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

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ABSTRACT : Microbial growth efficiency in the rumen was studied in sheep given hourly, 31.25 g oaten chaff with either 0.31 and 0.88 g urea or 1.88 and 5.63 g casein (exp. 1) and 33.33 g oaten chaff with 1.04 casein or 0.3, 0.6 and 0.9 g urea or the mixture of the casein and urea (exp. 2). Concentrations of ruminal fluid ammonia increased with increasing nitrogenous supplements. Organic matter digestibility *in sacco* in the rumen was not different irrespective of N sources. Isoacids and valeric acid increased with increasing ingested casein but decreased with increasing urea intake. Peptide and amino acid pools in ruminal fluid increased with increasing ammonia concentrations (exp. 2) suggesting that proteolytic activity and transportation of peptides and amino acids across microbial membrane of rumen microbes may be regulated by the metabolite mechanism (intracellular amino acids and NH_4^+, respectively). Densities of total viable and cellulolytic bacteria in ruminal fluid increased with increasing ammonia levels but that of small *Entodinia* decreased. The density of fungal sporangia growth on oat leaf blades decreased with increasing ammonia concentrations but appeared to remain constant in the presence of casein. Efficiency of net microbial cell synthesis was 15-28% higher when ammonia concentrations increased from 100 to above 200 mgN/l regardless of N sources. In conclusion, supplementation of preformed protein had no effect on rumen digestion and microbial growth efficiency. This could not be accounted for its effect on ruminal fluid ammonia. Increased microbial growth efficiency with increasing ammonia levels may be due to a reduction in the turnover of microbial cells within the rumen.

(Key Words: Ruminal Fluid Ammonia, Preformed Protein, Microbial Growth Efficiency)

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 (Cotta & Hespell, 1984) and fungi (Wallace & Joblin, 1985; Yanke et al., 1993) in the rumen occurs extracellularly 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, the main objective of any supplementation of dietary true protein or preformed protein (peptides and amino acids) is 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).

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 (Kanjanapruthipong & Leng, 1998). From studies with ^{15}N of the dynamics of nitrogen in the rumen of sheep given a roughage based diet tracers, 50-95% of microbial nitrogen appears to be derived from ammonia in ruminal fluid (Pilgrim et al., 1970; Al-

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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 populations 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.

MATERIALS AND METHODS

Experiment 1

Animals and housing

Twelve First Cross Merino × 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 a room controlled at 20℃. The room was well ventilated and lit at all times.

Diets and feeding

Oaten chaff containing 0.8% N from the same source and batch, was used as the basal diet throughout the study. To this was added 2% of minerals mix consisting of 1 part of Pfizer vitamin-minerals (Pfizer Agricare, NSW), 1 part of Na_2SO_4, 1 part of NaCl and 2 parts of Ca_2HPO_4. 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 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.

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 wk prior to commencement of the experiment. On the last day of the 2 wk adaptation period, samples of ruminal fluid from each animal were taken at 09:00 h, 13:00 h and 17:00 h for analysis of NH_3-N. 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 day 1-16, the animals were left undisturbed and on day 17-21, they were subjected to the experimental procedures. On day 17-19, the daily urine voided by each animal was collected into a container with 500 ml of 2% (v/v) CH_3COOH and 1% (v/v) H_2SO_4. The urine sample was diluted by 3-4 times just after collection and then stored at −20℃. Equal portions of the daily urine samples from individual animal from each day were pooled prior to analysis of purine derivatives. On day 20, samples of ruminal fluid were collected via the rumen fistula with a probe covered with a double layers of nylon stocking material at 09:00 h, 13:00 h and 17:00 h. The sample of 15 ml was acidified with 5 drops of concentrated H_2SO_4 and stored at −20℃ prior to analysis of VFA and NH_3-N. On the last day, three nylon bags with a pore size of 44 μm and measured 7 × 14 cm containing ground dietary materials (2 mm sieve) were suspended in the rumen in order to assess organic matter digestibility (OMD) over 24 h. The technique used to estimate the *in sacco* digestibility of feed is similar to that reported by Orskov et al. (1980).

Experiment 2

Animals and housing

Seven First Cross Merino × Border Leicester wethers, $2^1/_2$-3 years of age, weighing 30-34 kg and with permanent rumen cannulas were held in the same conditions as described above.

