Manipulating Ruminal Fermentation: A Microbial Ecological Perspective[1]

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ABSTRACT: The essential role of ruminal microflora in ruminant nutrition provides the potential for improvement in animal production via altering the numbers or activities of specific classes of microorganisms. Successful alterations will be facilitated by an understanding of the microbial ecology of the rumen based on its mechanistic underpinnings. Demonstrated improvements in ruminal fermentation can be traced to their consonance with well-established principles of microbial ecology (niche occupancy, selective pressure, adaptation, and interactions) and the thermodynamics and kinetics of substrate utilization. Application of these principles to several proposed alterations of the ruminal bacterial population allows a prediction of their relative feasibility. Improving fiber digestion, decreasing protein degradation, and detoxifying feed components that are present in low concentrations will be difficult to achieve in the rumen and are best approached by altering the feed, either genetically or with postharvest treatment. By contrast, the detoxification of feed components present in high concentration, and redirection of electron disposal away from methanogenesis, are more productive targets for microbiological research.

Key Words: Acetate, Bacteria, Fiber, Methane, Rumen, Toxins

 J. Anim. Sci. 1998. 76:3114–3122

Introduction

The rumen is an open, self-contained ecosystem in which feed consumed by the ruminant is fermented to volatile fatty acids and microbial biomass that serve the animal as sources of energy and protein, respectively. Individual microbial species that have developed in the rumen over eons interact in a complex manner and provide some of nature's best examples of microbial symbioses. However, modern feeding practices geared toward high production have presented some novel challenges to ruminal microflora. The microbial response to these challeges has not always been satisfactory to the animal owner, leading to a desire to improve ruminal fermentation to enhance production.

This article examines some demonstrated or potential manipulations of ruminal microflora in terms of their desirability, their practicality, and their effects on the overall microbial ecology. The role of currently used, direct-fed microbials will not be addressed, because these agents often have inconsistent effects on animal performance and because evidence that these agents establish themselves as significant, viable members of the ruminal microflora is equivocal (Williams and Newbold, 1991; Martin and Nisbet, 1992). Instead, this examination focuses on four other areas: metabolism of plant toxins, improvement of fiber fermentation, reduction in proteolysis, and reduction in methanogenesis. The salient themes of this discussion are that the rumen is subject to the same ecological principles that govern other habitats and that an understanding of these principles facilitates further advances in this area. Molecular probe technology now permits characterization and quantification of microbial populations in situ (Stahl et al., 1988; Amann et al., 1990). These techniques have revolutionized the study of ruminal microbial ecology, but the basic ecological principles are likely to be reinforced, rather than shaken, by the use of these methods in future studies.

Metabolism of Plant Toxins

Plants contain a variety of chemicals that have negative effects on either the ruminant animal, its gastrointestinal microflora, or both. Consumption of these forages often causes reduced performance or even fatal toxicoses, an economic hardship exacerbated by the tendency for a large fraction of a herd to

[1]Presented at a symposium titled "Manipulation of the Rumen Fermentation for Enhanced Animal Performance" at the 75th ASAS South. Sec. Mtg., January, 1998, Little Rock, AR.

Received February 17, 1998.
Accepted July 8, 1998.

Table 1. Standard free energy changes for reactions discussed in the text

Process	Overall reaction	$\Delta G^{\circ\prime}$, kJ[a]	Comments
Oxalate dismutation	$HOOC\text{-}COO^- \rightarrow HCOO^- + CO_2$	–44.4	Detoxification
Methanogenesis	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	–139.1	Source of ruminal methane
	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	–27.5	Not significant in rumen
Sulfate reduction	$4H_2 + 2H^+ + SO_4^= \rightarrow H_2S + 4H_2O$	–152.3	Undesirable reaction in rumen owing to toxicity of H_2S
Nitrate reduction	$4H_2 + 2H^+ + NO_3^- \rightarrow NH_4^+ + 3H_2O$	–599.8	Undesirable reaction in rumen owing to possible accumulation of toxic nitrite
Acetogenesis	$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+$	–111.6	Desirable, but currently not a significant ruminal reaction

[a]Values calculated at pH 7 and 25°C at 1 atm of gas or 1 *M* nongaseous component (except for water). Data were calculated from standard free energies of formation for each chemical species, using the tabular data of Thauer et al. (1977). In accord with convention, the more negative free energy change indicates a more thermodynamically favorable reaction. 1 kJ = .239 kcal.

be affected. As a result, there is considerable interest in the ability of the ruminal microflora to detoxify these plant components. Plant toxicoses have far more impact on beef production than on dairy production, owing to the abundance of toxic plant species on grazing lands used for beef production, particularly in arid regions.

