Abstract

The aim of the overall project was to develop new plants or plant extracts as dietary supplements for ruminants to replace chemical additives and growth-promoting antibiotics. The plant materials were collected from botanical and industrial collections, and evaluated for their ability to alleviate nutritional stress in ruminants, by preventing lactic acidosis and bloat, and to decrease pollution, by preventing methane formation and decreasing nitrogen excretions. Bloat and acidosis are distressing disorders which result from malfunction of microbial digestion in the rumen. Methane, a potent greenhouse gas, and ammonia, which forms urinary urea, arise from normal rumen fermentation. The project would deliver plant-based, sustainable solutions to these problems. The results would benefit European biotechnologal and agricultural industries, and the new plants would increase the diversity of crops used in agriculture. The main objective to be completed within the first 12 months was to assemble a 500-sample collection of plants and plant extracts, together with brief descriptions of their traditional use, possible secondary compounds and an assurance of their cultivability in the EU. This objective was achieved on schedule.

Each of the partners visited local university departments of botany and national or local botanical collections in order to discuss the project with experts and to collect samples. One hundred samples per academic partner and 50 samples per commercial partner were selected on the basis of their agronomic properties and known nutritional properties. The plants were able to be cultivated within the EU. The taxonomic name of the material was determined unambiguously, and common names also included. A literature search was performed on nutritional and anti-nutritional properties of the samples. A database was set up to organise this information, and to provide an organised repository for the vast amounts of data to be generated during screening and determination of more specific effects of the plants. A printed report describing the collection was compiled. The first milestone to be completed at this stage, namely M1 Annotated collection of specimens, was achieved. The collection is located at the Rowett Research Institute. The academic partners also have subsamples of the complete collection.

The next broad objective was to screen all samples for their effectiveness in inhibiting rumen ciliate protozoa, rumen proteolysis, methane formation, microbial protein synthesis, lactic acidosis and bloat. The samples were also investigated to ensure that potentially useful samples had no detrimental effect on the other basic functions of the fermentation, such as fibre digestion and volatile fatty acid production. All analyses were complete at 18 months, and a report was prepared and submitted to the Commission. The consortium has no plans to exploit the data on general effects, or the samples excluded from later phases. Nevertheless, we wish to highlight the data generated as a true European resource of tremendous value. More than 25 samples were identified in the different categories to be potentially useful, with no detrimental influence on general properties (Milestone M2). An investigation was sub-contracted to botanical specialists to ascertain if these samples were available commercially or could be obtained by commercial horticulture within the time-frame of the Rumen-up project. Subsequently, based on re-examination of methods and results, the list of most promising samples was changed. The partners re-evaluated the lists, basing their choices on all known properties and potential availability, and a refined list of eight was drawn up as being the most promising samples. Two each

from the categories antiprotozoal, antiproteolytic, antilactic acidosis and antibloat were selected.

A total of 23 samples was identified to have sufficient potential for development as feed additives which could manipulate fermentation in one or more of the target areas without having detrimental effects on overall fermentation. A patent was filed on 16 April 2004 to protect the intellectual property which had been generated on these 23 samples.

A smaller number of samples was then taken forward for more detailed experimentation, with the aim of producing a short list of two samples that would be tested in production trials in the last six months of the project. *In vivo* testing of acceptability and toxicity of this smaller number of samples was carried out in sheep. Only *Knautia arvensis* appeared to have a possible problem with feed intake, and this was not severe. None of the short-listed samples gave any indication of toxicity.

Difficulties were anticipated in securing sufficient materials for testing in the final phase of the project, because of the seasonal availability of plant foliage. A six-month extension was therefore requested by the consortium, and granted by the Commission, in order to maximise the effectiveness of the animal trials.

The academic partners carried out dose-response and persistency investigations on the target function which they had earlier investigated using the samples they had identified to carry most potential. Generally, the materials were not potent at low concentrations. Most would have to be included in the diet at 3-5%. Partners reviewed all data available to date in June 2004. The budget was examined carefully, together with the potential of the different samples which had hitherto proved successful. A decision was made that the budget could be stretched to cover the investigation of three rather than two samples by animal trial, namely *Bellis perennis*, *Knautia arvensis* and *Lactuca sativa*, thus enhancing the chance of overall success in the project. Subsequently, when drying problems were encountered with *L. sativa*, it was decided to replace *L. sativa* with *Urtica dioica*, the other sample with greatest potential as an antiacidosis agent. Sheep trials were commissioned at the University of León and the University of Hohenheim to begin September/October 2004. Then, in November 2004, an outbreak of Blue Tongue occurred in sheep in Spain. A further 4-month extension was therefore granted by the Commission.

Animal trials with the three selected samples showed some success. There were no negative effects, and there were signs of positive responses with *Bellis perennis* and *Knautia arvensis*. The effects were smaller than had been hoped for, such that further trials would have to be carried out to determine the magnitude and variability of response with different diets, animal species, etc.

Differential solvent extraction was carried out to begin the process of identifying the likely active components. Activity of antiprotozoal agents was retained in fractions likely to correspond to saponins. Low activities of antimethane and antiacidosis samples were lost on fractionation. The antiproteolysis factor of *K. arvensis* was associated with the methanol fraction. Unlike other antiproteolytic samples identifed in the collection, *K. arvensis* contained no tannins. The agent, whicvh could not be identified, affected proteolysis in an adaptive fashion. Unlike with the tannins-containing plants, no immediate inhibitory effect was observed, but significant inhibition was observed after a 12-h incubation. The effect was similar to that found with the feedlot ionophore, monensin, suggesting simliar effects on ruminal bacteria undertaking ammonia production from dietary protein.

Cultural analysis indicated that caution would be required in the use of some of the selected plants, because the growth of cellulolytic bacteria was often sensitive

to the plants. This was particularly tre of plants observed to contain high concentrations of tannins. Molecular analysis revealed changing community structure as the result of feeding the plants; however, this was difficult to distinguish from large changes resulting from inter-animal variation and level of feeding. The chemical nature of the antiprotozoal activity of the different plants was different; the two best candidates, *Bellis perennis* and *Gentiana ascledipea*, both contained saponins, but the former had high haemolytic activity while the other had none. The chemical identity of the antiproteolytic plant, *Knautia arvensis*, proved elusive, in spite of many differential solvent extractions.

Evaluation of the project by the commercial partners was highly positive, with the main obstacle to commercialization being identified as the hurdles to be surmounted in the regulatory process. This was considered to be a major disincentive to any of the partners or any other company wishing to take any of the vast quantity of promising data generated in Rumen-up to commercialization in Europe. Nevertheless, the information, when made available at the end of the consortium's confidentiality period, will be of value to many small businesses who, during the course of the project have enquired about results relating to products which they wish to promote using scientific data generated by Rumen-up.