Chapter 6

A Comparison of Ammonia and Preformed Protein as a Source of Nitrogen for Microbial Growth in the Rumen of Sheep Given Oaten Chaff.

6.1 Introduction

Dietary protein ingested in the rumen is subjected to microbial attack depending upon its solubility and structure (Annison, 1956). Protozoa in the rumen ingest particulate protein, digest and degrade it intracellularly to smaller molecules (Mangan & West, 1977). Degradation of protein by bacteria (presumably also fungi) in the rumen occurs extracellularly (Cotta & Hespell, 1984) with the protein molecules being absorbed on the surface of the bacteria (Nugent & Mangan, 1981). Small peptides (<5 amino acids residue) and free amino acids are the important intermediates (Russell et al. 1991). These can be taken up and incorporated directly into microbial protein or fermented as an energy source for organisms in the rumen. With respect to the incorporation of peptides and amino acids, it is the main objective of any supplementation of dietary true protein or preformed protein (peptides and amino acids) in order to maximise efficiency of microbial cell synthesis in the rumen. In regards of the fermentation of peptides and amino acids, it is wasteful in terms of use of dietary true protein. Peptides are converted to free amino acids which undergo further deamination. Consequently, ammonia, VFA and CO_2 are the products of the protein fermentation (Annison, 1956).

Most *in vivo* studies have shown that microbial protein synthesis in the rumen appears to be increased with increasing ingestion of degradable protein (Redman et al.

1980 ; Sadik et al. 1990 ; Hoover & Stokes, 1991 ; Cecava et al. 1993). The degradable protein effect in the rumen could be due to provision of peptides and/or amino acids (Hungate, 1966 ; Bryant, 1970) or alternatively to increased ammonia (see Chapter 4 and 5) all of which may be required for microbial growth in the rumen. Irrespective of cultures and bacterial sources, however, a number of *in vitro* studies have shown on diets containing starch or sugar that there is a substantial increase in bacterial yields when a small proportion of peptides or amino acids or both peptides and amino acids is added to the cultures (Maeng et al. 1976 ; Maeng & Baldwin, 1976a,b ; Cotta & Russell, 1982 ; Russell, 1983 ; Russell & Sniffen, 1984 ; Argyle & Baldwin, 1989 ; May et al. 1993).

Nonetheless, ammonia and not peptides and free amino acids is the primary nitrogen source of microbial protein synthesis particularly that of fibrolytic bacteria (Bryant & Robinson, 1962). The optimum requirement of ammonia for maximum microbial synthesis in the rumen may be much higher than that previously suggested by Satter & Slyter (1974) of 50 mgN/l. This is now shown to be above 200 mgN/l of ammonia concentration in the rumen of forage-fed sheep (see Chapter 4 and 5). From studies with ^{15}N tracers of the dynamics of nitrogen in the rumen of sheep given a roughage based diet, 50-95 % of microbial nitrogen appears to be derived from ammonia in ruminal fluid (Pilgrim et al. 1970 ; Al-Rabbat et al. 1971 ; Nolan & Leng, 1972 ; Nolan & Stachiw, 1979 ; Neutze, 1985) and thus 5-50 % of the microbial nitrogen can be derived from nitrogenous compounds (such as peptides and amino acids) of feed and endogenous nitrogen, that have not passed through the ammonia pool in the rumen. To further increase microbial protein synthesis in the rumen, particularly when ruminal fluid ammonia concentrations are high, the addition of peptides and amino acids may be essential.

The research reported here was conducted to examine the effects of supplementation of preformed protein (peptides and amino acids) over a wide range of ammonia concentrations on rumen function, microbial mix and the efficiency of net microbial cell synthesis in the rumen of sheep given oaten chaff as a basal diet supplemented with urea or casein or both urea and casein.

6.2 Experiment 6-1: A Comparison of Urea and Casein as a Source of Nitrogen for Microbial Growth in the Rumen of Sheep.

6.2.1 Materials and Methods

6.2.1.1 Animals:

Twelve First Cross Merino x Border Leicester Wethers, $1^1/_2$-2 years of age, weighing 27-35 kg and with permanent rumen cannulas were held in individual metabolism crates in an animal house (see section 3.2.1).

6.2.1.2 Diets and Feeding:

Oaten chaff (0.8% N) from the same source and batch, was used as the basal diet throughout the study. To this was added 2% of a minerals mix (see section 3.2.2.2). The oaten chaff plus the additive and urea or casein were offered together at a restricted intake of 750 g/d. Urea solution was prepared by dissolving 7.5 or 21 g of urea in 135 ml of tap water and was sprayed onto the diets. Casein (45 or 135 g) was initially sprinkled over the diet following tap water (135 ml). After spraying, the mixtures were left for about 30 min and then thoroughly mixed. The ration was delivered in 24 equal portions at hourly interval by an automatic feeding machine.

6.2.1.3 Experimental Procedure:

The experiment was divided into two 21-d periods and the wethers allowed to become accustomed to the diets and metabolism crates for 2 w prior to commencement of the experiment. On the last day of the 2 w adaptation period, samples of ruminal fluid from each animal were taken at 9.00 h, 13.00 h and 17.00 h for analysis of NH_3-N (see section 3.8.2). The amounts of urea and casein used were then adjusted so that the ruminal fluid ammonia concentrations would stabilise at approximately 100 and 200 mgN/l. Within the experimental period, on days 1-16, the animals were left undisturbed and on days 17-21, they were subjected to the experimental procedures. On days 17-19, the daily urine voided by each animal was

collected (see section 3.2.6) for analysis of purine derivatives (see section 3.8.5). On day 20, samples of ruminal fluid were collected via the rumen fistula with a probe covered with a double layer of nylon stocking material placed in the rumen at 9.00 h, 13.00 h and 17.00 h. The samples were acidified (see section 3.2.4) for analysis of NH_3-N (see section 3.8.2) and VFA (see section 3.8.3). On day 21, 24 h *in sacco* digestibility of the diet was measured (see section 3.7).

6.2.1.4 Statistical Analysis

This experiment was divided into two 21-d periods according to a Half Change Over design. The wethers were allocated randomly (only in the first period) into 4 groups in order to receive each of the treatments composed of 2 levels of ruminal fluid ammonia arising from either urea or casein in the diet. To minimise variations in individual animal within the same level of ammonia, the changeover between urea and casein treatment groups was made at the end of the first period. The statistical significance of the data was analysed as described in section 3.9.

6.2.2 Results

6.2.2.1 Feed intake

The feed was delivered hourly and consumed by the animals within a few minutes of its presentation.

6.2.2.2 Effects of urea and casein supplements on NH_3-N, pH and 24 h *in sacco* digestibility

Concentrations of ruminal fluid ammonia did not differ between the urea and casein treatment groups ($p>0.05$) but increased with increasing levels of ingested urea or casein ($p<0.01$; Table 6-1).

The pH in ruminal fluid and 24 h organic matter digestibility *in sacco* in the rumen (OMDR) were not significantly different irrespective of sources of nitrogenous supplements ($p>0.05$; Table 6-1).

6.2.2.3 The patterns of VFA i n ruminal fluid

The molar proportion (%) c f acetic (C2), propionic (C3), butyric (C4) acids and the ratio of acetogenic (C2+C4) to glucogenic (C3) acids was affected with nitrogenous supplements ($p<0.05$; $p<0.01$) and that of isoacids (isobutyric and isovaleric acids) and valeric acid was significant difference between ingested urea and casein ($p<0.01$). The concentration of total volatile fatty acids (VFA) did not differ between treatments ($p>0.05$; Tabl e 6-2). The concentrations of isoacids tended to increase with increasing levels of ir gested casein but decreased with increasing urea intake (Figure 6-1).

6.2.2.4 Net microbial cell synthesis in the rumen

Urinary excretion of purine derivatives was increased with increasing levels of urea and casein intake ($p<0.01$) bu t it did not differ within the same levels of the nitrogenous supplements ($p>0.05$. Figure 6-1). The calculated efficiency of net microbial cell synthesis in the rume (gN/kg OMDR) was 26% greater for the higher levels of ingested urea and casein (se e Table 6-1).

Table 6-1. Effects of different levels of ammonia concentrations arising from urea (U) or casein (C) intake (g/d) on pH, 24 h organic matter digested *in sacco* in the rumen (OMDR), microbial-N outflow from the rumen (MCO ; gN/d) and calculated efficiency of net microbial cell synthesis in the rumen (ENMS ; gN/kg OMDR).

	Urea or Casein intake (gN/d)				
Parameters	U-3.2	C-6.2	U-9.5	C-18.5	S.E.
NH_3-N (mgN/l)	118^a	119^a	272^b	265^b	8.50
pH	6.66	6.59	6.61	6.60	0.06
OMDR (%)	58.1	57.5	57.9	57.1	0.06
MCO (gN/d)	8.1	8.2^a	10.0^b	10.0^b	0.52
ENMS (gN/kg OMDR)	20.6	21.0^a	26.0^b	26.3^b	1.40

Different superscripts in numbers or letters in the same row indicate the difference between treatments at $p<0.05$ or $p<0.01$, respectively.

Table 6-2. Effects of different levels of urea (U) or casein (C) intake on the profiles of volatile fatty acids in ruminal fluid (VFA).