Microbial metabolism of plant toxins exemplifies a number of ecological concepts. The central principle is that the ability of ruminal microbes to detoxify these compounds is governed by whether such metabolism confers a selective advantage to a microbe in the ruminal environment. Selective advantage could arise from two sources: 1) through utilization of the toxic agent as an energy source or electron acceptor in microbial metabolism; or 2) through detoxification of a compound that inhibits one or more species of the microbial population. In the first case, metabolism will not provide a selective advantage unless the agent is present at concentrations sufficient to justify the development of the biochemical machinery necessary for its metabolism. This machinery includes the enzymes required for the biotransformations, any proteins that may be required to transport the agent into the cell, and the corresponding genes and regulatory elements. Several outstanding examples have been reported in which the toxic agent is metabolized by ruminal microbes as an energy source. The most famous example is provided by mimosine, an unusual amino acid found in large quantities in the tropical woody forage *Leucaena leucocephala*. Mimosine is readily converted by certain unidentified members of the ruminal population to the toxic goiterogen 3-hydroxy-4(1H)-pyridone (**DHP**). Jones and Megarrity (1983) noted that Australian goats that consumed leucaena developed toxicoses, but Hawaiian goats consuming the same plant species at equivalent mimosine intakes (~20 g/d) did not. Moreover, these workers demonstrated that inoculation of enrichment cultures derived from ruminal contents of Hawaiian goats into Australian ruminants conferred resistance to leucaena poisoning (Jones and Megarrity, 1986). The case was closed when Allison et al. (1992) isolated from resistant goats a new bacterial species, *Synergistes jonesii*, that was capable of metabolizing DHP. This metabolically specialized bacterium is capable of growth only on DHP and two less-common protein amino acids, arginine and histidine (McSweeney et al., 1993). Cultures of this organism have subsequently been used as inoculants to protect ruminants in other parts of the world from mimosine toxicity (Quirk et al., 1988; Hammond et al., 1989), and, once established, the organism seems to spread interuminally within a herd under conditions of normal contact. This example is often cited as one of the few known examples of a geographical localization of a bacterium. The rapid colonization of the rumens of leucaena-intolerant ruminants by *S. jonesii* also serves as a classic example of a microbe being able to fill a specific niche in response to selection pressure (namely, the availability of the metabolizable substrate DHP).

A second example of plant detoxification involves oxalic acid, which can represent up to 30% by weight of certain forages (e.g., halogeton; James and Butcher, 1972). Oxalic acid can be metabolized by *Oxalobacter formigenes*, a gastrointestinal bacterium that gains energy by dismutation of oxalic acid to formic acid and CO_2 (Table 1; Allison et al., 1985). Like *S. jonesii*, *O. formigenes* is extremely specialized, in this case by being able to grow only on oxalate. Because oxalic acid is a highly oxidized compound, its metabolism generates relatively little energy, and thus considerable amounts of this compound must be metabolized to satisfy the growth requirements of the organism. As a result, a fairly small population of this species in the rumen (10^4 to 10^6 cells/mL) is sufficient to metabolize considerable amounts of ingested oxalate. This is dramatically illustrated by the success of adapting sheep to halogeton-based diets in which oxalate represented nearly 5% of dietary dry matter (Daniel et al., 1989). Viewed through the lens of microbial

ecology, the microbial metabolism of both mimosine and oxalate share common determinants of success: dietary abundance (selective pressure) and a specialized degradative population (niche specialization).

A third case of toxin metabolism is provided by cicer milkvetch (*Astragalus cicer*), a forage that often exhibits poor digestibility despite its low lignin content (Kephart et al., 1990). This species does not contain toxic agents characteristic of some other *Astragalus* species (e.g., alkaloids, nitrotoxins, cyanogenic glucosides, or selenium). However, leaves of *A. cicer* contain a compound, now identified as an arabinogalactan protein, that prevents cellulolytic bacteria from adhering to cellulose (Weimer et al., 1993). The susceptible cellulolytic species are unable to degrade this arabinogalactan protein–not surprising, perhaps, given the extreme nutritional specialization of these bacteria. However, other members of the ruminal microflora can readily remove this agent upon adaptation, which probably reflects its significant concentration (~1% of dry matter) and the ease in which the component sugars and amino acids can be fed into intermediary metabolic pathways.