Diets and feeding

Oaten chaff containing 1.35% N from the same source and batch was used as the basal diet throughout the study. To this was added 2% of the minerals mix (see experiment 1). 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 urea in 40 ml of tap water. The solution was sprayed onto the chaff whereas 25 g of casein was initially sprinkled over the oaten chaff following the 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.

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 wk 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 as previously described and stored at −20℃ for later analysis of purine derivatives. On day 18, rumen digesta was collected via a core sampling probe. Bacteria in ruminal fluid and particulate-microbes were then isolated as described by Kanjanapruthipong et al. (1998) for analysis of purine and nitrogen 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 5,000 g for 10 min. The upper phase was kept and stored at −20℃ 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 (1 mg Cr/kg BW) used as a ruminal fluid marker according to the method of Downes & McDonald (1964) was injected into the rumen at 07:00 h. From 3-27 h after the injection, 10 samples of ruminal fluid were taken periodically via the rumen fistula with a probe covered with the stocking material. Prior to acidification, samples were removed to a vial for enumeration of protozoa and for measurement of pH. The rest was acidified as indicated in the previous study for analysis of Cr, VFA and NH_3-N. On the last day, 24 h *in sacco* digestibility of the diet was assessed and fungal sporangia growth was assessed from the sporangial density on oat leaf blades incubated for 24 h in the rumen.

Chemical analysis

Enumeration of protozoa and fungi, analysis of VFA, NH_3-N and Cr in ruminal fluid and purine derivatives in urine were made as described in the study of Kanjanapruthipong & Leng (1998). Purine content of rumen microbes was determined as described in the study of Kanjanapruthipong et al. (1998).

Prior to the analysis of free amino acids and peptides in the ruminal fluid, the supernatant was thawed and centrifuged at 5,000 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 710 B) connected with the Pico-Tag column (3.9 × 300 mm, Waters, Millipore Corp., MA, USA) and L-norleucine (Sigma, MO, USA) was used as an internal standard.

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, 1,000), with excitation at 396 nm and emission at 479 nm.

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) and cellulose broth tubes (Halliwell & Bryant, 1963) were used for the determination of the densities of total viable and cellulolytic bacteria (most probable numbers) in ruminal fluid, respectively.

Statistical analysis

The first experiment was divided into two 21-d periods according to a Half Change Over design or a Crossover 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 the urea and casein treatment groups was made at the end of the first period. In the second experiment, a 7 × 7 Latin Square design was used for the analysis of variance. The statistical significance of the data was analysed by SAS (1989). The difference between treatments means was assessed by the Least Squares Means.

RESULTS

Feed intake

In both experiments, the feed was delivered hourly and consumed by the animals within a few minutes of its presentation.

Experiment 1

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 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 1).

Table 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)

Items	Urea or Casein intake (gN/d)				
	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[a]	8.2[a]	10.0[b]	10.0[b]	0.52
ENMS (gN/kg OMDR)	20.6[a]	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.

The patterns of VFA in ruminal fluid

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

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

Molar Proportion	Urea or Casein Intake (gN/d)				
	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.

