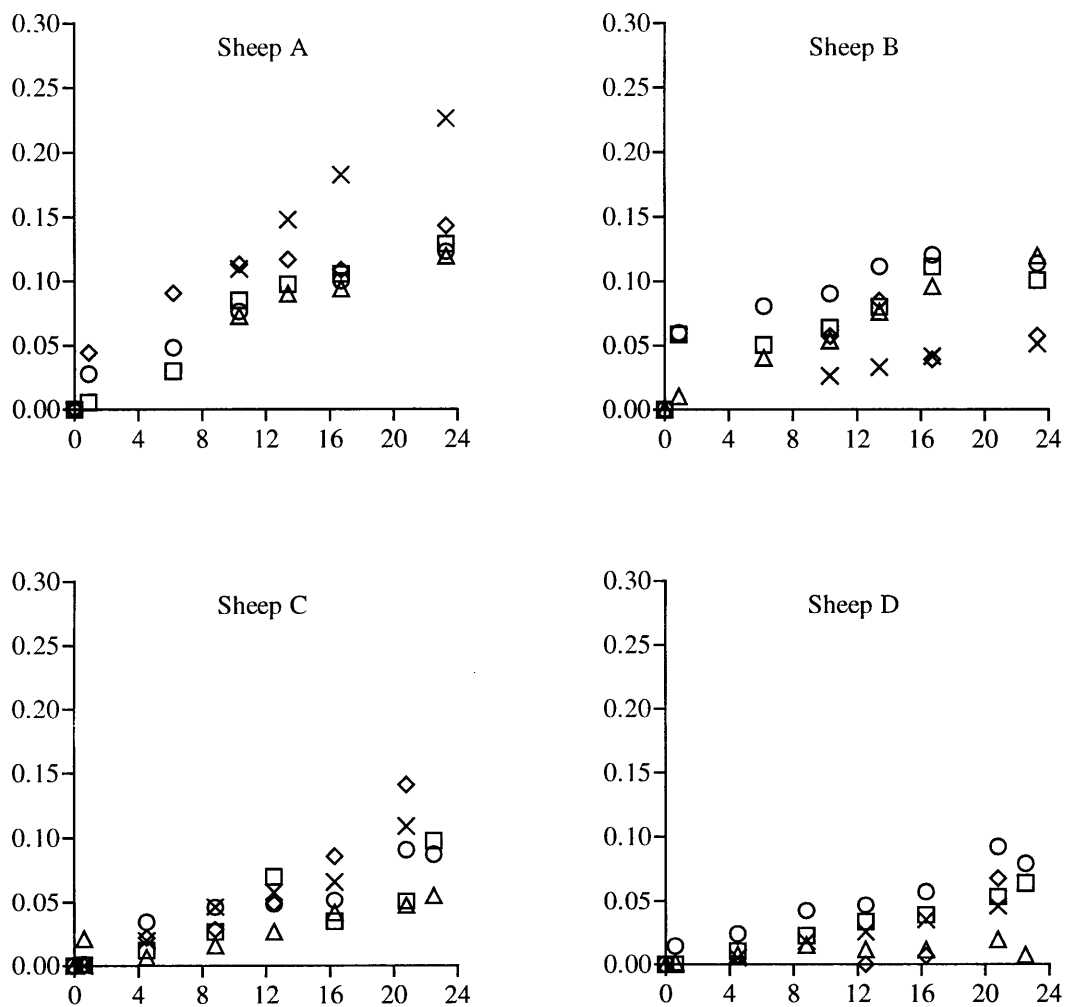


Figure 27. Enrichments (y axis; mol $^{15}\text{N}/100$ mol total N) versus time (x axis; h) in rumen fluid ammonia ($\circ$), rumen fluid NAN ($\square$) rumen bacterial-N ($\triangle$), reconstituted abomasum ammonia ($\times$) and abomasum NAN ($\diamond$) of sheep during a 24 h period of ingestion of $2 \mu\text{mol } ^{15}\text{N}/\text{min}$ as ^{15}N -labelled duckweed.

5.4 Experiment 4.3: Continuous ^{15}N administration (2)

5.4.1 Introduction

Results of Experiments 4.1 and 4.2 showed clear differences in the enrichment of rumen bacterial-N relative to that of rumen ammonia-N and NAN when the ^{15}N label was provided either in ammonia or duckweed. When ^{15}N -ammonia was infused, the enrichment of bacterial-N was far lower than that of rumen ammonia-N but higher than that of NAN in rumen fluid. In contrast, the enrichment of bacterial-N was much closer to that of ammonia-N and NAN when the tracer was provided as ^{15}N -labelled duckweed. These results indicated that the rumen bacteria not only assimilated ammonia-N but also incorporated the intermediary products of duckweed breakdown, *i.e.* peptides and amino acids, for the synthesis of their cellular proteins.

With the different modes of administration of ^{15}N in Experiment 4.2, there were also marked differences in the enrichment of abomasum NAN which was always lower than that of bacterial-N when ^{15}N -ammonia was infused into the rumen. On the other hand, the enrichment of abomasum NAN was higher than that of bacterial-N when ^{15}N -labelled duckweed was ingested. The higher relative enrichment of abomasum NAN in the latter case must have been due to the contribution of labelled duckweed NAN that was escaping to the abomasum without being degraded. However, further interpretations of the data were made difficult by the absence of plateau values due to the short period when sheep were ingesting ^{15}N -labelled duckweed (24 h).

The next experiment (Experiment 4.3) was carried out to extend the results of Experiment 4.2 and to obtain more direct information on the extent to which duckweed protein escapes rumen fermentation *in vivo*. To this end, the ^{15}N tracer, either as ammonia or duckweed, was provided to experimental animals as before, but for a longer period (72 h).

5.4.2 Materials and methods

5.4.2.1 Experimental animals, diets and procedures

The materials and methods were similar to those in Experiment 4.2. Two of the 4 sheep used in Experiment 4.2 (Sheep C and D) were returned to metabolism crates and allowed to become accustomed to the hourly feeding of a mixture of 400 g oaten chaff and 300 g lucerne chaff for about 10 d before the experiment commenced.

Again, a cross-over design was used. During two experimental periods, 1 week apart, the sheep received either an intra-ruminal continuous infusion of ^{15}N -ammonia or continuous feeding of ^{15}N -duckweed. For the sheep receiving ^{15}N -ammonia, a solution containing 1.48 mg N/ml (2.96 mol ^{15}N /100 mol total-N above natural abundance) and 116.5 mg Co / ml, was continuously infused into the rumen at a mean rate of 0.82 g/min. For the other sheep, 16 g ^{15}N -labelled duckweed (oven-dried and not milled) containing 2.81 g N/100 g DM was included in the diet and delivered hourly by automatic, overhead belt feeders. To ensure an equal and steady delivery of the ^{15}N tracer, the ^{15}N -duckweed was divided into 4 portions of 4 g each, and each 4 g was distributed evenly over the feed allocated for each 8-h period on the delivery belt. A Co solution (115.9 mg Co/ml) was also continuously infused into the rumen while the sheep was being offered the ^{15}N -duckweed.

During the ^{15}N -ammonia infusion, rumen fluid samples were collected, processed and analysed for rumen ammonia-N, NAN and bacterial-N as described in Experiment 4.2. To avoid effects on digesta flow due to sampling and possible disturbance of the animals, only 5 samples of abomasum digesta were collected. The abomasum digesta were separated into particle and fluid fractions by squeezing them through a layer of polyester cloth.

In Period 2, the routes of administration of ^{15}N were reversed. After the period of ^{15}N administration was terminated, 5 more samples of rumen contents and abomasum digesta were collected.

5.4.2.2 Analysis and calculation

All enrichment versus time curves for both animals were normalised, as previously, to represent a rate of administration of ^{15}N into the rumen of $2\ \mu\text{mol } ^{15}\text{N}/\text{min}$ above natural abundance (either as ^{15}N -ammonia or ^{15}N -duckweed).

The concentrations of AIA and Co were determined on both particle and fluid fractions of the abomasum digesta: the concentrations of DM, NAN and ammonia-N were also determined as well as the enrichments of each of the N fractions. The flow of 'true' abomasum digesta was calculated mathematically (Faichney, 1975) using the AIA and Co as the particle and fluid digesta markers, respectively. A similar mathematical reconstitution was done to enable estimation of flows of individual digesta constituents.

The difference between the total ^{15}N administered and the total ^{15}N flowing to the abomasum was a measure of the net ^{15}N absorption from the rumen. It was assumed that this ^{15}N was removed by diffusion of ammonia through the rumen wall, and thus ammonia-N absorption rate (mmol/d) was determined by dividing net ^{15}N absorption ($\text{mol } ^{15}\text{N}/\text{d}$) by ammonia-N enrichment ($\text{mol } ^{15}\text{N}/\text{mol total N}$). The total ^{15}N flow through the abomasum was calculated as the sum of ^{15}N in the outflowing ammonia-N and NAN (the latter being bacterial and non-bacterial [dietary and endogenous] ^{15}N). The bacterial- ^{15}N flow was calculated from the rumen bacterial-N enrichment and the flow of the bacterial-N fraction in the abomasum NAN. The latter was calculated from the enrichment ratio, abomasum NAN : rumen bacterial-N, when the animals were infused with ^{15}N -ammonia. The ^{15}N found to be flowing out in the non-bacterial NAN fraction when the ^{15}N -duckweed was being ingested was considered to represent the rate at which duckweed was escaping undegraded from the rumen: the latter rate divided by the rate of ^{15}N ingestion gave the proportion of duckweed-N that was escaping degradation in the rumen. (For this calculation, it was assumed that ^{15}N of endogenous origin in the non-bacterial NAN entering the abomasum was negligible.)

5.4.3 Results

The DM contents (g/kg air-dry) of the oaten chaff and the lucerne chaff were 912 and 894 g/kg, respectively, while the corresponding N contents (g/kg DM) were 11.4 and 34.0.

