1. 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 (Fig. 1.1). 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.

Protein degradation processes in rumen microorganisms

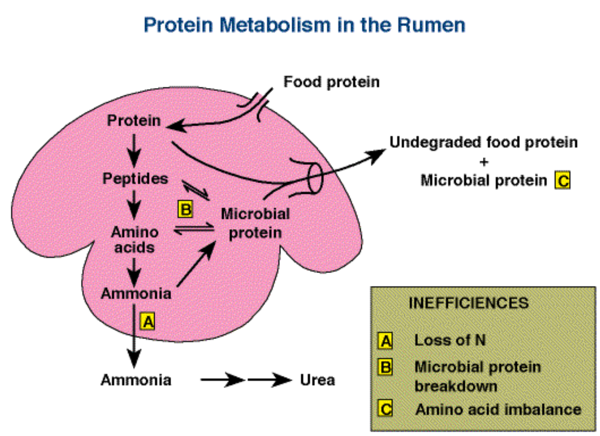

Fig. 1.1. Protein metabolism in the rumen. Inefficiencies are introduced by the breakdown pathways leading from protein to ammonia, by the rapid turnover of microbial protein, and by the amino acid composition of the protein flowing from the rumen not meeting the animal's needs. This project will investigate 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 (Morrison & Mackie, 1996; Wallace *et al.* 1997). 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 (Aerts *et al.* 1999; Barry & McNab, 1999), but they frequently impair other aspects of rumen fermentation, including fibre breakdown (Makkar *et al.* 1988). At the beginning of the Rumen-up project, 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, was considered timely. It was hoped that a new approach would 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 (Nagaraja *et al.* 1997) , which suppress the growth of the bacteria responsible. Finding substances which decrease ammonia formation was considered possibly to 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% (Ushida *et al.* 1991; Williams & Coleman, 1992). If the protozoa could be suppressed, there would be less ammonia formation and less need for dietary protein supplementation. Removal of rumen ciliate protozoa, or defaunation, has been an objective of rumen microbiologists for a generation. A meta-analysis has recently demonstrated that the benefits of defaunation outweigh any disadvantages (Eugene *et al.* 2004). Antiprotozoal agents, such as surface-active agents, that have been investigated in attempts to apply defaunation at the farm level have been hampered by problems with toxicity, either to other ruminal microorganisms (Eadie & Shand, 1981; Orpin, 1977; Bird & Leng, 1978; Bird *et al.* 1979) or to the host (Lovelock *et al.* 1982). Lipids are toxic to protozoa (Machmuller *et al.* 1998; Matsumoto *et al.* 1991; Newbold & Chamberlain, 1988) but also to fibre digestion (Broudiscou *et al.* 1994). Thus, there has been until now no reliable, safe on-farm method available for suppressing ruminal protozoa.Some tropical plant materials were known to suppress protozoa (Navas-Camaco *et al.* 1994; Wallace *et al.* 1994), but their potential does not seem tyo have been investigated fully. A novel aim of the Rumen-up project was 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 (Gibbs *et al.* 1989; Crutzen *et al.* 1995). Its concentration in the atmosphere has doubled over the last century and continues to increase alarmingly (Gibbs *et al.* 1989). Ruminants are major contributors to biogenic methane formation, and it has been estimated that preventing methane formation from ruminants would stabilise atmospheric methane concentrations (Gibbs *et al.* 1989; Crutzen *et al.* 1995; Johnson & Johnson, 1995). Furthermore, recent re-assessment of the Kyoto protocol places increased priority in decreasing methane emissions as part of a multi-gas strategy (Reilly *et al.* 1999).

Research in this area has failed to find a chemical inhibitor of methane formation whose effectiveness persists for more than several days (Clapperton, 1977; Van Nevel & Demeyer, 1996). 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 (Nagaraja *et al.* 1997). Methane formation is decreased by only up to 25%, however (Van Nevel & Demeyer, 1996). Some hydrogen sink compounds may be effective (Fig. 1.2). One of the aims of Rumen-up was to undertake the first systematic study of plant species to discover more effective and more sustainable means of inhibiting methanogenesis.

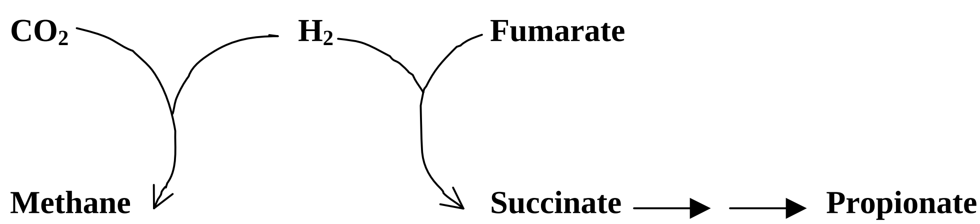

Fig. 1.2. Alternative routes for disposal of H_2 by ruminal microorganisms. When methane formation is inhibited, fumarate becomes an alternative H_2 sink. More propionate is formed, which is beneficial to the animal because propionate, but not acetate or butyrate (the other main fermentation products) is glucogenic.