Forage alkaloids represent a different nutritional situation. A given plant species may contain several classes of alkaloids, with each class represented by a variety of structurally distinct compounds. Each of these compounds are usually present in the plant at fairly low concentrations. For example, the concentrations of individual ergopeptide alkaloids in endophyte-infested tall fescue (*Festuca arundinacea*) are typically <100 μg/kg dry matter. Total dietary intake of each alkaloid compound may be only a few milligrams per day. This would not be expected to provide sufficient selective pressure to maintain a specialist population, even though it may provide additional metabolizable substrate for a more nutritionally versatile species.

On the other hand, because some alkaloids that are less toxic to ruminants themselves (e.g., perloline, perlolidine, and phenanthridine) have been shown to inhibit certain ruminal microbial processes (namely, cellulose digestion) (Bush et al., 1976), selective pressure for metabolism of these alkaloids may facilitate development of the ability to degrade them. Indeed, there is microbial evidence for the metabolism, by mixed ruminal microflora, of individual alkaloids (Moyer et al., 1993) or mixtures of alkaloids isolated directly from plant material (Craig et al., 1992). In all cases reported thus far, metabolism yields only partially degraded products rather than typical fermentation end products. Partial degradations also have been reported for some other toxins (e.g., the deacetylation of tricothene mycotoxins by *Butyrivibrio fibrisolvens*, apparently via nonspecific esterases; Westlake et al., 1987). In the case of the alkaloids, ergopeptines are metabolized in the rumen to remove the peptide or amino acid substituents, without affecting the lysergic acid ring. Because selective pressure is insufficient to favor complete metabolism of the alkaloids, the most productive strategy to reduce alkaloid toxicoses may be modification of plant germplasm to reduce alkaloid content, without sacrificing the desirable agronomic properties of the plant; in tall fescue these include enhanced root production that facilitates drought resistance.

A final, and somewhat enigmatic, example of the role of selection in microbial detoxification is provided by fluoroacetate. This compound, present in some tropical forages, is toxic to mammals via its conversion to fluorocitrate, an inhibitor of aconitase, a key enzyme in the citric acid cycle. Gregg (1995) has reported the introduction, into *Butyrivibrio fibrisolvens* OB156, of a plasmid containing a gene for hydrolytic dehalogenation of fluoroacetate. The plasmid was stably maintained in this strain in vitro, and the bacterium was maintained in measurable numbers for over 5 mo following inoculation into the rumens of two sheep, although protection of these sheep from fluoroacetate poisoning has not yet been reported. Because fluoroacetate is not acutely toxic to anaerobic bacteria and its metabolism does not yield energy, Gregg points out that the source of positive selection pressure for maintenance of dehalogenase function is not obvious. However, dehalogenation of fluoroacetate, by preventing inhibition on aconitase, would permit synthesis of α-ketoglutarate, a precursor to the glutamate family of amino acids. This may benefit *B. fibrisolvens* in animals grazing poor-quality tropical forages, in which amino acids may be present in ruminal concentrations low enough to challenge this bacterial species, whose amino acid uptake capabilities have been described by Hungate (1966) as “fair to poor.”

Manipulation of the Fibrolytic Population

Plant structural carbohydrates are major contributors to the energy requirements of the ruminant. As a result, there is considerable interest in optimizing the rate and extent of fiber digestion by the ruminal microflora. Because cellulose is the major component of forage fiber and is readily available in purified form, its degradation has received far more study than has that of other plant polysaccharides. Cellulose digestion in the rumen, unlike that in other habitats, proceeds primarily via a relatively few bacterial species that adhere directly onto the surface of the fibers. This mode of attack has several advantages. Localization of enzymes at or very near the cell surface permits both the hydrolysis of cellulose and ready access of the microbes to soluble hydrolytic products (cellodextrins). Moreover, the surface-bound nature of the process probably reduces loss of cellulases to proteolysis, or removal of cellulolytic bacteria by grazing protozoa. As a result of these factors, the predominant ruminal cellulolytic species (*Fibrobacter*

succinogenes, *Ruminococcus flavefaciens*, and *R. albus*) digest cellulose at rate constants of .05 to .10 h^{-1}, faster than nearly any other known cellulolytic species (Weimer, 1996).

