Background to RUMEN-UP

Introduction

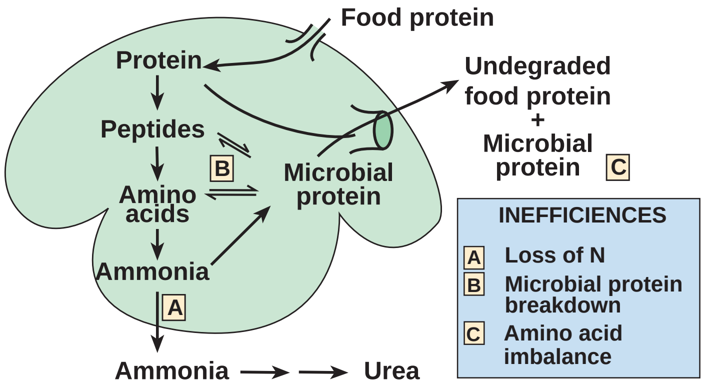
The rumen is the first stomach of cattle, sheep and goats. It enables these animals to digest high-fibre plant materials unsuitable for most non-ruminant animals. The rumen is a huge organ, containing in excess of 150 kg of digesta in dairy cows, in which all of the digestion is carried out by micro-organisms. The rumen evolved in the way it has done in order to benefit from the fibre-digesting activities of the microbes. The end-products, the volatile fatty acids, are absorbed across the rumen wall and used for energy and protein synthesis. The principal benefit of the rumen foregut fermentation in comparison with hindgut fermentation is that the microbial cells formed during the fermentation pass to the abomasum, where they are digested and their amino acids are absorbed. Unlike the hindgut fermentation, therefore, microbial amino acids become available to the host animal. The rumen is therefore a highly efficient organ in the context of the evolution of a herbivore subsisting on poor pasture. The ruminant is an essential component of utilising marginal land in the EU in a sustainable way.

Rumen fermentation also brings some disadvantages. Methane is produced as a natural consequence of the anaerobic fermentation; it is a potent greenhouse gas, so in this sense, ruminants damage the environment. The ruminant is also less efficient than other species in the utilisation of dietary protein. Nitrogen losses from ruminants are exceptionally high, particularly in grazing animals. This is an environmental problem as well as an economic one, because of the impact of nitrogen-rich excreta on the environment. Furthermore, there are digestive disorders unique to ruminants which occur as a direct consequence of rumen microbial fermentation being impaired. **This project will address these problems collectively, by combining the expertise of different research groups and biotechnology companies in Europe in a coordinated programme**.

Chemical and antibiotic feed additives have in the past been used to alleviate some of these problems. The presence of such materials in the food chain is becoming ever less acceptable to regulatory authorities and the consumer. Thus, solutions to the problems of ruminant livestock production must be natural and sustainable. **The proposed project will explore novel plants and plant materials as solutions to the problems**.

Protein degradation processes in rumen microorganisms

Fig. 1. Protein metabolism in the rumen. Inefficiencies are introduced by the breakdown pathways leading from protein to ammonia, by the rapid turnover of microbial protein, and by the amino acid composition of the protein flowing from the rumen not meeting the animal's needs. This project will investigate new means of minimising the first two inefficiencies.



Breakdown of dietary protein to ammonia. Dietary protein entering the rumen is broken down in an apparently uncontrolled way, resulting in ammonia formation and subsequent loss of N in the urine. The low efficiency of nitrogen retention which results represents a major economic loss, causes metabolic stress in the animal, and also places a burden on the environment, by way of nitrogen-rich wastes. If means of slowing the breakdown process at any of the individual steps, these problems would be decreased.

Many different microbial species, employing a range of proteolytic enzymes, carry out the initial step of protein breakdown (1, 2). The variety of proteolytic microbes present has made rational manipulation of the initial proteolytic step impossible, and solutions have generally required treatment of the protein before feeding, by heating for example. Tannins have been explored as a means of decreasing protein breakdown (3, 4), but they frequently impair other aspects of rumen fermentation, including fibre breakdown (5). A rational search for new plant materials which bind to proteins and prevent their digestion, or preferably which inhibit the proteinases directly, yet which are not otherwise detrimental, is timely. **This is a new approach which may lead to the discovery of much more specific, and therefore more useful, inhibitors than the tannins.** The subsequent processes of peptide and amino acid breakdown are carried out by more defined populations. The only methods available for altering these activities are dietary addition of antibiotics and ionophores (6) , which suppress the growth of the bacteria responsible. **Finding substances which decrease ammonia formation could lead to more acceptable ways of inhibiting the processes leading to ammonia formation in the rumen.**

Breakdown of microbial protein.