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 (Dawson *et al.* 1997). Bloat is an extremely distressing condition which has a high incidence in alfalfa and some clover pastures in North America (Reid *et al.* 1975). 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 (Kohler & Doll, 1996; Zentek, 1997). The foam-inducing material is sometimes of plant origin, as with some clovers (Reid *et al.* 1991), and sometimes polysaccharides of bacterial origin (dawson *et al.* 1997). Preventative measures used at present principally involve breeding plants which are bloat-safe (Cheng *et al.* 1976; Cheng 1988). Microbially derived or induced bloat has received less attention. New means of preventing and treating this disorder were required and were an objective of Rumen-up.

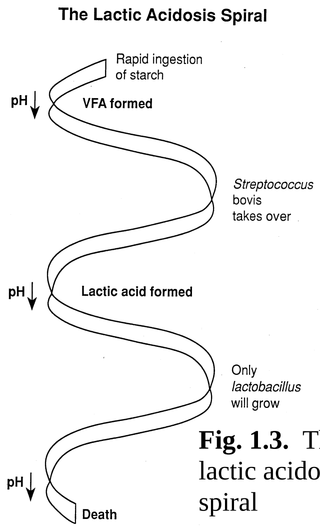

Fig. 1.3. The lactic acidosis spiral

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 (Fig. 1.3; Russell & Hino, 1985). 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 (Hart & Polan, 1984), 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 (Nagaraja *et al.* 1997). More sustainable methods were needed, however.

Antimicrobial properties of plant secondary metabolites
A huge variety of secondary compounds is produced by plants as natural protection against microbial and insect attack. Some are toxic to animals too, but others may not be, indeed many have been used in the form of whole plants or plant extracts for food or medical applications in man.

Essential oils. Essential oils are steam-volatile or organic-solvent extracts of plants, used traditionally by man for many centuries for the pleasant odour of the essence, or its flavour, or for its antiseptic and/or preservative properties. Although commonly thought of as being derived from herbs and spices, they are present to some degree in many plants for their protective role against bacterial, fungal or insect attack. They comprise mainly monoterpenes, cyclic hydrocarbons and their alcohol, aldehyde or ester derivatives. In a series of experiments carried out at the Rowett Research Institute (McIntosh *et al*. 2000, 2003; McEwan *et al*. 2002a,b; Wallace *et al*. 2002; Newbold *et al*. 2004), the effects of essential oils on ruminal fermentation have been investigated. Dietary inclusion of a commercial blend of essential oils caused significantly decreased NH_3 production from amino acids in ruminal fluid taken from sheep and cattle. This effect was mediated partly by effects on hyper-ammonia-productng (HAP) bacteria and on the protein and starch fermenting ruminal bacterium, *Ruminobacter amylophilus*. The potential therefore existed to find different essential oils which effected different benefits on ruminal fermentation, by preventing one or more of the problems described above.

Saponins. Saponins, like essential oils, cover a wide variety of chemical compounds and, also like essential oils, their properties have been used by man for centuries (Hostettmann & Marston 1995). The word 'saponin' is derived from the Latin word *sapo*, meaning soap, and traditionally saponins-containing plants have been used for washing. Chemically, saponins are high-molecular-weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety (Hostettmann & Marston (1995).
Van Nevel & Demeyer (1990) found no indication of any toxic effects or effects of sarsaponin on microbial growth or protein breakdown *in vitro*. In contrast, Lu *et al.* (1987) discovered that alfalfa saponins appeared to suppress fermentation in continuous culture. Subsequent *in vivo* investigation (Lu & Jorgensen, 1987) confirmed a general decrease in fermentative activity when alfalfa saponins were supplied to the sheep rumen, of which decreased VFA concentrations and decreased cellulose digestion were symptomatic. Significantly, Lu & Jorgensen (1987) also noted large decreases in protozoal numbers in sheep receiving alfalfa saponins. Goetsch & Owens (1985) concluded that the benefits of sarsaponin would be diet-dependent, increasing the digestion of sorghum silage and other fibrous feeds but apparently decreasing digestion of cereal and protein meals. Fairly recently, some tropical plants were found to have the potential to be used as a safe possible means of suppressing or eliminating protozoa from the rumen (Diaz *et al.* 1994; Navas-Camacho *et al.* 1993; Newbold *et al.* 1997; Odenyo *et al.* 1997). These plants all had the characteristic that they were rich in saponins.

Changes in legislation
Since the Rumen-up proposal was approved, legislators in Europe have moved to prohibit the use of growth-promoting antibiotics in animal feeds from the end of 2005 (Chesson, 2005). This decision was based on public and political concerns that the heavy use of antibiotics in general can give rise to transmissible resistance factors that can compromise the potency of therapeutic antibiotics in man. Growth promotion was a clearly avoidable use. US legislators may soon follow suit. Whether many of the commonly used growth promoters present such a threat is the subject of intense debate, nevertheless livestock producers in many countries must face a future without antibiotic growth promoters. Problems may be more acute in pig and poultry production, but ruminants will also be affected in the sense that existing and potential new strategies for manipulating rumen fermentation must avoid selective antimicrobials. Organically produced meat and milk are increasing in demand by consumers, and organic farmers therefore face the same problems. Thus, there is increasing interest in exploiting natural products, which have no similar public health hazard, as feed additives to solve problems in animal nutrition and livestock production.