Feed DM intake and the flows of DM and water through the abomasum are presented in Table 10.

Table 10. Intake of DM and flows of DM and water out of the rumen and through the abomasum of sheep given a diet of oaten chaff (400 g/d as fed) and lucerne chaff (300 g/d as fed).

	¹⁵ N-ammonia		¹⁵ N-duckweed		Mean	SEM	P
	Sheep C	Sheep D	Sheep C	Sheep D			
<u>DM</u>							
Intake (g/d)	633	633	649	649	641	4.57	
Abomasum flow (g/d)	416	408	472	439	434	14.4	>0.05
Apparent digestibility (%)	34.2	35.6	27.3	32.4	32.4	1.81	>0.05
<u>Water</u>							
Rumen volume (l)	4.7	3.9	3.3	6.0	4.5	0.57	>0.05
Rumen outflow (l/d)	7.1	7.8	6.0	8.6	7.4	0.55	>0.05
Abomasum flow (l/d)	7.4	8.7	8.8	9.5	8.6	0.45	>0.05
Net water addition (l/d)	0.3	0.9	2.8	0.9	1.2	0.56	>0.05

The DM intake (g/d) averaged 641 ($\pm$ SEM 4.57) g DM/d during the periods when ¹⁵N was administered. The mean DM content of the 'true' abomasum digesta was about 50 g/kg digesta. Between 64 and 73 % of the dietary DM intake was apparently recovered in the digesta flowing through the abomasum (Table 10), but the mean true feed DM digestibility in the forestomachs was calculated to be 45-71 %. (after allowance for the contributions of bacterial and endogenous DM to the abomasal DM, made by assuming the concentration of N in microbial materials was 0.8 g/kg DM and the endogenous N flow was 2 g N/d in materials containing 16 g N/kg DM).

Rumen water volume averaged $4.5 \text{ l} \pm \text{SEM } 0.57$ and the mean water flow through the abomasum ($8.6 \pm 0.45 \text{ l/d}$) was higher than outflow from the rumen ($7.4 \pm 0.55 \text{ l/d}$), indicating there was a net post-ruminal addition of water to the digesta (Table 10)

N intake tended to be higher, but total-N passing the abomasum tended to be lower when the sheep were infused with ^{15}N -ammonia than when they were ingesting ^{15}N -duckweed (Table 11). The ammonia concentration in abomasal digesta was about 93 mg N/kg and of NAN was about 1.58 g N/kg . The rates of flow of total-N as ammonia-N and as NAN did not differ ($P>0.05$) between animals or diets, with means ($\pm \text{SEM}$) of 57.7 ± 0.96 and $964 \pm 2.13 \text{ mmol/d}$ (or 0.81 and 13.5 g N/d), respectively. About 34-59% of the dietary N was removed from the rumen as ammonia (absorbed and in digesta).

Enrichments *v.* time in rumen fluid ammonia-N, NAN and bacterial-N, and ammonia-N and NAN in abomasal digesta are presented for Sheep C and D in Figures 28 and 29, respectively. As found in Experiment 4.2, the enrichments of rumen ammonia-N appeared to have reached plateau values after about 10 h of ^{15}N -ammonia infusion, although considerable variation in the enrichments of ammonia was again apparent. The percentage of bacterial-N derived from ammonia-N (from the period of ^{15}N -ammonia infusion) was 53-63 % (ratio of plateau enrichments) and thus 37-47% of bacterial-N was derived from NAN sources in the rumen.

Table 11. Intake of N and kinetics of various N-containing fractions in the rumen and abomasum of sheep given a diet of oaten chaff (400 g/d) and lucerne chaff (300 g/d) and either ^{15}N -ammonia or ^{15}N -labelled duckweed.

	^{15}N -ammonia	^{15}N -duckweed	SEM	P
N intake (g/d)	14.9	13.6		
<u>Rumen ammonia-N</u>				
Concentration (mg N/l)	131	137	4.53	>0.05
Compartment size (g N)	0.57	0.65	0.08	>0.05
Absorption rate (g N/d)	7.09	3.92	0.82	>0.05
Outflow rate (g N/d)	0.98	0.99	0.06	>0.05
<u>Abomasum ammonia-N</u>				
Concentration (mg N/l)	98.6	91.7	4.57	>0.05
Flow rate (g N/d)	0.81	0.79	0.01	>0.05
Proportion from rumen ammonia-N	0.73	0.97	0.07	>0.05
<u>Bacterial-N</u>				
Proportion from rumen ammonia-N	0.50	0.50¶		
Proportion of abomasum NAN	0.81	0.81¶		
Abomasum flow (g N/d)	10.4	9.98	0.02	>0.05

¶ values determined when ^{15}N -ammonia was infused into the rumen

The total ^{15}N flow through the abomasum was higher ($P < 0.001$) when ^{15}N -duckweed was given rather than ^{15}N -ammonia (2.40 ± 0.02 versus 1.29 ± 0.02 mmol/d). The ^{15}N in NAN flowing to the abomasum (mmol/d) was also significantly higher ($P < 0.001$) when ^{15}N -duckweed was given rather than ^{15}N -ammonia, with means of 2.31 ± 0.02 and 1.16 ± 0.00 , respectively. The flow of ^{15}N in ammonia, on the other hand, was lower ($P < 0.01$) when sheep ingested ^{15}N -duckweed than when they were infused with ^{15}N -ammonia (0.09 ± 0.00 v. 0.13 ± 0.09 mmol/d).

Table 12. Amounts of ^{15}N flowing in various N materials through the abomasum (mmol/d) during a continuous provision into the rumen of ^{15}N -ammonia or ^{15}N -labelled duckweed.

	^{15}N -ammonia	^{15}N -duckweed	SEM	P
^{15}N administered	2.88	2.88		
^{15}N flow in abomasum NAN	1.16	2.31	0.33	<0.001
^{15}N flow in bacterial-N	1.16	1.46	0.09	<0.01
^{15}N flow in non-bacterial-N	0.00	0.85		
^{15}N flow in abomasum ammonia-N	0.13	0.09	0.01	<0.01
Total ^{15}N flow through the abomasum	1.29	2.40	0.32	<0.001
^{15}N absorbed from the rumen	1.59	0.48	0.32	<0.05

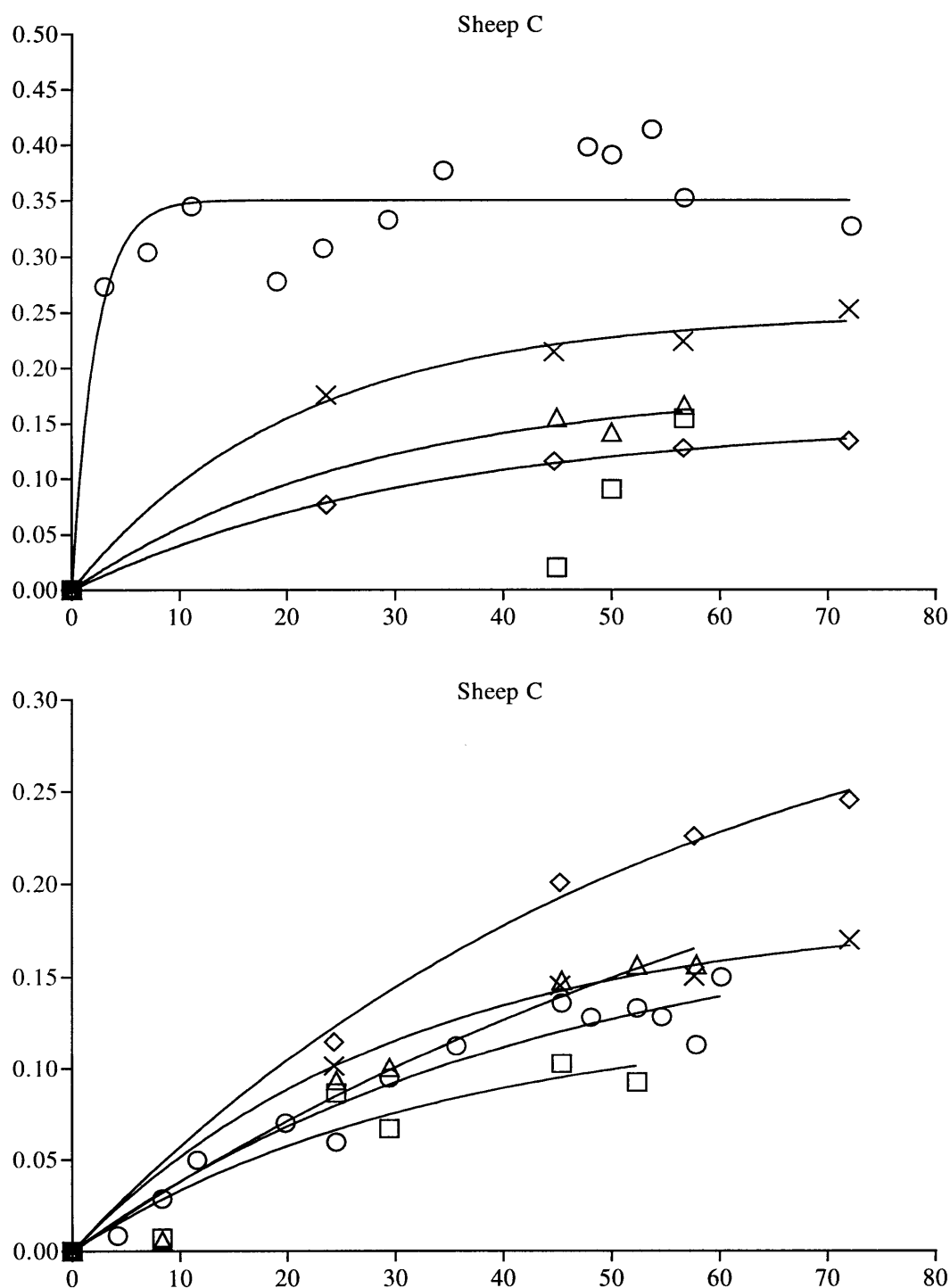


Figure 28. Enrichments (y axis; mol ^{15}N /100 mol total) versus time (x axis; h) in rumen fluid ammonia (○), rumen fluid NAN (□) rumen bacterial-N (△), abomasum ammonia (×) and abomasum NAN (◇) during a 72 h administration of $2\ \mu\text{mol}\ ^{15}\text{N}/\text{min}$ as ^{15}N -ammonia (top) or ^{15}N -duckweed (bottom) to Sheep C.

