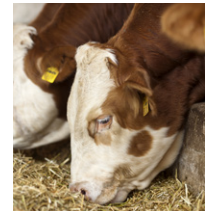


# Dietary impacts on rumen microbiota in beef and dairy production

J.J. Loor, A.A. Elolimy, and J.C. McCann

Department of Animal Sciences, Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801



## Implications

- The rumen harbors diverse microorganisms including bacteria, protozoa, fungi, archaea, and viruses. They play a key role in the breakdown and utilization of feedstuff carbohydrate and protein (from forage, grain) through the process of fermentation, resulting in the production of volatile fatty acids (or short-chain fatty acids) and microbial protein.
- The volatile fatty acids provide the ruminant with readily available sources of energy and substrates for the synthesis of lipid and glucose by the animal. Microbial protein provides a highly digestible source of amino acids for muscle growth in beef and milk protein in dairy cows. Therefore, rumen microorganisms play an essential role in optimizing nutrient utilization from feed.
- Historically, knowledge about the rumen microbial ecology and the nutrition of microorganisms was obtained using classical culture approaches, which by the 1990s, allowed the characterization of at least 22 major bacteria.
- Rapid advances in molecular biology and phylogenetic techniques (e.g., multiple sequence alignment) and the rise of “omic” approaches (e.g., high-throughput sequencing) have allowed for understanding the ecology and function of microbial ecosystems in the rumen.
- These technical advances have enabled scientists, for the first time, to have a holistic view of the rumen microbiota and enhanced our ability to explore some of the hidden relationships between an “altered” rumen environment and the development of a disorder such as sub-acute ruminal acidosis or milk fat depression (“low fat milk syndrome”).
- Here we briefly discuss recent examples demonstrating how age and nutrition can alter the structure, composition, and diversity of the rumen microbiota in beef and dairy production. We also highlight areas where there are knowledge gaps for further research.
- Applying an integrative approach, i.e., systems biology, encompassing nutritional management effects on the rumen microbiota, the tissue responses, and the production outcomes will provide added value to nutritionists attempting to optimize further the production of high-quality beef and milk to meet the demands of a growing population worldwide.

**Key words:** cattle, microbiome, ruminant nutrition

## Background

The reticulo-rumen is the largest compartment of the ruminant digestive tract, and it harbors a complex anaerobic microbial community capable of producing a wide array of enzymes, some of which are important for the breakdown of plant lignocellulosic and non-structural carbohydrate (starch, sugars) material through the process of fermentation (Russell and Rychlik, 2001). Important outcomes of microbial fermentation are the production of volatile fatty acids (or short-chain fatty acids) that serve as fuels for the animal's tissues and the synthesis of microbial protein that provides amino acids for the animal to produce high-quality protein for human consumption, i.e., meat and milk. Studies over the last few decades have conclusively demonstrated that the ruminal microbial ecosystem (both of the neonate and mature ruminant) can be altered by nutritional management (McCann et al., 2014b). Therefore, nutrition represents an important tool for manipulating the microbial ecosystem to optimize rumen function while producing high-quality meat and milk for meeting the demands of a growing human population.

The recent development of omic technologies, e.g., sequencing of the 16S and 18S ribosomal RNA gene, metagenomics, and metatranscriptomics, along with bioinformatics tools, have not only enhanced the understanding of the rumen ecology, but also our ability to predict the functional capacity of the microbiota (McCann et al., 2014b). From a production standpoint, it is believed that further gains in knowledge that in the medium-to-long term could lead to practical applications will arise from the integration of taxonomic and functional data with “classical” parameters of rumen digestion, metabolism, and performance (Morgavi et al., 2013). Our aim in this review is to provide recent examples demonstrating how nutrition can alter the structure, composition, and diversity of the rumen microbiota in beef and dairy production. In addition, we attempt to highlight points where there are knowledge gaps for further research.