The last two decades have witnessed increased efforts to more fully understand the enzymology of fiber (particularly cellulose) digestion, and to genetically engineer ruminal bacteria with enhanced cellulolytic capabilities. The implicit principle driving such work is that cellulose digestion is limited by the cellulolytic capabilities of the resident microflora. Yet this principle does not stand up to close scrutiny. There is abundant evidence that the kinetics of cellulose digestion is first-order with respect to cellulose concentration or available surface area (Waldo et al., 1972; Van Soest, 1973; Fisher et al., 1989; Weimer et al., 1990; Maglione et al., 1997). In other words, cellulose digestion is limited not by the population or activity of the cellulolytic microbes, but rather by the amount of cellulose available for microbial attack. Strategies to modify the plant to make fiber more available (e.g., reducing crosslinking between lignin and hemicelluloses) may permit improvements in the rate or extent of fiber digestion while retaining the desirable fermentation product mix of the native cellulolytic population, as long as such improvements do not negatively affect yield or environmental fitness of the plant (Jung et al., 1993).

Because cellulose digestion in the rumen does not seem to be limited by the density or activity of the native cellulolytic species, it is unlikely that a genetically engineered "hypercellulolytic" strain would increase the rate of cellulose digestion, and it may not even effectively compete with the native cellulolytic population for the limiting amount of cellulose that is available. The difficulty in establishing alternative cellulolytic species has been dramatically demonstrated by Varel et al. (1995), using *Clostridium longisporum*, a highly cellulolytic bacterium that is occasionally present in the rumen but that never reaches the abundance of more predominant cellulolytic species, such as *F. succinogenes* or the ruminococci. Varel et al. (1995) introduced 6 L of a fermentor-grown culture of *C. longisporum*, along with 20 L of buffer, directly into each of the emptied rumens of three fistulated cows. Despite its ruminal origin and its active cellulolytic capabilities in vitro, this strain was completely undetectable in all three rumens within 48 h after its introduction. These results indicate that the native, or so-called autochthonous, population provides a strong barrier to invasion by foreign (allochthonous) species, even those of apparently similar functionality.

A more promising direction may lie in improving fiber fermentation under acidic ruminal conditions arising from diets geared to high production. Ruminal fiber digestion is known to be inhibited by low pH, via inhibition of growth of cellulolytic bacteria (pH minimum for growth ~5.9; Russell and Dombrowski, 1980). Engineering of cellulolytic functions into ruminal bacteria that tolerate low pH (e.g., *Prevotella ruminicola*) may provide a route to enhance fiber digestion under acidic conditions (Russell and Wilson, 1996). Such an engineered organism would be unlikely to digest cellulose as rapidly as the predominant cellulolytic specialist species at pH > 6, but it should be able to grow at lower pH by degradation of accessible (uncolonized) fiber particles. The degree to which fiber digestion may be improved is dependent not only on the cellulolytic capabilities of the engineered strains, but also on the dynamics of ruminal pH during the feeding cycle (i.e., what fraction of the time pH is below the levels that permit the growth of cellulolytic specialists), and whether the cellulolytic specialists, which physically colonize fiber, restrict the access of engineered strains to fiber even when the former are not actively growing. Because the cellulolytic specialists are in considerable excess of the cell concentration needed to digest fiber, they may retain prominence as long as pH remains above 6 long enough during the feeding cycle to permit growth at rates equal to or greater than the rate of passage of fiber from the rumen.

If we cannot improve the kinetics of fiber digestion, is there anything to be gained by manipulating the native cellulolytic population? One potential benefit may be the productive manipulation of the VFA ratios in the rumen. Each of the three major species of cellulolytic bacteria in the rumen produces a mixture of fermentation end products that is fairly characteristic for that species. Unlike the more rapidly growing sugar-fermenting species (e.g., *Streptococcus bovis* or *Selenomonas ruminantium*), the ratios of these end products for a particular cellulolytic species do not vary much with growth rate or pH (Pavlostathis, 1988; Shi and Weimer, 1992; Weimer, 1993). In principle, controlling the relative population of these major cellulolytic species may allow us to modify VFA ratios in the rumen.

