VIEWPOINT

Factors That Alter Rumen Microbial Ecology

James B. Russell[1]* and Jennifer L. Rychlik[2]

Ruminant animals and ruminal microorganisms have a symbiotic relationship that facilitates fiber digestion, but domestic ruminants in developed countries are often fed an abundance of grain and little fiber. When ruminants are fed fiber-deficient rations, physiological mechanisms of homeostasis are disrupted, ruminal pH declines, microbial ecology is altered, and the animal becomes more susceptible to metabolic disorders and, in some cases, infectious disease. Some disorders can be counteracted by feed additives (for example, antibiotics and buffers), but these additives can alter the composition of the ruminal ecosystem even further.

Ruminants and humans have shared a long history. Prehistoric, and often nomadic, hunters realized that they could exploit the photosynthetic potential of grasslands by domesticating ruminants, and subsequent increases in food supply allowed them to form stable social communities. The Bible recognized the value of ruminants as livestock, and the Old Testament states, in Leviticus 11:3, "Whatsoever parteth the hoof, and is clovenfooted, and cheweth the cud among the beasts, that shall ye eat." (*1*). Much of our meat and virtually all of our milk is still produced by domestic ruminants, but humans have modified the type of feed that these animals consume.

The success of wild ruminants can be largely explained by their ability to digest fibrous plant materials (*1*). Ruminants themselves do not produce fiber-degrading enzymes, but they harbor bacteria, fungi, and protozoa that can. The host provides the microorganisms with a suitable habitat for growth, and the microbes supply protein, vitamins, and short-chain organic acids for the animal. Microbial protein accounts for as much as 90% of the amino acids reaching the small intestine, and energy from short-chain organic acids (primarily acetic, propionic, and butyric acids) drives animal metabolism (*2*).

Ruminal microorganisms can also ferment starch and sugars, and these nonfibrous materials increase fermentation rate and animal productivity (*2*). However, when ruminants are fed fiber-deficient rations, homeostatic mechanisms of digesta flow, gas removal, and pH regulation are disrupted, and the animal can be severely affected (*3, 4*).

Rumen Anatomy and Normal Physiology

The digestive tract of ruminants is ideally suited for fermentation (*1*). The rumen is a large chamber (Fig. 1), and the selective retention of large feed particles by the omasum increases the residence time of fibrous feed materials. When large feed particles are ruminated, surface area and fermentation rate are both increased. Rumination also triggers saliva flow, which maintains a favorable pH for the microbes and the animal. Muscular contractions mix fresh feed with microorganisms and wash the epithelium with fermentation fluids so the microbial short-chain organic acids can be absorbed. Specialized contractions hold feed materials away from the esophagus so that fermentation gases can be expelled by a process known as eructation. Rumen physiology is largely dictated by the presence of fibrous materials in the rumen and the pharynx. If ruminants are fed fiber-deficient diets, then mixing motions, eructation, rumination, and saliva flow decrease; fermentation acids accumulate; and ruminal pH declines (*1, 3*).

Fermentation Schemes

Because the rumen is an anaerobic habitat, substrates are only partially oxidized (Fig. 2), and reducing equivalent disposal [e.g., NADH (reduced form of nicotinamide adenine dinucleotide) reoxidation] is a critical feature of the fermentation (*5*). Acetate is the dominant end product, but acetate production is dependent on the ability of hydrogenases to produce hydrogen gas from reduced cofactors. Hydrogen production is a thermodynamically unfavorable reaction, but methanogens scavenge hydrogen and relieve this inhibition. If carbohydrates are converted to propionate, butyrate, or lactate, dehydrogenase reactions provide alternative sinks for the reducing equivalents. Adenosine 5′-triphosphate (ATP) availability is determined by the method of reducing equivalent disposal. When hydrogenase activity is coupled to acetate production, the ATP yield from hexose can be as great as 4 mol/mol, but ATP yields for butyrate and lactate are only 3 and 2 mol/mol, respectively. For many years, it was assumed that strict anaerobes did not have respiratory schemes; however, many ruminal bacteria have fumarate reductase reactions that are coupled to cytochromes, and the ATP yield from propionate production can be as high as that from acetate.