## The Symbiosis between Rumen Microbiota and the Host

Rumen microorganisms, termed rumen microbiota, encompass a diverse class diverse class of bacteria, archaea, protozoa, fungi, and viruses. Bacteria compose an estimated 95% of the whole ruminal microbial community (Pitta et al., 2014a). Hence, these microorganisms contribute the most to feedstuff (i.e., forage or forage-grain mixtures) digestion and the conversion of plant materials to volatile fatty acids and microbial protein. Before fermentation can take place, colonization or adhesion by microbiota to feed particles should occur to allow for the formation of enzymatic complexes that break down plant indigestible polymers, e.g., cellulose and hemicellulose, into monomeric and dimeric simple sugars (Russell and Rychlik, 2001). The major end-products of microbial metabolism of feedstuffs are

the volatile fatty acids along with microbial protein. The most quantitatively important volatile fatty acids are acetate, propionate, and butyrate, which are used by the animal for fatty acid synthesis, glucose, production of energy (ATP), and ketone bodies. The volatile fatty acids are readily absorbed across the rumen wall and enter the systemic circulation where tissues like the liver, adipose, mammary gland, and muscle could utilize them. Therefore, the volatile fatty acids serve as the main metabolic fuels in tissues of ruminants. The microbial mass that leaves the rumen into the small intestine serves as an important source of amino acids for animal tissues (Storm and Orskov, 1983). In some instances, e.g., at maintenance, microbial protein can supply the animal with 50 to 80% of the amino acid requirements.

## Manipulation of Ruminal Fermentation

Sustaining the high levels of productivity in modern dairy and beef cattle cannot be achieved solely with forage. Therefore, nutritionists have long sought methods to enhance beneficial processes (e.g., increase volatile fatty acids production, microbial protein synthesis, and bypass protein), alter inefficient processes such as limit methane and ammonia production, or enhance protection processes in the rumen (e.g., maintain rumen pH using buffers) (Weimer, 1998; Bach et al., 2005; Jeyanathan et al., 2014). As an example, rapidly-fermented grains increase energy (ATP) availability to microbes resulting in volatile fatty acid production; non-protein nitrogen (NPN) inclusion provides rumen microbes with essential nitrogen required for microbial protein synthesis; feeding of heat-treated proteins decrease the ability of microbes to degrade plant amino acids, and hence, those amino acids remain intact and travel directly to the small intestine for absorption; ionophores inhibit gram-positive bacteria that produce hydrogen, ammonia, or lactic acid; and buffers (additives to neutralize acid production; e.g., sodium bicarbonate) counteract the decrease in grain-dependent pH that can be harmful to fiber-degrading microorganisms (Nagaraja et al., 1997; Russell and Rychlik, 2001).

Transfaunation, a common long-practiced medical method used to treat indigestion in ruminants where rumen microorganisms are collected from a healthy donor animal to be transferred to a sick recipient animal to replace the unhealthy microbial ecosystem in the sick recipient with a healthy microbial profile, helps to re-establish a normal rumen fermentation process (DePeters and George, 2014). Classical rumen microbiology work during the 1960s through the 1990s allowed for the discovery and gross characterization of the predominant bacterial species (22 main species; Table 1) that inhabit the rumen, their main nutritional source, and the main fermentation products (see Russell and Rychlik, 2001). Clearly, those studies (mostly using pure cultures of microorganisms) laid the foundation for ruminant nutritionists seeking approaches to optimize feedstuff utilization by the microbes and the animal.

## Microbial Methodological Advancements

While rumen function has always been a focal point of cattle nutrition, the growing understanding and interest in ruminal microbiota is directly tied to rapid development in culture-independent techniques. After developments and improvements in polymerase chain reaction (PCR) technology, evaluation of marker genes began being used for microbial community analysis. While the marker genes are present in all members of a population interest, variation in regions of the DNA sequence allows for differentiation among classes of microbes. Observed in all bacterial and archaeal genomes, the 16S rRNA gene is widely used and is sup-

**Table 1. Major characteristics of predominant cultured bacteria in the rumen (Russell and Rychlik, 2001).**

Species	Ruminal niche	Fermentation products
<i>Fibrobacter succinogenes</i>	CU	S, F, A
<i>Ruminococcus albus</i>	CU, HC	A, F, E, H2
<i>Ruminococcus favefaciens</i>	CU, HC	S, F, A, H2
<i>Eubacterium ruminantium</i>	HC, DX, SU	A, F, B, L
<i>Ruminobacter amylophilus</i>	ST	S, F, A, E
<i>Streptococcus bovis</i>	ST, SU	L, A, F, E
<i>Succinomonas amylolytica</i>	ST	S, A, P
<i>Prevotella ruminicola, albensis, brevis, and bryantii</i>	ST, PC, XY, SU	S, A, F, P
<i>Butyrivibrio fibrisolvens</i>	ST, CU, HC, PC, SU	B, F, A, H2
<i>Selenomonas ruminantium</i>	ST, DX, SU, L, S	L, A, P, B, F, H2
<i>Megasphaera elsdenii</i>	L, SU	P, A, B, Br, H2
<i>Lachnospira multiparus</i>	PC, SU	L, A, F, H2
<i>Succinivibrio dextrinosolvens</i>	PC, DX, SU	S, A, F, L
<i>Anaerovibrio lipolytica</i>	GL, SU	A, S, P
<i>Peptostreptococcus anaerobius</i>	AA	Br, A
<i>Clostridium aminophilum</i>	AA	A, B
<i>Clostridium sticklandii</i>	AA	A, Br, B, P
<i>Wolinella succinogenes</i>	OA, H2, F	S
<i>Methanobrevibacter ruminantium</i>	H2, CO2, F	CH4