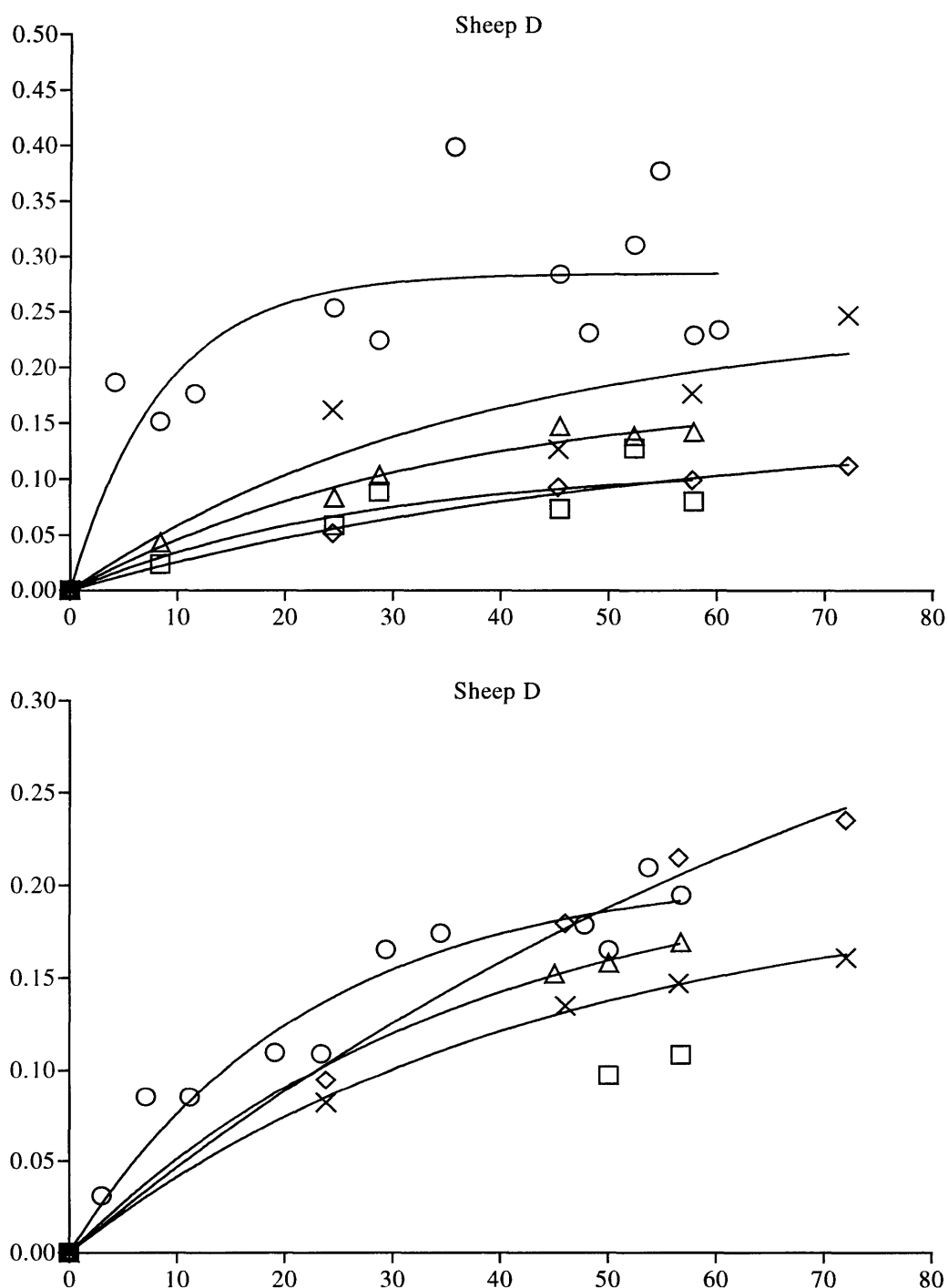


Figure 29. Enrichments (y axis; mol ^{15}N /100 mol total) versus time (x axis; h) in rumen fluid ammonia (○), rumen fluid NAN (□) rumen bacterial-N (△), reconstituted abomasum ammonia (×) and abomasum NAN (◇) when 2 $\mu\text{mol } ^{15}\text{N}/\text{min}$ as ^{15}N -ammonia (top) or ^{15}N -duckweed (bottom) was continually supplied to the rumen of Sheep D.

5.5 Discussion

5.5.1 Methodology – isolating a pure sample of bacteria

The isolation of a pure sample of bacteria from rumen fluid was a critical step in these experiments. Contamination of bacterial-N by undegraded feed-N when ^{15}N -ammonia was infused would have caused under-estimation of the true enrichment of bacterial-N and lead to under-estimation of the bacterial assimilation of ammonia. On the other hand, if the bacterial-N were contaminated by duckweed N when the animal was ingesting ^{15}N -duckweed, this would have increased the apparent bacterial-N enrichment resulting in over-estimation of the extent of bacterial utilisation of NAN. For these reasons, it was important to verify that the procedures used were giving an uncontaminated bacterial sample. Accordingly, the bacterial samples obtained in these studies (isolated using an established procedure - see Forster, 1989), were inspected under a light microscope on various occasions and, according to this assessment, found to be free of visible feed materials. Microscopic examination was supplemented and extended by a second checking procedure. This involved mixing ground ^{15}N -labelled duckweed with rumen fluid containing background levels of ^{15}N . The bacterial-N was then isolated by the procedures used for experimental samples, and its enrichment was determined. The isolated bacterial-N was found to remain at background abundance levels, indicating that there was no labelled duckweed in the bacterial preparation. Both checks engendered confidence that the procedure used in these studies to isolate the bacteria provided bacterial-N that was free from feed contamination.

5.5.2 Concentrations of rumen fluid and abomasal metabolites

The mean concentration of ammonia-N in the rumen fluid throughout the experiment was at least 100 mg N/l. This is higher than the minimum concentration of 50 mg N/l (Satter and Slyter, 1974) considered necessary to support microbial growth and so the availability of ammonia-N in the rumen should not have been a limiting factor for microbial growth in the sheep given the daily ration of 400 g oaten chaff and 300 g lucerne chaff. The mean NAN concentration in the rumen fluid from which the

bacterial-N had been removed was found to be at least 120 mg/l. Demeyer and Van Nevel (1980) reported that the rumen concentration of peptide and amino acid was less than 20 mg N/l, while that of soluble protein was about 40 mg N/l. These values were probably obtained when sheep were fed once per day, so that their low rumen NAN concentration was probably due to rapid deamination of amino acids rather than to their assimilation for microbial protein synthesis (Wallace and Cotta, 1988). The higher rumen NAN concentrations observed in my study probably occurred because, in the rumen of animals fed more frequently, there would have been a more constant release of NAN intermediates from dietary protein.

5.5.3 Flows of DM and nutrients through the abomasum

In Experiment 4.3, the mean DM intake and DM flow through the abomasum was 641 and 434 g/d, respectively. Thus, about 32.4 % of the ingested DM was apparently digested in the rumen. However, further calculations suggested that the true digestibility of DM in the rumen was about 71 %, a coefficient that is similar to values obtained in comparable published studies (SCA, 1990). The rumen water outflow rate, calculated from the rate of decline in Co concentration after the infusion was terminated, was 7.4 l/d. There was a slightly higher flow rate of water (8.6 l/d) through the abomasum suggesting a *net* post-ruminal water addition, probably as gastric secretions, of about 1.2 l/d: the actual water input may have been higher than the net estimate if water is also absorbed in this part of the gut of the sheep (*e.g.* in the omasum). The total N flowing through the abomasum was 15.2 g/d which when compared with a mean N intake of 14.3 g N/d indicated that there was a net addition (*i.e.* the excess of endogenous input of N over that absorbed as ammonia or other N) between the mouth and abomasum of about 0.9 g N/d. Harrop (1974) estimated that the daily total N secreted in gastric juice was 0.51–2.86 g N, but this amount would not explain the 0.9 g N/d net gain if, as my results suggest, there was also an absorption of 5–8 g N/d.

About 95 % of the total N flowing through the abomasum in this experiment was NAN, probably consisting mainly of dietary 'escape' protein and microbial protein formed in the rumen, but there would also have been a small fraction of endogenous N. Because there was a net loss of ammonia from digesta leaving the rumen and passing through the abomasum (about 0.2 g N/d), the endogenous N added to the abomasum may have been mostly in the form of NAN, *e.g.* urea and pepsinogen.

5.5.4 Rumen fluid NAN

When ^{15}N -ammonia was administered into the rumen, the rumen bacteria assimilated the ^{15}N while synthesising new cellular protein: in this case, the enrichment in rumen fluid NAN samples was therefore entirely due to release of bacterial-N into the medium. However, when the ^{15}N tracer was provided as ^{15}N -labelled duckweed in the diet, its soluble proteins and degradation products (peptides, amino acids and ammonia) would all contribute directly to the presence of label in rumen fluid NAN, with a secondary contribution also occurring from products derived from viable or lysed bacteria that had become labelled by assimilating the labelled peptides, amino acids or ammonia. The enrichment of rumen fluid NAN samples when the ^{15}N -labelled duckweed was the source of the tracer could, therefore, be due to a mixture of microbial-N products, as well as soluble duckweed protein and intermediates of protein degradation.