Molar	Urea or Casein Intake (gN/d)				
Proportion	U-3.2	C-6.2	U-9.5	C-18.5	S.E.
Acetic (%)	72.2^a	71.9^a	72.1^a	67.6^b	0.52
Propionic (%)	17.4^1	$16.9^{1,a}$	$19.0^{2,b}$	17.2^1	0.40
Isobutyric (%)	0.5^a	1.4^b	0.4^a	2.3^c	0.11
Butyric (%)	$9.0^{1,a}$	$7.6^{1,b}$	$7.5^{1,b}$	8.2^1	0.26
Isovaleric (%)	$0.6^{1,a}$	$1.3^{2,b}$	$0.4^{1,a}$	2.7^c	0.16
Valeric (%)	0.5^a	0.8^b	0.5^a	2.0^c	0.06
(C2+C4)/C3	4.7^1	4.7^1	4.2^2	$4.4^{1,2}$	0.12
Total VFA (μm/ml)	67.8	73.7	76.4	65.4	4.11

Different superscripts in numbers or letters in the same row indicate the difference between treatments at $p<0.05$ or $p<0.01$, respectively.

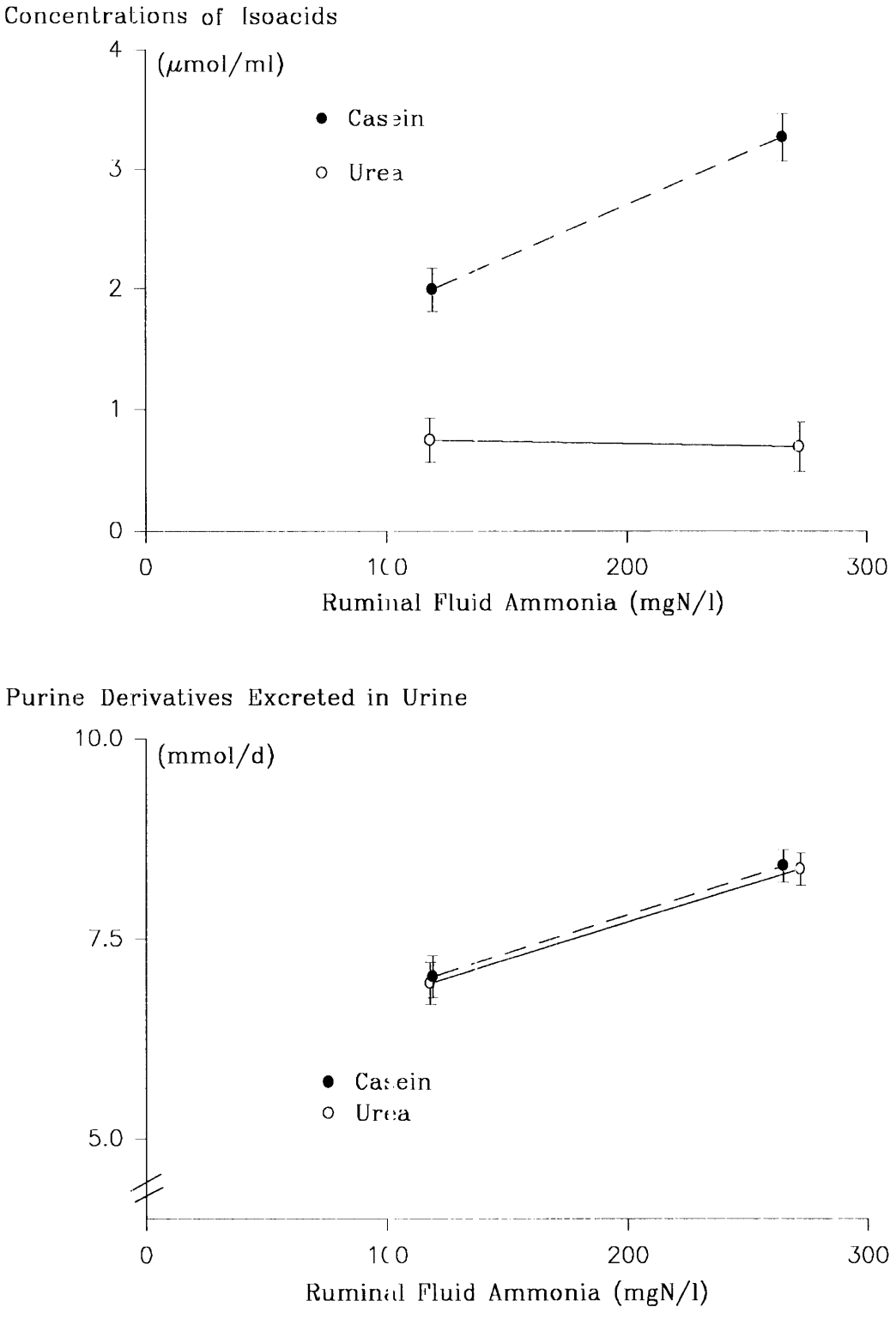

Figure 6-1. Effects of concentrations of ruminal fluid ammonia arising from urea or casein on concentrations of isoacids (isobutyric and isovaleric acids) and urinary excretion of purine derivatives.

6.3 Experiment 6-2: A Comparison of Urea and Urea plus Casein as a Source of Nitrogen for Microbial Growth in the Rumen of Sheep.

6.3.1 Materials and Methods

6.3.1.1 Animals:

Seven First Cross Merino x Border Leicester wethers, $2^1/_2$-3 years of age, weighing 30-34 kg and with permanent rumen cannulas were held in individual metabolism crates (see section 3.2.1 .

6.3.1.2 Diets and Feeding:

Oaten chaff (1.35% N) from the same source and batch was used as the basal diet throughout the study. To this was added 2% of a minerals mix (see section 3.2.2.2). Dietary intake of each animal was restricted at 800 g/d of the oaten chaff plus the minerals mix and urea or casein or both urea and casein. Urea solutions were prepared by dissolving 7.2, 14.4 and 21.6 g of urea in tap water (see section 3.2.2.1). The solution was sprayed onto the chaff whereas 25 g of casein was initially sprinkled over the oaten chaff following tap water (40 ml). After the sprinkling, the diets were left for about 20 min and then thoroughly mixed. The diet was delivered by an automatic feeding machine in equal portions each hour.

6.3.1.3 Experimental Procedure:

The study was divided into seven 21-d periods and the wethers were allowed to become accustomed to the diets and metabolism crates for 2 w prior to commencement of the experiment. Within the 21-d experimental period, the first 3-d was regarded as a transitional period, following 11-d allowed for adaptation period and over the last 7-d, intensive sampling was undertaken. On days 15-17, daily urine voided by each animal was collected into a container (see section 3.2.6) for later analysis of purine derivatives (see section 3.8.5). On day 18, rumen digesta was collected via a core sampling probe (see section 3.2.5). Bacteria in ruminal fluid and

particulate-microbes were then isolated (see section 3.3) for analysis of nitrogen (see section 3.8.1) and purine (see section 3.8.6) content. After harvesting bacteria in ruminal fluid, 40 ml of the supernatant was then added with 30% (w/v) $HClO_4$. This was then placed in ice for 10 min and centrifuged at 5000 g for 10 min. The upper phase was kept and stored at -20^0 prior to analysis of amino acids and peptides. On day 19, ruminal fluid was sampled via a probe covered with a double layers of nylon stocking material for total viable and cellulolytic bacteria counts by use of the agar roll tubes and cellulose broth tubes. On day 20, Cr-EDTA was injected into the rumen at 07.00 h. From 3-27 h after the injection, 10 samples of ruminal fluid were taken periodically (see section 3.2.4). Prior to acidification, samples were removed to a vial for enumeration of protozoa (see section 3.5) and for measurement of pH (see section 3.4). The rest was acidified (see section 3.2.4) for analysis of NH_3-N (see section 3.8.2), VFA (see section 3.8.3) and Cr (see section 3.8.4). On the last day, 24 h *in sacco* digestibility of the diet was assessed (see section 3.7). Fungal sporangia growth was assessed from the sporangial densities on oat leaf blades in nylon bags incubated for 24 h in the rumen (see section 3.6).

6.3.1.4 Chemical Analysis:

6.3.1.4.1 Analysis of free amino acds in the ruminal fluid

Prior to the analysis of free amino acids in the ruminal fluid, the supernatant was thawed and centrifuged at 5000 g for 10 min and the upper phase was extracted. Free amino acid concentrations were determined according to the analysis of physiological samples of the Pico-Tag method (Cohen et al. 1989) using a dual-pump HPLC system (Waters associates, USA) with an automatic injector (WISP, model 710B) connected with the Pico-Tag column (3.9 x 300 mm, Waters, Millipore Corp., MA, USA) and L-norleucine (Sigma, MO, USA) was used as an internal standard.

6.3.1.4.2 Analysis of peptides in the ruminal fluid

Prior to the analysis of peptides in the ruminal fluid, the supernatant was thawed and centrifuged at 5000 g for 10 min and the upper phase was extracted. Peptide concentrations were assayed using fluorescamine similar to the procedure used

by Perrett et al. (1975), Nisbet & Payne (1979) and Broderick & Wallace (1988). In this study, the equimolar mixture of di- and tetra-alanine (Sigma, MO, USA) was used as a standard. Fluorescence was measured using a fluorescence spectrophotometer (Ferkin-Elmer, 1000), with excitation at 396 nm and emission at 479 nm.

6.3.1.4.3 Total viable count

Fresh ruminal fluid (0.1 ml) was used as inoculum and serially diluted in a bicarbonate buffer containing L-α cystine-HCl as a reducing agent. Medium 98-5 agar roll tubes (Bryant & Robinson, 1961) were used for the determination of the densities of total viable (most probable numbers) in ruminal fluid.

6.3.1.4.4 Cellulolytic bacteria count

Fresh ruminal fluid (0.1 ml) was used as inoculum and serially diluted in a bicarbonate buffer containing L-α cystine-HCl as a reducing agent. Cellulose broth tubes (Halliwell & Bryant, 1963) were used for the determination of the densities of cellulolytic bacteria (most probable numbers) in ruminal fluid.

6.3.1.5 Statistical Analysis:

A 7 x 7 Latin Square design with 7 animals and 7 periods was used for the analysis of variance. The statistical significance of the data was analysed as indicated in section 3.9.

