A Microbiologist's View on Improving Nutrient Utilization in Ruminants

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Introduction

The rumen is an open fermentation chamber, inhabited by microorganisms that anaerobically digest complex components of feedstuffs and generate fermentation products (mainly acids) and microbial cell mass for utilization by the host. The absorption of fermentation products, mainly VFA, from the rumen into the blood, and outflow of microbial cells to the abomasum and small intestine provide the animal energy and protein, respectively. The microbial ecosystem of the rumen is probably the most extensively described gut microbial ecosystem because of the importance of ruminants as a source of meat and milk. The rumen environment, created by interactions between the animal and the microbes, operates as an efficient continuous culture system with optimal physical and chemical conditions for microbial growth and activities, more or less continuous availability of substrate (at least in grazing cattle), removal of end products (by absorption, eructation or passage), and passage of undigested feed (Figure 1).

Ruminal Microbes

The microbial population of the rumen is complex and includes members that belong to the three domains of life: Eubacteria (bacteria), Archaea (methanogens), and Eukarya (protozoa and fungi). Bacteria constitute the most significant member of the microbial population based on cell mass (>50%), number (10^{10} to 10^{11}/g of contents), small subunit ribosomal RNA content (Figure 2, Lin et al., 1997) and contribution to ruminal fermentation. Methanogens were once considered as bacteria because of morphological resemblance. However, based on evolutionary lineage and distinct molecular features (no more related to bacteria than protozoa or fungi), methanogens are placed in a new domain, called Archaea (meaning antiquity). There are two types of protozoa in the rumen, flagellated and ciliated. The flagellated protozoa do not exceed 10^3 per g of contents and their contribution to ruminal fermentation is insignificant. Ciliated protozoa constitute a significant portion of the microbial cell mass (range from 0 to 50%) and include a variety of morphological types that are broadly grouped under holotrichid and entodiniomorphid ciliates. In some animals, ciliated protozoa may be completely absent (defaunation), naturally or they could be deliberately eliminated.

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Fungi in the rumen are characterized by a specialized, two-stage life cycle. There is the zoosporic stage consisting of actively motile, flagellated spores that attach to feed particles, germinate, and develop into vegetative stage consisting of mycelia structure. The mycelial structures, which are intimately attached to feed particles, are responsible for the production of hydrolytic enzymes. Because of the two-stage life cycle and the ability of the mycelial structures to grow extensively on feed particles, it is not possible to quantify fungal cell mass in the rumen. However, based on some indirect estimates, fungi are believed to account for about 10% of the microbial mass. The rumen also has bacteriophages (viruses that infect bacteria) that were first recognized by electron microscopic observations of ruminal contents. As many as 10^{11} phage particles have been counted per g of ruminal contents and more than 125 different morphological types have been described. They belong to both lytic (cause lysis of the bacterial cell) and temperate (become integrated into the bacterial chromosome) types, but majority of them in the rumen are temperate phages. The lytic phages may potentially have an influence on bacterial numbers and types in the rumen. However, the population dynamics, biology, and overall functional significance of bacteriophages in the rumen have not been determined.

Because microbes share the same habitat and compete for the same substrates, there are a number of simple and complex interactions, both positive and negative for the microbes and the host. Additionally, there is evidence of transfer of genetic material (horizontal gene transfer) between bacteria and ciliated protozoa in the rumen (Ricard et al., 2006), which could provide novel functions to the recipient organisms. Because ciliates engulf and digest bacteria, some of the DNA may be taken up by the ciliates and incorporated into their genomes. For example, a xylanase produced by *Polyplastron multivesiculatum* shows close sequence similarity to the family of xylanase enzymes from gram positive bacteria (Devillard et al., 1999).

Molecular Techniques and Diversity of Ruminal Microbes

Historically, ruminal bacteria and fungi have been studied, in terms of their activities and products produced, by isolation and cultivation in pure culture. Because ciliated protozoa are difficult to cultivate *in vitro*, much of the information on their contribution to ruminal function has been derived by microscopic examination of number and kinds in relation to dietary changes and by complete or partial defaunation of the rumen. Cultivation-based techniques or microscopy in case of ciliated protozoa have identified approximately 60 to 70 genera and 300 to 400 species of bacteria, protozoa, and fungi in the rumen and metabolic activities of many of those species, particularly of bacteria, have been described. However, with the development and application of a variety of cultivation-independent, molecular techniques, it has become clear that cultivation-based methods have only identified approximately 10 to 20% or less of the total microbial population harbored in the rumen (Edwards et al., 2008). Nucleic acid-based techniques primarily through the use of the RNA sequence associated with the small subunit ribosome, 16S rRNA for bacteria and methanogens, 18S rRNA for protozoa and fungi, and analyses of genes (metagenomics and genomics) and gene

expressions (mRNA; transcriptomics) have been employed to understand the structure and function of the microbial community of the rumen. Also, rapid high-throughput technologies (microarray analysis) and the next generation nucleic acid sequencing technologies (pyrosequencing) are now being deployed to analyze the microbial ecology of the rumen. Some of these techniques can also be used to quantify a specific microbe in the rumen (real time, quantitative PCR). Relatively fewer nucleic acid-based analyses of fungi and ciliated protozoa have been published compared to studies on ruminal bacteria. An extensive suite of molecular techniques have been applied to study the microbial diversity, community structure, monitor changes associated with dietary changes and microbial interactions of the rumen. An advantage of microbial community analysis with nucleic acid-based techniques is that ruminal content samples need not be processed immediately to maintain viability and can be archived and processed at convenience.

The use of small subunit rRNA sequence or the sequence of genes that codes for rRNA, called rDNA, has allowed a more complete description of the rumen microbiome. Based on 16S rDNA analyses, the rumen is inhabited by about 300 to 400 different bacterial species (Edwards et al., 2004). Many of the 'species' identified are categorized as unknown because they have not been cultivated yet and do not match with any of the known cultured organisms. The term used by microbiologists to describe genomic uniqueness of an organism is 'operational taxonomic unit (OTU)' or 'phylotype'. The obvious question is why the discrepancy in number of species or phylotypes between culture-dependent and culture-independent methods? Culture-independent methods that detect nucleic acids do not distinguish between live and dead microbes. It is either that the rumen contains a large number of dead bacteria or simply rumen microbiologists do not yet know how to culture the most numerous, and potentially important bacteria. Another complication of quantification of ruminal bacteria by culture-based methods is intimate adherence of bacteria to feed particles, which is not an issue for nucleic acid-based techniques. Molecular techniques have focused on identifying and enumerating populations and monitoring population changes. The key is to link analysis of the community structure to ruminal function by measuring the gene expression, which is translated into protein and metabolic function (fiber degradation, amino acid deamination, methanogenesis etc.). The ultimate challenge is to understand and describe the biology at a molecular level so that the information can be used to manipulate feeding systems to maximize efficiency of digestion in the rumen.

Genomics and Rumen Microbiology

Genomics is the discipline of sequencing, mapping, and analyzing the entire complement of genetic information of an organism. It is a genetic blueprint that provides complete information on the lifestyle of the organism in an ecosystem. The first organism to be sequenced was a virus in 1976 and the first bacterium to be sequenced was *Haemophilus influenzae* in 1995. To date, thousands of bacterial species have been sequenced and the number is growing on a daily basis. Although the total number of sequenced rumen microbial genomes is relatively low, there are

sequence data available on phylogenetically related species of microbes of other gut habitats, mainly human, that could provide information about processes in the rumen. In 2000, the North American Consortium for the Genomics of Fibrolytic Ruminal Bacteria (FibRumBa database; http://jcvi.org/rumenomics/) in collaboration with the J. Craig Venter's Institute of Genomics Research (http://www.jcvi.org), formerly the Institute of Genomic Research (TIGR) initiated collaborative projects to sequence the genomes of the major ruminal fibrolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus, R. flavefaciens, Prevotella bryantii, Prevotella ruminicola*). In 2011, a global research alliance representing 27 institutes and universities from 11 countries was formed to accelerate Rumen Microbial Genomics research, called 'The Hungate 1,000' project (named after Robert E. Hungate, the Father of Rumen Microbiology). The goal is to generate a reference set of rumen microbial sequences (approximately 1,000 cultures) which will support international efforts to understand rumen function and develop strategies to improve fermentation efficiency and minimize environmental impact, such as mitigation of methane production.