Aims of the project
The aims of the project are encapsulated in the 'Rumen-up diagram', which has prefaced all periodic reports and presentations (Fig. 1.4). The plan for achieving the objectives (Fig. 1.5) involved alternating phases in which the partners made a collection of plants and plant extracts, then pooled the knowledge; the general effects of the collected samples on ruminal fermentation were determined by different partners using different diets, and the knowledge was again pooled; determining effects on specific target functions was carried out by different partners, then the knowledge was pooled; plants

were selected for acceptability and toxicity determination; then, the final production trials were planned and carried out.

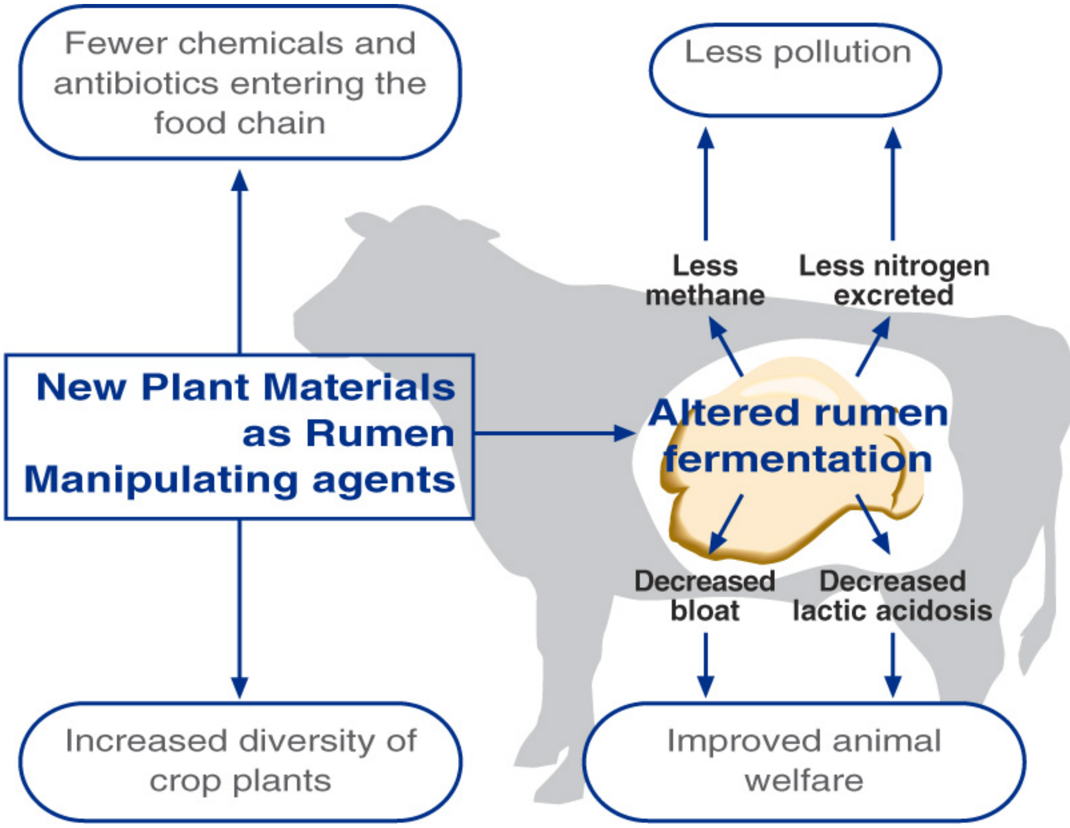

Fig. 1.4. The Rumen-up diagram. This diagram explains in a schematic way the aims of the project.

The consortium The groups comprising this consortium (Table 1) each had several years' experience in one aspect of the work. Partner 1 had focussed on suppressing ciliate protozoa using plants and plant extracts, with the primary focus on developing countries (Wallace *et al.* 1994; El Hassan *et al.* 1995; Teferedegne *et al.* 1999); partner 1 also had extensive experience of feed additives (Newbold & Wallace, 1988; Frumholtz *et al.* 1989; Wallace *et al.* 1997; Nagaraja *et al.* 1997). Partner 2 had also focussed primarily on the developing world, using plant materials rich in saponins and tannins (Makkar *et al.* 1988; Makkar *et al.* 1998; Sen *et al.* 1998). Partner 3 was 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 was expert in the evaluation of feed additives. This project coordinated these activities by creating a common resource of plant materials and pooling the expertise of the different partners. It also involved commercial partners with a product profile and new in-house materials which were entirely consistent with the project objectives. The final phase of the project, which was to be commissioned principally by the industrial partners, was committed to use state-of-the-art techniques for evaluating the impact of feeding the new materials on meat and/or milk quality.

Fig. 1.5. Project plan, Rumen-up