The second possibility which exists for improving the efficiency of fibre breakdown in the rumen is the suppression of the population of rumen ciliate protozoa. Protozoa consume large quantities of bacteria in the rumen, and the protein breakdown which results causes the nett yield of microbial protein resulting from rumen fermentation to be decreased by up to 50% (7, 8). If the protozoa could be suppressed, there would be less ammonia formation and less need for dietary protein supplementation. Some tropical plant materials are known to suppress protozoa (9, 10). **One novel aim of this project is to examine plant materials applicable to and cultivable in Europe for their usefulness as inhibitors of ciliate protozoa.**

Methane formation

Methane is a greenhouse gas many times more potent than CO_2 (11, 12). Its concentration in the atmosphere has doubled over the last century and continues to increase alarmingly (11, 12). Ruminants are major contributors to biogenic methane formation, and it has been estimated that preventing methane formation from ruminants would stabilise atmospheric methane concentrations (11-13). Furthermore, recent re-assessment of the

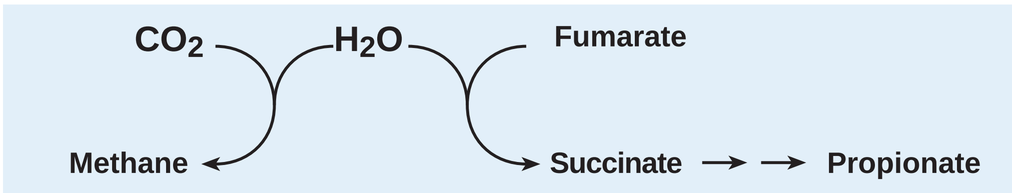


Fig. 2. Alternative routes for disposal of H_2 by ruminal microorganisms. When methane formation is inhibited, fumarate becomes an alternative H_2 sink. More propionate is formed, which is beneficial to the animal because propionate, but not acetate or butyrate (the other main fermentation products) is glucogenic.

Kyoto protocol places increased priority in decreasing methane emissions as part of a multi-gas strategy (14).

Research in this area has failed to find a chemical inhibitor of methane formation whose effectiveness persists for more than several days (16, 17). The only effective method in common use is again feed additive antibiotics and ionophores, which inhibit H_2 formation by species which provide H_2 to the methanogens (6). Methane formation is decreased by only up to 25%, however (17). As far as we are aware, **this will be the first systematic study of plant species to discover more effective and more sustainable means of inhibiting methanogenesis.**

Digestive disorders

Bloat. Under some dietary circumstances, a disorder occurs whereby the ruminal microbes ferment their food, but the gases formed are prevented from excaping because a stable foam forms in the rumen. This condition is known as bloat (18). Bloat is an extremely distressing condition which has a high incidence in alfalfa and some clover pastures in North America (19). The incidence of bloat in Europe is less clear, but some studies have clearly identified many cases where bloat was a cause of severe stress in calves (20, 21). The foam-inducing material is sometimes of plant origin, as with some clovers (19), and sometimes polysaccharides of bacterial origin (18). Preventative measures used at present principally involve breeding plants which are bloat-safe (22). Microbially derived or induced bloat has received less attention. **New means of preventing and treating this disorder are required.**

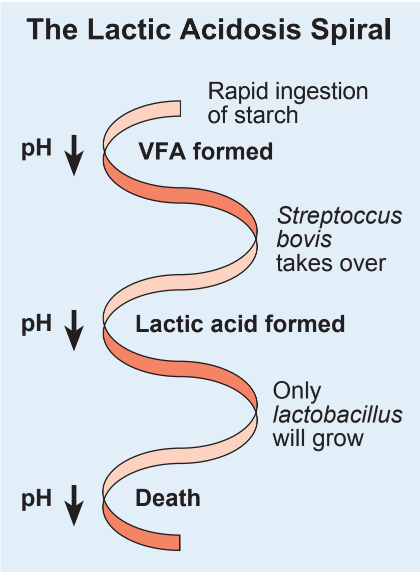


Fig. 3. The sequence of events which leads to acute lactic acidosis

Lactic acidosis. Lactic acid is normally only a minor product of rumen fermentation. However, when a rapidly degraded feed is introduced too quickly, or when concentrates form a high proportion of the diet, volatile fatty acid production exceeds the buffering capacity of the rumen, rumen pH falls, and only lactic acid-producing bacteria can grow (23). As lactic acid is a stronger acid than the volatile fatty acids, pH falls even more, the recovery of the normal fermentation becomes impossible, and the animal dies. More typically, however, ruminants on high-concentrate diets suffer sub-clinical acidosis, which is distressing although not life-threatening. Chemical buffers can reverse this condition (24), but it would be better if the growth of the lactic acid-producing bacteria could be suppressed. To some extent, ionophores and growth-promoting antibiotics achieve this (6, 23). **More sustainable methods are needed, however, and the proposed experiments represent a novel approach to the problem.**