The above phenomena could clearly be seen in Experiment 4.1 when the ^{15}N was provided as a single injection. The shape of the enrichment curve for rumen fluid NAN was similar to that of bacterial-N but the enrichment values for the NAN were always lower than for the bacterial-N. This indicated that any ^{15}N found in the NAN when ^{15}N -ammonia was administered was synthesised by rumen microbes. When ^{15}N -duckweed was dosed into the rumen, the shape of rumen fluid NAN enrichment curve for the first 5 h after dosing was similar to, but the enrichment values were lower than, those of ammonia-N. Most of the ^{15}N in the rumen fluid NAN found during this time was probably present as soluble proteins released from the duckweed. The enrichments of rumen ammonia-N, which were higher than of rumen fluid NAN

during this period, were probably mostly contributed directly by the ^{15}N -ammonia already present in the duckweed.

5.5.5 Rumen fluid ammonia flux and recycling

In the rumen, there is continuous entry and removal of ammonia in the rumen fluid compartment. The sources include: degradation of dietary N, N recycled into the rumen from the body via the rumen wall and saliva, and recycling of microbial-N substances within the rumen itself (see Chapter 2). Ammonia leaves the compartment in the rumen in bacterial-N flowing into the lower gut after being assimilated from rumen fluid, by ammonia absorption through the rumen wall and by ammonia outflow in digesta. In Experiment 4.1, when ^{15}N -ammonia was given to Sheep 1 as a single injection into the rumen, about 70 % of the daily total ammonia flux left the compartment irreversibly, and about 30 % recycled. This recycling rate was within the range of 10-50 % suggested by Nolan (1975). A higher value (57 % of the total ammonia flux) has also been reported for defaunated sheep (Nolan and Stachiw, 1979). Most of this recycling probably occurred in the rumen itself, mainly through the degradation of microbial-N, as the probability of ^{15}N returning via extra-ruminal routes is relatively low, for reasons discussed previously (Nolan and Leng, 1972; Nolan *et al.*, 1976; Nolan and Stachiw, 1979).

The percentage of ^{15}N in duckweed passing through the ammonia compartment in the rumen of Sheep 2 in Experiment 4.1 was estimated by assuming that the rates of ammonia entering and leaving the ammonia compartment in the rumen would be similar in both Sheep 1 and 2. The result was taken to be an index of the rumen degradability of duckweed protein. The calculated entry rate of ^{15}N from labelled duckweed into the ammonia compartment for Sheep 2 - *i.e.* the product of the net flux rate (mol N/d) and the area under curve for the ammonia enrichment v. time ($[\text{mol } ^{15}\text{N}/\text{mol N}] \times \text{d}$) - was about 50 % of that administered. It can, therefore, be concluded from this preliminary study (Experiment 4.1) that the rumen degradability of protein in dried duckweed was at least 50 %.

The percentage of bacterial-N newly synthesised from ammonia-N in Sheep 1 in Experiment 4.1, which was calculated from the ratio of the area under the bacterial-N enrichment curve to that under the rumen fluid ammonia-N enrichment curve, was about 70 %. This indicates that 70% of the N in bacteria was derived from rumen ammonia and the other 30% was derived from NAN. The corresponding ratio for Sheep 2 was higher (92 %) probably because the enrichment of bacterial-N was not only due to the assimilation of ^{15}N -ammonia but was also due to the direct incorporation of other ^{15}N -labelled NAN (*e.g.* peptides and amino acids) released during the degradation of ^{15}N -labelled duckweed. This finding provides evidence of direct uptake of NAN from degraded duckweed in forms that are more complex than ammonia. This result suggests that peptides and amino acids may be extensively used by rumen microbial populations when they are available, even though it is well established that healthy populations of bacteria can also exist in the rumen of animals given diets containing almost no true protein (Virtanen, 1966).

5.5.6 Rumen fluid ammonia enrichments

Results obtained in Experiments 4.2 and 4.3 when ^{15}N -ammonia was infused into the rumen indicated that the rumen ammonia enrichment had reached a plateau value 10-20 h after the start of infusion. There was a considerable between-sample variation, however, in the rumen ammonia enrichments. The most likely reason for this variation is the high fractional rate of turnover of ammonia: this is related to the small compartment size and the high rate of flux of ammonia through the compartment. To some extent, the problem could also have been due to non-ideal mixing of the tracer with unlabelled N, but precautions were taken to minimise this effect, *e.g.* the sampling probe was positioned at a distance from the infusion line to prevent the samples taken being contaminated by the ^{15}N in the infusion solution. Variation due to the inaccuracies in the mass spectrometer analyses for determining enrichment can be discounted because the variation was apparent only in the rumen ammonia results (*i.e.* not in other samples) and, in addition, checks showed that the ^{15}N analyses were both precise and repeatable (see Appendix 3).

There was considerable variation between animals in the estimates of the proportions of bacterial-N synthesised from ammonia-N when ^{15}N -ammonia was infused into the rumen. This was probably mainly due to uncertainties about the plateau ammonia enrichments, as already discussed. Additional variation may have been due to different bacterial populations present in the rumen - which can occur even when animals ingest the same diet (Armstead and Ling, 1993) - or to possible errors associated with predicting the true plateau enrichment of rumen ammonia-N. The proportion of bacterial-N derived from ammonia-N was also lower when ^{15}N -ammonia was infused than when it was injected into the rumen. The recycling of the absorbed ^{15}N -ammonia and ^{15}N -bacteria during the longer period of infusion may have been responsible for lower estimates of the ammonia-derived bacterial-N for the tracer infusion experiments as compared to the tracer injection experiment.

When the sheep were ingesting ^{15}N -duckweed, the enrichment of rumen ammonia-N also increased exponentially with time, but the rate of increase in enrichment (*i.e.* from the tangent to the fitted curve) was slower, and the absolute values were much lower than those obtained when the same quantity of ^{15}N was infused in ammonia. It is, therefore, clear that the ^{15}N ingested in duckweed was not quantitatively degraded all the way to ammonia-N. The ^{15}N must have been utilised for microbial protein synthesis in NAN forms, or it escaped either undegraded or partially degraded to the abomasum. The ammonia-N enrichment in the sheep ingesting duckweed would have been due, in part, to the direct contribution of ammonia present in the ingested duckweed at the time of its ingestion (about 8 % of total N). However, the slow rate of increase in enrichment also suggests there was a delayed contribution, probably via intra-ruminal (and to a lesser extent extra-ruminal) recycling of labelled microbial-N.

5.5.7 Balance of ^{15}N across the rumen (ammonia absorption/urea)

The ^{15}N administered that was not recovered in the abomasum must have been ^{15}N absorbed from the rumen. The non-recovered fraction was significantly higher ($P < 0.001$) when ^{15}N -ammonia was infused than when ^{15}N -duckweed was ingested because ammonia absorption across the rumen wall is an important route of loss of

ammonia that is not assimilated by bacteria. Also, because ingested duckweed N was only partially degraded in the rumen, the undegraded portion escaped to the intestines. Assuming that all the non-recovered ^{15}N ($\text{mol } ^{15}\text{N/d}$) was removed from the rumen as absorbed ammonia, the ammonia absorption rate (mol N/d) could be calculated from knowledge of rumen ammonia-N enrichment at plateau ($\text{mol } ^{15}\text{N/mol total N}$). The estimate of ammonia absorption when ^{15}N ammonia was administered (0.51 mol/d , 7.1 g N/d) tended to be higher than that obtained when ^{15}N -duckweed was ingested (4.0 g N/d), but the former value may be more nearly correct because of the earlier and more complete attainment of plateau enrichment in rumen fluid ammonia-N during the ^{15}N -ammonia infusion.

The flow rate of ammonia-N out of the rumen was about 0.2 g/d higher than the rate of ammonia-N flow through the abomasum (Table 11), indicating there was a net loss of ammonia from digesta passing through the omasum or abomasum. The omasum is the likely absorption site as absorption of ammonia from the abomasum is unlikely (Williams *et al.*, 1968). At the same time, however, the ammonia-N enrichment in the abomasum was lower than the rumen ammonia-N enrichment indicating that some unlabelled ammonia-N was added to digesta in one or both of these organs. The proportion of the abomasal ammonia-N derived from the rumen was about 85 % implying that about 15 % of the ammonia flowing through the abomasum was added post-ruminally, probably from the degradation of blood urea entering the abomasum in gastric juices (Harrop, 1974).

5.5.8 Abomasal bacterial flow

The proportion of bacterial-N in the abomasum NAN was estimated from the ratio of enrichments in abomasum NAN and bacterial-N isolated from the rumen fluid. This ratio was only applicable when ^{15}N -ammonia was infused into the rumen (not when the tracer was provided by the ^{15}N -duckweed as, in this case, labelled NAN in the abomasum could be non-bacterial in origin). The ratio, which was about 81 %, was assumed to apply throughout the experiment and was also used in calculations for the duckweed-ingesting animals. The ratio was higher than that obtained for sheep with similar intakes of DM by Siddons *et al.* (1982), and the estimates of flow of

bacterial-N through the abomasum (10.5 g N/d), and the efficiency of bacterial-N synthesis (39-45 g N/kg DM apparently digested in the rumen, or 22-45 g N/kg DM truly digested in the rumen) also seemed to lie at the high end of the range of published values (SCA, 1990).