The genomic sequence of an organism can provide comprehensive information on the metabolic potential. The genome of *Fibrobacter succinogenes,* a dominant fibrolytic organism, was the first ruminal bacterium to be sequenced and annotated (identification and analysis of the genes). The organism contains 3,252 genes coding for proteins and of those at least 104 genes were identified as coding for enzymes involved in plant cell wall degradation, including 33 genes for cellulose enzymes (Suen et al., 2011). Biochemical studies before genomic sequencing had only identified a dozen or so enzymes in *F. succinogenes* involved in cell wall digestion. The information gleaned from genomics of fibrolytic bacteria not only provides more information on fiber digestion in the rumen, but could potentially lead to identification of novel fibrolytic enzymes for commercial exploitations such as exogenous enzymes as feed additives or their use in biofuel production (Hess et al., 2011). Researchers in New Zealand (Attwood et al., 2011; Leahy et al., 2010) have sequenced and analyzed the genome of *Methanobrevibacter ruminantium,* a major ruminal methanogen, and have identified methanogen-specific genes that code for critical enzymes for methane production, which can potentially be targeted for mitigation. The organism contains a large number of genes that encode for surface adhesion like proteins, which may be involved in mediating close association with hydrogen- producing bacterium or protozoa in the rumen. These proteins can potentially be used as antigens in a vaccine to induce antibodies to inhibit ruminal methanogens.

Linking Microbial Ecology to Animal Physiology

Microbes provide energy, protein and vitamins to the host. The question often asked is whether ruminal microbial composition and activities could be linked to specific host physiology, particularly productivity, growth, milk, and wool production. In the past 5 to 6 years, significant progress has been made in analyzing colonic microbiota of laboratory mice (as a human model) and humans, based on the premise that without understanding the interactions between human and microbial genomes, it is impossible

to obtain a complete picture of human biology (Gordon, 2011; Walters and Ley, 2011). Much of the understanding comes from studies of germ-free mice with defined components of the normal mouse or human gut microbiota (Tsai and Coyle, 2009). Such studies have shown that the gut microbiota helps regulate energy balance both by extracting calories from otherwise indigestible dietary components and by controlling host genes that promote storage of energy in adipocytes. Association between gut microbial structure and obesity traits have been demonstrated in mice and humans (Ley et al., 2006). The gut 'microbial trait' is transmissible because colonization of germ-free mice with an "obese-gut-derived" microflora results in a much greater increase in total body fat and leads to obesity. Studies in obese and lean human twins suggest that a core gut microbiome exists, and that obese twins exhibit reduced diversity and an altered representation of metabolic pathways in their microbiota (Ley et al., 2006). Potentially, future treatments for obesity may involve modulation of gut microbiota using probiotics or prebiotics.

Few studies have been done in ruminants to relate rumen microbial community structure to host phenotypical traits. Guan et al. (2008) reported differences in ruminal microbial profile (based on a molecular technique) and fermentation products between cattle with low residual feed intake (RFI; more efficient) and high residual feed intake (less efficient). In terms of fermentation products higher concentrations of butyrate and valerate were detected in rumens of more efficient cattle compared to less efficient cattle (Table 1), which does suggest differences in microbial activities. Although it is difficult to relate butyrate and valerate concentrations to feed efficiency, there is evidence that butyrate, for example, could have a regulatory role in gene expressions of bovine cells, including adipocytes (Li and Li, 2006). Cattle selected for lower RFI have been shown to produce less methane (Hegarty et al., 2007). The size of the methanogenic populations in cattle with low-RFI and high-RFI were not different, but the composition of methanogens (genus, species, strains, and phylotypes) was different between the two groups (Zhou et al. 2009; 2010). This suggests that that any attempt to link ruminal microbes to host physiology or production parameters should consider differences in community structure at the species or strain level.

Improving Nutrient Utilization

The manipulation of ruminal fermentation to maximize efficiency of feed utilization to increase ruminant productivity, i.e., increase milk, meat, and wool production, continues to be of great interest to Rumen Microbiologists and Ruminant Nutritionists. In simplistic terms, the objectives of ruminal manipulation are to enhance ruminal fermentation processes that are beneficial to the host, minimize, alter, or delete inefficient or deleterious ruminal fermentation processes (Nagaraja et al., 1997). The activities of ruminal fermentation targeted for manipulations include:

1. Increased microbial degradation of fiber.
2. Decreased protein degradation and ammonia production in the rumen, not only to improve efficiency of nitrogen metabolism, but also to decrease the overall nitrogen

excretion by the animal, which would contribute to decreased ammonia emissions from cattle manure.
3. Decreased methane production. Again, to improve fermentation efficiency and at the same time contribute to a reduction in greenhouse gas emissions.
4. Decreased production and increased fermentation of lactate.
5. Decreased production of *trans*-fatty acids in dairy cows to offset depressed milk fat syndrome.
6. Increased production of conjugated linoleic acids.
7. Partial or total reduction in protozoal population or activities to increase fermentation efficiency and possibly increase ruminal escape of feed protein and microbial protein production.

In recent years, another activity targeted as part of the gut manipulation is the reduction of gut pathogens, particularly food-borne organisms like *Escherichia coli* O157, *Salmonella* and *Campylobacter,* to improve animal health and increase food safety.

There are two aspects of ruminal environment that have significant influence on ruminal microbial activities and fermentation products. One is ruminal pH and the other is the production and utilization of hydrogen. The focus of this paper is to address the importance of ruminal pH and hydrogen production and utilization to ruminal fermentation and discuss targets and approaches available to modify ruminal fermentation to enhance the overall efficiency of ruminal fermentation.

Ruminal pH, Ruminal Microbes and Microbial Activities

The pH of the ruminal contents is probably the most important ruminal factor affecting the microbial population and their activities. Ruminal fermentation products, particularly acetate, propionate, lactate, and methane, are strongly affected by ruminal pH, mediated in large part by the effect of pH on the microbes (Lana et al., 1998). Among microbes, ciliated protozoa and fungi are more sensitive to pH than bacteria. The pH sensitivity of bacteria varies depending on the functional groups, and bacteria involved in fiber degradation, lactate utilization, and methane production are more susceptible to pH changes. The pH sensitivity of ruminal bacteria is dictated by the pH gradient across the cell membrane and the ability of the bacterial cell to regulate intracellular pH. Typically, in cattle fed once or twice a day, ruminal pH decreases after feeding for a period of few hours, reaching a nadir between 2 to 8 h depending on the diet, and then increases to reach or come close to the prefeeding value. The increase is mainly because of removal of VFA by absorption, and other factors like saliva flow, buffering capacity of feeds, and rate of passage of contents to the omasum.

The effect of ruminal pH on microbial activity depends on the magnitude of the reduction and more importantly duration of optimal or suboptimal pH. It is difficult or almost unfeasible to design and conduct *in vivo* studies to determine the duration of suboptimal pH on ruminal fermentation. *In vitr*o systems, both batch culture and continuous culture systems, have been used to delineate the pH effects on ruminal

fermentation (Calsamiglia et al. 2002, 2008; Cerrato-Sanchez et al., 2008). In a study published by Cerrato-Sanchez et al. (2008), digestibility values and concentrations of VFA and ammonia were not affected by maintaining pH 5.6 for 4 h or fluctuating pH between 5.1 (2 h/d) and 7.1 (2 h/d), but were affected to some extent by maintaining a pH of 5.1 for 4 h (Table 2). This shows that the effects of low pH on ruminal microbial activity are due not only to the magnitude but also to the duration of low pH. Therefore, the area below pH 5.6 in a pH curve, which measures the duration, may be the most appropriate measure to assess ruminal pH effects. De Veth and Kolver (2001) showed that a duration of 4 h at pH 5.4 was sufficient to reduce digestibility of DM, OM, and NDF. However, longer periods (>8 h) of suboptimal pH were required to reduce microbial protein synthesis, which suggests that suboptimal pH may only affect the activity of the microbes but not the cell numbers (Table 3). Because reduction in ruminal pH is associated with feeding diets rich in highly-fermentable carbohydrate, the changes in fermentation products are confounded by availability of substrates. For example, low ruminal pH favors production of propionate over acetate and availability of starch or sugars as substrates also favors propionate production. Calsamiglia et al. (2008) varied forage to concentrate ratio (60:40 vs. 10:90) to determine the effects of different pH (4.9 to 7.0) on fermentation in a continuous culture system. The data indicated that nutrient digestibility and fermentation products were due to the combined effects of pH and diet in different proportions depending on the measure. The pH was a major determinant of organic matter or NDF digestibility, and acetate and butyrate concentrations. In contrast, total VFA and propionate concentrations were affected by the combined effects of pH and substrate type (diet). The difference between the pH and diet effect on variables may be reflective of changes in microbial population.