Normal Ecology and Microbial Interactions

Bacterial numbers in the rumen are very high ($>10^{10}$ cells per g of contents), and bacteria play a dominant role in all facets of ruminal fermentation (*1*). Early work indicated that the complexity of ruminal bacteria was great

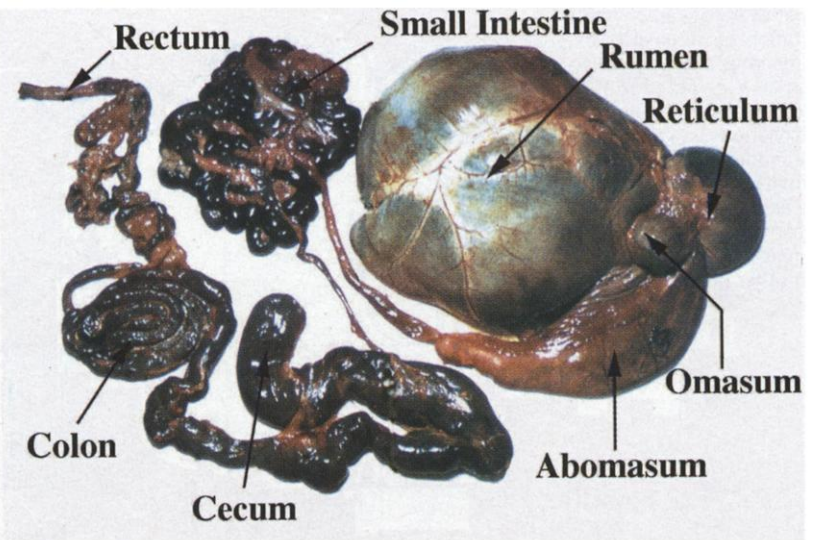

Fig. 1. The digestive tract of a healthy adult ruminant showing the various compartments (reticulum, rumen, omasum, abomasum, small intestine, cecum, colon, and rectum) [from (*54*)].

[1]Agricultural Research Service, U.S. Department of Agriculture, [2]Department of Microbiology, Cornell University, Ithaca, NY 14853, USA.

*To whom correspondence should be addressed.

(*1, 6*), and molecular techniques have revealed even more diversity (*7*). Recent work indicates that bacterial strains once thought to be the same species are phylogenetically distinct [i.e., prevotellas (*8*) (Table 1)], and clonal libraries prepared from mixed ruminal bacteria had an abundance of previously unrecognized 16*S* ribosomal DNA (rDNA) sequences (*9*). The taxonomy of ruminal bacteria is continually changing, and the possibility of nonculturable or previously unrecognized ruminal bacteria still exists (*7*).

The study of ruminal ecology has been confounded by the lack of truly selective media or unique end products (Table 1). Because most ruminal bacteria are Gram-variable and often have similar morphologies, rapid identification has not been possible (*1*). Certain species have been detected with 16*S* rRNA probes, but the ability of these probes to react with a broad range of strains in the rumen has not been shown (*7*). Given the fact that 16*S* rRNA probes have, in some cases, predicted low population densities, this technology may need additional refinement and improvement (*10, 11*).

Bacteria capable of performing all the major transformations have been isolated, and these organisms have provided models for ruminal ecology and interactions (*12*). Early work showed that a variety of ruminal bacteria produced end products that could not be detected in ruminal fluid (*1*), and these intermediates (e.g., succinate and lactate) are subjected to secondary fermentations by other species (e.g., *Selenomonas ruminantium* and *Megasphaera elsdenii*) (*12*) (Fig. 2). The most active cellulolytic ruminal bacteria (*Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*) require ruminal fluid, and subsequent work has shown that this requirement was due to branched-chain volatile fatty acids (*1*). These latter acids are derived from a distinctly different population of amino acid fermenting bacteria (*M. elsdenii* and *Peptostreptococcus anaerobius*) (*1, 13*) (Table 1). Feedstuffs are composed of large, insoluble, and sometimes complex polymers that must be degraded by extracellular enzymes, but enzyme production is not always correlated with end product utilization. Some polymer-degrading bacteria discard the soluble end products, and these substrates are available to other bacteria (*12*) (Table 1).

