Rumen microbiology, biotechnology and ruminant nutrition: The application of research findings to a complex microbial ecosystem

R.J. Wallace

Rowett Research Institute, Bucksburn, Aberdeen, UK

Received 7 June 1992
Accepted 22 June 1992

Key words: Rumen; Ionophore; Biotechnology; Probiotics

1. SUMMARY

Research on rumen microorganisms has contributed greatly to our understanding of anaerobic microbial ecosystems, and has also influenced feeding practices and nutritional modelling with ruminants. However, it can be argued that rumen microbiology has not yet fulfilled its true potential. Growth-promoting ionophores, antibiotics and microbial feed additives were introduced before their microbiological effects had been determined. A more pro-active role for the microbiologist was predicted with the advent of recombinant DNA technology. Whether ventures in molecular biology can be applied successfully to benefit nutrition and health is likely to depend on whether means can be found for maintaining new strains in vivo.

2. INTRODUCTION

The classic monograph by Hungate describing ‘The rumen and its microbes’ [1] is a wonderful account of the birth and early years of rumen microbiology. When it was realised that rumen bacteria were more strictly anaerobic than other ‘anaerobes’ and that most of the species had fastidious nutritional requirements, it became possible to study these bacteria in depth [2,3]. By analogy, studying obligate anaerobes from other anaerobic habitats, including the human intestine, also became possible [4,5]. These advances took place at about the same time as it was being revealed that ruminants depend for their energy needs on the products of the fermentation, namely the volatile fatty acids, acetic, propionic and butyric acids [6]. The potential for altering ruminant nutrition by understanding and manipulating rumen fermentation was then obvious. This paper explores how effectively this potential has been realised.

3. RUMEN FUNCTION AND DYSFUNCTION

Nutritional modelling for ruminants depends on understanding the nature of both energy and protein metabolism by rumen microorganisms. Rumen microbiology has been able to provide values for microbial growth yields and rates, fer-
mentation stoichiometries, cell composition and catabolic activities which enable quantitative models of normal rumen fermentation to be set up [7,8]. Fundamental studies also help to understand, and therefore avoid, nutritional disorders. Cellulolytic bacteria are sensitive to low pH [9,10], so transient excesses of rapidly degraded starch are to be avoided if fibre breakdown is to be maximal [11]. Excessive starch consumption leads to potentially fatal lactic acidosis, a well-described downward spiral of rumen pH as first Streptococcus bovis, then Lactobacillus spp. replace the normal rumen flora [12]. Bloat-causing feedstuffs can be identified in advance from in vitro tests with rumen microorganisms [13]. In addition to these specific examples, it cannot be doubted that simply an awareness of the nature of rumen digestion has benefited nutrition. But what about deliberate intervention, or manipulation?

3. IONOPHORES AND ANTIBIOTICS

Monensin is the best-known of the ionophores and antibiotics which have been used as growth promoters for ruminants. Others include narasin, salinomycin, lysocellin, lasalocid, tetronasin and avoparcin. All of the compounds are selectively toxic, particularly to Gram-positive bacteria [14–18]. Their effects on the fermentation have generally been consistent with their antimicrobial activity (Table 1). Furthermore, the ionophores have proved useful in probing normal rumen function, including the microbial ecology and biochemistry of amino acid catabolism [19].

Explanations which invoke the elimination of certain organisms by ionophores and the proliferation of resistant species as their mode of action may oversimplify their effects, however. Bacteria which are initially sensitive to monensin and other ionophores soon become resistant [14,18], most likely by mutation. Ruminococcus flavefaciens