Manipulation of VFA ratios to improve animal production already has a precedent in beef production, in which monensin has long been used to improve feed efficiency and live weight gain (Goodrich et al., 1984). These effects were originally attributed to the inhibition of ruminal methanogenesis. However, monensin is a proton ionophore that affects many nonmethanogenic species as well, and is a particularly strong inhibitor of Gram-positive bacteria, not methanogens (Chen and Wolin, 1979). The observed decrease in ruminal acetate:propionate ratio that accompanies monensin feeding (Ushida et al., 1985) is consistent with the selective reduction of Gram-positive ruminococci, which produce primarily acetate, and the likely proliferation of the Gram-negative bacteria, such as *Fibrobacter succinogenes*, which produces primarily succinate, and *Selenomonas ruminantium*, which converts succinate to propionate.

By analogy, it may be useful to identify stategies to selectively enhance the ruminococci at the expense of *F. succinogenes* and *S. ruminantium* in lactating cows, in which elevated acetate:propionate ratios should translate to higher milkfat (Van Soest, 1963). Because of regulatory constraints and consumer preferences, alteration of the microbial population would ideally be achieved in ways that do not involve the addition of unnatural chemicals to diets. The obvious alternative is through the use of feeding or management strategies that take advantage of natural competitive features of individual microbial species. Thus, studies of competition among the fibrolytic species (including both the outcome of competition and the mechanisms that underlie these outcomes) may have practical significance. A number of such studies have recently been reported (Odenyo et al., 1994; Shi et al., 1997). The data indicate that the requirements for successful competition for cellulose are complex and may include rate and extent of adherence to cellulose, affinity for soluble products of cellulose hydrolysis, ability to tolerate periods of nutrient unavailability, and production of inhibitory compounds. It is likely that each species represents a combination of adaptations that allows it to compete successfully with other species, without clear dominance of any one strain. We would expect that forages, which are composed of several types of structural polysaccharide and assembled into a complex architecture, would provide even more opportunities for unique adaptations of each species and more complex interactions among different species (Osborne and Dehority, 1989). However, despite large differences in fermentation products among the cellulolytic species, it is unlikely that the populations of individual species can be altered dramatically enough, by themselves, to substantially affect VFA ratios because the cellulolytic population makes up less than 10% of the bacterial population in the rumen (Van Gylswyk, 1970; Dehority et al., 1989).

Inhibition of Ruminal Protein Degradation

Modern dairy diets contain high concentrations of protein necessary to sustain production of large volumes of high-protein milk. Unfortunately, most of this protein is degraded by the ruminal microflora before it can reach the abomasum, where its hydrolysis would yield peptides and amino acids that are absorbed from the intestine. It has been estimated that over 70% of the protein in such common feed ingredients as alfalfa and corn is degraded in the rumen (NRC, 1989). Even though a considerable amount of this protein is recovered postruminally by digestion of microbial cells, the net losses of protein are high and are expensive for the animal owner. Moreover, the loss of protein N as urea or ammonia in urine and feces is a major contributor to groundwater pollution in areas of intensive animal agriculture, a fact that has not gone unnoticed by regulatory agencies. There is some potential to recapture NH_3 as microbial protein via better balancing of carbohydrate and protein fermentation, but inhibition of feed protein hydrolysis seems to have attracted more interest.

Several strategies are available for reducing ruminal proteolysis. One strategy is heat or chemical treatment of feeds (particularly soybeans and other protein supplements) to increase their resistance to proteolytic enzymes (Broderick et al., 1991). Alternatively, feeds can be supplemented with tannins or tannin-rich forages, which bind proteases and thus inhibit their activity (Broderick et al., 1991). Modifying the feed is probably a more productive approach than are attempts to inhibit proteolytic microbes. Proteolysis is exhibited in a broad taxonomic distribution among the more versatile ruminal microbes, and the number of different functional classes of proteases is sufficient to ensure that chemical inhibition will be incomplete.