The study of ruminal ecology is further complicated by the observation that approximately three-quarters of the bacteria are tightly attached to feed particles or are found in biofilms (*1*). Bacterial attachment is critical for cellulose digestion, and the ruminococci appear to have a multienzyme complex that is similar to the cellulosome of *Clostridium thermocellum* (*14*). Even free-floating bacteria have an abundance of lipopolysaccharide or lipoteichoic acid (*15*), and this material can protect the cells from toxic agents (e.g., bacteriocins or antibiotics) (*16*).

Some ruminal bacteria (*R. albus*, *Butyrivibrio fibrisolvens*, *Streptococcus bovis*, and *Lactobacillus fermentens*) are armed with bacteriocins, and these small peptides can form pores in the cell membranes of closely related bacteria that compete for the same substrates (*17, 18*). The impact of bacteriocins on ruminal ecology has yet to be fully explained, but mixed culture studies indicate that bacteriocins may affect cellulose digestion (*19*), amino acid degradation (*20*), and even starch fermentation (*18*).

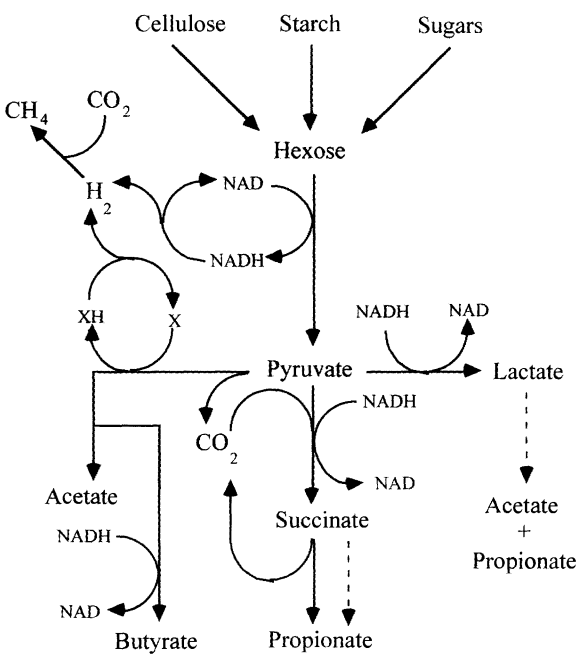

Fig. 2. A schematic showing the major pathways of carbohydrate fermentation by ruminal bacteria. "X" denotes alternative electron carrier (e.g., ferredoxin). In some ruminal bacteria, pyruvate decarboxylation is coupled to formate production, but most of this formate is converted to hydrogen and carbon dioxide by hydrogen formate lyase. The dashed lines show pathways that occur in other organisms.

The ruminal ciliate protozoa are complex and often ornate creatures, but they have not been grown axenically for long periods of time (*1, 21*). Until recently, ruminal protozoa were only classified according to morphological traits (location of the cilia, the number of skeletal plates, presence or absence of spines, etc.), but phylogenetic relationships based on small subunit ribosomal RNA sequences are being developed (*22*). Large protozoa consume smaller ones, but in vitro studies have shown that some smaller ones grow spines to resist predation (*21*).

Significant numbers of ruminal bacteria can be consumed by protozoa, resulting in an inverse relationship between protozoal and bacterial densities (*21*). Ruminal protozoa can reach numbers as high as 10^6 cells/g and account for half of the microbial mass in the rumen, but defaunation studies indicate that the ruminal protozoa are not essential to their host's nutritional status (*21, 23*). Nevertheless, many protozoa take up and store small starch granules, thereby modulating the fermentation rate and protecting the animal from acidosis. However, bacterial predation and protozoal lysis can deprive the animal of microbial protein and increase excess ruminal ammonia (*24*). Some protozoa appear to digest cellulose, and up to one-third of fiber digestion can be protozoal (*1, 23*).