5.5.9 Duckweed protein escape

The proportion of duckweed-N that escaped rumen fermentation was estimated from the data describing the ^{15}N balance across the rumen (Table 12). The ^{15}N flowing through the abomasum would have been present as both ammonia-N and NAN, the latter consisting of microbial-N and non-microbial-N. The non-microbial-N probably also contained relatively small amounts of labelled endogenous NAN. From the knowledge of bacterial-N enrichment and the proportion of bacterial-N in the abomasal NAN, the amount of ^{15}N in bacterial-N flowing through the abomasum was calculated. Subtracting the result from the total ^{15}N flow gave an estimate of the non-bacterial ^{15}N -labelled NAN flow (0.85 mmol/d). If the non-bacterial-N flow through the abomasum is assumed to be solely from labelled escape duckweed, then about 30 % of the duckweed N ingested escaped digestion in the rumen which also implies that about 70 % of the dietary duckweed-N was degraded in the rumen. About 25 % of the ^{15}N in the ruminally degraded component of the duckweed was absorbed through the rumen wall, probably as ammonia as mentioned above. The majority of the ^{15}N in duckweed digested in the rumen, however, was converted to, and recovered as bacterial-N flowing through the abomasum: this bacterial-N would then be available for intestinal absorption and utilisation by the host.

Chapter 6 The Effects of Duckweed Supplementation on Wool Growth and Characteristics in Fine-Wool Merinos

6.1 General

Dietary protein ingested by ruminants is either digested and fermented by microbes in the rumen or escapes these ruminal processes and becomes substrate for digestion and absorption in the small intestine. The dietary proteins that escapes rumen fermentation and the microbial protein synthesised in the rumen are the sources of the amino acids required for the maintenance and synthesis of tissue protein in ruminants. The earlier studies in this thesis indicate that duckweed protein may be a useful source of escape protein for ruminants.

Microbial protein constitutes the majority of the amino acids absorbed by ruminants (AFRC, 1992) and with its well-balanced amino acid profile (Storm and Orskov, 1983), the microbial protein alone is able to satisfy the amino acid requirements of ruminants at maintenance levels (Virtanen, 1966). However, the amino acids requirements of animals that are in highly productive states (fast growing, late pregnancy or early lactation) are higher than that can be supplied by microbial protein (Orskov, 1970). For these animals, the provision of a dietary protein such as

duckweed or cottonseed meal that will escape rumen fermentation is necessary to augment the supply of microbial amino acids.

Wool growth has been shown to be responsive to the amounts and the amino acid composition of the protein available for absorption in the small intestine (Ferguson, 1975; Reis, 1979). Increasing the supply of protein available for absorption increased wool growth and Reis (1969) found that wool growth was at near maximal rates when there was about 150 g/d protein casein infused into the abomasum. On this basis, wool growth may therefore be used as an indicator of the amounts of protein leaving the rumen and available for intestinal absorption (Leng *et al.*, 1983). In other words, wool growth may be used to predict the undegradability of protein supplement in the rumen, if it is assumed that the amounts and the composition of amino acids supplied by both microbial and endogenous protein are not affected.

The following two experiments were carried out to enable assessment of the nutritional value of duckweed as a potential escape protein supplement by using wool growth and wool characteristics as a means of evaluating the amounts of protein available for absorption from the small intestine of sheep.

6.2 Experiment 5.1: The Effects of Duckweed Supplementation on Wool Growth and Characteristics

6.2.1 Introduction

In a study, which assessed the rumen degradability of sun-dried duckweed using the *in sacco* technique, it was reported that the protein in duckweed was about 80 % degradable in the rumen (Huque *et al.*, 1996). Leng *et al.*, (1995) speculated that the major role duckweed may play in the nutrition of ruminants is to provide readily available nutrients for the growth of microbes in the rumen. Results of the experiments reported in Chapter 5, however, indicated that the rumen degradability of duckweed protein was about 70%. The remaining protein apparently passed through the abomasum and was expected to be digested and absorbed from the small intestine. It appears, therefore, that in addition to providing N in different forms (ammonia,

peptides and amino acids) and other nutrients for the rumen microbes, a substantial proportion of duckweed protein would be available for absorption in the small intestine of ruminants and for utilisation for different forms of production.

Having assessed the rumen degradability of duckweed, experiments were required to investigate the effect of feeding duckweed on the production of ruminants to determine whether ruminant production may be enhanced when duckweed is used as a supplement. This has not been done in the past, with the exception of a study reported by Rusoff *et al.* (1978) who found that the use of duckweed to supplement a corn silage-based diet increased the daily weight gain of Holstein heifers.

In the following experiment, both dried and fresh duckweed were offered as a supplement to sheep given a hay diet and its effects on the ruminant productivity (wool growth and characteristics) were investigated. The hypothesis tested was that sheep offered a hay diet supplemented with duckweed would exhibit a higher rate of wool growth than the animals fed on the basal diet alone.

6.2.2 Materials and Methods

6.2.2.1 Experimental animals

Thirty-six fine-wool Merino sheep (castrated males, aged about 6 months, mean initial live weight of 32.2 ± 0.57 kg and in good body condition) were obtained from a flock at the University of New England. During the experiment, the animals were confined in individual pens (0.9 x 1.2 m). The pens had a feed trough at the front and a water trough at the back from which fresh water was always freely available. New feed was given each day at 09.00 h. The sheep were weight (at 14.00 h) at strategic times throughout the experiment.

6.2.2.2 Duckweed

Fresh and sun-dried duckweed (*Spirodela punctata*) was obtained from the Bio-Tech Waste Management, Armidale.

6.2.2.3 Experimental procedures

a Pre-experimental phase

From 21st November to 3rd December 1997, the sheep were housed indoors and offered a mixed diet consisting of oaten chaff (600 g/d) and lucerne chaff (50 g/d), and allowed to become accustomed to their new situation. On 3rd December 1997, by which time all the animals were eating the oaten chaff/lucerne chaff mixture, the diet was changed to a mixture of sun-dried duckweed (50 g/d) and oaten chaff (700 g/d) as the pre-experimental diet. On the same day, a mid-side patch was clipped on the left side of each animal and the wool that was removed was retained for detailed description. A dye-band was placed in the wool next to the skin surface about 5 cm posterior to the mid-side patch.

During the next 7 weeks (3rd December 1997 – 27th January 1998), the sheep were offered the pre-experimental diet. This was done so that all of the sheep could experience the taste and metabolic consequences of ingesting duckweed. On 27th January, the wool that had grown on the mid-side patch was clipped close to the skin surface, and retained for detailed description and a second dye-band was placed in the wool at the same site as the original dye-band. The characteristics of wool produced by individual sheep in the period of 3rd December 1997 – 27th January 1998 were used later as the covariate in an analysis of covariance when comparing wool production characteristics during the experimental period (27th January - 11th March 1998).

b Experimental phase

On 27th January 1998, the animals were allocated to 4 groups of 9 sheep, each group being assigned to one of the 4 experimental diets. The allocation was done on the basis of the animals' live weight and the mean fibre diameter of their wool obtained from the mid-side patch. The diets, live weights and diameter of wool fibre from the sheep in each of the treatment groups on the first day of experimental period are given in Table 13. The diets consisted of oaten chaff supplemented with different amounts of fresh or sun-dried duckweed. They were designed to be iso-energetic and

to provide a metabolisable energy intake (MEI) of 5.4 MJ (1.3 Mcal)/d. This MEI was expected to be just adequate to enable the sheep to maintain live weight (SCA, 1990). Diet 1 (OC) provided 700 g oaten chaff /d, and was estimated to provide about 9 g N/d. Diet 2 (630 g OC + 50 g sun-dried duckweed /d; OC + 50DW) and diet 4 (630 g OC + 1000 g fresh duckweed /d; OC + 1000DW) were expected to provide the same amounts of N and DM, with sun-dried or fresh duckweed (4.9 % DM; 33 % CP) providing an additional 2.6 g N/d, respectively. Diet 3 (540 g OC + 100 g sun-dried duckweed; OC + 100DW) would have duckweed that provided an additional 5.2 g N/d. When predicting MEI, it was assumed that the ME content of duckweed was 9.7 MJ (2.3 Mcal)/kg DM (Harris *et al.*, 1982).

At the end of the experimental period, on 11th March 1998, the sheep were weighed and wool was again clipped from the mid-side patches, and a third dye-band was placed in the wool close to the skin surface and below the previous two dye-bands. The animals were then put out onto pasture to allow time for the third dye-band to grow away from the skin surface. Dye-banded wool staples were removed from the animals on 27th April 1998 and the sheep were returned to pasture and allowed to graze as one group until the end of July 1998 when they were returned to the animal house in preparation for Experiment 6.2.

Table 13 Dietary treatments, live weights and wool fibre diameters of the treatment groups at the start of the experiment on 27th January 1998 (mean $\pm$ SEM, n = 9)

Diet	Ingredients	Live weight (kg)	Mean fibre diameter (μ m)
1	OC*	35.7 $\pm$ 1.1	17.8 $\pm$ 0.63
2	OC + 50DW**	37.5 $\pm$ 0.9	17.9 $\pm$ 0.59
3	OC + 100DW	39.2 $\pm$ 1.3	17.7 $\pm$ 0.76
4	OC + 1000DW	38.9 $\pm$ 1.3	18.1 $\pm$ 0.83

*OC = oaten chaff

**DW = duckweed

6.2.2.4 Laboratory analysis and calculation

a Feed analysis

DM content of feed was determined by drying a sample of that feed (air-dried) in a forced-draught oven at 105°C for 24 h. OM was estimated by burning the oven-dried feed sample at 600°C for 6 h in an ashing oven. DM and OM contents were determined by the difference. Total-N content finely ground feed sample (1 mm sieve) was determined using a N analyser (Leco FP 2000).

b Characteristics of wool

Rate of fibre elongation

The first dye-band (inserted on the 3rd December 1997) indicated the beginning of pre-experimental phase and the second (middle) dye-band (inserted on the 27th January 1998) marked the end of pre-experimental phase and the beginning of experimental phase. The third (last) band (inserted on the 11th March 1998) showed the end of experimental phase. The distance on the staple from the bases of two adjacent bands, divided by the number of days, gives the daily rate of fibre elongation. Thus, the distance from the base of the first dye-band to the base of the second band, and then from the base of the second band to the base of the third band indicated the length of the wool staple grown during the pre-experimental and experimental periods, respectively. Measurements were made using 5 randomly chosen wool staples from each sheep.