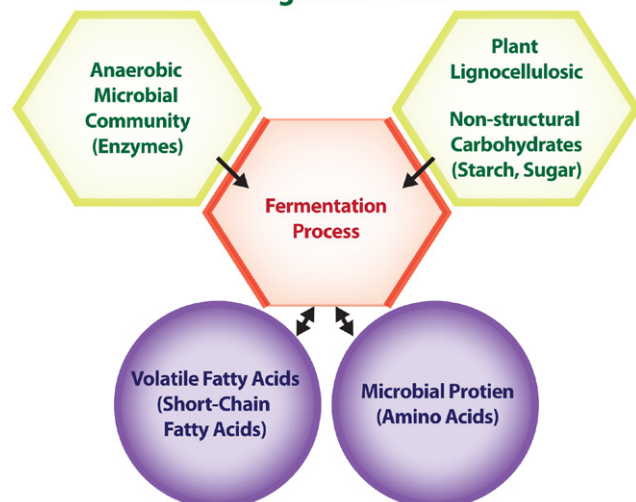
Abbreviations are as follows: CU, cellulose; HC, hemicellulose; DX, dextrins; SU, sugars; ST, starch; PC, pectin; XY, xylans; L, lactate; S, succinate; GL, glycerol; AA, amino acids; OA, organic acids; H2, hydrogen; F, formate; CO2, carbon dioxide; A, acetate; E, ethanol; B, butyrate; L, lactate; P, propionate; Br, branched-chain volatile fatty acids; and CH4, methane.

ported by the most robust databases. Using primers to amplify a portion of the marker gene facilitated gel-based community “fingerprinting” techniques, which recently have been replaced by high-throughput sequencing the PCR products on “next-generation” DNA sequencers. The increasing ability of these machines to output millions to billions of reads and the capability to multiplex samples with unique barcodes has dramatically reduced the cost and led to the widespread adoption of this technology.

Current challenges include different sampling methods (e.g., cannulated vs. gastric tube vs. epimural microbial communities), diverse DNA and RNA extraction methods, DNA- vs. RNA-based comparisons, known biases within the PCR step that may lead to underrepresentation of some bacteria (e.g., Proteobacteria and Actinobacteria are not well represented in the 16S rRNA PCR-based approach compared with metagenomic analysis) as well as varying results from other regions of the 16S rRNA gene (V1/V2 versus V3/V4) for amplicon-based sequencing. Furthermore, the variation in bioinformatics analysis and presentation of the data can make comparisons between studies difficult. It is important to remember that marker gene techniques not only determine the presence of a microbe, but also provide information on its metabolic capability. “Shotgun” metagenomics can be used to determine the genomic capability of all bacteria in a sample while metatranscriptomics evaluates genes that are currently being expressed. Challenges in sample preparation, analysis of bioinformatics, and data interpretation have limited the use of these methods to study rumen microbiota thus far.

Quantitative PCR also has been extensively used in studying ruminal bacteria, methanogens, fungi, and protozoa. This technique focuses on identifying specific, often culturable species, and is a more sensitive tool

## Ruminant Digestive Fermentation



to determine population changes. Additionally, efforts spearheaded by the Hungate 1000 initiative have sequenced the complete genome of more than 200 cultured microbial strains from the rumen (Creevey et al., 2014). Other techniques are currently available, and their further development and implementation to understand the microbiome within the rumen will improve beef and dairy production.