For many years, fungi were assumed to be obligate aerobes. However, some ruminal organisms that were previously thought to be flagellated protozoa have been revealed to be zoospores of chytrid fungi, and feed particles are sometimes seen covered by a dense fungal mycelium (*25*). The ruminal fungi have a relatively long cycle (24 to 32 hours), and only ruminants that are fed poor-quality forage appear to have large fungal populations (8% of ruminal biomass) (*25, 26*). In vitro studies indicate that fiber-digesting ruminal bacteria produce bacteriocins that can inhibit the fungi, which could explain the low fungal numbers in ruminants fed lush forage or grain-based diets (*27*).

Manipulation of Ruminal Fermentation

High levels of animal productivity cannot be sustained by forage alone (*2*), and ruminant nutritionists have sought methods for decreasing fermentation losses (e.g., methane or ammonia) or increasing the rate of fermentation acid formation (*28*). In the 1980s, several labs used recombinant DNA techniques to improve fiber digestion, but success was thwarted by (i) the diversity of ruminal strains, (ii) their inability to clone critical enzymes (e.g., native cellulases), (iii) the production of truncated proteins in *Escherichia coli*, (iv) novel promoters and transcriptional machinery, (v) a lack of shuttle vectors to move genes into ruminal bacteria, and (vi) the poor fitness of genetically altered bacteria

(*29, 30*). However, ruminants are fed a variety of additives to alter fermentation, and these supplements are widely used (*28*). Heat-treated proteins decrease ruminal deamination and provide an additional source of amino acids; rapidly fermented grain supplements increase energy availability; buffers counteract grain-dependent declines in ruminal pH; ionophores (e.g., monensin) inhibit Gram-positive bacteria that produce hydrogen, ammonia, or lactic acid; and other antibiotics inhibit pathogenic bacteria in the gut and animal tissues.

Domestic ruminants in developing countries are still fed forage-based diets, but the diets of cattle in developed countries are often supplemented with 50 to 90% grain. When cattle are switched abruptly from forage to grain, the rumen can become severely acidic (ruminal pH $<$ 5.5), and this acute acidosis is caused by the overgrowth of starch-fermenting, lactate-producing bacteria (*S. bovis* and *Lactobacillus* ssp.) (*31*). If the dietary shift is gradual, *M. elsdenii* and *Sel. ruminantium* (Table 1) can convert lactic acid to acetate and propionate (Fig. 2), the ruminal pH is not as severely affected (*31*), and the ruminal ecology is not so drastically altered (*32*). However, even high concentrations of volatile fatty acids can cause subacute ruminal acidosis (*31*), and pH-sensitive ruminal bacteria (e.g., cellulolytics) are inhibited if the ruminal pH is $<$6.0 (*33*).

The pH sensitivity of ruminal bacteria can be explained by differences in intracellular pH regulation. When the extracellular pH of acid-sensitive bacteria like *F. succinogenes* declines, the intracellular pH is relatively stable, but the increase in the transmembrane pH gradient causes a logarithmic accumulation of intracellular fermentation acid anions (*33*). In contrast, pH-resistant ruminal bacteria (*S. bovis*, *Prevotella ruminicola*, *Clostridium aminophilum*, and *Sel. ruminantium*) allow their intracellular pH to decline, which protects them from the influx and accumulation of fermentation acid anions (*34*).

The ruminal epithelium is not protected by mucous, thus even brief periods of subacute acidosis can cause inflammation, ulceration, and scarring (*31*). Lactate accumulation promotes the growth of *Fusobacterium necrophorum*, a lactating utilizing bacterium that infects ruminal ulcers (*35*). If *Fus. necrophorum* passes from the rumen and colonizes the liver, abscesses develop. *Fus. necrophorum* also produces leukotoxin and endotoxic lipopolysaccharide (*35*). If pH is chronically acidic, the epithelium releases metalloproteinases that cause tissue degradation (*4*). If these metalloproteinases enter the bloodstream, the laminae above the hoof become inflamed, the animal becomes lame, and, in extreme cases, the hoof can fall off.