| Table 1 |
| Effects of ionophores on rumen fermentation |

<table>
<thead>
<tr>
<th>In vivo observation</th>
<th>Species/function affected</th>
<th>Other consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased acetate production</td>
<td>Butyribrio, Ruminococcus suppressed; acetate producers</td>
<td>Enhanced growth of propionate producers</td>
</tr>
<tr>
<td>Decreased tendency to lactic acidosis</td>
<td>S. bovis, Lactobacillus suppressed; lactate producers</td>
<td>Increased rumen pH</td>
</tr>
<tr>
<td>Increased flow of amino acids</td>
<td>S. bovis, Peptostreptococcus inhibited; proteolytic and amino acid deamination</td>
<td>Decreased ammonia production</td>
</tr>
<tr>
<td>Decreased methane production</td>
<td>Butyribrio, Ruminococcus suppressed; formate, hydrogen producers</td>
<td>Methanogens unaffected, only substrate supply decreased</td>
</tr>
</tbody>
</table>
eventually tolerates 100 times the dose of tetronasin to which it was originally sensitive (Fig. 1). Presumably fibre digestion would be compromised if this adaptation did not happen. Furthermore, species which appear initially to be resistant to an ionophore may yet be affected by and indeed mutate in the continued presence of the compound. Sub-lethal concentrations of monensin caused growth rates and yields of monensin-resistant rumen bacteria to be depressed [14,15] and ionophore-resistant mutants of *B. ruminicola* can be isolated which have decreased cell-envelope permeability [20] and amino acid catabolic activity [21]. Thus the long-term effects of ionophores may not be explained simply by the elimination of initially sensitive Gram-positive bacteria. Furthermore, although much has been written about the ion-translocating properties of ionophores [22,23], it is their ability to permeate the cell envelope of different species to different extents that is the basis of their selective action. For example, monensin is a Na⁺,K⁺/H⁺ antiporter, tetronasin is a Ca²⁺/H⁺ antiporter, and avoparcin is an antibiotic which inhibits cell-wall synthesis, yet they have an almost identical spectrum of antimicrobial activity against rumen bacteria [14–16,18], and bacteria which become resistant to one of these compounds usually become more resistant to others (ref. 20; C.J. Newbold, unpublished results).

Our description of the mode of action of ionophores remains incomplete, therefore. It should also be recognised that, despite their success, these compounds were introduced initially in the mid-70s in an empirical way. Monensin already had wide application as a coccidiostat in poultry. The contribution of the microbiologist, although valuable, was not a predictive, instigating one, but rather he explained microbiologically the effects which had already been observed in vivo.

Other chemical modifying agents, including inhibitors of methane production and compounds toxic to ciliate protozoa, have been investigated extensively. None of these is in general use at the present time, although several are highly promising. Detailed accounts of these materials are given elsewhere [24,25].

4. INTRODUCING NEW OR IMPROVED SPECIES

The idea that an improved understanding of rumen microorganisms would lead to the development of improved species which could then be introduced in vivo to improve ruminant nutrition and health was not new, but it was given fresh impetus by the molecular biology revolution of the 1970s and 80s. Several excellent articles pointed out areas in which recombinant organisms with certain properties could benefit ruminant nutrition [26–31].

Three objectives would have to be achieved in order to sustain an organism which would benefit the host animal. Firstly, a useful new property would have to be identified. There is no shortage of suggestions, which include improved cellulolysis at low pH, increased lactate utilisation, detoxification of antinutritional factors in plants, and increasing the methionine and lysine content of microbial protein [26–31]. Secondly, techniques for inserting new genetic information into rumen bacteria must be available. Again there is no lack of potential mechanisms. Shuttle vectors and naturally occurring, self-transmissible plasmids have been developed for *B. ruminicola* [32,33]. Streptococcal transposons have been introduced into *Butyribrio fibrisolvens* [34] and *Streptococcus bovis* [35]. Phage systems were also reported to be undergoing development for *S. bovis* [36]. In other species, similar experiments have proved less successful, but there is no reason to suppose that genetic modification of any desired species may not be achieved eventually.

