ganisms are considered responsible for herbage protein degradation in the rumen. However, the proteolytic activity of the rumen microbial population is only moderate when compared with other proteolytic microorganisms and the animal's own gastric and pancreatic secretions [2]. Thus we conclude from this and much anecdotal evidence that plant enzyme mediated proteolysis is involved in protein breakdown in the rumen of grazing animals [3].

To test the above hypothesis, we have studied plant enzyme mediated proteolysis *in vitro* in fresh grasses incubated under anaerobic conditions. Four treatments were used: 1) herbage plus buffer, 2) herbage plus filter-sterilized rumen fluid, 3) herbage plus heat-treated rumen fluid and 4) herbage plus strained fresh rumen fluid. Herbage proteins were extracted after 0, 6, 12 and 24h of incubation and protein degradation profiles compared using SDS-PAGE. During the initial 12h of incubation, no difference was found in protein band profiles between the four treatments; the large Rubisco fraction (MW *ca.* 55kDa) was partially degraded to smaller sub-units. By 24h of incubation, the large Rubisco fraction had further been degraded into smaller bands in treatments 1, 2 and 3, whereas for the treatment involving fresh rumen fluid most bands including the large Rubisco fraction and its derivatives had disappeared completely. To monitor the presence or absence of microbial activity in each treatment, volatile fatty acids from culture supernatants were measured. With treatments 1, 2 and 3, acetate, propionate and butyrate concentrations were negligible over 24h of incubation, indicating an absence of effective microbial growth. With fresh rumen fluid, however, high levels of acetate (*ca.* 40 mM), propionate (*ca.* 40mM) and butyrate (*ca.* 8mM) did accumulate. This significant microbial activity in treatment 4 probably made a major contribution to the disappearance of peptides between 12 and 24h of incubation. Moreover, studies with up to 15 different grass species showed that plant proteolytic activity varies according to age and cultivar. In conclusion our results (a) indicate that there is scope to manipulate proteolysis in plants and (b) support the hypothesis that plant proteases are involved in initial cleavage of plant proteins in the rumen, whereas rumen micro-organisms make their contribution during the later stages of proteolysis.

1. Wetherall JA, Armstrong DG, Finlayson, HJ, Rooke, JA (1995) *J Sci Food Agric* 68, 497-505
2. Wallace RJ (1995) Rumen Microbiology Satellite Symposium of IV International Symposium on the Nutrition of Herbivores Sept 16-17, Clermont-Ferrand, France
3. Theodorou MK, Merry RJ, Thomas H (1996) *Brit J Nutr* 75, 507-508

Factors affecting de novo synthesis of amino acids by mixed microorganisms from the sheep rumen in vitro and by pure cultures of rumen bacteria. C Atasoglu[1], C Valdés[2], ND Walker[1], CJ Newbold[1], RJ Wallace[1] *([1]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [2]Departamento de Producción Animal I, Facultad de Veterinaria, Universidad de León, E-24007 León, Spain)*

Ammonia has an important role in providing nitrogen for protein synthesis by rumen microorganisms. Estimates of the contribution of ammonia versus preformed amino acids to protein synthesis by the

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