Fibre diameter and wool volume

Measurement and calculation of wool fibre diameter and wool volume were carried out using the procedures described in Chapter 3 (General Materials and Methods).

6.2.2.5 Statistical analysis

Intakes of nutrients and yields of greasy wool were subjected to analysis of variance. Differences in the rates of wool fibre elongation, in fibre diameter and wool

volume during the experimental phase were evaluated by analysis of covariance with adjustments for these same parameters determined in the pre-experimental phase.

6.2.3 Results

6.2.3.1 The edibility of duckweed

When the sheep were first brought into the animal house in, they were allowed to become accustomed to their new surroundings and to learn to eat oaten chaff and lucerne chaff as well as duckweed. The sheep were offered a basal diet of oaten chaff, but were encouraged to investigate their feed bins and their contents by placing some lucerne and common salt in the bins. The animals took about 4-5 days to become accustomed to eating all of these feed ingredients.

During the pre-experimental phase when duckweed was first introduced to the animals in either dried or fresh forms, there were occasional feed refusals when fresh duckweed was mixed in the diet, but the amounts refused were small and the duckweed containing diets were readily accepted within a few days. During the experimental phase, duckweed was completely ingested by the sheep each day. No clinical ill-effects associated with the ingestion of duckweed by the sheep were observed in this experiment.

6.2.3.2 Feed intakes

The intakes of nutrients and calculated digestible protein leaving the stomach (DPLS) are presented in Tables 14.

6.2.3.3 Live-weight change

The live weights of animals (Figure 30) were generally constant throughout the experiment. During the pre-experimental phase, the sheep lost live-weight from a mean of 39.1 kg on 1st December 97 to be 1.4 kg lighter on 26th January 98. During the experimental phase (26 Jan – 11 March 98), there was a slight tendency for animals in Diet OC+50DW to regain weight slightly (mean 0.8 kg; $p = 0.08$).

Table 14. Least-square means for intakes (g/d) of OM and CP based on analysis of ingredients, and estimated MEI (MJ (Mcal)/d) and the amounts (g/d) of digestible protein leaving the stomach during the experimental phase.

Nutrients	Diets			
	OC	OC+50DW	OC+100DW	OC+1000DW
OM (g/d)	644	624	611	630
ME* (MJ (Mcal)/d)	5.5 (1.3)	5.4 (1.3)	5.3 (1.3)	5.3 (1.3)
CP (g/d)	42.5	51.9	61.9	49.4
DPLS** (g/d)	27	29	32	28

*Estimated using published analysis (Harris *et al.*, 1982)

**Digestible protein leaving stomach estimated using *GrazFeed* (Freer *et al.*, 1997)

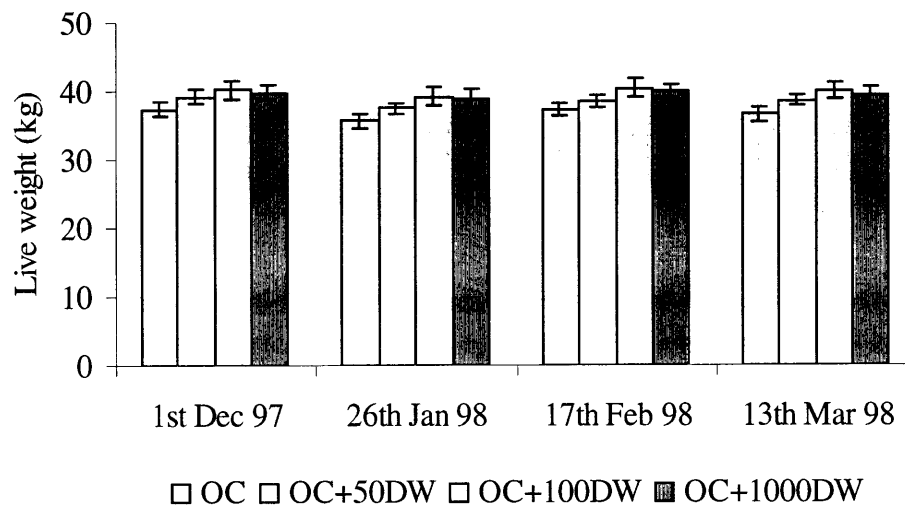


Figure 30. Live weights of animals (kg) offered the four experimental diets on different dates during the experiment.

6.2.3.4 Wool yield, fibre elongation and fibre diameter

Yield of greasy wool on mid-side patches (62 – 80 g/m² per d) and rate of wool fibre elongation (0.171 – 0.192 mm/d) were higher, before covariate adjustment, in the pre-experimental phase than in the experimental phase (46 – 60 g/m² per d and 0.157 – 0.177 mm/d, respectively). During the experimental phase, none of the adjusted wool parameters differed significantly between diets ($P > 0.05$; Table 15).

Table 14. Least-square means for intakes (g/d) of OM and CP based on analysis of ingredients, and estimated MEI (MJ (Mcal)/d) and the amounts (g/d) of digestible protein leaving the stomach during the experimental phase.

Nutrients	Diets			
	OC	OC+50DW	OC+100DW	OC+1000DW
OM (g/d)	644	624	611	630
ME* (MJ (Mcal)/d)	5.5 (1.3)	5.4 (1.3)	5.3 (1.3)	5.3 (1.3)
CP (g/d)	42.5	51.9	61.9	49.4
DPLS** (g/d)	27	29	32	28

*Estimated using published analysis (Harris *et al.*, 1982)

**Digestible protein leaving stomach estimated using *GrazFeed* (Freer *et al.*, 1997)

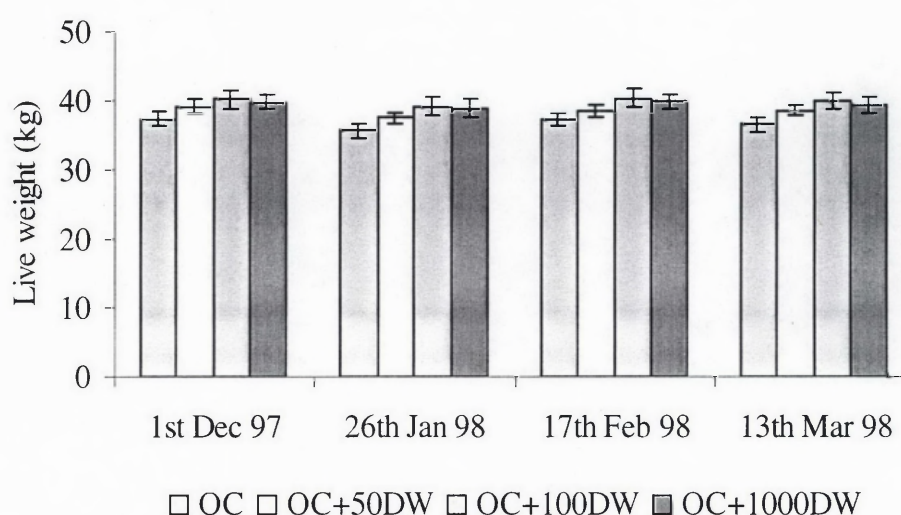


Figure 30. Live weights of animals (kg) offered the four experimental diets on different dates during the experiment.

6.2.3.4 Wool yield, fibre elongation and fibre diameter

Yield of greasy wool on mid-side patches ($62 - 80 \text{ g/m}^2$ per d) and rate of wool fibre elongation ($0.171 - 0.192 \text{ mm/d}$) were higher, before covariate adjustment, in the pre-experimental phase than in the experimental phase ($46 - 60 \text{ g/m}^2$ per d and $0.157 - 0.177 \text{ mm/d}$, respectively). During the experimental phase, none of the adjusted wool parameters differed significantly between diets ($P > 0.05$; Table 15).

Table 15. Least-square means ($\pm$ SEM) for yield of greasy wool (mid-side patch), rate of wool fibre elongation (dye-band), fibre diameter and fibre volume production in the experimental phase (adjusted for same characteristics in the pre-experimental phase)

Items	Diets			
	OC	OC+50DW	OC+100DW	OC+1000DW
Yield of greasy wool (g/m^2 per d)	53 ± 2.9	53 ± 3.0	57 ± 2.9	53 ± 3.0
Rate of fibre elongation ($\mu\text{m}/\text{d}$)	178 ± 5.4	160 ± 5.0	163 ± 5.4	169 ± 5.4
Fibre diameter (μm)	15.9 ± 0.24	15.8 ± 0.24	16.0 ± 0.24	15.7 ± 0.24
Fibre volume ($\mu\text{m}^3/\text{d} \times 10^{-3}$)	35.5 ± 1.6	33.9 ± 1.5	32.3 ± 1.6	31.7 ± 1.6
Fibre diameter, 2 mm snippet* (μm)	15.4 ± 0.42	15.0 ± 0.29	15.4 ± 0.75	15.5 ± 0.62

*Fibre grown in the last 10 d of the period of supplementation

6.3 Experiment 5.2: The Effects of Urea, Duckweed or Cottonseed Meal Supplementation on Wool Growth and Characteristics

6.3.1 Introduction

Results of Experiment 5.1 showed that supplementation of duckweed to the basal diet of oaten chaff did not affect the rate of wool growth and the characteristics of the wool fibre. It was thought, however, that this was due to the relatively small increase in the total digestible protein leaving the stomach ($1 - 5 \text{ g/d}$) between the control and the duckweed-supplemented animals. Such increase in the total digestible protein available for wool protein synthesis was unlikely to promote any observable differences in wool growth and characteristics.