Ruminal pH and Fiber Degradation

Ruminal pH is the most important ruminal factor affecting fiber digestion in the rumen. The effect of pH is related to the growth of the bacteria, and a pH < 6.0 significantly slows down the growth. Rumens of both beef and dairy cattle fed for peak production generally spend a substantial amount of time below pH 6. Even though prolonged exposure of cellulolytic bacteria to low pH has little effect on subsequent ability to digest cellulose, ruminal pH needs to remain >6.0 long enough to permit growth rates that exceed the passage rate. A recent study (Palmonari et al., 2010) has shown that cows with low pH can maintain normal population of cellulolytic bacteria. Even in cows with grain-induced acidosis, the abundance of cellulolytic bacteria did not reduce, unless it progressed to more severe and sustained acidosis (Khafipour et al., 2009c). Also, cellulotyic bacteria can provide breakdown products (cellobiose, cellodextrins, etc.) to non-cellulolytic bacteria that are more acid tolerant, which help to moderate the effect of pH on cellulose digestion. Complete cessation of cellulose digestion only occurs at pH below 5.3, and generally, ruminal pH of dairy cows does not reach such low values for significant amounts of time. Inhibition of the growth is because of the inability of the bacteria to regulate intracellular pH. There is also evidence that low ruminal pH could reduce binding of fibrolytic bacteria to feed particles (Mourino et al., 2001).

Ruminal pH and Subacute Acidosis

Feeding of energy dense diets in order to meet the production demand results in accumulation of organic acids, mainly VFA, in the rumen and if ruminal buffering capacity is unable to keep pace with VFA accumulation, ruminal pH will be depressed. When ruminal pH is depressed below 5.6 and sustained for an extended period of time, generally > 3 h per day, subacute ruminal acidosis (SARA) results. This is a major nutritional disorder, particularly in dairy cows, because the syndrome depresses feed intake, milk production, and milk fat content, and can lead to inflammatory problems, such as rumenitis, laminitis, and liver abscesses. In SARA, unlike acute acidosis, there is no accumulation of lactic acid because ruminal pH is high enough for lactic acid fermenting bacteria, particularly *Megasphaera elsdenii,* to actively metabolizing lactic acid to keep pace with production. In contrast to acute acidosis, not much is known about the microbial changes associated with subacute acidosis (Goad et al., 1998). In acute acidosis, the well accepted information on the initial increase in *Streptococcus bovis,* which paves the way for acid-tolerant lactobacilli and destruction of lactic acid-fermenting bacteria responsible for lactic acid accumulation is derived from culture-based studies. The potential contributions of unculturable bacteria in acute acidosis have not been determined. Recently, the ruminal microbial population associated with grain-induced subacute acidosis was determined by a culture-independent method (Khafipour et al., 2009a). The analysis indicated a general increase in gram positive bacteria (includes amylolytic bacteria) and a decrease in gram negative bacteria (except for *M. elsdenii*) with SARA. The increase in *M. elsdenii* paralleled the increase in *S. bovis* numbers, indicating that any lactic acid produced is effectively utilized by *M. elsdenii*. Maybe the most interesting observation was the increased abundance of *E. coli* in the rumen (Khafipour et al., 2011), which is probably because of the availability readily fermentable sugars. Also, many of the isolates were shown to carry genes that code for major virulence factors. Besides being a source of lipopolysaccharide (LPS) in the rumen, *E. coli* may exert pathogenic effects by attaching to the ruminal epithelium, invading the tissue, injuring the host tissue and stimulating an inflammatory response.

The LPS, also called endotoxin because of potent biological activities, is part of the cell wall of all gram negative bacteria and is typically released when cells die or get lysed. Ruminal endotoxins have long been suspected to contribute to the pathogenesis of ruminal acidosis (Nagaraja and Titgemeyer, 2007). The clinical and blood biochemical changes associated with ruminal acidosis are somewhat similar to those observed following endotoxin administration. In both acute and subacute acidosis, there is an increase in concentration of LPS or endotoxin in the rumen, which may get absorbed and trigger systemic inflammatory responses (Plaizier et al., 2008). The absorption may be facilitated by the compromised barrier function of the ruminal epithelium. The absorption is evidenced by detection of LPS in peripheral blood (Khafipour et al., 2009a, 2009b), although not in all instances (Li et al., 2011). Systemic LPS induces an inflammation cascade that results in the production of acute phase proteins, such as serum amyloid A, haptoglobin, (Table 4) and LPS-binding proteins in the peripheral blood (Khafipour et al., 2009a; Zebeli and Amtej, 2009).

Ruminal pH and Biohydrogenation

The process of hydrogenation of unsaturated fatty acids is referred to as biohydrogenation and represents one of the hydrogen-sink reactions in the rumen. The topic of biohydrogenation has garnered a lot of attention because of the association of the intermediates, particularly conjugated linoleic acid in human health and *trans*-fatty acids that cause milk fat depression in cows. Bacteria play a major role in biohydrogenation, although both ciliated protozoa and fungi make contributions. One of the earliest bacteria to be identified to hydrogenate unsaturated fatty acids, except the terminal step to produce stearate, was *Butyrivibrio fibrisolvens,* a fibrolytic organism. Two other species of *Butyrivibrio* that have now been identified to produce stearate are *hungatei* and *proteoclasticus* (formerly, *Clostridium proteoclasticum*). Both hydrogenate the final step to produce stearate. Biohydrogenation of linoleic acid by *Butyrivibrio* does not produce the *trans*-10 isomers implicated in depressed milk fat syndrome. In dairy cows fed a low-fiber diet, the change in microbial fermentation is characterized by a decline in ruminal pH, reduction in acetate:propionate ratio, and increase in *trans*-10 C18:1 concentration. Not much is known about the bacterial species that produce *trans*-10 fatty acids, although certain strains of *Megasphaera elsdenii* have been shown to produce *trans*-10 fatty acids (Kim et al., 2002). Weimer et al. (2010) reported major changes in bacterial community structure in cows experiencing depression milk fat content. A conducive ruminal pH is one of the factors necessary for production of intermediates of biohydrogenation that induce milk fat depression. In continuous culture fermentation studies, maintaining fermenter pH at 5.6 increased the production of 18:1 *trans*-10 compared to pH 6.4 (Fuentes et al., 2009). The pH effect on *trans*-10 fatty acid production explains the mechanism of action of ruminal buffers in offsetting depression in milk fat synthesis.

Ruminal pH and Deamination

The decreased ruminal ammonia concentration associated with feeding starch-based diets is explained by increased assimilation of ammonia by ruminal bacteria. However, low ruminal pH can also decrease production of ammonia by decreasing deamination of amino acids (Lana et al., 1998). Some of the obligate amino acid-fermenting bacteria (*Clostridium aminophilum* and *Peptostreptococcus anaerobius*) are strongly inhibited by pH lower than 6.0.

Ruminal pH and Methanogenesis

Among ruminal organisms, methanogens are particularly sensitive to even modest decreases in ruminal pH. Based on *in vitro* incubations, ruminal methanogens are unable to take up H_2 when pH is below 5.5 (Van Kessel and Russell, 1996). Low ruminal pH in cattle is typically associated with starch-based diets and low acetate to propionate ratio in the rumen. The latter is due to metabolic characteristics of the fiber-digesting (tend to produce acetate) and starch-digesting (tend to produce propionate) bacteria. However, some of the major cellulolytic bacteria (*F. succinogenes* and *R.*

flavefaciens) produce mostly succinate, an intermediate of propionate. The mirroring of increased propionate and decreased methane suggests that they both compete for the available H_2. At pH lower than 5.5, propionate production will also decrease indicating that propionate-producing bacteria may also be sensitive to ruminal pH (< 5.3; Russell, 1998).

Hydrogen production and utilization

The utilization of H_2 in an ecosystem that does not have oxygen is critical to prevent increases in the partial pressure of H_2, which could disrupt the normal functioning of microbial enzymes involved in oxidation-reduction reactions. The production of H_2 by one species and utilization by another species, referred to as 'inter species H_2 transfer', is a major microbial interaction in the rumen. Although methanogens account for 1 to 4% of the total microbial population in the rumen, methanogenesis represents a major pathway to utilize hydrogen. The methanogens in the rumen are distributed free in ruminal fluid, attached to feed particles, associated with ciliated protozoa, and attached to ruminal epithelium. Based on culture-dependent and independent analyses, ruminal methanogens are placed in three genus-level groups: *Methanobrevibacter* (62%), *Methanobacterium* (15%), and an as-yet uncultured group (16%), called rumen cluster C (Janssen and Kirs, 2008). Methanogens associated with protozoa and epithelium are novel phylotypes, and the role of methanogens associated with ruminal epithelium has not been identified. Methanogens associated with ciliated protozoa can be intracellular, called endosymbionts, or on the surface, called ectosymbionts. Intracellular methanogens are found inside most of the common protozoal species. In contrast, the extracellular methanogens are less numerous and only 30 to 50% of the protozoan cells carry them. Protozoa produce hydrogen in large amounts in a specialized organelle called hydrogenosomes (similar to mitochondria). This hydrogen is utilized by methanogens that are inside or outside the protozoan cell, and the association represents an important microbial interaction in the rumen. Removal of hydrogen by methanogens allows the fermentation of organic matter to mainly acetate instead of butyrate or lactate resulting in more ATP production by the protozoan cell. A single protozoan could produce methane that ranges from trace amounts to up to 3 nmol/day. Therefore, eliminating ciliated protozoa will reduce methane production. In a meta-analysis of published data, defaunation resulted in an increase in molar proportion of propionate (P <0.05) and a decrease in concentrations of acetate (P = 0.08) and butyrate (P <0.05; Eugene et al., 2004). Based on stoichiometry, such a shift in acetate to propionate should result in a decrease in methane production. Based on *in vivo* studies, removal of protozoa resulted in a 10.5% decrease in methane production.