6.3.2 Results

6.3.2.1 Feed intake

The feed was delivered hourly and consumed by the animals within a few minutes of its presentation.

6.3.2.2 Effects of urea and casein or both urea and casein supplements on NH_3-N, pH, 24 h *in sacco* digestibility and the kinetics of ruminal fluid

Concentrations of ruminal fluid ammonia were significantly increased with increasing levels of nitrogenous supplements ($p<0.01$) but they did not differ within the same levels of nitrogen intake irrespective of nitrogen sources ($p>0.05$; Table 6-3).

The pH in ruminal fluid and 24 h organic matter digestibility *in sacco* in the rumen (OMDR) was not different ($p>0.05$) regardless of levels and sources of nitrogen intake (Table 6-3).

Of the kinetics of ruminal fluid, only rumen volume was significantly smaller when the highest level of urea or urea plus casein was supplemented ($p<0.01$; Table 6-3).

6.3.2.3 The pattern of VFA in ruminal fluid

Of the molar proportion (%) of each volatile fatty acids (VFA), only butyric acid and the concentration of total VFA were not affected by increasing levels of nitrogenous supplements ($p>0.05$). The molar proportion (%) of isoacids (isobutyric and isovaleric acids) and valeric acid significantly increased with ingested casein ($p<0.01$; Table 6-4). The tendency of concentrations of isoacids with different levels and sources of nitrogenous supplements is shown in Figure 6-2.

6.3.2.4 Microbial mix

Both concentrations of total viable and cellulolytic bacteria in ruminal fluid were affected by nitrogenous supplements ($p<0.01$) and tended to increase with increasing levels of nitrogenous supplements irrespective of the nitrogen sources (Table 6-5).

The number of fungal sporangia growth on oat leaf blades decreased with increasing levels of urea intakes ($p<0.01$) but it was not affected when casein was supplemented ($p>0.05$; Table 6-5).

The density of small *Entodinium sp.* in ruminal fluid was significantly decreased with increasing intake of urea or urea plus casein but that of *Holotrich sp.* was greater

when casein was presented ($p<0.01$; Table 6-5). However, the density of large *Entodinium sp.* was not affected by either levels or sources of nitrogen intakes ($p>0.05$; Table 6-5).

6.3.2.5 Purine:total-N ratio of rumen microbes and net microbial cell synthesis in the rumen

In both fluid- and particle-associated bacteria, the purine:total-N ratio was not affected by increasing levels nor sources of the nitrogenous supplements ($p>0.05$; Table 6-6).

Urinary excretion of purine derivatives from sheep with the ruminal fluid ammonia concentration being in excess of 200 mgN/l was significantly greater than that from other sheep ($p<0.01$; Figure 6-2) and the calculated efficiency of net microbial cell synthesis in the rumen (gN/kg OMDR) was also 27% greater in relation to that in the rumen of the control sheep ($P<0.01$) irrespective of nitrogen sources (Table 6-6).

6.3.2.6 Concentrations of free amino acids and peptides in ruminal fluid

Valine, tyrosine, isoleucine and leucine (essential amino acids ; EAA) and alanine, glycine, serine, cysteine, aspartic and glutamic acids (non essential amino acids ; NEAA) were consistently found in ruminal fluid. The concentration of EAA in ruminal fluid was not affected ($p>0.05$) but tended to increase with increasing levels of the nitrogenous supplements (Table 6-6). Both concentrations of NEAA and total amino acids (TAA) in ruminal fluid were significantly greater when casein was the major N source ($p<0.05$) and the concentrations increased with increasing levels of urea intake (Table 6-6).

The concentration of peptides in ruminal fluid was also significantly greater when casein was supplemented ($p<0.05$) and it increased with increasing levels of urea intake (Table 6-6).

Table 6-3. Ammonia concentrations, pH, organic matter digestibility *in sacco* (24 h) in the rumen (OMDR) and rumen volume (RV), outflow rate (RF) and fractional turnover rate (RT) in sheep given oaten chaff as a basal diet (Control) with casein (C ; 25 g/d) or different levels of urea (U1, U2, U3 ; 7.2, 14.4 and 21.6 g urea/d, respectively) or both urea and casein (U1+C, U2+C) supplements.

	Treatments							
Items	Control	U1	C	U2	U1+C	U3	U2+C	S.E.
NH_3-N (mgN/l)	54.5^{a}	112.1^{b}	102.0^{b}	169.9^{c}	158.7^{c}	258.9^{d}	236.7^{d}	9.82
pH	6.59^{1}	6.58^{1}	6.57^{1}	6.39^{1}	6.59^{1}	6.66^{2}	$6.63^{1,2}$	0.02
OMDR (%)	59.5	59.3	59.6	59.1	59.1	59.4	59.2	0.33
RV (l)	$6.5^{1,a}$	$6.4^{1,a}$	$6.5^{1,a}$	$6.2^{1,2}$	6.0^{2}	$5.8^{2,b}$	$5.8^{2,b}$	0.16
RF (l/d)	13.4^{1}	$13.3^{1,2}$	13.4^{1}	13.4^{1}	$12.9^{1,2}$	12.3^{2}	$12.8^{1,2}$	0.37
RT (/d)	2.16	2.12	2.09	2.16	2.15	2.14	2.20	0.07

Different superscripts in numbers or letters in the same row indicate the difference between treatments at $p<0.05$ or $p<0.01$, respectively.

Table 6-4. The patterns of volatile fatty acids (VFA) in ruminal fluid in sheep given oaten chaff as a basal diet (Control) with casein (C ; 25 g/d) or different levels of urea (U1, U2, U3 ; 7.2, 14.4 and 21.6 g urea/d, respectively) or both urea and casein (U1+C, U2+C) supplements.

% Molar	Treatments							
proportion	Control	U1	C	U2	U1+C	U3	U2+C	S.E.
Acetic	$72.1^{1,4,a}$	$71.7^{1,2,4,a}$	$70.4^{3,b}$	$72.4^{1,a}$	$71.3^{4,5}$	$71.1^{2,3,5,b}$	$70.7^{3,5,b}$	0.30
Propionic	17.5^{a}	18.0^{a}	18.0^{a}	17.6^{a}	17.5^{a}	19.4^{b}	17.6^{a}	0.24
Isobutyric	0.6^{a}	0.6^{a}	1.2^{b}	0.5^{a}	1.2^{b}	0.4^{a}	1.1^{b}	0.05
Butyric	8.6	8.6	8.3	8.3	8.0	8.0	8.5	0.22
Isovaleric	0.7^{a}	0.6^{a}	1.2^{b}	0.6^{a}	1.2^{b}	0.5^{a}	1.1^{b}	0.04
Valeric	0.5a	0.5a	0.9b	0.5a	0.9b	0.5a	0.9b	0.02
(C2+C4):C3	$4.6^{1,a}$	$4.5^{1,2,a}$	$4.4^{2,a}$	$4.6^{1,a}$	$4.5^{1,2,a}$	4.0^{b}	$4.5^{1,2,a}$	0.08
Total VFA (μm/ml)	$77.3^{1,a}$	82.8^{2}	82.6^{2}	$80.5^{1,2}$	84.0^{b}	$79.4^{1,2}$	$79.9^{1,2}$	1.63

Different superscripts in numbers or letters in the same row indicate the difference between treatments at $p<0.05$ or $p<0.01$, respectively.

Table 6-5. Total viable count (TVC) and cellulolytic bacteria count (CBC), fungal sporangia growth on leaf blade, small and large *Entodinium sp.*(SEn and LEn), *Holotrich sp.* (Hol) and protozoa (Ptz) in ruminal fluid in sheep given oaten chaff as a basal diet (Control) with casein (C ; 25 g/d) or different levels of urea (U1, U2, U3 ; 7.2, 14.4 and 21.6 g urea/d, respectively) or both urea and casein (U1+C, U2+C) supplements.

Microbes in ruminal fluid	Treatments							
	Control	U1	C	U2	U1+C	U3	U2+C	S.E.
TVC(10^{-10}/ml)	$6.96^{1,2,a}$	$6.50^{1,a}$	$6.99^{1,2,a}$	$8.01^{2,3}$	$7.49^{1,2}$	$9.26^{3,b}$	$9.20^{3,b}$	0.49
CBC (10^{-8}/ml)	1.21^{a}	1.30^{a}	1.35^{a}	1.67^{b}	1.67^{b}	1.82^{b}	1.81^{b}	0.08
Fungi (/mm^2)	47^{a}	46^{a}	31^{b}	32^{b}	31^{b}	22^{c}	33^{b}	1.28
SEn (10^{-5}/ml)	3.1^{a}	2.9^{a}	2.4^{a}	2.3^{a}	2.3^{a}	1.1^{b}	1.3^{b}	0.13
LEn (10^{-2}/ml)	$4.5^{1,a}$	$5.7^{1,2,3}$	$6.2^{1,2,3}$	$5.3^{1,2}$	$7.2^{2,3}$	$4.9^{1,2}$	$7.8^{3,b}$	0.78
Hol (10^{-3}/ml)	4.5^{a}	$4.0^{1,a,c}$	7.6^{b}	$3.7^{a,c}$	7.0^{b}	$2.8^{2,c}$	7.0^{b}	0.36
Ptz (10^{-5}/ml)	3.20^{a}	2.96^{a}	2.44^{a}	2.36^{a}	2.36^{a}	1.11^{b}	1.32^{b}	0.13

Different superscripts in numbers or letters in the same row indicate the difference between treatments at $p<0.05$ or $p<0.01$, respectively.