Beyond the inhibition of proteolysis, it is also desirable to reduce the deamination of amino acids that precedes their fermentation. Amino acid degradation was originally blamed on nutritionally versatile organisms, such as *Prevotella ruminicola* (formerly *Bacteroides ruminicola*), but these bacteria produce ammonia in vitro at rates well below the rates observed in whole rumen contents. In the past decade, several species of specialist, amino acid-fermenting ruminal bacteria have been described that display much more rapid rates of amino acid fermentation and ammonia production (Paster et al., 1993). The monensin sensitivity of these new isolates may explain monensin's protein-sparing effect. Further control of these ammonia-hyperproducing species provides dairy producers with the opportunity to reduce both the expensive loss of protein and the discharge of nitrogenous wastes. It should be remembered, however, that amino acid fermentations are an essential part of the microbial ecology of the rumen. Degradation of branched-chain amino acids (i.e., valine, leucine, and isoleucine) provides branched-chain volatile fatty acids (isobutyrate, isovalerate, and 2-methylbutyrate, respectively) required by the fibrolytic bacteria for growth (Bryant, 1973). The extent to which protein degradation might be inhibited without limiting the growth of the fibrolytic bacteria merits further study. A logical first step would be to determine the affinity constants of each cellulolytic species for individual branched-chain VFA for comparison to concentrations of these branched-chain VFA in the rumen.

Reduction of Ruminal Methanogenesis

Methane production has been estimated to result in a loss of 2 to 12% of feed energy from cattle and is thought to be most serious in forage-fed animals

Table 2. Kinetic parameters for H_2 consumption by anaerobic bacteria[a]

Microbial group	Range of values			Median values		
	H_2 threshold[b]	K_m[c]	V_{max}[d]	H_2 threshold[b]	K_m[c]	V_{max}[d]
Sulfate-reducing bacteria	.0002–.04	.7–1.9	.88–79	.0013	.1	.3
Methanogenic archaea	.0088–.205	1.1–78	4.2–215	.067	6.6	6.0
CO_2-reducing acetogenic bacteria	.42–4.03	NR[e]	NR[e]	1.26	NR[e]	NR[e]

[a]Data compiled from reviews of Widdel (1988) and Mackie and Bryant (1994).
[b]Threshold values of H_2 in millimoles per liter are the minimum concentrations of H_2 in pure cultures, or measured concentrations in natural environments in which the indicated organisms are the dominant electron-accepting species.
[c]Michaelis constant in millimoles per liter for pure cultures.
[d]Maximum specific rate of H_2 consumption ($mmol \cdot g^{-1}$ cells$\cdot h^{-1}$) for pure cultures and natural environments where the indicated organisms are the dominant electron-accepting species.
[e]Not reported.

(Johnson and Johnson, 1995). Reduction or elimination of methanogenesis in domestic livestock has been touted as a way of improving animal production and may marginally contribute to control of anthropogenic release of methane, a potent greenhouse gas.

Methane is produced exclusively by the methanogenic archaea, a unique microbial group phylogenetically distinct from eubacteria (the "true" bacteria). In habitats such as lake sediments, in which unidirectional mass flow does not occur, or sewage digesters, in which mass flow occurs but retention times are long, most methane is produced from acetic acid by slow-growing (generation time ~130 h) "aceticlastic" methanogens, while some methane is produced by other, faster-growing (generation time 4 to 12 h) methanogenic species that reduce CO_2 with H_2 (Table 1). In the rumen, methanogenesis occurs exclusively by the latter pathway because ruminal retention times are too short to permit establishment of the slow-growing aceticlastic species.

It is important to recognize that CO_2 reduction to methane provides the same important function in the rumen as it does in other anaerobic habitats. As the terminal electron-accepting process in the rumen, methanogenesis continually removes H_2, a product of fermentations whose accumulation would prevent further degradation of organic matter (namely, ruminant feeds; Russell and Jeraci, 1984). Consequently, reduction or elimination of methanogenesis would require the establishment of other routes of electron disposal. Use of common feed additives as alternative electron acceptors cannot be expected to significantly reduce methanogenesis. For example, in a cow that is producing 90 kg of methane yearly, complete redirection of reducing equivalents lost as methane to the reduction of unsaturated fats, even if microbiologically feasible, would require the feeding of > 4 kg of unsaturated fats/d. Among chemicals that are not feed additives, nitrate and sulfate are two examples of energetically feasible electron acceptors that are undesirable in practice (Table 1). Nitrate would have to be fed, and in the rumen would be reduced primarily to ammonia (exacerbating N excretion) or to toxic nitrite, rather than to N_2. Sulfate is an electron acceptor for the nutritionally versatile sulfate-reducing bacteria (**SRB**). These bacteria are normally present in low numbers in the rumen, where sulfate limitation probably confines their metabolism to oxidation of acids and alcohols, producing H_2 that is consumed by methanogens (Bryant et al., 1977). The SRB have a higher affinity for H_2 (Table 2), and sulfate reduction with H_2 provides more energy than does CO_2 reduction to methane (Table 1; Widdel, 1988). Grazing ruminants in some parts of the western United States, where water supplies are naturally high in sulfate, often develop polioencephalomalacia resulting from H_2S production by the increased ruminal populations and activities of SRB (Cummings et al., 1995). Again, this illustrates both the powerful impact of thermodynamics and kinetics on microbial competition and the adaptability of microbes in response to available nutrients.