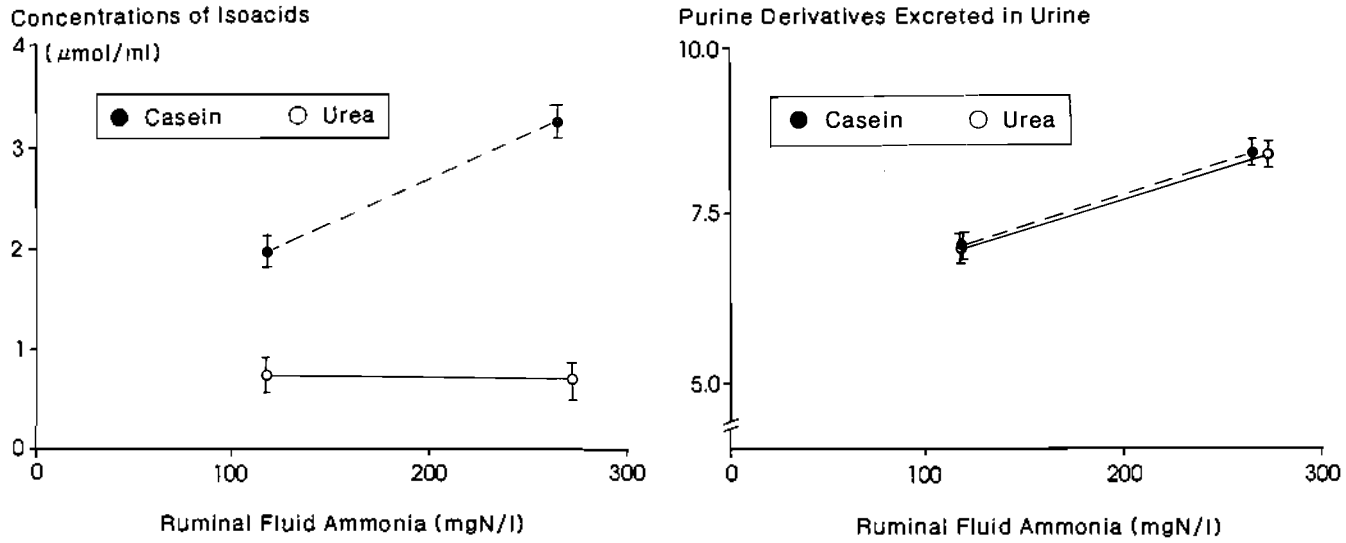

Figure 1. Effects of concentrations of ruminal fluid ammonia arising from urea or casein on concentrations of isoacids (isobutyric and isovaleric acids) and urinary excretion of purine derivatives.

Net microbial cell synthesis in the rumen

Urinary excretion of purine derivatives was increased with increasing levels of urea and casein intake ($p < 0.01$) but it did not differ within the same levels of the nitrogenous supplements ($p > 0.05$; figure 1). The calculated efficiency of net microbial cell synthesis in the rumen (gN/kg OMDR) was 26% greater for the higher levels of ingested urea and casein (see table 1).

Experiment 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 nitrogenous supplements irrespective of nitrogen sources ($p > 0.05$; table 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 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 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

Table 3. Ammonia concentrations, pH, organic matter digestibity *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

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

nitrogenous supplements ($p > 0.05$). The molar proportion (%) of isoacids and valeric acid significantly increased with ingested casein ($p > 0.01$; table 4). The tendency of concentrations of isoacids with different levels and sources of nitrogenous supplements is shown in figure 2.

Microbial populations

Both concentrations of total viable and celluloytic 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 5).

The number of fungal sporangia growth on oat leaf blades decreased with increasing levels of urea intake ($p > 0.01$) but it was not affected when casein was supplemented ($p < 0.05$; table 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 5). However, the density of large *Entodinium* sp. was not affected by either levels or sources of nitrogen intakes ($p > 0.05$; table 5).

Table 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) suppelements

% Molar proportion	Treatments							
	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	0.5^{a}	0.5^{a}	0.9^{b}	0.5^{a}	0.9^{b}	0.5^{a}	0.9^{b}	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.