## Dietary Impacts on Rumen Microbiota in Cattle

### Dairy calves

The establishment of a microbial ecosystem in the rumen begins early after birth and continues rapidly through weaning to become an “adult-like” rumen microbiome (Jami et al., 2013). The early colonization of the rumen with microbes induces microbial fermentation of dietary complex carbohydrates resulting in the production of volatile fatty acids, which play a vital role in the development and growth of rumen papillae, leading to increase papillae capacity for nutrient absorption and metabolism (Lane and Jesse, 1997). Therefore, an optimal development of the rumen microbiome early in life directly impacts the ability of the calf to become a “fully functioning” ruminant. Interactions among diet, microbiome, and the host are complex but in large part dictate overall metabolic and productive response of the animal.

Various factors can affect the composition of the rumen microbiome in calves including age and nutrition (Tajima et al., 2001). In one of the first studies of its kind, Li et al. (2012) used next-generation sequencing to explore the changes in microbiome composition between 2 and 6 wk of age in dairy calves. The study underscored an age-dependent change in the microbiome. For example, the abundance of Bacteroidetes increased by 1.5-fold from 2 to 6 wk of age. Furthermore, *Bacteroides* dominated the microbial community at 6 wk while *Prevotella* was dominant at 2 wk of age. Other subsequent studies revealed that microbiome diversity increases in a sequential fashion with age (Jami et al., 2013; Rey et al., 2014). Such effect is partly due to the gradual introduction of solid feed (e.g., “starter” grain) to the diet of pre-wean calves (Malmuthuge et al., 2013). As a result, volatile fatty acid production in the rumen increases (Laarman et al., 2012) and accelerates rumen epithelium development, which facilitates the weaning transition.

Other research has provided evidence that colostrum can induce quick changes in the microbial composition within the small intestine of the

calf. For example, Malmuthuge et al. (2015) used qPCR technology to determine that feeding heat-treated colostrum (60°C, 60 min) to dairy calves increased *Bifidobacterium* in the small intestine within 12 h after birth. *Bifidobacterium* is considered a beneficial bacteria that plays a role in enhancing the immune system, improving gut barrier, and reducing enteric pathogens (Fukuda et al., 2011). Heat treatment increases the availability of sialylated oligosaccharides in the colostrum, which can be utilized by *Bifidobacterium* as an energy source (LoCascio et al., 2007). Heat-treated colostrum also decreased the colonization of the small intestine by *Escherichia coli* (Malmuthuge et al., 2015), which is a common pathogen associated with diarrhea in calves (Foster and Smith, 2009). A better understanding of the response of the digestive tract microbiota to dietary changes in neonatal calves would not only help optimize rumen development and function but also overall health.

### The peripartum or ‘transition’ period in dairy cows

The “transition” period in dairy cows is defined as the stage between 3 wk before parturition through the first 3 wk postpartum. This physiological stage is characterized by dramatic physiologic and metabolic adaptations, and it is now well established that the cow is at the highest risk of succumbing to health disorder during this time (Lor et al., 2013a). From a rumen microbiota standpoint, nutrition-induced changes during the transition period could play a vital role in providing optimal amounts of volatile fatty acids, propionate in particular, to help alleviate the demands of the mammary gland for glucose and energy. The shortfall in glucose availability after parturition is one key driver of ketosis, a potentially deadly metabolic disorder (Bobe et al., 2004). Because the diversity, richness, and composition of ruminal microbiota are diet specific (McCann et al., 2014b), nutritionists can manipulate the proportions of forage and grain fed and, for example, increase fibrolytic bacteria or amylolytic bacteria.

A common approach to manage high-producing pregnant dairy cows during the last 3 wk of the non-lactating period is to shift from a high-forage, low-energy diet to a high-grain, high-energy diet (Roche et al., 2013) to enhance the population of microorganisms capable of fermenting starch and sugars (non-structural carbohydrates), and hence, produce more volatile fatty acids and propionate. Such an approach also could enhance ruminal epithelium development and potentially increase the volatile fatty acids absorptive capacity after parturition when cows will normally take a week or longer to reach appropriate levels of feed intake.

A recent study detected increases after parturition in abundance of *Lactobacillus* spp., *Streptococcus bovis*, *Megasphaera elsdenii*, *Ruminobacter amylophilus*, *Prevotella ruminicola*, and *Selenomonas ruminantium* (Minuti et al., 2015). A decrease in fibrolytic bacteria such as *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens* also was detected after parturition. Such responses were primarily driven by the feeding of a higher-grain, higher-energy diet after parturition, which is a common management practice in the field. Similar results were reported by others utilizing PCR or sequencing approaches (Fernando et al., 2010; Wang et al., 2012) and agree with the characteristics of these predominant bacteria identified in vitro (Russell and Rychlik, 2001).