It is well known that inhibiting methane production will have a positive effect on feed efficiency. In the past few years, there has been renewed interest due to concerns about the amounts of methane generated from domestic ruminants. The ruminant-derived methane accounts for about one-quarter of all anthropogenic methane emissions and is implicated in human-induced global climate change. Methane is a

potent greenhouse gas, with a global warming potential 21 times that of CO_2 and it accounts for 16% of total global greenhouse gas emissions. Because methane accounts for 2 to 12% of the ingested gross energy, reducing methane emissions will make more energy available to the animal and therefore increase efficiency of production.

A number of attempts have been made to inhibit the activity of methanogens using a variety of interventions but most have failed or have had limited success, due to low efficacy, poor selectivity, toxic effects to the host or development of resistance to antimethanogenic compounds. The key to reduce methane production is to find alternative ways to utilize H_2, particularly if the products produced are beneficial to the host (Figure 3). Introduction of substances like fumarate and malate that could utilize H_2 and are subsequently converted to propionate has shown variable effects on methane production. Reductive acetogenesis is an alternative H_2 utilizing pathway carried out by homoacetogens. These bacteria use CO_2 and H_2 to produce acetate, but attempts to establish acetogens in the rumen have failed because of a lower affinity of acetogens to H_2 compared to methanogens (LeVan et al., 1998). Acetogens and methanogens do coexist in the digestive tracts of humans, swine and rodents, and it is not known why they do not coexist in the rumen. Nitrate is another alternative H_2 utilizer and conversion of nitrate to the final product, ammonia, requires 4 moles of H_2, which means each mole of nitrate could reduce 1 mole of methane. However, the intermediate product, nitrite, is a toxic compound. Similarly, sulfate could use H_2 to become hydrogen sulfide, another potentially toxic compound. Sulfate reduction to hydrogen sulfide also utilizes 4 moles of H_2 and thus offers the same potential to decrease methane as nitrate. Hydrogen utilization by both nitrate and sulfates are energetically favorable reactions and they have a higher affinity for H_2 than CO_2 reduction to methane. In fact, inclusion of both nitrate and sulfate in diets of sheep has shown additive effects on methane reduction and if they can be fed in a safe way, they could be useful feed additives (van Zijderveld, et al., 2010).

Methods to Manipulate Microbial Activity in the Rumen

Basically, the ruminal microbial fermentation processes can be modified by intervention at three levels: feed, animal, and microbial. The first two approaches impact ruminal fermentation indirectly by altering the feedstuffs or the physiology of the ruminant animal. Intervention at the microbial level is more direct, whereby the fermentation pattern is altered through the action on microbes by certain additives included in the diet. Research on approaches to microbial manipulation of ruminal fermentation began in 1940's with the discovery that feed supplementation of antibiotics improved animal growth and efficiency of feed utilization. The growth in popularity of antibiotics in feed paralleled the progress in intensive management of cattle. Use of antibiotics to reduce subclinical infections and improve growth resulted in efficient beef and milk production. Monensin, an ionophore antibiotic, has been in use as a feed additive for decades to increase efficiency of both beef and dairy cattle production.

In many developed countries, consumers are increasingly expecting good quality and safe meat and milk, as well as their production under optimal conditions for animal health and welfare with minimal environmental impact. Issues like development of microbial resistance, transfer of resistance to human pathogens, decrease in the efficacy, possible residues in meat and milk products, and a general awareness of risks to public safety have led to the decrease and in some countries, outright ban of subtherapeutic uses of antibiotics. Consumers and public health authorities in many countries, particularly in Europe, Australia, and New Zealand are demanding the ban or phasing out of the use of chemical feed additives, and replacing them, if possible, with natural products and practices in animal production. Therefore, there is new impetus to exploit or develop new natural products as feed additives to manipulate ruminal fermentation and solve nutritional problems, essentially to replace antibiotics in the feed. The common natural products include probiotics or direct-fed microbials (DFM), prebiotic oligosaccharides, exogenous enzymes, and plant extracts or metabolites.

Probiotics or Direct-fed Microbials

The beneficial effect of using live microbial cells or their products in livestock was reported even before the discovery of antibiotics. The basis for the concept of feeding probiotics stems from the understanding that normal and healthy gut flora are required to protect the host animal against gastrointestinal colonization by non-indigenous microorganisms and thereby optimize animal production. Probiotics are hypothesized to exert their beneficial effects on the host by modifying the composition or activity of gut microflora (Krehbiel et al., 2003). Probiotics used in cattle can be broadly categorized into bacterial or fungal types. Most bacterial probiotics have maximum efficacy in preruminant calves, whereas fungal products have shown greater benefits in adult dairy or beef cattle. Bacterial probiotics are generally believed to exert their effects in the lower gut. Among, bacterial probiotics, lactic acid producing bacteria (LAB; Lactobacilli and Enterococci) are commonly used. It is not known whether LAB can overcome the ruminal microbial barrier to exert any effects in the intestine and whether they even affect ruminal fermentation. There are some reports that feeding LAB alters ruminal fermentation products, but the results are highly inconsistent. *Enterococcus,* particularly *E. faecium,* has been shown to prevent a decline in the ruminal pH of lactating dairy cows (Nocek et al., 2002). Published studies have shown that feeding *E. faecium* to dairy cows raised the pH nadir and increased mean daily ruminal pH. The concept behind elevation of ruminal pH with a lactic acid producing probiotic, such as *Enterococcus,* is that production of a 'tonic' concentration of lactic acid may stimulate and sustain an active population of lactic acid utilizers, which in turn would result in elevated ruminal pH (Nocek *et al*., 2002, 2003; Nocek and Kautz, 2006). It is difficult to believe that feeding a probiotic is needed to produce a tonic concentration of lactic acid, when the rumen is loaded with lactic acid-producing bacteria, particularly in cattle that are supplemented with grain. However, there is evidence of reduced ruminal acidosis with feeding of *E. faecium* or *Lactobacillus* sp*.,* alone or in combination with yeasts, *Saccharomyces cerevisiae* or *Propionibacterium* (Ghorbani *et al*., 2002; Nocek *et al*., 2002, 2003; Oetzel *et al*., 2007).

Fungal cultures used in ruminant diets include yeasts, generally *S. cerevisiae,* and a mold, generally *Aspergillus oryzae.* In adult cattle, dietary addition of yeasts and *Aspergillus oryzae* (AO) extract have been shown to increase feed efficiency and weight gain, and slightly increase milk production in lactating dairy cows. Based on peer-reviewed published studies, fungal cultures seem to exert their effect more at the ruminal level than postruminally. There are a number of yeast products available commercially for use in cattle. Most research on AO has been with a commercial product called Amaferm™ (Biozyme Inc., St. Joseph. MO). Amaferm is a fermentation extract from a select strain of AO*,* manufactured in a two-step process*.* The product is not labeled to contain live cells, therefore it is a true DFM. It is generally believed that fungal cultures mediate their effects on ruminal microbes, primarily bacteria and fungi. Among ruminal bacteria, two functional groups, the fiber digesting and lactate utilizing bacteria, are stimulated by addition of fungal cultures (Callaway and Martin, 1997; Martin and Nisbet, 1992). How exactly yeasts and AO stimulate microbial growth is unknown. It is theorized that yeasts and AO provide micronutrients that act as growth factors for bacteria. The stimulation of fibrolytic bacteria could explain the increase in fiber digestibility reported from *in vitro, in sit*u and *in vivo* studies (Chaucheyras-Durand et al., 2008). Additionally, increased ruminal lactic acid utilization in the rumen because of increased growth of lactic acid fermenters could result in higher ruminal pH thus favoring increased fiber digestibility (Nisbet and Martin, 1991). More importantly AO extract stimulates growth of ruminal fungi, and it is well established that ruminal fungi are active in digesting fiber. Fungal cultures may stabilize the ruminal environment by alleviating the depression in ruminal pH in lactating cows or feedlot cattle fed fermentable grain-based diets. The higher pH is often associated with lower lactate concentration in the ruminal fluid, particularly in *in vitro* fermentation studies with rapidly fermentable substrates. The reason for increased lactate utilization is probably because yeasts or AO extract increase lactate uptake by stimulating bacteria, such as *Selenomonas ruminantium,* and *Megasphaera elsdenii,* which are two dominant lactate utilizing bacteria in the rumens of grain-fed cattle (Martin and Nisbet, 1992).