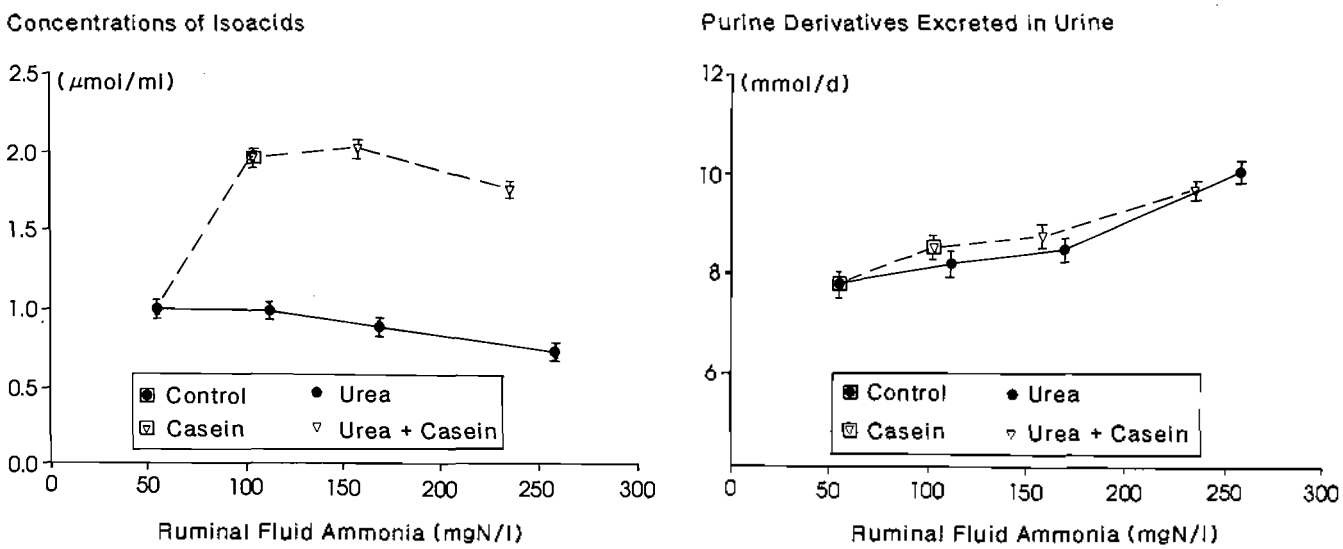

Figure 2. Effects of concentrations of ruminal fluid ammonia arising from urea or casein or urea plus casein on concentrations of isoacids (isobutyric and isovaleric acids) and urinary excretion of purine derivatives.

Table 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.

Purine: total-N ratio of rumen microbes and net microbial 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).

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 2) and the calculated efficiency of net microbial cell synthesis in the rumen (gN/kg OMDR) was also 72% greater in relation to that in the rumen of the control sheep ($p < 0.01$) irrespective of nitrogen sources (table 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). Both concentra-

Table 6. Essential (EAA), non-essential (NEAA) and total amino acids (TAA), peptides in ruminal fluid, purine: toal-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/kg OMDR) 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/kg OMDR)	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.

tions of NEAA and total amino acids (TAA) in ruminal fluid were significantly greater when casein was the major nitrogen source ($p < 0.05$) and the concentrations increased with increasing levels of urea intake (table 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).

DISCUSSION

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.

Casein was a source of peptided 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.

The quality of oaten chaff (1.35 vs 0.6% N and 59.5 vs 43.2% OMDR *in sacco*) as a basal diet in this study is much higher than that in the study of Kanjanapruthipong & Leng (1998). Consequently, urea supplementation in the study reported here appears to have smaller effect on the microbial ecosystem in the rumen. However, the effect of dietary urea on the microbial populations of both studies is similar when ruminal fluid ammonia concentrations are above 50 mgN/l.

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

Increased digestion of dry matter in the rumen and therefore feed intake (partly influenced by increasing digestibility; Oldham, 1984) often occurs as a result of supplementation of dietary protein that enhances ruminal fluid ammonia concentrations (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; MeAllan & 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 than nitrogen is dependent primarily on the concentration of ruminal fluid ammonia over a 24 h period (see table 1).

Ingested protein and rumen fermentation

A number of papers have reported that isoacids appear to be essential and required by fibrolytic bacteria in the rumen (Van Gylswyk, 1970; Hume, 1970; 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 1 and 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.

Protein degradation an ammonia concentrations in the rumen

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, 1967) which accords with a result shown in table 6. This is due to the rapid uptake of the substrated 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). 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 avaiable 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 requlated by the end-products mechanism.

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 (Broderick et al., 1981; Broderick & Wallace, 1988). This is consistent with a result shown in table 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 reguiatory mechanism for the transportation of peptides and amino acids across the membrane of rumen microbes.

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 or strained rumen 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 1; figure 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 relatively 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.

Preformed protein and ammonia-N and net microbial synthesis in the rumen

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 Soto 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). 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 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 Soto et al., 1994). Similarly, in sheep fed mashed feed containing 40:60 rice straw: maize starch-molasses and additives, the quantity of net microbial cells entering the small intestine was not affected either the mixture of amino acids or urea (Fujimaki et al., 1994). A similar result was also observed in the present study as shown in figure 2. This indicates that peptides and amino acids are not limited for microbial growth in the rumen.

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 or 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 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 (Demeyer & Van Nevel, 1979). 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 (see table 1 and 6).

CONCLUSION

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

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