The final obstacle may be more difficult to overcome. In a sense the introduction of a new strain is attempting to override evolution. Russell and Wilson [30] and Gregg and Sharpe [31] stressed that unless the new organism had a selective advantage, it would struggle to survive. How great a problem this might pose is difficult to predict. Attwood et al. [37] found that a marked laboratory strain of *B. ruminicola* disappeared rapidly when re-introduced into the rumen. In contrast, Flint et al. [38] found that a rifampicin-resistant *Selenomonas ruminantium* persisted for 30 days in the rumen of sheep. The problem
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Weight gain (kg/day)</th>
<th>Urinary DHP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated steers</td>
<td>0.52</td>
<td>0.28</td>
</tr>
<tr>
<td>Inoculated steers</td>
<td>1.03</td>
<td>0</td>
</tr>
</tbody>
</table>

(Data from Quirk et al., ref. 43).

might be overcome by creating a new ecological niche, by adding to the feed a compound which could be used selectively by the new strain [39]. Attempts to introduce a non-rumen, sorbitol-utilising Escherichia coli were unsuccessful, partly because of the toxic effects of the high volatile fatty acid concentrations on E. coli [40]. Similar experiments with rifampicin-resistant (as a marker), sorbitol- and xylitol-utilising Selenomonas ruminantium were also unsuccessful, largely because the sugar alcohols chosen as selective growth substrates were absorbed rapidly from the rumen (R.J. Wallace and N.D. Watt, unpublished results). One has therefore to conclude that the problem of establishing new organisms, unless their new property gives them an intrinsic selective advantage (as cellulolysis at low pH might, for example), remains to be overcome.

The most notable success in this area concerns the introduction into Australia of bacteria capable of metabolising 3-hydroxy-4(1H)-pyridone (DHP). Australian cattle were poisoned when they consumed the leguminous forage Leucaena leucocephala, because the non-protein amino acid, mimosine, was converted to DHP, but no further, and the DHP was a goitrogen [41]. However, ruminants in Hawaii and Indonesia grazed on Leucaena without ill effects. When rumen fluid from goats receiving Leucaena was used to inoculate the Australian cattle, DHP accumulation and the toxicity of the legume were overcome (Table 2) [42,43]. The bacteria responsible for DHP breakdown were subsequently isolated and characterised [44].

5. MICROBIAL FEED ADDITIVES

Fermentation extracts of Saccharomyces cerevisiae and Aspergillus oryzae are used as growth promoters in ruminants [45]. Like the ionophores, they were introduced before their mode of action at the microbiological level was known. Their effects are more variable than the ionophores, but there is little doubt that they can have an important influence on fermentation by the mixed rumen population. Most studies find that the viable count of bacteria is increased, sometimes several fold, by these additives [46–50], and it seems that the improved stability of the bacterial population gives rise in turn to other benefits, such as better fibre breakdown [45]. Neither S. cerevisiae nor A. oryzae grows in the rumen, but their metabolic activity seems to be vital for their effect. In both instances, autoclaving the extracts destroyed their stimulatory activity [48,50], but gamma-irradiation did not with A. oryzae [50].

Much remains to be disclosed about the mode of action of the present generation of microbial feed additives. However, it seems that here is an opportunity for the rumen microbiologist. Dawson and his colleagues [51] are experimenting with yeasts that have been selected on the basis that they stimulate specific properties of rumen bacteria, such as cellulolysis or lactate utilisation (Fig. 2). It is a relatively small step from there to suggest that the yeasts or fungi may in fact be much better organisms, at least in the short term, for implementing the benefits of biotechnology.
than indigenous rumen bacteria. The genetics of *S. cerevisiae* and *A. oryzae* are much better known than any species of rumen microorganism; the microbial cultures are fed daily, so no selection pressure is necessary; the technology exists for their large-scale production; and, as food products already, they are perceived to be safe. Of course, the safety of using recombinant DNA in this form would still have to be demonstrated, but nevertheless an opportunity exists here, even using mutants selected by non-recombinant means.

5. CONCLUSIONS

The rumen microbiologist has made a major contribution to our understanding of ruminant nutrition, but his role has been mainly descriptive. Prospects for a more pro-active future role in manipulating rumen fermentation are bright. Microbial feed additives offer a new opportunity for rational manipulation, and possibly also may be the best vehicle for exploiting the potential benefits of biotechnology in improving ruminant nutrition, until problems of maintaining modified strains of bacteria in vivo are solved.

REFERENCES