In recent years, there has been considerable interest in food safety implications of probiotics (Jacob and Nagaraja, 2011). The underlying concept, called ‘competitive exclusion’, is basically to displace or inhibit food-borne pathogens in the hind gut. Feeding of cattle with probiotics to reduce prevalence of *E. coli* O157:H7, a serotype that has emerged as a major food-borne pathogen, is a promising preharvest intervention strategy.

Prebiotic Oligosaccharides

These feed additives increase growth of ‘health promoting bacteria’, such as *bifidobacteria* and *lactobacilli*, in the intestinal tracts of humans and animals. These oligosaccharides (fructo-, Manno-, Galacto-, etc.) are often only digestible by microbial enzymes and hence offer a degree of selective stimulation. This poses a special problem in ruminants because of the microbial fermentation in the reticulo-rumen. The oligosaccharides will be utilized by ruminal microbes, therefore, may not reach the lower

gut to stimulate the beneficial bacteria. In animals, oligosaccharides have been used mainly in monogastrics, particularly pigs and chickens (Mul and Perry, 1994). The prebiotic concept is a much more recent development in dietary intervention of stimulation of gut function. However, current research efforts are focused towards developing 'second generation prebiotics' that have multiple biological activities beyond the stimulation of bifidobacteria and lactobacilli. The area of research, called 'Glycobiology' (developments in carbohydrate chemistry in relation to biological activity), applies biotechnological approaches to develop oligomers that stimulate lactic acid bacteria at the species, rather than genus level and may include receptor sites for certain gut pathogens and their toxins. In humans, prebiotics are targeted to have activities in the distal colon, which is the frequent region of the colon prone to dysfunctions and disorders, such as irritable bowel syndrome, Crohn's disease, etc.

Exogenous Enzymes

Enzyme feed additives, primarily fibrolytic enzymes, are intended to increase diet digestibility and improve the productive efficiency of ruminant animals. The enzyme preparations are relatively concentrated and often purified, containing specific enzymes. Enzymes that are commercially available are generally products of microbial fermentation. Many have non-feed applications, such as food, pulp, paper, textile, and chemical industries. Enzymes used are fungal (*Trichoderma, Aspergillus,* etc.) or bacterial (*Bacillus* sp.) origin. Because enzymes are proteins, a frequently raised issue is about their longevity in the rumen that is loaded with proteolytic activity. Surprisingly, exogenous enzymes are much more stable in the rumen than previously thought, particularly if applied to the feed prior to ingestion (Morgavi et al., 2001). Possibly, binding of the enzymes to the substrate in the feed affords protection against proteolysis and prolongs the residence time within the rumen. The effects of exogenous enzymes on digestibility of feedstuffs can be categorized as preingestive, ruminal or postruminal (Beauchemin et al., 2006). Application of exogenous enzymes onto feeds causes a release of sugars, and in some cases, cause some degree of solubilization of ADF and NDF.

Plant Extracts or Products

There is interest in identifying natural bioactive extracts or products of plant origin which may beneficially modify ruminal fermentation and at the same time minimize the environmental impact of livestock production. The trend is particularly evident in Europe, Australia and New Zealand (Wallace et al., 2002; Calsmiglia et al., 2007). Plant extracts have been used for centuries for various purposes because of their antimicrobial activities. The use of plant extracts appears to be one of the most natural alternatives to antibiotic use in animals. It is possible that many of these phytochemicals may eventually find approval as antimicrobial or anti-infective drugs in animal or human medicine and have the potential to replace ionophores and other antibiotics in animal feeds. Plants contain multitude of compounds, often not identified or characterized, whose purpose is to protect them from attack by bacteria, fungi, insects and

vertebrates. Such compounds like saponins, tannins, lignins, flavanoids, and essential oils are particularly prevalent in many tropical plants. At low doses, they have the potential to improve ruminal fermentation, but at high doses, they have adverse effects on ruminal fermentation and animal health and immunity. The major phytochemicals that have been tested in *in vitro* ruminal fermentation and in *in vivo* studies include essential oils, tannins, and saponins. The effectiveness of plant compounds in modifying ruminal fermentation has not been consistent or conclusive. A better understanding the chemical structure and activity relationship is required to fully exploit the application of plant compounds in ruminant animal production.

Essential Oils

These are steam volatile or organic solvent extracts of plants traditionally known for their odor, fragrances, flavor, or antiseptic and/or preservative properties. The chemistry of such compounds is complex and they comprise mainly cyclic hydrocarbons and their alcohol, aldehyde or ester derivatives. In most of them, there is a mixture of several, even hundreds, of individual compounds. Many essential oils have been known to possess antimicrobial properties (Chao and Young, 2000). Similar to ionophore antibiotics, essential oils are typically more active against gram positive bacteria than gram negative bacteria. The main action of essential oils as an antimicrobial appears to be because of their activity on the cell membrane. The loss of the membrane stability results in the leakage of ions across the cell membrane, decreasing the ion gradient. These effects will be more effective against gram positive bacteria, in which the cell membrane can interact directly with hydrophobic compounds of essential oils. The lipophilic nature of cell membranes of gram negative bacteria does not allow essential oils to penetrate. Change in growth rates of bacteria affected by essential oils will result in changes in the proportion of rumen bacterial populations. Additionally, essential oils could coagulate some cell constituents, likely by denaturation of proteins. Some compounds may interact with chemical groups of proteins and inhibit enzymes (Wendakoon and Sakaguchi, 1995).

More than 25 different plant extracts have been tested on *in vitro* rumen microbial fermentation (Calsamiglia *et al*., 2007). There is considerable variation in the content of active compounds in these extracts. Only recently have the effects of pure, active components of essential oils on ruminal microbial fermentation have been studied. Busquet *et al*. (2006) screened 12 plant extracts, including nine essential oils, for ruminal effects in *in vitro* fermentations. Some had antimethanogenic effect, some reduced ammonia concentration and some negatively affected fermentation. Research has indicated that garlic oil, cinnamaldehyde (the main active component of cinnamon oil), eugenol (the active component of the clove bud), capsaicin (the active component of the hot pepper), cervacol (active component of oregano), and anethol (the active component of anise oil) improve the fermentation profile in *in vitro* and, in some cases, in vivo (Cardozo et al., 2006). Many species of ruminal bacteria have been tested for growth in a range of concentrations of essential oils (Wallace *et al*., 2002). Interestingly, only the hyperammonia producing (HAP) bacteria (*Clostridium sticklandii,*

Peptostreptococcus anaerobius), Prevotella ruminicola, and a methanogen were prevented from growth. The effect on HAP explains the reduction in ruminal ammonia production that is sometimes associated with essential oil addition. There is also some evidence of decreased colonization of readily degradable substrates in the rumen by essential oils (Wallace et al., 2002). The exact mode of action for inhibition of colonization is not known.

Other experimental approaches, which are not necessarily newer technologies, that could be employed to manipulate ruminal fermentation include vaccination, and use of bacteriophages or bacteriocins.

Vaccination

An alternative approach to modify ruminal fermentation is to induce the animal to produce antibodies against ruminal microbial antigens (*S. bovis* to control ruminal acidosis, ciliated protozoa to defaunate the rumen, methanogens to mitigate methane production) that would be delivered to the rumen. The ruminal epithelium is immunologically inert and is not secretory. Therefore, the only route for humoral antibodies to reach rumen is via salivary secretion. The effectiveness of the approach depends on survival of antibodies in the rumen and binding of the antibodies to the target microbes. Antibodies are glycoproteins and glycoproteins are somewhat resistant to proteolysis, hence are likely to survive in the rumen and to bind to the target cells (Williams et al., 2007). Based on published studies, none of the vaccines that have been tried to control acidosis, defaunate, or inhibit methane production has shown any promise. Although the exact reason for the lack of efficacy is not known, it is likely that effective antigens have not been identified. Another approach to deliver antibodies to the rumen is to use preformed antibodies as a feed additive (passive immunity). Chickens have been immunized to generate IgY (egg yolk) antibodies and either liquid egg or dried egg powder can be added to the diets (Cook et al., 2008; DiLorenzo et al., 2008).

Bacteriophages

Bacteriophages or bacterial viruses are obligate parasites that infect bacterial cells and cause lysis. The rumen is inhabited by a large number of bacteriophages and it is likely that these contribute to the 'homeostasis' of the microbial population. Generally, phages are highly host specific and the specificity could extend to the strain level within a species, which could be either an advantage or disadvantage, depending on the target. An obvious advantage with phage treatment is they are self-replicating units. However, there is also a possibility of resistance development in bacteria to phages. Bacteriophage therapy to eliminate specific microbes like *S. bovis,* methanogens, hyperammonia-producing bacteria, or pathogens like *E. coli* O157:H7 or *Salomonella* may have obvious benefits.