Some starch-fermenting ruminal bacteria secrete polysaccharides and produce a foam that causes "feedlot bloat." Because gas bubbles entrapped in the foam cannot be eructated, the rumen expands. Mild cases of bloat only depress feed intake, but severe bloat can kill the animal. If the gas pressure is very high, the rumen compresses the lungs and the animal suffocates (*36*).

Because ruminants evolved as grazing herbivores and their diets had little if any starch, there was little need for intestinal amylases (*4*). If large amounts of starch escape from the rumen and pass to the intestines, as can happen in grain-fed animals, overgrowth of *Clostridium perfringens*, a bacterium that produces a powerful enterotoxin, may occur and cause sudden death in feedlot cattle (*37*).

Less than 0.3% of the fattening beef cattle in feedlots die from grain-related problems (*38*), but chronic acidosis continues to plague the cattle industry (*31*). Cattle that bloat or have subacute acidosis consume less feed or must be culled (*31, 36*), and ~13% of the livers are condemned owing to bacterial abscesses (*38*). Dairy cattle rations contain less grain than feedlot rations, but the duration of grain feeding is longer (*35*). Dairy cattle are also susceptible to founder, chronic acidosis, rumen ulceration, and liver abscesses (*4*), and herds fed an abundance of grain can have a substantial mortality in comparison with those that have slower ruminal fermentation rates (*38, 39*).

In the 1970s, ruminant nutritionists added bicarbonate to cattle rations, to neutralize acids, stimulate water intake, increase the fluid dilution rate, and increase ruminal pH (*3, 40*). However, bicarbonate responses are only significant if the rumen is moderately acidic (e.g., dairy cattle rations), and bicarbonate alone cannot counteract feedlot acidosis. Calcium carbonate (limestone) and magnesium oxide are more effective at low pH than bicarbonate, but even these buffers cannot counteract grain-dependent increases in acid-resistant *E. coli* (*41*).

Safety Concerns

Definitive links between antibiotic use in animal feed and human health have yet to be shown, but the prevalence of antibiotic-resistant bacteria has increased in recent years (*42*). Feedlot cattle in the United States are still fed a variety of antibiotics (tylosin, bacitracin methylene disalicylate, chlortetracycline, oxytracycline, and virginiamycin) to reduce the incidence of liver abscesses (*35*), and some of these antibiotics are used to treat human diseases. Beef cattle are routinely fed the ionophore monensin, but this antibiotic is not employed in human medicine, and the only known resistance mechanism is provided by the outer membrane of Gram-negative bacteria (*43*). Avoparcin was once marketed as an alternative to ionophores, but it is a vancomycin analog, and after vancomycin-resistant enterococci were isolated from animals fed avoparcin, its use was discontinued (*44*).

Most strains of *E. coli* are harmless, but enterohemorrhagic *E. coli* (e.g., O157:H7) can cause acute human illness. When cattle are slaughtered, carcasses can be contaminated with fecal material, and hamburger is a source of *E. coli* O157:H7 (*45*). *E. coli* O157:H7 is not a problem if food is thoroughly cooked, but ~62,000 people are infected each year in the United States (*46*). It had generally been assumed that only 0 to 3% of

Table 1. The characteristics of predominant ruminal bacteria. Abbreviations are as follows: CU, cellulose; HC, hemicellulose; DX, dextrins; SU, sugars; ST, starch; PC, pectin; XY, xylans; L, lactate; S, succinate; GL, glycerol; AA, amino acids; OA, organic acids; H_2, hydrogen; F, formate; CO_2, carbon dioxide; A, acetate; E, ethanol; B, butyrate; L, lactate; P, propionate; Br, branched-chain volatile fatty acids; and CH_4, methane.