Table 6-6. Essential (EAA), non-essential (NEAA) and total amino acids (TAA), peptides in ruminal fluid, purine:total-N ratio of bacteria in ruminal fluid (F) and particle-associated microbes (P), microbial-N outflow from the rumen (MCO ; gN/d) and calculated efficiency of net microbial cell synthesis in the rumen (ENMS ; gN/kgOMDR) in sheep given oaten chaff as a basal diet (Control) with casein (C ; 25 g/d) or different levels of urea (U1, U2, U3 ; 7.2, 14.4 and 21.6 g urea/d, respectively) or both urea and casein (U1+C, U2+C) supplements.

Parameters	Treatments							
	Control	U1	C	U2	U1+C	U3	U2+C	S.E.
EAA (nM)	12^{1}	$9^{1,a}$	$30^{1,2}$	$10^{1,a}$	$39^{1,2}$	23^{1}	$59^{2,b}$	1.90
NEAA (nM)	$62^{1,a}$	82^{1}	$86^{1,2}$	$76.^{1}$	$121^{2,b}$	$98^{1,2}$	$120^{2,b}$	12.9
TAA (nM)	$74^{1,a}$	91^{1}	$116^{1,2}$	$86^{1,a}$	160^{2}	$121^{1,2}$	$179^{2,b}$	15.2
Peptides(μM)	58.6^{1}	70.5^{1}	100.2^{2}	55.3^{1}	137.6^{2}	51.6^{1}	155.3^{2}	17.2
F-purine:total-N	0.177	0.169	0.177	0.180	0.174	0.172	0.168	0.02
P-purine:total-N	0.151	0.155	0.154	0.156	0.152	0.153	0.154	0.02
MCO (gN/d)	9.4^{a}	9.9^{a}	10.3^{a}	10.0^{a}	10.4^{a}	12.2^{b}	11.6^{b}	0.39
ENMS (gN/kgOMDR)	22.3^{a}	23.2^{a}	24.2^{a}	23.6^{a}	24.5^{a}	28.6^{b}	27.9^{b}	0.94

Different superscripts in numbers or letters in the same row indicate the difference between treatments at $p<0.05$ or $p<0.01$, respectively.

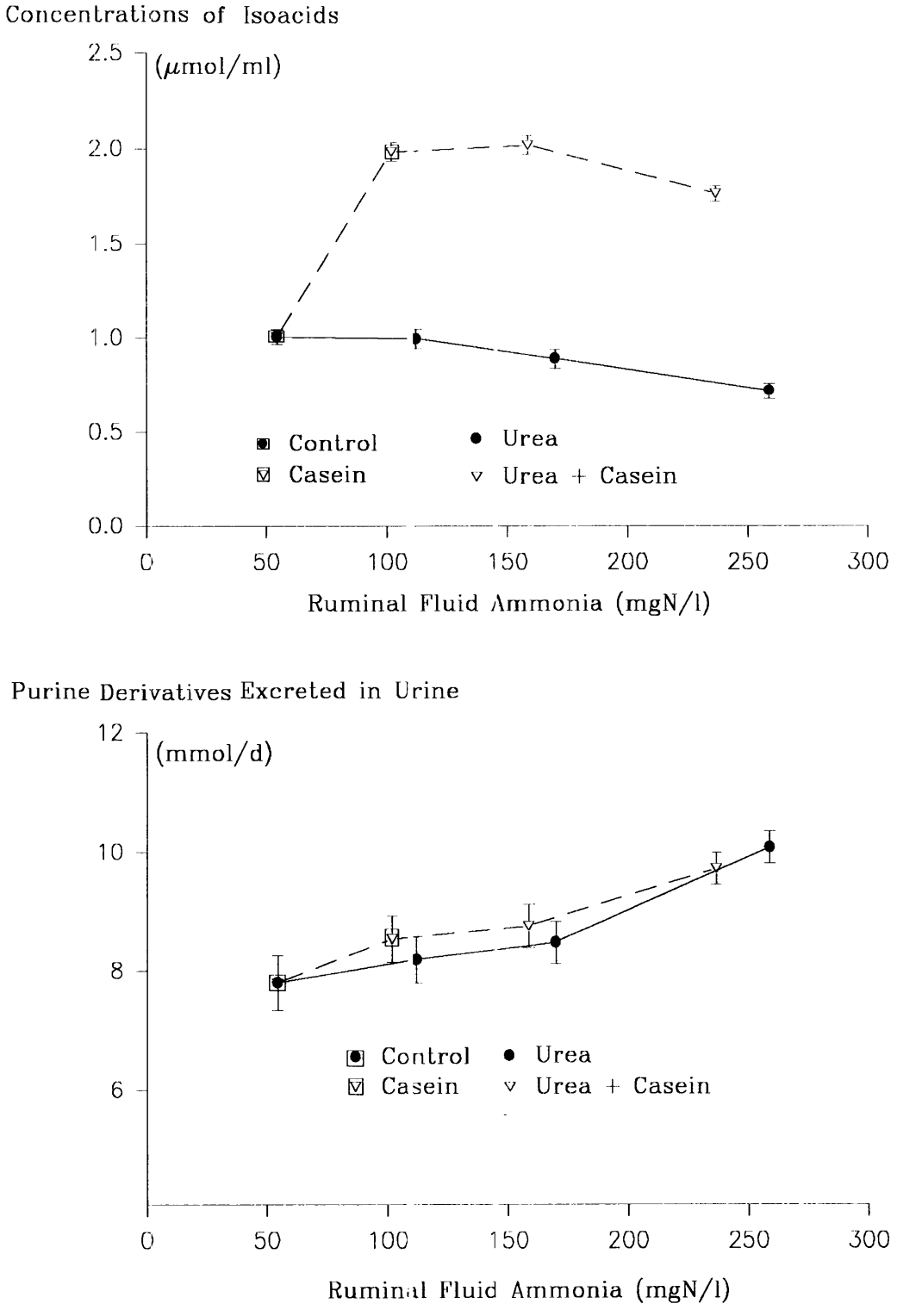

Figure 6-2. Effects of concentrations of ruminal fluid ammonia arising from urea or casein or urea plus casein on concentrations of isoacids (isobutyric and isovaleric acids) and urinary excretion of purine derivatives.

6.4 Discussion

It was apparent that at ruminal fluid ammonia concentrations above 200 mgN/l, there was a substantial increase in net microbial cell synthesis in the rumen of sheep (see Chapter 4). This has been argued to be a result of a reduction in the lysis of microbial cells within the rumen rather than a higher specific growth rate (as indicated by purine:total-N ratio) of rumen microbes. However, it may also argued that on an oaten chaff diet the availability of peptides and amino acids may have been insufficient to meet the requirements of apparent increased density of microbes in ruminal fluid. Therefore microbial growth efficiency in the rumen of sheep on diets low in protein and high in NPN could be further increased by supplying extra sources of peptides and amino acids from degradable protein. One of the objectives of the present study was to investigate the efficiency of net microbial cell synthesis in the rumen with increasing levels of ruminal fluid ammonia concentrations in the presence or the absence of added dietary degradable protein (the experiment 6-1). Ruminal fluid ammonia was increased by supplementation with urea or casein or urea plus casein so as to be able to compare urinary excretion of purine derivatives when extra peptides and amino acids were present in the rumen at different ammonia concentrations (the experiment 6-2) in sheep given oaten chaff as a basal diet.

Casein was a source of peptides and amino acids supplemented in this study because of its ready solubility and degradability in the rumen. Although *Holotrich sp* may be able to extracellularly coagulate soluble protein in ruminal fluid which then is ingested by themselves or by *Entodiniomorphid sp* (Onodera, 1990), the numbers of protozoa in the rumen appeared to decrease with increasing the solubility of dietary protein (Michalowski, 1989). Bacteria (Annison, 1956 ; Wright, 1967) and fungi (Orpin & Greenwood, 1986 ; Gulati et al. 1989) in the rumen are expected to be more capable of taking up soluble peptides and amino acids particularly at a high ruminal fluid ammonia concentration. Therefore, any response of net microbial cell synthesis to soluble protein in the rumen is expected to be mostly contributed by bacterial fraction.

In order to be able to feed to ensure ruminal fluid ammonia concentrations of approximately 100 and 200 mgN/l n the first experiment, sheep were fed estimated amounts of urea and casein and ruminal fluid samples were taken and ammonia concentrations were assessed. The amounts of dietary urea and casein used were then adjusted to ensure that the level of ammonia was within ±10%. The urea or casein was sprinkled onto the oaten chaff with other additives and given in equal portions at hourly interval to the animals. To provide variable levels of ammonia with a small quantity of peptides and amino acids in the rumen, the following experiment was designed using three isonitrogenous diets based on mixtures of urea and casein.

From statistical analysis of the repeat measurement of a dietary treatment over a period of time in both experiments, there were no differences in ammonia and VFA concentrations and pH in ruminal fluid. This indicates that the dietary urea and casein were well mixed in the feed and the organisms in the rumen of the animals were in a steady-state.

6.4.1 Ingested protein and digestion and the kinetics of digesta in the rumen

Increased digestion of dry matter in the rumen and therefore feed intake (partly influenced by increasing digestibility ; see Oldham, 1984) often occurs as a result of supplementation of dietary protein that enhances ruminal fluid ammonia concentrations (see Van Gylswyk, 1970 ; Hoover, 1986 ; Coomer et al. 1993 ; Keery et al. 1993). With the exception of supplementation of fish meal, the digestibility of fibre in a diet (Van Gylswyk, 1970 ; McAllan & Smith, 1983 ; Sultan et al. 1992) particularly at 1 h post-feeding (Grummer & Clark, 1982) appears to increase with increasing degradability of dietary protein in the rumen. The effects on digestibility may be through provision of peptides, amino acids, ammonia, isoacids and some other growth factors supplied by the protein meal in the rumen. These have all been suggested to be essential and required for microbial growth depending upon the mix of rumen microbes (Hungate, 1966 ; Bryant, 1970 ; Van Gylswyk et al. 1992). However, it has been reported that peptides and amino acids added to the rumen appear to give no benefit of organic matter digested in the rumen over urea added regardless of diet (Redman, et al.