The groups comprising this consortium each have several years' experience in one aspect of the work. Partner 1 has focussed on suppressing ciliate protozoa using plants and plant extracts, with the primary focus on developing countries (10, 28, 29); partner 1 also has extensive experience of feed additives (2, 6, 25, 30). Partner 2 has also focussed primarily on the developing world, using plant materials rich in saponins and tannins (5, 31, 32). Partner 3 is actively involved in the exploitation of indigenous browse species and (with Partner 1) in the inhibition of methane formation (3 references in partner profiles). Partner 4 is expert in the evaluation of feed additives (see profile for references). This project will for the first time coordinate these activities by creating a common resource of plant materials and pooling the expertise of the different partners. It will also involve commercial partners with a product profile and new in-house materials which are entirely consistent with the project objectives. The final phase of the project, which will be carried out principally by the industrial partners, will be **to use state-of-the-art techniques for evaluating the impact of feeding the new materials on meat and/or milk quality.**

References

1. Morrison, M. and Mackie, R.I. (1996) Nitrogen metabolism by ruminal microorganisms: current understanding and future perspectives. *Australian Journal of Agricultural Research* **47,** 227-246.

2. Wallace RJ, Onodera R, Cotta MA. ; Hobson PN, Stewart CS, editors.The rumen microbial ecosystem. 2nd ed. London: Chapman & Hall, 1997; 7, Metabolism of nitrogen-containing compounds. p. 283-328.

3. Aerts, R.J., Barry, T.N., McNabb, W.C. (1999) Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agriculture Ecosystems & Environment* **75,** 1-12.

4. Barry, T.N. and McNabb, W.C. (1999) The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* **81,** 263-272.

5. Makkar, H.P.S., Singh, B., Dawra, R.K. (1988) Effect of tannin-rich leaves of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. *British Journal of Nutrition* **60,** 287-296.

6. Nagaraja TG, Newbold CJ, Van Nevel CJ, et al. Hobson PN, Stewart CS, editors.The rumen microbial ecosystem. 2nd ed. London: Chapman&Hall, 1997; 13, Manipulation of ruminal fermentation. p. 523-632.

7. Ushida K, Jouany JP, Demeyer D. ; Tsuda T, Sasaki Y, Kawashima R, editors.Physiological aspects of digestion and metabolism in ruminants. London: Academic Press, 1991;Effects of presence or absence of rumen protozoa on the efficiency of utilization of concentrate and fibrous feeds. p. 625-54.

8. Williams AG, Coleman AG. The rumen protozoa. New York: Springer-Verlag; 1992.

9. Navas-Camacho, A., Laredo, M.A., Cuesta, A., Ortega, O., Romero, M. (1994) Evaluation of tropical trees with high or medium saponin content as dietary alternative to eliminate ciliate protozoa from the rumen. *Proceedings of the Society of Nutrition Physiology* **3,** 204.

10. Wallace, R.J., Arthaud, L., Newbold, C.J. (1994) Influence of *Yucca shidigera*

extract on ruminal ammonia concentrations and ruminal microorganisms. *Applied Environmental Microbiology* **60,** 1762-1767.

11. Gibbs MJ, Lewis L. ; Hoffman JS, editor.Reducing Methane Emissions from Livestock: Opportunities and Issues. U.S. Environmental Protection Agency; 1989.

12. Crutzen PJ. ; Engelhardt WV, Leonard-Marek S, Breves G, Giesecke D, editors.Ruminant physiology: digestion, metabolism, growth and reproduction. Stuttgart, Germany: Ferdinand Enke Verlag, 1995;The role of methane in atmospheric chemistry and climate. p. 291-331.

13. Johnson, K.A. and Johnson, D.E. (1995) Methane emissions from cattle. *Journal of Animal Science* **73,No.8,** 2483-2492.

14. Reilly, J., Prinn, R., Harnisch, J., Fitzmaurice, J., Jacoby, H., Kicklighter, D., Melillo, J., Stone, P., Solokov, A. & Wang, C. (1999) Multi-gas assessment of the Kyoto Protocol. Nature 401, 549-555.