Species	Ruminal niche	Fermentation products
Fibrobacter succinogenes	CU	S, F, A
Ruminococcus albus	CU, HC	A, F, E, H_2
Ruminococcus flavefaciens	CU, HC	S, F, A, H_2
Eubacterium ruminantium	HC, DX, SU	A, F, B, L
Ruminobacter amylophilus	ST	S, F, A, E
Streptococcus bovis	ST, SU	L, A, F, E
Succinomonas amylolytica	ST	S, A, P
Prevotella ruminocola, albensis, brevis, and *bryantii*	ST, PC, XY, SU	S, A, F, P
Butyrivibrio fibrisolvens	ST, CU, HC, PC, SU	B, F, A, H_2
Selenomonas ruminantium	ST, DX, SU, L, S	L, A, P, B, F, H_2
Megasphaera elsdenii	L, SU	P, A, B, Br, H_2
Lachnospira multiparus	PC, SU	L, A, F, H_2
Succinivibrio dextrinosolvens	PC, DX, SU	S, A, F, L
Anaerovibrio lipolytica	GL, SU	A, S, P
Peptostreptococcus anaerobius	AA	Br, A
Clostridium aminophilum	AA	A, B
Clostridium sticklandii	AA	A, Br, B, P
Wolinella succinogenes	OA, H_2, F	S
Methanobrevibacter ruminantium	H_2, CO_2, F	CH_4

feedlot cattle were asymptomatic carriers (*47*), but recent work indicates that incidence of *E. coli* O157:H7 is at least 10 times the assumed amount (*48, 49*).

The pathogenicity of *E. coli* O157:H7 is enhanced by its ability to survive the low pH of the gastric stomach, and extreme acid resistance is an inducible trait triggered by undissociated fermentation acids and decreasing colonic pH (*50*). When cattle were fed hay, the concentration of fermentation acids in the colon was low, and virtually all of the *E. coli* were killed by a pH 2.0 acid shock that mimicked the human gastric stomach (*51*). However, when cattle were fed an abundance of grain, fermentation acids accumulated in the colon, colonic pH declined, and 10,000- to 1,000,000-fold more *E. coli* survived the acid shock (*41, 51*).

Because grain feeding is a practice that promotes both the rate and the efficiency of production, it is unlikely that fattening beef cattle will ever be fed diets consisting only of forage. However, even brief periods of hay feeding immediately before slaughter decreased the *E. coli* O157:H7 shedding (*49*) and acid resistance of *E. coli* from cattle previously fed grain (*41, 51*). Vaccinations have been an effective method for combating many pathogens, but vaccines against predominant gut bacteria (e.g., *F. necrophorum* and *E. coli* O157:H7) have not yet been highly successful (*35, 52*). In newborn animals, probiotic bacteria and yeast can colonize the gut and exclude potential pathogens and offer another avenue for altering ruminal and intestinal ecology (*53*). However, probiotics have not yet supplanted the use of ionophores and other antibiotics, and further work will be needed to see if these products can be used to combat the ruminal and intestinal disorders of mature cattle.

Conclusions

Ruminant animals and ruminal microorganisms have evolved together for millions of years, and the rumen is inhabited by diverse and interdependent populations of bacteria, protozoa, and fungi. Because ruminal microorganisms are highly competitive, the ruminal community is normally quite stable. However, in the past 50 years, humans have drastically altered the diet that ruminants consume. The use of grain-based diets has increased animal productivity and improved the economics of animal agriculture; however, grain-dependent changes in ruminal pH and ruminal ecology have created a variety of disorders and, in turn, have increased the need for feed additives (ionophores, buffers, and antibiotics) to counter these problems.

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55. The authors thank M. S. Allen, M. J. Allison, M. A. Cotta, J. F. Firkins, T. J. Klopfenstein, T. G. Nagaraja, G. R. Oetzel, F. N. Owens, L. F. Richardson, R. A. Stock, V. H. Varel, and P. J. Weimer for their helpful comments.