mixed rumen population have been highly variable. Published ^{15}N studies using ^{15}N-ammonia or urea (which rapidly releases ammonia) infused into the rumen or added as a single dose indicated values of microbial N derived from ammonia that range from 18 to 100%. Most microbial species can use NH_3, although peptides and amino acids are generally stimulatory. The present study was undertaken to determine the influence of peptides and amino acids on ammonia uptake by pure and mixed cultures of rumen microrganisms, and to identify which amino acids are formed de novo under different nutritional circumstances.

Strained rumen fluid from four sheep receiving a mixed grass hay/concentrate diet was diluted three-fold in buffer and incubated with a mixture of starch, cellobiose and xylose (each at 2.2mg ml^{-1}) in the presence of added ^{15}N-ammonium chloride (1.33mg ml^{-1}), either alone or with added Trypticase (10mg ml^{-1}) or amino acids (10mg ml^{-1}). The proportion of cell N derived from NH_3 was 0.99, 0.38 and 0.47 (SD 0.19, 0.17 and 0.03) for the control, Trypticase and amino acids treatments respectively. Most cellular glutamate, aspartate and alanine continued to be synthesised *de novo* in the presence of Trypticase but the synthesis of lysine, threonine and valine was greatly decreased and proline biosynthesis was abolished. In pure cultures, *Fibrobacter succinogenes* S85, *Prevotella bryantii* B_14, *Selenomonas ruminantium* and *Streptococcus bovis* all grew using NH_3 in minimal medium with added methionine, but shut down de novo amino acid synthesis almost completely when Trypticase was added. In contrast, *Ruminococcus albus* SY3 and *Ruminococcus flavefaciens* still formed the majority of cellular N from NH_3 when Trypticase was present. Again, however, proline biosynthesis was depressed more than the other amino acids. The results imply that non-protein N will be used inefficiently by the mixed population if preformed amino acids are present, and that proline may be a key amino acid in determining the response to pre-formed amino acids in both pure and mixed cultures.

Ammonia production by rumen microorganisms and the enumeration and isolation of bacteria capable of growth on peptides and amino acids from the sheep rumen. S Eschenlauer, CJ Newbold, N McKain, ND Walker, RJ Wallace (*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK*)

Rumen fluid was taken from rumen-fistulated sheep and a cow receiving a mixed hay-concentrate diet and analysed in vitro for rates of NH_3 production and for numbers and characteristics of NH_3-producing bacteria. The rate of NH_3 production from Trypticase varied from 2.8 to 19.7 nmol (mg protein)$^{-1}$ min^{-1} depending on the method used. The lowest value was obtained under conditions considered to be most representative of those prevailing in the rumen. Ammonia production from Trypticase and soya peptone by the mixed rumen population was inhibited by 43 and 41% on addition of 5μM monensin, while ammonia production from proteins decreased by 46-61% and from amino acids fell by 49%. Thus less than half of ammonia production from peptides and amino acids in the mixed rumen population was sensitive to monensin. Bacteria capable of growth on Trypticase or amino acids as sole energy source were assessed by a most-probable-numbers

method. A small proportion (1.5%) of the total bacterial population grew weakly on Trypticase alone, a smaller number (0.7%) grew strongly on Trypticase alone, and many fewer (0.02%) grew on free amino acids. Monensin eliminated 89% of the Trypticase fermenters. The most numerous Trypticase utilisers were characterised and all but one of 18 isolates obtained from four sheep were spore-forming anaerobic rods from a number of different *Clostridium* spp. However, unlike previous isolates, none was obligately peptidolytic: their growth was stimulated strongly by soluble sugars. Their deaminative activity from Trypticase varied from nil to 68 nmol (mg protein)$^{-1}$ min^{-1}. It was calculated that even the highest-activity isolate could carry out only 17% at most of NH_3 production by the mixed ruminal microbial population, and therefore by far the majority of deamination was carried out by the more numerous, low-activity bacteria typical of the main species of rumen bacteria.

Influence of peptides on the growth of mixed rumen microoganisms on different carbohydrates *in vitro*. J Wang[1], RJ Wallace[2], CJ Newbold[2] (*[1]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094, China; [2]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK)*

Many studies *in vivo* and in pure and mixed cultures *in vitro* have shown that pre-formed amino acids and peptides increased the rate or efficiency of rumen microbial growth. However, Cruz Soto *et al.* [1] observed that the response of cellulolytic bacteria to peptides depended on the energy source: peptides would stimulate growth on rapidly but not on slowly degraded carbohydrates. The aim of the present experiment was to test this hypothesis in mixed rumen microorganisms in vitro. Rumen fluid was taken from two rumen-fistulated sheep receiving a mixed grass hay/concentrate diet immediately before the morning feeding. The samples were mixed and diluted three-fold in buffer to provide the inoculum for incubations with glucose, xylose, potato starch, rice straw or wheat straw (0.2g in 30ml) with added 0.17g NH_4Cl or 0.3g Trypticase. Gas and VFA production were determined by standard methods, and microbial growth was determined by adding 0.033 μCi ^{32}P-phosphate and measuring incorporation into particulate material. Results were calculated as the difference between incubations containing carbohydrate and those without carbohydrate, with or without added peptides.

Fermentation of wheat and rice straw was weak at 8h, although some stimulation by peptides was apparent, and peptides increased all measurements at 24h. The other carbohydrates were fermented more rapidly. Xylose and starch fermentation at 8 and 24h were stimulated by peptides. Gas and VFA production from glucose was unaffected by peptides after 8h, whereas ^{32}P incorporation increased by 46%, suggesting that energy spilling was decreased. Thus these results are not consistent with peptides stimulating growth only on rapidly degraded substrates, as was suggested by Cruz Soto *et al.* [1] and Russell *et al.* [2]. Alternative explanations must be found to reconcile many in vivo and other in vitro results. A better microbiological description may be required. For example, glucose would be fermented by a variety of species, many without a re-