Bacteriocins

These are peptides naturally produced by some bacteria that are inhibitory to other, generally related bacteria by affecting their cell membranes. These substances may play an important role in microbial competition and other interactions in the rumen. Bacteriocin or bacteriocin-like inhibitory substance (BLIS) production has been detected in certain strains of *Butyrivibrio, Ruminococcus, Streptococcus,* and *Lactobacillus.* Some of the bacteriocins have been shown to be inhibitory to methanogens, hyperammonia producers, etc. Because bacteriocins are peptides there is always a question of their persistence in the rumen, but they are generally resistant to gut proteases. Instead of using bacteriocins as a feed additive, one could potentially use the organism (or clone the gene) that produces the bacteriocin as a probiotic (Rychlik and Russell, 2002; Whitford et al., 2001).

Conclusion

Since the initiation of the study of this subject area in the 1940s by Robert Hungate, considered as the father of Rumen Microbiology, the rumen has become by far the most thoroughly investigated anaerobic microbial ecosystem. Despite the progress in our understanding of the microbiology of the rumen, the description that the rumen is a 'black box' is still unchallenged. Traditional, culture-dependent microbiological methods had indicated that the rumen is inhabited by diverse and complex groups of microbes belonging to all three domains of life. The newer molecular techniques, which are culture-independent, have indicated that ruminal microbes are much more diverse and complex than previously known and the number and types of microbes that have been studied so far represent only a minor component of the ecosystem. The full potential of these techniques to relate the microbial population to metabolic activities and to describe their contribution to ruminal fermentation and host nutrition is just beginning to be realized. A more comprehensive understanding of the players involved and processes that occur in the rumen (more important than players) will lead to additional insight into the biology of the system and provide potential targets for manipulation. Because of the public health concerns associated with the use of subtherapeutic levels of antibiotics, there is considerable interest in the use of natural products, such as DFM, prebiotic oligosaccharides, enzymes, and plant extracts or metabolites to modulate ruminal fermentation to enhance efficiency. Newer technologies (vaccination, bacteriocins, bacteriophages, etc.) are being investigated but none of them has achieved commercial application. It is feasible to genetically manipulate organisms to increase production of beneficial products or antibacterial substances that modify ruminal fermentation, or to manipulate microbial features to enhance the colonization potential of the organism in the rumen. Organisms resulting from such manipulation could be construed as being genetically modified and may consequently face regulatory restrictions. However, if the antibacterial metabolite is identified, it may be possible to develop a nonviable preparation to be used as a feed additive rather than the live cells.

References

Attwood, G. T., E. Altermann, W. J. Kelly, S. C. Leahy, L. Zhang, and M. Morrison. 2011. Exploring rumen methanogen genomes to identify targets for methane mitigation strategies. Anim. Feed Sci. Technol. 166-167:65-75.

Beauchemin, K. A., C. R. Krehbiel, and C. J. Newbold. 2006. Enzymes, bacterial direct-fed microbials and yeast: principles for use in ruminant nutrition. *p.* 251-284. In: Biology of Nutrition in Growing Animals. R. Mosenthin, J. Zentek, and T. Zebrowska (eds.), Elsevier Ltd., NY.

Busquet, M., M., S. Calsamiglia, A. Ferret and C. Kamel. 2006**.** Plant extracts affect in vitro rumen microbial fermentation. J. Dairy Sci. 89:761-771.

Callaway, E. S., and S. A. Martin. 1997. Effects of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Calsamiglia, M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J Dairy Sci. 90:2580-2595.

Calsamiglia, S., P. W. Cardazo, A. Ferret, and A. Bach. 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. J. Anim. Sci. 86:378-383.

Calsamiglia, S., A. Ferret, and M. Devant. 2002. Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. J. Dairy Sci. 85:574-579.

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Effects of alfalfa extract, anise, capsicum and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high concentrate diet. J. Anim. Sci. 84:2801-2808.

Cerrato-Sanchez, M., S. Calsamiglia, and A. Ferret. 2008. Effect of the magnitude of the decrease of rumen pH on rumen fermentation in a dual-flow continuous culture system. J. Anim. Sci. 86:419-431.

Chao, S. C., and D. G. Young. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J. Essent. Oil Res. 12:639-649.

Chaucheyras-Durand, F., N. D. Walker, A. Bach. 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed Sci. Technol. 145:5-26

Cook S. R., P. K. Maiti, A. V. Chaves, C. Benchaar, K. A. Beauchemin, and T. A. McAllister. 2008. Avian (IgY) anti-methanogen antibodies for reducing ruminal methane production: *in vitro* assessment of their effects. Aust. J. Exp. Agric. 48:260-264.

DeVeth, M. J. and E. S. Kolver. 2001. Diurnal variation in pH reduces digestion and synthesis of microbial protein when pasture is fermented in continuous culture. J. Dairy Sci. 84:2066-2072.

Devillard, E., C. J. Newbold; K. P. Scott, E. Forano, R. J. Wallace, J-P. Jouany, and H. J. Flint. 1999. A xylanase produced by the rumen anaerobic protozoan

Polyplastron multivesiculatum shows close sequence similarity to family 11 xylanases from Gram-positive bacteria. FEMS Microbiol. Lett. 181:145-152.

DiLorenzo, N., C. R. Dahlen, F. Diez-Gonzalez, G. C. Lamb, J. E. Larson and A. DiCostanza. 2008. Effects of feeding polyclonal antibody preparations on rumen fermentation patterns, performance, and carcass characteristics of feedlot steers. J. Anim. Sci. 86:3023-3032.

Edwards, J. E., S. A. Huws, E. J. Kim, M. R. F. Lee, A. H. Kinston-Smith, and N. D. Scollan. 2008. Advances in microbial ecosystem concepts and their consequences for ruminant digestion. Animal 2:653-660.

Edwards, J. E., N. R. McEwn, A. J. Travis, and R. J. Wallace. 2004. 16s rDNA library-based analysis of ruminal bacterial diversity. Ant. Van Leeuwenhoek 86:263-281.

Eugène, M., H. Archimède, and D. Sauvant. 2004. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. Livestock Prod. Sci. 85: 81-97.

Fuentes, M. C., S. Calsamiglia, P. W. Cardozo, and B. Vlaeminck. 2009. Effect of pH and level of concentrate in the diet on the production of biohydrogenation intermediates in a dual-flow continuous culture. J. Dairy Sci. 92:4456-4466.

Ghorbani, G. R., D. P. Morgavi, K. A. Beauchemin, and J. A. Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977-1980.

Goad D. W., C. L. Goad, and T. G. Nagaraja. 1998. Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. J. Anim. Sci. 76:234-241.

Gordon, J. I. 2011. Dining in with trillions of fascinating friends: Exploring our human gut microbiome in health and disease. J. Biomol. Tech. 22:S5.

Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore. 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol. Lett. 288:85-91.

Hegarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85:1479-1486.

Hess, M., A. Sczyrba, R. Egan, T-W. Kim, H. Chokhawala, G. Schroth, S. Luo, D. S. Clark, F. Chen, T. Zhang, R. I. Mackie, L. A. Pennacchio, S. G. Tringe, A. Visel, T. Woyke, Z. Wang, E. M. Rubin. 2011. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331:463-467

Jacob, M. E. and T. G. Nagaraja. 2012. Use of direct-fed microbials as a preharvest food safety intervention in cattle. p 189-202. In: Direct-Fed Microbials and Probiotics for Animals: Science and Mechanisms of Action. T. R. Callaway and S. C. Ricke (eds.) Springer Publ., NY.

Janssen, P. H. and M. Kirs. 2008. Structure of the archaeal community of the rumen. Appl. Environ. Microbiol. 74:3619-3625.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009a. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060-1070.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009b. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. J**.** Dairy Sci*.* 92:1712-1724.

Khafipour, E., S. Li, J. C. Plaizier, and D. O. Krause. 2009c. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. Appl. Environ. Microbiol. 75:7115-7124.

Khafipour, E., J. C. Plaizier, P. C. Aikman, and D. O. Krause. 2011. Population structure of rumen *Escherichia coli* associated with subacute ruminal acidosis (SARA) in dairy cattle. J. Dairy Sci. 94:351-360.

Kim, Y. J., R. H. Liu, J. L. Rychlic, and J. B. Russell. 2002. The enrichment of a ruminal bacterium (*Megasphaera elsdenii* YJ-4) that produces the *trans*-10, *cis*-12 isomer of linoleic acid. J. Appl. Microbiol. 92:976-982.

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81:E120-E132.

Lana, R. P., J. B. Russell, and M. E. Van Amburgh. 1998. The role of pH in regulating ruminal methane and ammonia production. J. Anim. Sci. 76:2190-2196.

Leahy, S. C., W. J. Kelly, E. H. Altermann, R. S. Ronimus, C. Yeoman, D. M. Pacheco, D. Li, Z. Kong, S. McTavish, C. Sang, S. C. Lambie, P. H. Janssen, D. Dey, G. T. Attwood. 2010. The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. PLOS One 5:e8926.