The experiment reported below was designed with an objective to compare the effects on wool growth and characteristics of supplementing the experimental animals with the same quantities of total-N provided either as urea, cottonseed meal or duckweed. The hypothesis tested was that wool growth would be higher for the

animals supplemented with cottonseed meal or duckweed than for those supplemented with urea.

6.3.2 Materials and Methods

6.3.2.1 Experimental animals

Twenty-one of the 36 sheep used in the Experiment 5.1 were returned to the animal house and used in the current experiment. Management of the animals was generally the same as that applied in previous experiment (Experiment 5.1).

6.3.2.2 Duckweed

Sun-dried duckweed (*Spirodela punctata*) was obtained from the Bio-Tech Waste Management, Armidale.

6.3.2.3 Experimental procedures

a Pre-experimental phase

The sheep were individually penned indoors, and from 30th July 1998 were given a daily ration of oaten chaff (800 g) and lucerne chaff (50 g) mixed with urea (8 g) until 18th October 1998. A dye-band was placed next to the skin in a wool staple on the right side of the sheep on 12th August 1998.

b Experimental phase

On 19th October 1998, the animals were allocated to 3 groups of 7 sheep on the basis of their mean wool fibre diameter and live weight at the end of Experiment 5.1 as shown in Table 16. The sheep were assigned to receive, from 19th October to 22nd November 1998, one of three diets, viz. 800 g oaten chaff + 8 g urea (OC+U), 800 g oaten chaff + 60 g cottonseed meal (OC+CSM) or 800 g oaten chaff + 100 g sun-dried duckweed (OC+DW). These diets were intended to provide approximately similar amounts of total-N (17 - 18 g N/day) and of supplementary N (4 - 5 g N/day) in the form of urea, cottonseed meal or duckweed. Analyses of the ingredients in these diets are given in Table 17.

Table 16. Dietary treatments, mean $\pm$ SEM ($n = 7$) of live weight and wool fibre diameter of sheep in treatment groups at the start of pre-experimental period (19th October 1998).

No.	Diets	Live weight (kg)	Mean fibre diameter (μm)
1	OC+U	41.3 $\pm$ 1.72	16.2 $\pm$ 0.88
2	OC+CSM	38.7 $\pm$ 1.30	16.3 $\pm$ 0.65
3	OC+DW	38.6 $\pm$ 0.65	16.5 $\pm$ 0.94

Table 17. DM (% air-dry), OM and CP (% DM) contents of dietary ingredients.

Feed component	Chemical composition		
	DM	OM	CP*
Oaten chaff	91.6	91.3	10.4
Lucerne chaff	92.5	94.1	19.6
Duckweed (sun-dried)	88.5	84.5	35.5
Cottonseed meal	92.0	92.9	46.5

* total-N (% DM) $\times$ 6.25

A second dye-band was placed next to the skin below the first dye-band on the 21st October 1998. Rumen fluid was collected using a stomach tube on the 20th November 1998 at 0.5, 4.5 and 7.5 h after feeding and the pH of the rumen fluid was determined immediately. A sub-sample of rumen fluid (16 ml) was transferred into a wide-neck McCartney bottle containing 0.3 ml of 18 M H₂SO₄ and stored at -18°C pending analysis to determine ammonia-N concentration as described in section 3.3.4.2.

A third dye-band was inserted close to the skin surface below the second one at the end of the experimental period (22nd November 1998) after which animals were again put out on pasture to allow time for the dye-band to grow away from the skin. Dye-banded wool samples were removed from the sheep on 29th January 1999, at the time of this sampling, one sheep from the (OC+U) group and two from the (OC+DW) could not be found and associated data were treated as missing values for statistical analysis.

6.3.2.4 Laboratory analysis and calculation

Nutrients content of feed, technique in dye-banding, and analyses of wool fibre and calculations of volume and digestible protein leaving the stomach were carried out as for Experiment 5.1.

6.3.2.5 Statistical analysis

An analysis of covariate was applied to evaluate the effects of experimental diets on feed intakes, rates of fibre elongation, fibre diameter and wool volume with the corresponding pre-experimental data for the same parameter were used as the covariate. Treatment means for ammonia-N concentration in rumen fluid and rumen fluid pH were evaluated with an analysis of variance.

6.3.3 Results

6.3.3.1 Feed intakes

Intakes of DM, OM, CP and estimated metabolisable energy (adjusted for intakes in the pre-experimental phase) are presented in Table 18. Animals given Diet OC+U ingested less ($P<0.05$) DM, OM and ME than animals given Diets OC+CSM or OC+DW. CP intake of animals in Diet OC+DW was less ($P<0.05$) than that of animals in Diet OC+U or Diet OC+CSM.

6.3.3.2 Rates of wool fibre elongation and fibre diameter

The adjusted rate of wool fibre elongation in the experimental phase was lower ($p<0.05$) for the sheep supplemented with urea than for those offered duckweed or cottonseed meal (Table 19).

Table 18. Table 19 Least-square means for intakes of DM, OM and CP and estimated ME (MEI) and digestible protein leaving the stomach (DPLS) during the experimental phase.

Nutrients	Diets		
	OC+U	OC+CSM	OC+DW
DM (g/d)	723 ^a	789 ^b	797 ^b
OM (g/d)	659 ^a	721 ^b	722 ^b
CP (g/d)	102 ^a	102 ^a	93.0 ^b
Estimated* MEI (MJ (Mcal)/d)	6.5 (1.6) ^a	7.2 (1.7) ^b	7.2 (1.7) ^b
DPLS** (g/d)	42 ^a	50 ^b	50 ^b

Means within a row without a common superscript differ ($P < 0.05$)

*Estimated using published analysis (Harris *et al.*, 1982)

**Calculated using GrazFeed (Freer *et al.*, 1997).

Table 20. Least-square means $\pm$ SEM for rate of wool fibre elongation, fibre diameter and volume in the experimental phase (adjusted for the corresponding wool characteristics in the pre-experimental period).

Items	Diets		
	OC+U	OC+CSM	OC+DW
Fibre elongation ($\mu\text{m}/\text{d}$)	137 ± 6.4^a	155 ± 5.3^b	159 ± 6.1^b
Fibre diameter (μm)	16.2 ± 0.30^a	16.0 ± 0.28^a	16.7 ± 0.33^a
Fibre volume ($\mu\text{m}^3/\text{d} \times 10^{-3}$)	28.9 ± 1.6^a	31.3 ± 1.5^{ab}	35.1 ± 1.8^b

Means within a row without a common superscript differ ($P < 0.05$)

6.3.3.3 Rumen ammonia concentration and pH

Ammonia-N concentration in the rumen fluid increased from 96.3 to 176 mg N/l between 0.5 and 4.5 h after feeding and then decreased to 84.4 mg N/l at 7.5 h after feeding (Figure 31). The rumen ammonia-N concentrations did not differ ($P > 0.05$) between diets in samples collected at 0.5 h after feeding, but were higher ($P < 0.05$) for Diet OC+U at 4.5 and 7.5 h than for the other two diets, which did not differ ($P > 0.05$). Rumen fluid pH decreased significantly ($P < 0.001$) from 7.0 ± 0.03 h at 0.5 h after feeding, to 6.5 ± 0.05 at 4.5 h and 6.4 ± 0.04 at 7.5 h after feeding, but did not differ significantly between diets (Figure 32).

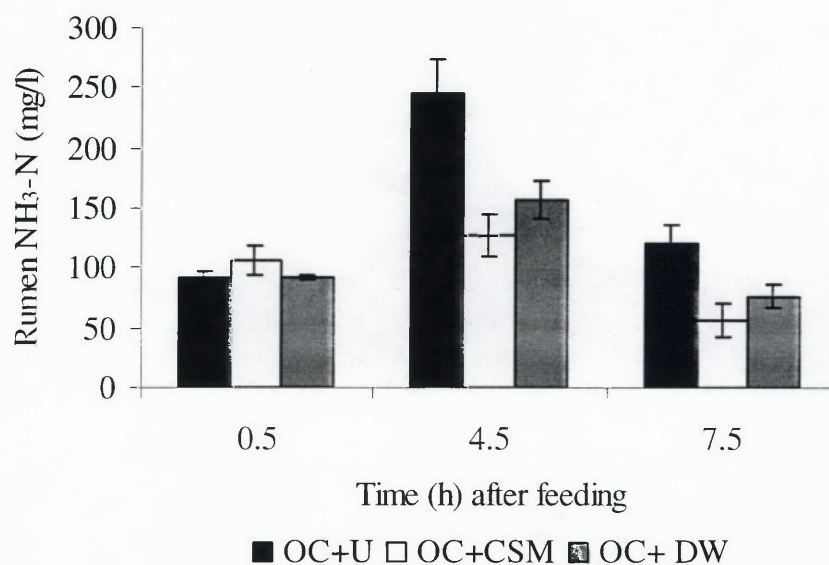


Figure 31. Rumen ammonia concentration (means $\pm$ SEM; mg N/l) of animals on the three different diets at 0.5, 4.5 and 7.5 h after feeding (119.1 ± 5.20 mg N/l).