1980 ; Cruz Soto et al. 1994 ; Fujimaki et al. 1994). The present study also strongly supported the same concept which suggests that efficient fermentation and digestion in the rumen that is supplied with all growth factors other that nitrogen is dependent primarily on the concentration of rumenal fluid ammonia over a 24 h period (see Table 6-1).

There appears to have been a reduction in digestion in the rumen at increasing levels of feed intake (Staples et al. 1984 ; Colucci et al. 1982). Feed intake, rumen digestion, salivary secretion and retention time of the feed in the rumen, all affect the kinetics of rumen digesta. In general, increasing feed intake results in increasing pools of rumen digesta (see Owens & Goetsch, 1986) and the opposite effects would be expected with increasing the digestibility in the rumen at constant feed intake (Colucci et al. 1982). Feed intake and organic matter digested in the rumen were similar, when ammonia was provided by either urea or casein and therefore it may be assumed that added peptides and amino acids did not influence digestion, microbial growth and the outflow rate of fluid and digesta (see Table 6-3). The concept is strongly supported by results reported by Fujimaki, et al. (1994) and Cruz Soto et al. (1994). This indicates that ammonia concentrations appear to have a direct effect on the kinetics of rumen digesta and the presence of degradable true protein even at a low ammonia concentration appears to have no effect on fermentative digestion.

6.4.2 Ingested protein and rumen fermentation

Degradable protein in the rumen undergoes hydrolysis and deamination of amino acids to form ammonia, VFA and CO_2 (see Annison, 1956). Regardless of populations of rumen microbes and types of basal diets, the proportion of individual VFA in ruminal fluid is affected by the amino acid profiles of the protein that are deaminated in the rumen (Wohlt et al. 1976). From C^{14}-labelled glycine, 90 % of radioactivity in total VFA was recovered in acetic acid (Wright & Hungate, 1967b). Equimolar of acetic and butyric acids was observed from lysine fermentation (Dohner & Cardon, 1954). However, it is generally accepted that isoacids (isobutyric and isovaleric acids) are derived from valine, leucine and isoleucine (see Van Den Hende et al. 1963 ; Allison, 1970).

A number of papers have reported that isoacids appear to be essential and required by fibrolytic bacteria in the rumen (Van Gylswyk, 1970 ; Hume, 1970a ; Russell & Sniffen, 1984) and they can be incorporated into the microbial biosynthesis as branched chain amino acids (Bryant, 1973). However, an increase in isoacids arising from supplemental protein (Ciszuk & Eriksson, 1973 ; Perdok & Leng, 1990) over a range of ammonia concentrations from 102 to 237 mgN/l did not appear to influence the net microbial cell synthesis in the rumen (see Figure 6-1 and 6-2) as measured by urinary excretion of purine derivatives. This indicates that isoacids did not limit microbial cells synthesis on these diets even though the levels derived from the protein in the oaten chaff were extremely low.

6.4.3 Protein degradation and concentrations of ruminal fluid ammonia

Protein catabolism by micro-organisms in the rumen commences with microbial proteases, followed by membrane transportation of small peptides and free amino acids (bacteria and fungi but not protozoa) and further deamination or fermentation of free amino acids.

Although the rate of protein degradation in the rumen and the solubility of dietary protein appear to have a positive correlation (Hungate, 1966), solubility is not always a valid criterion for degradation rate and with some protein its structure is important. Soluble albumin containing high levels of disulphide bonds is relatively slowly degraded (Annison, 1956). On the other hand, some apparently insoluble oilseedcakes are relatively highly degraded (Tamminga, 1983). Therefore, the rate of dietary protein breakdown in the rumen is most likely dependent upon its solubility as well as its structure.

Various species of protozoa, bacteria and fungi in the rumen are all responsible for proteolysis of ingested protein in the rumen (Nolan, 1993). Proteolytic activity of bacteria in the rumen may be more active for soluble protein, particularly fraction 1 leaf proteins (Nugent & Mangan, 1981) where the major soluble protein is in the chloroplasts of green leaves (Lyttleton & Ts'o, 1958). Proteolytic activities of

protozoa and fungi are apparent for insoluble particulate protein (Wallace & Munro, 1986 ; Onodera, 1990).

Proteolytic enzymes of rumen microbes are mostly periplasmic-bound or associated with extracellular capsular materials chrymotrypsin and trypsin-like proteases activity (Cotta & Hespell, 1984) and there appears to be a small proportion found in cell-free ruminal fluid (Nugent & Mangan, 1981 ; Brock et al. 1982). Little is known of the mechanisms of proteolysis in the rumen. Degradation of dietary protein in the rumen generally gives rise to polypeptides which are further cleaved into smaller molecules. Dipeptides appear to be the predominant residues of oligopeptides hydrolysed by peptidases activity of rumen microbes (Wallace & McKain, 1989). Therefore, the dipeptidyl peptidases mechanism (aminopeptidase and carboxypeptidase) would be the major process of polypeptides breakdown possessed by proteolytic or peptinolytic organisms in the rumen (Wallace & McKain, 1989). Unlike that by protozoa, the breakdown of oligopeptides by bacteria in the rumen is more likely to occur extracellularly because molecules no larger than pentrapeptides equivalent to 650 daltons can be transported through the porin of bacterial membrane (Payne, 1980).

In general, there is a very low concentration of peptides and free amino acids in ruminal fluid of animals on normal diets (Annison, 1956 ; Wright & Hungate, 1967a) which accords with a result shown in Table 6-6. This is due to the rapid uptake of the substrates by organisms in the rumen (Annison, 1956 ; Wright, 1967) but not the rapid absorption across the rumen wall (Annison, 1956). However, peptides and amino acids can be transiently accumulated in ruminal fluid post-feeding in ruminants given soluble and highly degradable protein in the rumen (Annison, 1956 ; Chen et al. 1987 ; Broderick & Wallace, 1988). A similar result was observed in this study (see Table 6-6). The rates of proteolysis (Nugent & Mangan, 1981 ; Tamminga, 1983) and uptake (Chen et al. 1987) of peptides and amino acids are hypothesised to be the rate-limiting step in protein degradation in the rumen. But little is know of the regulation of activity of the periplasmic-bound protease as well as the transportation of peptides and amino acids across the membrane of rumen microbes (bacteria and fungi).

From the available information on the degradation of ingested protein, it is apparent that the proteolytic activity of rumen microbes and the transportation of peptides and amino acids across the microbial membrane may be regulated by the end-products mechanism.

Peptides absorbed by bacteria can be rapidly hydrolysed to amino acids (Pittman et al. 1967), but the intracellular composition of the amino acids of rumen bacteria is fairly constant and is not influenced by the composition of amino acids of the dietary protein (see Purser, 1970 ; Storm & Orskov, 1983 ; Kobayashi et al. 1993). On the other hand, rumen microbes are able to synthesise *de novo* required amino acids (Allison, 1969) and they are unlikely to over produce them (Umbargen, 1969). However any excess could be released into the media in order to maintain the intracellular osmotic pressure (Pittman et al. 1967) or to maintain the intracellular metabolites against a concentration gradient across cell membrane. In the continuous culture of strained ruminal fluid, the inhibition of proteolysis of soluble protein was observed when free amino acids were added to the media (Veresegyhazy et al. 1993). In the same study when various VFA concentrations were increased, there was increased degradation of amino acids. A slight decrease in proteolytic activity was also demonstrated with a relatively high concentration of ammonia in a continuous culture of strained ruminal fluid (Veresegyhazy et al. 1993) and in a batch culture of *Butyrivibrio fibrisolvens* (Cotta & Hespell, 1986). These could suggest that the concentration of intracellular amino acids in rumen microbes may be a repressor of the regulatory phenomena of proteolytic activity in the rumen.

Rates of uptake as well as metabolism of peptides and amino acids are initially rapid within the first 3 min as their presentation and the rate remains fairly constant thereafter (Armstead & Ling, 1993) The intracellular peptides are converted to amino acids which undergo further deamination to ammonia in rumen microbes (Annison, 1956). An unionised but not ionised ammonia can diffuse passively in or out across the microbial membrane depending on the concentration gradients across microbial membrane (Russell & Strobel, 1987). In ruminal fluid with a relatively high concentration of ammonia arising from urea, however, peptides and amino acids can be

accumulated in ruminal fluid for 3 h at least post-feeding following the pattern of concentrations of ruminal fluid ammonia (see Broderick et al. 1981 : Broderick & Wallace, 1988). This is consistent with a result shown in Table 6-6. In contrast, ammonia concentrations appeared to follow the pattern of peptides concentrations in ruminal fluid only when dietary true protein was added (Robinson & McQueen, 1994). Similarly, in the continuous culture of strained ruminal fluid both intracellular and extracellular amino acids pools decreased under conditions of $^{+}NH_4$ limitation (Erfle et al. 1976). These indicate that $^{+}NH_4$ may be a feed-back substrate of the regulatory mechanism for the transportation of peptides and amino acids across the membrane of rumen microbes.

The rate of degradation of individual amino acids varied ranging from 5.3 to 10.5 nM/min in ruminal fluid (Chalupa, 1976) and this was also found to vary dependent upon the predominant type of rumen microbes (Scheifinger et al. 1976). Although there are a few bacteria in the rumen that are unable to use carbohydrate and use only protein as a source of energy (Blackburn & Hobson, 1962), recently, *Peptostreptococus anaerobius*, *Clostridium sticklandii* and *Clostridium aminophilum sp.* nov. were reported to be protein utilising bacteria and capable of producing up to 20-fold more ammonia than other ammonia-producing rumen bacteria (Paster et al. 1993). Protozoa in ruminal fluid have more active deaminases than bacteria (Wallace et al. 1987) and the specific activity of protozoa is 3 times higher than that of bacteria (Hino & Russell, 1987). However, the deaminase activity is apparently decreased with an increase in the specific growth rate of rumen microbes (Russell, 1983).