15. Van Nevel C, Demeyer D. ; Wallace RJ, Chesson A, editors.Biotechnology in animal feeds and animal feeding. Weinheim, Germany: VCH, 1995;Feed additives and other interventions for decreasing methane emissions. p. 329-49.

16. Clapperton, J.L. (1977) The effect of a methane-suppressing compound, trichloroethyladipate, on rumen fermentation and the growth of sheep. *Animal Production* **24,** 169-181.

17. Van Nevel, C.J. and Demeyer, D.I. (1996) Control of rumen methanogenesis. *Environ Monit Assess* 77-101.

18. Dawson KA, Rasmussen MA, Allison MJ. ; Hobson PN, Stewart CS, editors.The rumen microbial ecosystem. Chapman&Hall, 1997;Digestive disorders and nutritional toxicity. p. 633-60.

19. Reid CSW, Clarke RTJ, Cockrem FRM, et al. McDonald IW, Warner ACI, editors.Digestion and Metabolism in the Ruminant. Armidale, Australia: University of New England Publishing Unit, 1975;Physiological and genetic aspects of pasture (legume) bloat. p. 524-36.

20. Kohler, M. and Doll, K. (1996) Studies on the aetiology and treatment of recurrent bloat in calves and young cattle. *Tierarztliche Umschau* **51,** 340-345.

21. Zentek, J. (1997) Case report: bloat and diarrhoea in calves. *Deutsche Tierarztliche Wochenschrift* **104,** 153-155.

22. Cheng, K.-J. (1998) A review of bloat in feed cattle. *Journal of Animal Science* **76,** 1997.

23. Russell, J.B. and Hino, T. (1985) Regulation of lactate production in Streptococcus bovis: a spiraling effect that contributes to rumen acidosis. *Journal of Dairy Science* **68,** 1712-1721.

24. Hart, S.P. and Polan, C.E. (1984) Effect of sodium bicarbonate and disodium phosphate on animal performance, ruminal metabolism, digestion and rate of passage in ruminating calves. *Journal of Dairy Science* **67,** 2356-2368.

25. Frumholtz, P.P., Newbold, C.J., Wallace, R.J. (1989) Influence of *Aspergillus oryzae* fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). *Journal of Agricultural Science* **113,** 169-172.

26. Wallace, R.J. and McPherson, C.A. (1987) Factors affecting the rate of breakdown of bacterial

27. Cheng, K.-J., Hironaka, R., Jones, G.A., Nicas, T., Costerton, J.W. (1976) Frothy feedlot bloat in cattle: production of extracellular polysaccharides and development of viscosity in cultures of Streptococcus bovis. *Canadian Journal of Microbiology* **22,** 450-459.

28. Teferedegne, B., McIntosh, F.M., Osuji, P.O., Odenyo, A., Wallace, R.J., Newbold, C.J. (1999) Influence of the foliage from different accessions of the sub-tropical leguminous tree, *Sesbania sesban*, on ruminal protozoa in Ethiopian and Scottish sheep. *Animal Feed Science And Technology* 78, 11-20.

29. El Hassan, S.M., Lahlou-Kassi, A., Newbold, C.J., Wallace, R.J. (1995) Antimicrobial factors in African multipurpose trees. In Rumen Ecology Research Planning (ed. R.J. Wallace & A. Lahlou-Kassi), pp. 43-61. International Livestock Research Institute, Nairobi, Kenya.

30. Newbold, C.J. and Wallace, R.J. (1988) The effects of the ionophores monensin and tetronasin on the simulated development of lactic acidosis *in vitro*. *Applied and Environmental Microbiology* **54,** 2981-2985.

31. Sen, S., Makkar, H.P.S., Becker, K. (1998) Alfalfa saponins and their implication in animal nutrition. *Journal of Agricultural and Food Chemistry* **46,** 131-140.

32. Makkar, H.P.S., Sen, S., Blummel, M., Becker, K. (1998) Effects of fractions containing saponins from yucca schidigera, quillaja saponaria, and acacia auriculoformis on rumen fermentation. *Journal of Agricultural and Food Chemistry* **46,** 4324-4328.

33. BenSalem, H., Nefzaoui, A., BenSalem, L., Tisserand, J.L. (1999) Intake, digestibility, urinary excretion of purine derivatives and growth by sheep given fresh, air-dried or polyethylene glycol-treated foliage of acacia cyanophylla lindl. *Animal Feed Science and Technology* **78,** 297-311.