LeVan T. D., J. A. Robinson, J. Ralph, R. C. Greening, W. J. Smolenski, J. A. Z. Leedle, and D. M. Schaefer. 1998. Assessment of reductive acetogenesis with indigenous ruminal bacterial populations and *Acetitomaculum ruminis*. Appl. Environ. Microbiol. 64:3429-3436.

Ley R. E., P. J. Turnbaugh, S. Klein, and J. I. Gordon. 2006. Microbial ecology: human gut microbes associated with obesity. Nature 444:1022-23.

Li, S., E. Khafipour , D. O. Krause, A. Kroeker , J. C. Rodriguez-Lecompte, G. N. Gozho , and J. C. Plaizier. 2011. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. J. Dairy Sci. 95:294–303

Li, R. W. and C. Li. 2006. Butyrate induces profound changes in gene expression related to multiple signal pathways in bovine kidney epithelial cells. BMC Genomics **7**:234.

Lin, C., L. Raskin, and D. A. Stahl. 1997. Microbial community structure in gastrointestinal tracts of domestic animals: comparative analyses using rRNA-targeted oligonucleotide probes. FEMS Microbiol. Ecol. 22:281-294.

Martin, S. A., and D. J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

Morgavi, D. P., K. A. Beauchemin, V. L. Nsereko, L. M. Rode, T. A. McAllister, A. D. Iwaasa, Y. Wang, and W. Z. Yang. 2001. Resistance of feed enzymes to proteolytic inactivation by rumen microorganisms and gastrointestinal proteases. J. Anim. Sci. 79:1621-1630.

Mourino, F., R. Akkarawongsa, and P. J. Weimer. 2001. Initial pH as a determinant of cellulose digestion rate by mixed ruminal microorganisms in vitro. J. Dairy Sci. 84:848–859

Mul, A. J. and F. G. Perry. 1994. The role of fructo-oligosaccharides in animal nutrition. *Pages* 57-79. In: Recent Advances in Animal Nutrition. P. C. Garnsworthy and D. J. A. Cole (eds.) Nottingham University Press, London, UK.

Nagaraja, T. G., C. J. Newbold, C. J. Van Nevel, and D. I. Demeyer. 1997. Manipulation of ruminal fermentation. *Pages* 523–632 in The Rumen Microbial Ecosystem. P. N. Hobson and C. S. Stewart, ed. Blackie Acad. and Prof., London.

Nagaraja T. G. and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. J. Dairy Sci. 90(E. Suppl.):E17-E38.

Nisbet, D. J. and S. A. Martin. 1991. Effects of *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium.* J. Anim. Sci. 69:4628–4633.

Nocek, J. E., and W. P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. J. Dairy Sci. 89:260-266.

Nocek, J. E., W. P. Kautz, J. A. Z. Leedle, and J. G. Allman. 2002. Ruminal supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. J. Dairy Sci. 85:429-433.

Nocek, J. E., W. P. Kautz, J. A. Z. Leedle, and E. Block. 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. J. Dairy Sci. 86:331-335.

Oetzel, G. R., K. M. Emery, W. P. Kautz, and J. E. Nocek. 2007. Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: a field trial. J Dairy Sci. 90: 2058-2068.

Palmonari, A., D. M. Stevenson, D. R. Mertens, C. W. Cruywagen, and P. J. Weimer. 2010. pH dynamics and bacterial community composition in the rumen of lactating dairy cows. J. Dairy Sci. 93:279-287.

Plaizier J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences**.** Vet. J. 176:21-31.

Ricard, G., N. R. McEwan**,** B. E. Dutilh**,** J-P. Jouany**,** D. Macheboeuf**,** M. Mitsumori**,** F. M. McIntosh**,** T. Michalowski**,** T. Nagamine**,** N. Nelson**,** C. J. Newbold**,** E. Nsabimana**,** A. Takenaka**,** N. A. Thomas**,** K. Ushida**,** J. Hackstein and M. A. Huyn. 2006. Horizontal gene transfer from bacteria to rumen ciliates indicates adaptation to their anaerobic, carbohydrate-rich environment. BMC Genomics 7*:*22-35.

Russell, J. B. 1998. The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production in vitro. J. Dairy Sci. 81:3222-3230.

Rychlik, J. L. and J. B. Russell. 2002. Bacteriocin-like activity of *Butyrivibrio fibrisolvens* JL5 and its effect on other ruminal bacteria and ammonia production. Appl. Environ. Microbiol. 68:1040-1044.

Suen, G., P. J. Weimer, D. M. Stevenson, F. O. Aylward, J. Boyum, J. Deneke, C. Drinkwater, N. N. Ivanova, N. Mikhailova, O. Chertkov, L. A. Goodwin, C. R.

Currie, D. Mead, P. J. Brumm. 2011. The complete genome sequence of *Fibrobacter succinogenes* S85 reveals a cellulolytic and metabolic specialist. PloSOne 6:e18814.

Tsai, F. and W. J. Coyle. 2009. The microbiome and obesity: is obesity linked to our gut flora? Curr. Gastroenterol. Rep. 211:307-313.

Van Kessel, J. A. S., and J. B. Russell. 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiol. Ecol. 20:205-210.

van Zijderveld, S. M., B. Fonken, J. Dijkstra, W. J. J. Gerrits, H. B. Perdok, W. Fokkink, and J. R. Newbold. 2011. Effects of a combination of feed additives on methane production, diet digestibility, and animal performance in lactating dairy cows. J. Dairy Sci. 94:1445-1454

Wallace, R. J., N. R. McEwan, F. M. McIntosh, B. Teferedegne, and C. J. Newbold. 2002. Natural products as manipulators of rumen fermentation. Asian-Australas. J. Anim. Sci. 10:1458-1468.

Walters, J. and R. Ley. 2011**.** The human gut microbiome: ecology and recent evolutionary changes. Ann. Rev. Microbiol. 65:411-429.

Weimer, P. J., D. M. Stevenson, and D. R. Mertens. 2010. Shifts in bacterial community composition in the rumen of lactating dairy cows under conditions of milk fat depression. J. Dairy Sci. 93:265-278.

Wendakoon, C. N., and M. Sakaguchi. 1995. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Prot. 58:280-283.

Whitford, M. F., M. A. McPherson, R. J. Forster, and R. M. Teather. 2001. Identification of bacteriocin-like inhibitors from rumen *Streptococcus* spp. and isolation and characterization of bovicin 255. Appl. Environ. Microbiol. 67:569-574.

Williams Y. J., S. M. Rea, S. Popovski, C. L. Pimm, A. J. Williams, A. F. Toovey, L. C. Skillman, A-D. G. Wright. 2007. Responses of sheep to a vaccination of entodinial or mixed rumen protozoal antigens to reduce rumen protozoal numbers. Brit. J. Nutr. 99:100-109.

Zebeli, Q. and B. N. Ametaj. 2009. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows**.** J. Dairy Sci. 92:3800-3809.

Zhou, M., E. Hernandez-Sanabria, and L. L. Guan. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microbiol. 75:6524-6533.

Zhou M., E. Hernandez-Sanabria, and L. L. Guan. 2010. Characterization of variation in rumen methanogenic communities under different dietary and host feed efficiency conditions, as determined by PCR-denaturing gradient gel electrophoresis analysis. Appl. Environ. Microbiol. 76:3776–3786.

Table 1. Ruminal VFA concentrations in cattle with low or high residual feed intake [1]

Item	Low-residual feed intake	High-residual feed intake	*P*-value
Residual feed intake, Kg/day	-1.38	1.40	< 0.001
Total VFA, m*M*	96.7	55.4	0.06
Acetate, m*M*	52.7	31.2	0.07
Propionate, m*M*	25.0	18.0	0.4
Isobutyrate, m*M*	0.9	0.6	0.3
Butyrate, m*M*	14.5	3.4	< 0.001
Isovalerate, m*M*	2.0	1.8	0.8
Valerate, m*M*	1.7	0.7	0.006

[1] From Guan et al., 2008.

Table 2. Effect of the magnitude of pH decrease on digestibility, fermentation products, and microbial protein production in a dual-flow continuous culture system[1]

Item	pH treatment				SEM	*P* value
	6.4 for 24 h	5.6 for 4 h and 6.4 for 20 h	5.1 for 4 h and 6.4 for 20 h	5.1 for 2 h, 7.1 for 2 h and 6.4 for 20 h		
Digestibility, %						
True OM	52.4	49.1	45.5	47.7	1.8	0.09
NDF	33.8[a]	25.6[ab]	20.8[b]	26.3[ab]	4.4	0.02
Fermentation products						
Total VFA, m*M*	96.3[ab]	105.8[a]	86.6[b]	95.9[ab]	2.4	< 0.01
Ace:Pro ratio, %	3.42[a]	2.96[a]	1.74[b]	1.99[b]	0.2	< 0.01
NH_3-N, mg/dL	13.3	11.6	9.5	8.3	1.0	< 0.01
Flow of bacterial N, g/d	1.47	1.39	1.18	1.31	0.12	0.28

[1]From Cerrato-Sanchez et al., 2008.