With the information available on the deamination of ingested amino acids in the rumen, microbial deaminase activities are, nonetheless, apparently dependent upon quantities of amino acids presented and concentrations of ruminal fluid ammonia. An increase in quantities of amino acids in the batch culture of strained ruminal fluid was associated with increasing VFA and CO_2 production (Maeng et al. 1976) and presumably ammonia production was also increased. In sheep given various forage based diets, ammonia and isoacid concentrations in ruminal fluid appeared to increase with increasing digestible crude protein content in the diets (Ciszuk & Eriksson, 1973).

This was similar to ammonia and isoacid concentrations reported in this study (see Table 6-1 ; Figure 6-1). This result suggests that there is an increase in microbial deaminase activities with an increase in the quantities of ingested amino acids in the rumen. On the other hand, in a relative high ammonia concentration arising from urea with a small quantity of amino acids, there was a slight decrease in VFA and CO_2 production (Maeng et al. 1976) which might be attributable to an increased microbial growth efficiency. However it may also indicate that there is a reduction in microbial deaminase activity with a high concentration of ruminal fluid ammonia.

6.4.4 Preformed protein and ammonia and net microbial cell synthesis in the rumen

It has been recognised that peptides and amino acids are essential substrates required for the growth of some rumen microbes. This has been mostly demonstrated by a number of *in vitro* studies (Maeng et al. 1976 ; Maeng & Baldwin, 1976a,b ; Cotta & Russell, 1982 ; Russell, 1983 ; Russell & Sniffen, 1984 ; Argyle & Baldwin, 1989 ; May et al. 1993). The specific growth rate of microbes in the media containing urea plus amino acids was twice that when urea alone was supplied (Maeng & Baldwin, 1976a). In the batch culture of strained ruminal fluid when glucose or soluble starch or cellobiose was the carbohydrate source, the substitution of urea with a small amount of amino acids was able to substantially promote the growth of bacteria, but the incorporation of amino acids into microbial cells gradually declined from 48.8 to 9.2 % as the proportion of amino acids was progressively increased from 25 to 100 % (Maeng et al. 1976). To further stimulate the growth of bacteria in a similar culture, however, a small quantity of peptides was superior to the corresponding amount of free amino acids (Wright, 1967 ; Argyle & Baldwin, 1989).

The majority of *in vivo* studies, however, have shown that there is no benefit from feeding dietary true protein, peptides and amino acids over urea as nitrogenous supplements on net microbial protein synthesis in the rumen (Redman et al. 1980 ; Cecava & Parker, 1993 ; Susmel et al, 1994 ; Cruz Solo et al. 1994 ; Fujimaki et al. 1994). The specific growth rate as indicated by purine:total-N ratio of rumen microbes in the present study was fairly constant and was not affected by the source of nitrogen

(see Table 6-6). There was no advantage in soybean supplement (Susmel et al, 1994) over the corresponding nitrogen from urea on microbial biomass entering the duodenum which is the same as the result reported here (Figure 6-1). Irrespective of the basal diets, increased net microbial protein synthesis in the rumen was observed with urea rather than other true protein supplements (Redman et al. 1980 ; Cecava & Parker, 1993). Recently, in sheep given low nitrogen grass hay with an intraruminal infusion of water or urea or peptides or amino acids, there was no difference in microbial yields leaving the rumen (Cruz Solo et al. 1994). Similarly, in sheep fed mashed feed containing 40:60 rice straw:maize starch-molasses plus urea and additives, the quantity of net microbial cells entering the small intestine was not affected by either 10 essential or 18 mixed amino acids or the mixture of leucine, methionine and histidine or urea (Fujimaki et al. 1994). A similar result was also observed in the present study as shown in Figure 6-2. This indicates that peptides and amino acids are not limited for microbial growth in the rumen.

Peptides and free amino acids can be derived from the lysis of microbial cells within the rumen, endogenous substrates and the basal diets that undergo degradation in the rumen. This is likely to be sufficient for microbial growth in the rumen in most conditions. A possible explanation for the difference of the results between *in vitro* and *in vivo* studies is that microbial populations of both conditions are obviously different, particularly when long incubations are involved. The active organisms in an *in vitro* study may not reflect of those in the rumen. Interactions between rumen microbes particularly the predation of fungi and bacteria by protozoa, resulting in a substantial recycling of microbial cells within the rumen, are inevitable (see section 2.3). An increase in degradable protein in the rumen is associated with increasing proteolytic populations in the rumen (Briggs et al. 1964a). Protozoa in the rumen possess higher specific deaminase activity than bacteria (Hino & Russell, 1987). Therefore, microbial proteins are likely to be mostly synthesised from the nitrogen of degradable protein, passing through the ammonia pool in the rumen (see Figure 6-1 and 6-2).

6.4.5 Ingested protein and efficiency of net microbial cell synthesis in the rumen

Most of bacteria in the rumen can not grow on media consisting of only peptides or amino acids as a source of energy (Hungate, 1966). This is hypothesised to be due to the slower rate of ATP generated from fermentation of peptides of amino acids than of ATP needed for maintenance of microbes (Russell, 1983). However, any excess of peptides and amino acids from degradable protein over that are required for microbial protein synthesis can be utilised as ATP-yielding substrates. Fermentation of 1 kg amino acids in the rumen may give rise to 15 moles of ATP which are half that of a corresponding weight of carbohydrate (see Demeyer & Van Nevel, 1979a). Thus amino acids catabolised anaerobically are low ATP-yielding substrates and could contribute to energetic-spilling reactions occurring under the condition that the rate of ATP production by catabolism is in excess of the rate of ATP utilisation by anabolism (Stouthamer, 1979). This may be an explanation for a lack of response for the efficiency of net microbial cell synthesis to an excess of the preformed protein (Table 6-1).

Energetic expenditure for transportation between small peptides and amino acids across the microbial membrane would not be different (Wright, 1967). Theoretically, the ATP required for protein polymerisation from peptides would be less than that from amino acids (Hespell & Bryant, 1979). However, the polymerisation of protein from peptides by bacteria would give an advantage in the energetic expenditure over that from amino acids only when the sequence of amino acids of the uptake peptides is correspondent to that required for the polymerisation or otherwise the uptake peptides are degraded to amino acids.

The present study showed that the efficiency of net microbial cell synthesis in the rumen responded to a high concentration of ammonia rather than the quantity of peptides and amino acids in ruminal fluid (Table 6-1 and 6-6). In contrast, addition of small quantities of amino acids in a batch culture of strained ruminal fluid increased microbial yields between 36 and 62 % (Maeng & Baldwin, 1976a). This is suggested that the added amino acids lead to a reduction in energetic uncoupling (see Hespell &

Bryant, 1979). However, energetic expenditure for transport between amino acids and ammonium ion ($^{+}NH_4$) is similar (see Stouthamer, 1979). The ATP required for the formation of amino acids is calculated to be from 2 to 4 % of total ATP requirement for microbial cells synthesis depending upon energy sources (see Stouthamer, 1979). This energy is small but could be important only in the ammonia-limiting condition in the rumen. Possibly increased energy is expended for $^{+}NH_4$ that is assimilated via glutamine synthetase (see Tyler, 1978).

6.5 Conclusion

The question is: Do organisms in the rumen really need preformed protein for their growth and activity? There has been growing evidence that added true protein in the rumen does not give advantage to rumen digestion and microbial protein synthesis when the concentration of ammonia meets the requirement of the rumen microbes for nitrogen (Redman et al. 1980 ; Cruz Soto et al. 1994 ; Fujimaki et al. 1994). This is consistent with the results reported in Table 6-1, 6-3 and 6-6. The result of the present studies showed that 24 h organic matter digested in the rumen, the specific growth rate of rumen microbes (see purine:total-N ratio in Table 6-6) and efficiency of net microbial cell synthesis in the rumen did not respond to the preformed protein over a range of ruminal fluid ammonia concentrations. However, the efficiency of net microbial cell synthesis did respond to concentrations of ruminal fluid ammonia particularly when the ammonia concentration was in excess of 200 mgN/l irrespective of nitrogen sources.

Chapter 7

General discussion

The research reported in this thesis was aimed at determining the relative roles of ammonia and preformed protein (peptides and amino acids) or the mixture of ammonia and preformed protein in the rumen on the net availability for digestion of microbial cells to the host animals' intestines. A major objective was to assess the optimum level of these nitrogenous substrates required to maximise rumen function in terms of digestibility and the efficiency of net microbial cell synthesis. The outcomes of the level and balance of nitrogenous substrates in the rumen are dependent on interactions between bacteria, protozoa and fungi in the rumen. Changes in these interactions can result in changes in either the specific growth rate of bacteria as indicated by changing purine:total-N ratio in bacteria or the turnover of bacterial cells within the rumen as implied by a changing protozoal density in ruminal fluid. These changes were examined in the rumen of sheep fed on a low nitrogen forage. The contribution of the bacterial biomass that leaves the rumen was assumed to follow closely the relative changes in purine derivatives excreted in urine (see section 2.5).

The underlying principle of manipulating the rumen is to increase the efficiency of net microbial cell synthesis by optimising both digestibility and microbial yields. This results in maximising the extraction of nutrients and minimising the losses of energy in heat and CH_4 (see section 2.4). In general, the major objectives of manipulation in the rumen are:

1) To improve the digestibility of feedstuffs in the rumen. Digestibility primarily determines the amounts of nutrients extracted from the feed and therefore the amounts available for absorption by the host animal. Digestibility also determines the level of intake particularly of low quality forages. The availability of microbial protein and VFA for absorption is dependent on the availability of the monomers required for

microbial cell synthesis and therefore the utilisation of ATP produced from the feedstuff during its breakdown in the rumen. In many situation, where the rate and extent of ATP generated during VFA formation correspond to ATP required for anabolism, digestibility will also be highly related to microbial growth efficiency.