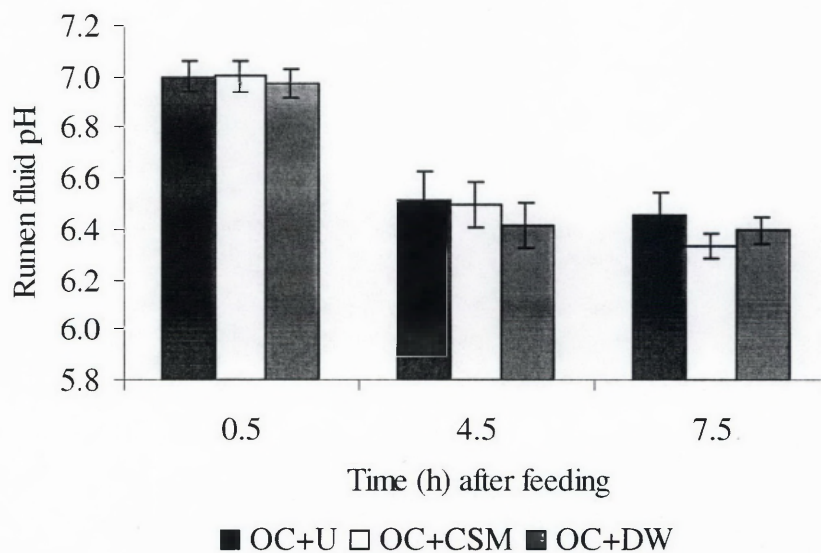


Figure 32. Rumen fluid pH (means $\pm$ SEM) of animals on the three different diets at 0.5, 4.5 and 7.5 h after feeding.

6.4 Discussion

These experiments demonstrated that duckweed, given as a supplement to a basal diet of oaten chaff in both fresh and sun-dried forms, was well accepted by the Merino sheep. No clinical ill-effects associated with the ingestion of duckweed were apparent in this experiment. When the sheep in Experiment 5.2 were re-introduced to the duckweed during the experimental phase, they recognised and immediately accepted the duckweed.

It is expected that animals will exhibit neophobia (fear of novelty) when first offered a food that they have not previously experienced (Domjan, 1977). Wong and McBride (1993) argue that flavor neophobia is a reason for animals not to ingest an unfamiliar feed that is known to be edible and non-toxic. These authors also suggested neophobia as a basis of an innate mechanism, which ensures that animals have the opportunity to learn from the post-ingestive consequences of eating novel and potentially toxic before being harmed by it. After the animals ingest small amounts of the new food and detect the metabolic consequences, they may form conditioned aversions or preferences for the food depending on whether the post-ingestive metabolic effects of the food ingredients are detrimental or beneficial (Tien *et al.*, 1999). The sheep in these experiments were observed to form a strong preference for duckweed after a short period of exposure, and it is therefore concluded that the beneficial nutritional properties of duckweed outweighed any toxic or other detrimental effects that may have been present.

Diets in Experiment 5.1 were formulated to provide a maintenance level of energy intake, and this was successfully achieved, as indicated by the relatively constant live weight of animals during the experiment. Under these conditions, it seemed likely that wool growth might be stimulated by an increase in the intestinal supply of amino acids when duckweed was included in the basal diet (Black *et al.*, 1973). However, despite duckweed-supplemented animals ingesting more CP than those receiving oaten chaff only, there were no significant effects of including duckweed in the diet on wool yield or characteristics.

The total amounts of amino acids available for intestinal absorption (derived from digestion of both microbial and dietary rumen-undegraded proteins) were predicted using the software model *GrazFeed* (Freer *et al.*, 1997) assuming that duckweed would be 80 % degradable in the rumen (Huque *et al.*, 1996) and oaten chaff 70 % degradable. *GrazFeed*, which uses equations from SCA (1990) predicted that, for sheep given oaten chaff only, 27.0 g/d total digestible protein would have passed out of the stomach, whereas in those offered oaten chaff supplemented with 50 or 100 g dried duckweed or 1 kg fresh duckweed, the values would have increased slightly to 29, 32 and 29 g/d, respectively. These values were similar to the *GrazFeed*-predicted maintenance requirement of about 30 g/d. An increase of 1-5 g/d in the amounts of total digestible protein leaving the stomach may have probably been too small to generate a detectable increase in wool growth in this experiment, especially as the additional protein may have been used in part to prevent tissue protein lost rather than to supply amino acids to the wool follicle.

The suggestion that the amounts of digestible CP leaving the stomach and potentially available for intestinal absorption (27 – 32 g/d) were insufficient to enhance a detectable wool growth is consistent with the continued decline of wool yield and fibre diameter observed throughout the experiment. Black *et al.* (1973) found that, under nutritional conditions that were closely similar to those applied in this experiment, wool production of fine-wool Merino sheep (initial body weight of 33 kg) declined from about 4 to 2 g/d over a period of 8 weeks during which they were given a complete diet by abomasal infusion that provided 5 MJ (1.2 Mcal)/d of gross energy and 20 g/d of CP.

Results of Experiment 5.2 showed that the rate of wool fibre elongation was higher when animals were given oaten chaff supplemented with cottonseed meal or duckweed than when they were supplemented only with urea. The estimated amount of digestible protein flowing to the small intestine of animals in Experiment 5.2 when they were supplemented with cottonseed meal or duckweed was 50 g/sheep per d while that of animals given urea was 40 g /sheep per d. Increasing rates of protein infusion (40 – 80 g protein/sheep per d) at the same rate of energy infusion, and in

nutritional conditions rather similar to this experiment, Black *et al.* (1973) found that the higher initial rates of wool growth (5-9 g wool/sheep per d) were just maintained or slightly increased over an 8-week period. Results of these experiments and those of Black *et al.* (1973) therefore suggest that wool growth is less responsive to an increase in intestinal protein supply if this supply is below that required to support live weight maintenance.

The rate of wool fibre elongation was higher in animals supplemented with cottonseed meal or duckweed than in those supplemented with urea, even though CP intake was lower for animals supplemented with duckweed than for those offered the other two diets. The faster rate of fibre elongation for animals supplemented with cottonseed meal and duckweed was probably because part of the protein in these supplements escaped intact from the rumen, increasing the intestinal availability of digestible CP above that by microbial cells passing from the rumen of sheep supplemented with urea. The predicted total digestible CP (of microbial and dietary origin) leaving the stomach in animals supplemented with cottonseed meal would be 50 g/d, assuming 60 % rumen degradability of cottonseed meal, and 50 g/d for sheep supplemented with duckweed, assuming 80 % rumen degradability, whereas for those supplemented with urea it would be 42 g/d. Based on these predictions, it is possible that the rumen degradability of the duckweed protein used in this experiment was lower than the assumed 80 % and perhaps closer to the 60 – 70 % as found in experiments reported in Chapter 5.

It is unlikely that MEI, which was also higher for animals supplemented with cottonseed meal and duckweed than for those receiving urea, contributed to the enhanced wool growth, as Black *et al.* (1973) and Reis *et al.* (1990) found that amino acid supply was the dominant factor affecting wool production and energy only played a minor role. Their studies showed that increasing gross energy intakes from 3.01 to 10.04 MJ/sheep per d (0.7-2.4 Mcal/sheep per d) in fine-wool Merino sheep stimulated wool growth only when the amount of protein infused into the abomasum was 100 g/d, but not when it was 20 or 60 g/d, *i.e.* there was no effect of energy when conditions were similar to those applied in these experiments.

It is concluded that the response in wool production found in Experiment 5.2 was likely primarily due to the enhanced supply of amino acids of dietary origin when duckweed or cottonseed meal rather than urea was used to supplement a basal diet of oaten chaff.

The lower concentration of rumen ammonia at 4.5 or 7.5 h after feeding sheep iso-nitrogenous amounts of duckweed and cottonseed meal in place of urea was probably a result of a slower rate of ammonia production from ruminal degradation of duckweed and cottonseed meal proteins relative to that from degradation of urea. Effects of pH, which can alter the rate of absorption of ammonia across the rumen wall (Siddons *et al.*, 1985), were unlikely to have generated differences in rumen ammonia concentration between diets in this experiment because there were no significant between-diet differences in the rumen pH. Degradation of dietary protein, intra-ruminal recycling of microbial-N and influx of endogenous N (including urea) all contribute to the ammonia present in rumen fluid, whereas microbial assimilation of ammonia for protein synthesis, outflow to the lower tract and absorption through the rumen wall account for ammonia disappearance (Nolan, 1993). Of these, however, degradation of dietary protein (influx) and microbial assimilation (removal) are often the most significant ammonia transactions in the rumen. Even though rumen ammonia concentrations were apparently affected by diet, the concentrations of rumen ammonia in all animals at 7.5 h after feeding (84.4–176.0 mg N/l) were above the minimum of 50 mg N/l thought to be required for optimum microbial protein synthesis (Satter and Slyter, 1974) or 80 mg/l needed for optimum feed digestion (Leng *et al.*, 1993). It therefore seems that the rumen ammonia concentration in the present study was not a major constraint to achieving optimum feed digestion or microbial protein synthesis.

Huque *et al.* (1996) reported that sun-dried duckweed proteins were extensively degraded in dacron bags placed in the rumen of bulls for 72 h (80 % for *Spirodela*; 87 % for *Lemna* and 94 % for *Wolfia*). If this had also been the case in the sheep used in Experiment 5.2, most of the protein present in duckweed would have been available to rumen microbes and only small amounts of amino acids would have been available from duckweed for absorption in the small intestine. Results, however,

showed that the concentrations of ammonia in the rumen of sheep supplemented with duckweed were similar to those supplemented with cottonseed meal. It therefore seems probable that, in this experiment, the resistance of proteins in the duckweed to degradation in the rumen was rather similar to that in cottonseed meal, which is considered to be a good source of escape protein for ruminants (Krysl *et al.*, 1987). The higher resistance of duckweed to ruminal degradation in this experiment compared with that of Huque *et al.* (1996) may be due to differences in (a) the residence time of duckweed in the rumen (perhaps considerably less than 72 h), (b) duckweed-drying conditions or (c) ruminal conditions between our sheep and their cattle. The *in sacco* method used in the study of Huque *et al.* (1996) also tends to overestimate the degradation of protein because all the materials that disappear from the bag are assumed to be completely degradable.

It can be concluded that the duckweed used in these experiments was readily eaten by sheep and that it was as effective, as a protein source for wool growth, as cottonseed meal – a supplement that is considered to be an excellent source of escape protein for ruminants (Leng *et al.*, 1983).