[ab] Within a row, means without a common superscript letter differ at $P < 0.05$.

Table 3. Effect of the duration of suboptimal pH on digestibility, fermentation products, and microbial protein production in a dual-flow continuous culture system[1]

Item	Hour at suboptimal pH of 5.4				SED	Linear effect *P* value
	0	4	8	12		
Digestibility, %						
True DM	65.5	61.2	59.0	57.6	1.1	0.001
True OM	65.9	61.8	59.4	58.2	1.0	0.001
NDF	76.0	72.0	68.7	67.4	1.1	0.001
Fermentation products						
Total VFA, m*M*	61.8	55.5	52.8	46.5	1.6	0.001
Ace:Pro ratio, %	2.9	2.1	2.0	2.0	0.05	0.001
NH_3-N, mg/dl	18.9	20.2	19.1	19.5	0.6	0.7
Flow of microbial N, g/d	0.39	0.37	0.34	0.30	0.04	0.3

[1] From de Veth and Kolver, 2001. The substrate was high quality rye grass.

Table 4. Ruminal pH and endotoxin concentration, and plasma endotoxin, serum amyloid A, and haptoglobin concentrations in dairy cows during a control period and grain-induced subacute acidosis (SARA)[1]

Period	Minutes spent < pH 5.6	Endotoxin concentration (EU/mL)		Serum amyloid A (µg/mL)	Serum haptoglobin (µg/mL)
		Rumen	Blood		
Control	118	29,492	< 0.05	164.4	0
SARA	279	151,985	0.81	446.7	484

[1]From Khafipour et al., 2009a.

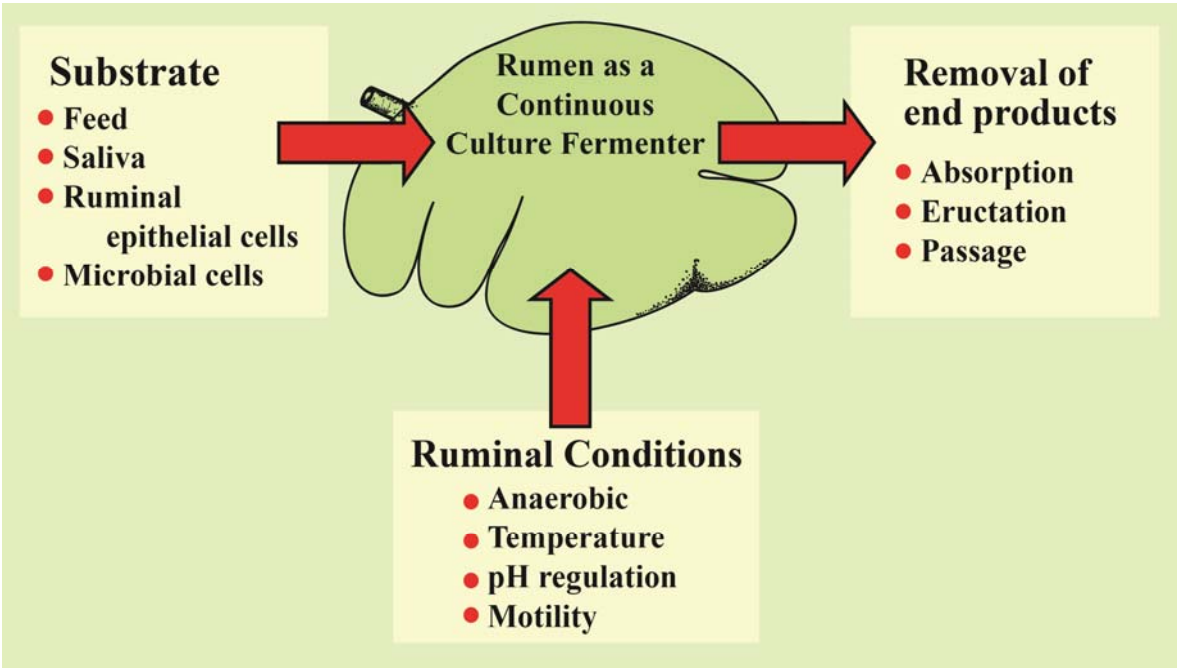

Figure 1. The rumen as a continuous culture fermenter

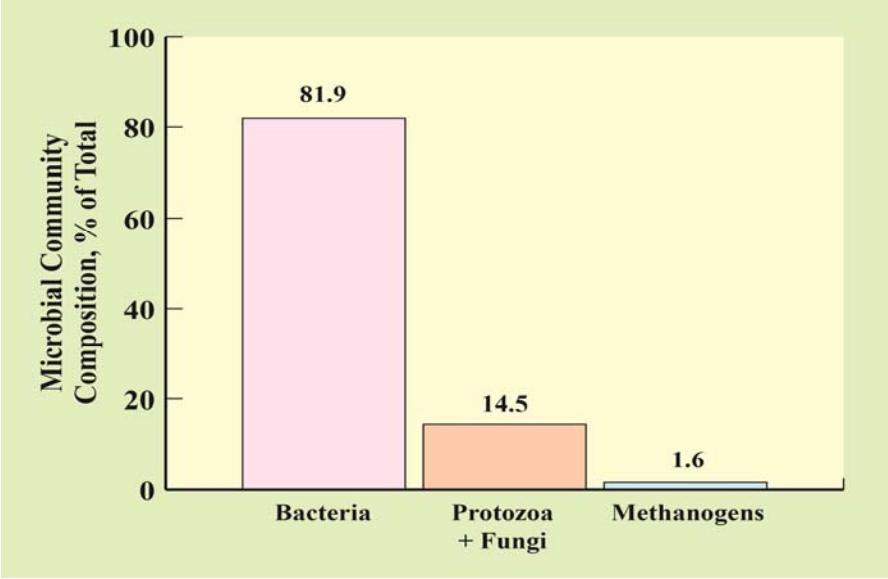

Figure 2. Proportions of bacteria, protozoa, fungi and methanogens in the rumen (From Lin et al., 1997)

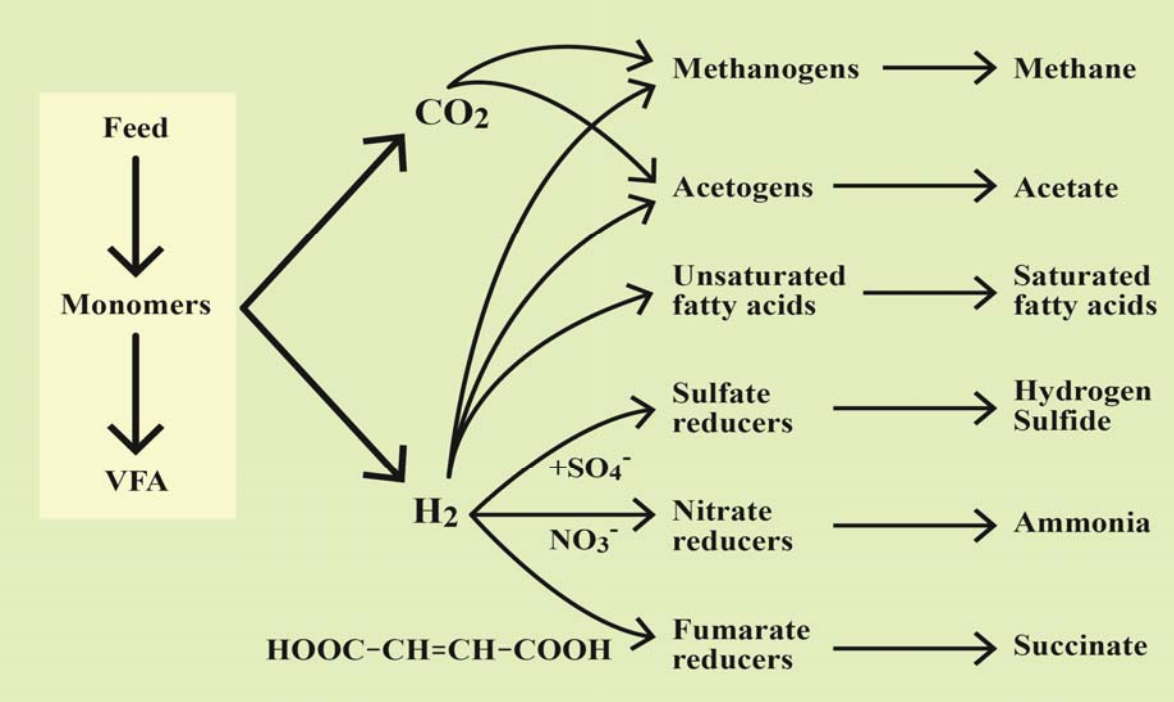

Figure 3. Potential hydrogen sink reactions in the rumen

SESSION NOTES