2) To reduce microbial cell turnover within the rumen. The lysis of microbial cells within the rumen is a major factor resulting in increased requirement for M_{ATP} and decreased ATP available for cell synthesis (i.e. a lower Y_{ATP}). The predatory activity of protozoa appears to be the main factor causing considerable turnover of bacterial and fungal cells within the rumen (see section 2.4.3.3).

3) To increase the specific growth rate of rumen bacteria by increasing the turnover rate of macro-molecule (RNA) in rumen bacteria. There is a close correlation between RNA:total-N ratio in bacteria and the specific growth rate of bacteria (see Koch, 1970 ; Bates et al. 1985). The idea is that a smaller bacterial pool growing at a rapid rate will be more efficient than a large pool turning over slowly.

4) To improve the nature and balance of nutrients bypassing reactions in the rumen but which are digestible in the intestines.

7.1 Research findings

An insufficient supply to the rumen of nitrogenous substrates will limit microbial activity and potentially impair digestion and therefore microbial growth efficiency. Changes in availability of nitrogenous substrates will result in a change in microbial populations in the rumen due primarily to differences in the capabilities of substrate assimilation among microbial species.

7.1.1 Relationship between dietary nitrogen and digestibility in the rumen

Supplementation to correct nutrient deficiency in feedstuffs ingested by ruminants is of importance for optimising microbial activity and therefore digestibility in the rumen. An increase in digestibility in the rumen allows feed intake to increase substantially (Minson, 1982), thus potentially increasing productivity of ruminants in particular those fed on low quality forages.

A supply of urea to correct nitrogen which is deficient in a forage obviously increased digestibility in the rumen (see section 4.3.4 and 5.4.5) while added preformed protein did not show any advantage in improving digestibility over a corresponding nitrogen from urea (see section 6.4.1). The requirement of rumen microbes for ammonia depends largely on the feedstuff ingested by ruminants as discussed in section 4.3.4 and the availability of ATP in its breakdown. The optimum requirement for ammonia for maximum digestibility of the oaten chaff occurred below 30 mgN/l (see section 4.2.2.4, 4.3.2.5 and 5.3.7).

The outstanding finding in this research was that protozoa appeared to have an important role in fibre digestion in the rumen only when the concentration of ruminal fluid ammonia was below 40 mgN/l (see section 5.4.5). On the other hand, the removal of protozoa from the rumen resulted in a critical reduction of available ammonia which consequently impaired fibre digestion in the rumen. The requirement for ammonia for maximum digestion in the rumen was higher in the fauna-free than in faunated sheep and was in excess of 160 mgN/l.

7.1.2 Relationship between dietary nitrogen and microbial mix in the rumen

An increase in concentrations of ruminal fluid ammonia was associated with decreasing protozoal pool in rumina fluid (see Figure 7-1). This tends to suggest that increasing concentrations of rumina fluid ammonia may have a detrimental effect on protozoal populations in the rumen.

The mechanism of how high concentrations of ruminal fluid ammonia affect protozoal cells is not known. An explanation could be that there is a feed-back mechanism on ammonia excretion by protozoa when ammonia concentration is high. This concept is supported by the findings of Nour et al. (1979) who found that increased amounts of urea ingested (ammonia concentrations were not available) were associated with increased turnover time (slower growth) of protozoa in the rumen but this was not observed when the diet contained molasses.

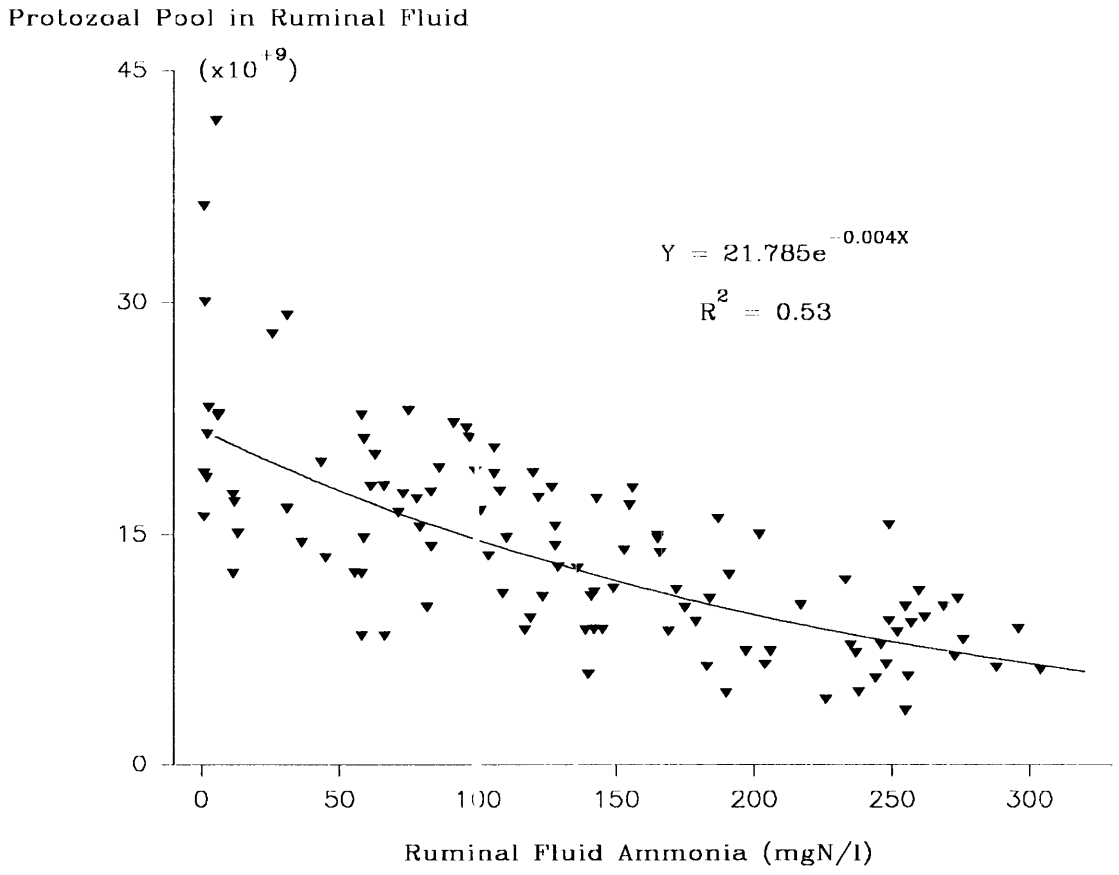

Figure 7-1. Relationships between concentrations of ruminal fluid ammonia and protozoal pool in ruminal fluid (density of protozoa x volume of ruminal fluid) in sheep given a low protein forage.

There is evidence that the numbers of bacteria in the ruminal fluid increase with increasing amounts of urea or ammonium salts ingested and therefore increasing concentrations of ruminal fluid ammonia (see Virtanen, 1966 ; Teather et al. 1980 ; Elliott & Armstrong, 1982). This is consistent with the results reported in this study. The relationship between concentrations of ruminal fluid ammonia (derived from both urea and casein) and urinary excretion of purine derivatives from sheep is shown in Figure 7-2 (taken from the individual data of the continuous feeding faunated sheep reported in this thesis). Urinary excretion of purine derivatives reported in this thesis is assumed to be contributed mainly from the bacterial biomass that leaves the rumen since bacterial and possibly fungal but not protozoal populations (see chapter 4) are

able to assimilate ammonia mainly via glutamine synthetase and glutamate dehydrogenase (see section 2.4.3.1.2).

Determination of fungal biomass in the rumen is difficult as pointed out by Orpin & Joblin (1988). The difficulty is associated with the lack of a precise indicator of fungal biomass and also with their life cycle. However, an indication of a relative change in fungal biomass in the rumen used in the literature is given by thallus or zoospore or sporangia.

Ammonia appears to be a growth-limiting nutrient for rumen fungi as indicated by increased fungal activity suggested by increased production of sporangia on oat leaf blades when concentrations of ruminal fluid ammonia were between 15 and 50 mgN/l (see chapter 4, 5 and 6). However, the numbers of sporangia progressively declined when ammonia concentrations were gradually increased above 50 mgN/l, while a marginal reduction in sporangial numbers was observed when a small amount of preformed protein was presented. These results indicate that ammonia concentrations above 50 mgN/l did not have any significant harmful effect on fungal population in the rumen even to concentrations above 200 mgN/l. A reduction in the sporangial number appearing on the oat leaf blade is possibly due to some competitive disadvantages in obtaining critical nutrients.

7.1.3 Relationship between dietary nitrogen and net microbial cell synthesis in the rumen

Concentrations of amino acids and peptides in ruminal fluid are usually low and a supply of a small amount of these substrates in addition to ammonia to a sugar or starch but not cellulose diet resulted in a substantial increase in net bacterial protein synthesis (Maeng & Baldwin, 1976b). A lack of response in the rumen conditions can be due primarily to a large diversity of rumen microbes and their interactions. Dietary true protein entering the rumen is mostly deaminated to ammonia and other end-products and a small proportion of preformed protein (amino acids and peptides) is assimilated into microbial protein (see chapter 6).