The most desirable alternative route for electron disposal is another CO_2-reducing process to produce acetate. Acetogenesis from CO_2 has two main advantages: abundance of available electron acceptor and production of acetate, an energy source readily utilizable by the ruminant. However, the barriers to achieving acetogenesis in the rumen are formidable. The most important of these is that acetogens have considerably poorer affinity for H_2 than do the methanogens (Table 2; Mackie and Bryant, 1994). As a result, methanogens can easily outcompete acetogens for the low concentrations of H_2 (~1 μM) normally encountered in the rumen (Mackie and Bryant, 1994).

Yet, one must not give up all hope. Chemical inhibition of methanogens, combined with the addition of a probiotic yeast, has been reported to stimulate acetogenesis in laboratory cultures under artificially high H_2 concentrations (80% vol/vol of the gas phase; Chaucheyras et al., 1995). Moreover, several natural habitats have been identified in which acetogenesis is a dominant disposal route for reducing equivalents. These include the hindgut of certain wood-eating termites (Breznak, 1994) and the colons of a consider-

Table 3. Some principles of microbial ecology relevant to attempts at manipulating ruminal fermentation, and ruminal examples that illustrate these principles (see text for discussion)

Principle	Ruminal example
Adaptation for utilization of a given compound requires that the compound be present at concentrations at which utilization will provide a selective advantage to the organism.	Cellulose digestion Amino acid fermentation Metabolism of plant toxins
Increasing the concentration of catalyst (namely, density of microbes) will not enhance the rate of a substrate-limited process.	Cellulose digestion
In an established ecosystem, invading (allochthonous) microbes will generally not compete effectively with native (autochthonous) microbial species.	Cellulose digestion
Allochthonous microbes can establish themselves (i.e., become autochthonous) by filling an unoccupied niche.	Metabolism of 3,4-DHP[a]
End products of metabolism from one group of microbes can often be utilized as energy sources or growth substrates for other groups of microbes.	Cellulose digestion Amino acid fermentation H_2 production/oxidation
For a given metabolic task, specialists will usually outcompete or outperform generalists.	Cellulose digestion Metabolism of oxalate Metabolism of 3,4-DHP Amino acid fermentation H_2 oxidation

[a]3,4-DHP stands for 3-hydroxy-4(1H)-pyridone.

able fraction of the human population (Wolin and Miller, 1994). In these habitats, methanogens are present in significant, but not dominating, numbers. The ability of the acetogens in these environments to compete effectively with, or even dominate, the methanogens is currently unexplained but is clearly a worthy target for future research. Acetogens are notoriously versatile in their energy metabolism (Mackie and Bryant, 1994), which would explain their poor affinity for H_2. The key solution to the problem may lie in the discovery or engineering of a specialist acetogen with a higher affinity for H_2, to compete effectively with methanogens for H_2 at natural ruminal concentrations (< .1%, vol/vol) or to use in conjunction with chemical suppression of the methanogens.

Summary

Ruminal microbes obey the laws of microbial ecology summarized in Table 3. From the above discussion, it is clear that the establishment of foreign species–whether naturally occurring or genetically engineered–will only be successful if they can outcompete the native microflora or can fill those few niches not already occupied. Even in these cases, the overall process of interest may not be improved if it is not limited by the microbial population. If alteration of the populations or activities of individual microbial species or particular physiological groups is desired, success will require applying the proper selective pressure to the native population. In the cases of enhanced fiber digestion and reduced proteolysis, this selective pressure will be difficult to apply, and the most likely improvements will be obtained from altering the characteristics of the feed. On the other hand, reducing methanogenesis might be achieved through the development of specialist acetogens for use in combination with chemical suppression of methanogenesis.

Implications

The bacterial flora of the rumen can be successfully manipulated if such manipulations are consistent with the principles of microbial ecology. Attempts to enhance the populations or activities of native ruminal strains or to establish populations on nonindigenous bacteria will be successful only if conditions are established that provide selective benefit to the bacteria of interest.

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