Studies reported in this thesis clearly showed that an increase in the net microbial cell synthesis that leaves the rumen is associated with an increase in concentration of ruminal fluid ammonia as shown in Figure 7-2. An increase in the net microbial cell synthesis appeared to be unrelated to the change in the specific growth rate as indicated by the purine:total-N ratio in bacteria. However, it negatively correlated to the protozoal pool in ruminal fluid as shown in Figure 7-3 (from the individual data of the continuous feeding faunated sheep reported in this thesis). These results suggest that a considerable increase in the net microbial cell synthesis could be a result of a substantial reduction in the lysis and turnover of bacterial cells within the rumen, due to lower rate of predation of protozoa. At the same time a high concentration of ammonia allows bacteria to grow continuously.

The outstanding finding in this study was that at ammonia concentration around 160 mgN/l when no protozoa were present in the rumen, the net microbial cell synthesis or Y_{ATP} was equal to 21.3 cells/mole ATP which is close to the maximum theoretical Y_{ATP} of 26 g cells/mole ATP from the rumen. The former Y_{ATP} was calculated from the data reported in Chapter 5 and assumed that 25 moles of ATP are generated from 1 kg organic matter digested in the rumen (see the detailed assumption in Leng, 1982b).

7.1.4 Conclusion

Research reported in this thesis clearly showed that when concentrations of ruminal fluid ammonia were below 40 mgN/l, protozoa appeared to contribute significantly to fibre digestion in the rumen but they decreased the availability of microbial cells for digestion in the intestine. An increase in the efficiency of net microbial cell synthesis with an increasing availability of ammonia in the rumen was associated with a decreasing protozoal pool in ruminal fluid while the microbial growth *per se* remained unchanged. This indicates that increasing the efficiency of net microbial cell synthesis is due to reduced turnover of microbial cells within the rumen.

It is now recommended that requirements for ammonia for optimal net microbial cell synthesis in the rumen are much higher than that previously recognised

with forage based diets. Since organic matter digested in the rumen is either fermented to VFA or used in the synthesis of microbial cells, an increasing efficiency of net microbial cell synthesis in the rumen will have large effects on the ratio of microbial protein to VFA available for digestion and for absorption, respectively. For a forage that is low in crude protein, the ratios of protein:energy and acetogenic:glucogenic substrates available for digestion and absorption from the digestive tract of ruminants can be optimised by adjusting the concentration of ruminal fluid ammonia to or above 200 mgN/l.

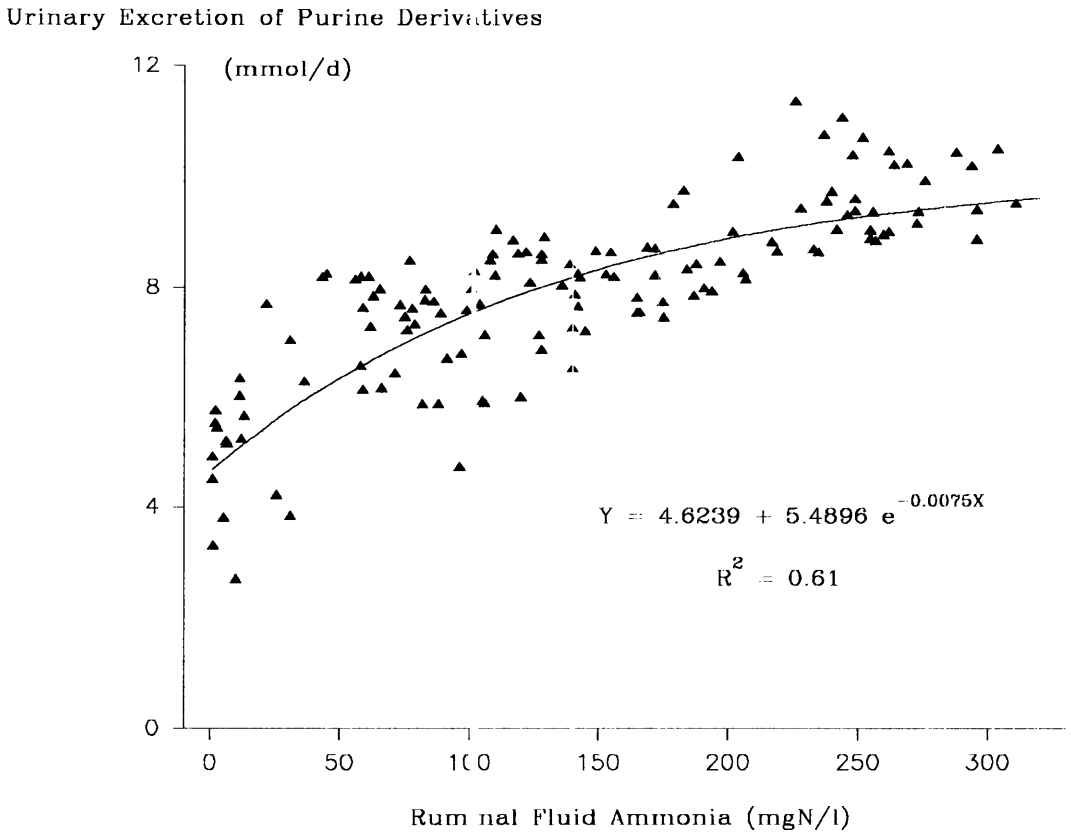

Figure 7-2. Relationships between concentrations of ruminal fluid ammonia and urinary excretion of purine derivatives from sheep given a low protein forage.

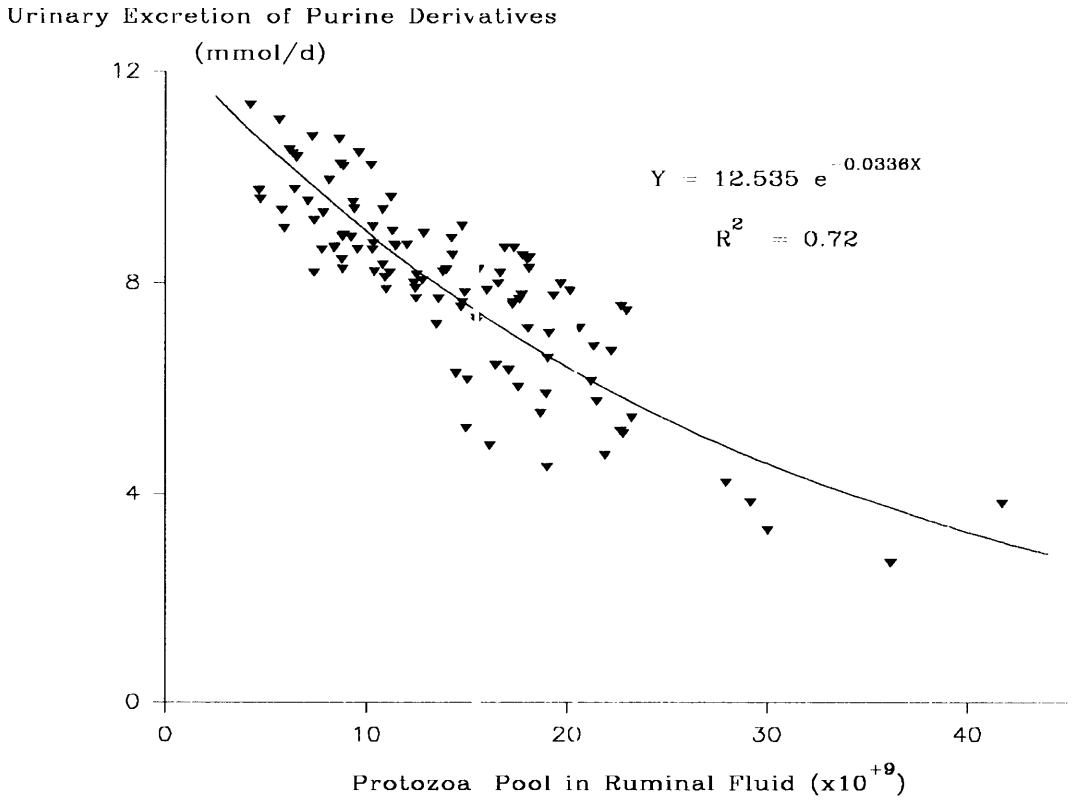

Figure 7-3. Relationships between protozoal pool in ruminal fluid (density of protozoa x volume of ruminal fluid) and urinary excretion of purine derivatives from sheep given a low protein forage.

7.2 Future research

Manipulation of the rumen to increase the efficiency of net microbial cell synthesis by increasing supply of ammonia from urea is important for ruminants fed on low nitrogen forage based diets. Ammonia in the rumen may be regarded as an anti-protozoal substrate at high levels and at the same time it is required for growth and development of the bacterial and fungal populations.

Microbial populations in the rumen will change due primarily to a change in feedstuffs ingested by ruminants. Carbohydrate sources and the availability of ATP from the reaction involved in its breakdown will primarily determine the requirement of rumen microbes for ammonia. The requirement for ammonia for both optimum

digestion and microbial growth is expected to be higher in animals fed on concentrate-based diets than those on roughage-based diets. However, the effects of increasing availability of ammonia on rumen function found in this study need to be tested on other diets.

The absence of protozoal fibrolytic activity together with the influence of diets used are often explained to be major factors causing a reduction in fibre digestion in the rumen of fauna-free relative to faunated animals. However the removal of protozoa results in a considerable reduction of available ammonia in the rumen which is a major constraint to the growth of bacteria and fungi. Thus, the effects of defaunation on fibre digestion in the rumen need to be re-examined on various diets with an sufficient supply of ammonia.

The extent to which ammonia concentrations influence the actual growth rate of protozoa in the rumen is not known. A study with the ^{14}C-choline labelled protozoa would allow for assessment of the turnover rate of protozoa in the rumen (Leng, 1982a).

Research is also suggested to verify the hypothesis that the reduced lysis and turnover of bacterial cells within the rumen are due to increasing ammonia concentrations. A study involved L-(^{14}C)leucine labelled bacteria (Wallace & McPherson, 1987) would allow for assessment of the rate of bacterial-protein breakdown in the rumen.