In addition to changes in bacterial populations, Kumar et al. (2015) and Lima et al. (2015) reported a decrease in fungi after parturition when a high-grain, high-energy diet was fed and suggested that fungi may play an important role in fiber degradation. Lima et al. (2015) revealed that *Litostomatea*, a ruminal protozoal taxa, was more abundant with the high-energy diet but the biologic role of this change could not be discerned.



## Subacute ruminal acidosis

The onset of subacute ruminal acidosis is common and somewhat difficult to diagnose in the field, but it causes substantial economic losses to dairy farmers because it decreases milk production, increases premature culling, and also could result in death if not detected in a timely fashion. Although shifting postpartum dairy cows to a high-grain, high-energy diet is a widely used feeding strategy in the dairy industry, grain-based diets can reduce rumen pH and dramatically alter the microbial ecosystem, which often leads to onset of subacute ruminal acidosis and, if uncontrolled, could lead to death (Nagaraja and Titgemeyer, 2007). Onset of subacute ruminal acidosis, which is not associated with accumulation of lactic acid, is often diagnosed when ruminal pH after feeding remains below 5.5 for a period of 4 h or more after feeding (Garrett et al. (1999).

Khafipour et al. (2009) and Mao et al. (2013) investigated the shift in the rumen microbiota associated with subacute ruminal acidosis incidence in lactating cows fed high-starch diets. Common to both studies was the marked reduction in the diversity of the rumen microbiota during subacute ruminal acidosis. Analyses revealed that 14 bacterial phyla (a taxonomic rank), including 5,664 species within 155 genera (a taxonomic rank), were affected during the onset of subacute ruminal acidosis. At the genus level, subacute ruminal acidosis increased numbers of microorganisms involved in starch fermentation such as *Ruminococcus*, *Bifidobacterium*, and *Atopobium*. In contrast, the growth of fibrolytic microorganisms such as *Prevotella*, *Treponema*, *Anaeroplasm*, *Papillibacter*, and *Acinetobacter* decreased in large part because of their intolerance to low rumen pH. In general, the low rumen pH associated with high-grain diets increases gram-positive bacteria such as the Firmicutes phylum members, which can readily ferment starch and sugars, while it reduces gram-negative bacteria such as the Bacteroidetes phylum.

The greater loss of gram-negative bacteria, due to cellular death and lysing with the low pH, can lead to the release of “endotoxin,” a large molecule consisting of lipid and a polysaccharide (e.g., lipopolysaccharide or LPS) into the ruminal fluid (Nagaraja and Titgemeyer, 2007). In addition, lowering rumen pH for extended periods of time because of volatile fatty acid production could damage the epithelial tight junctions of the rumen wall, allowing for transport of endotoxin to peripheral blood and stimulation of inflammation. In the short term, an uncontrolled inflammatory response reduces feed intake and compromises liver function, both of which are detrimental to the animal (Lor et al., 2013a).

## Milk fat depression

The lactating mammary gland of dairy cows is a “lipid-synthesizing machine,” and it relies primarily on the volatile fatty acids acetate and butyrate as substrates for “lipogenesis” (making a 16-carbon fatty acid from the 2-carbon acetate). The overall process of making milk fat includes not only the availability of acetate and butyrate, but also a number of enzymes that work in a coordinated fashion to synthesize fatty acids and use them to make “fat,” i.e., fatty acids attached to a glycerol backbone (“triacylglycerol”) (Bionaz and Lor, 2008). Milk fat synthesis also relies on the availability of dietary and rumen-derived fatty acids that are absorbed in the small intestine during digestion. Reducing fat yield could represent an economic loss to dairy producers because milk fat content is one important component that influences milk price.

Milk fat depression occurs for a number of reasons, e.g., heat stress and dietary forage chopped too fine, but elucidating fundamental biological mechanisms underlying milk fat depression from the standpoint of rumen

microbiota and diet interactions would be helpful to nutritionists. Perhaps the best-studied model of nutrition-induced milk fat depression is that involving rumen-derived “trans fatty acids” (Davis and Brown, 1970), i.e., unsaturated fatty acids with a *trans* configuration around a double bond on the carbon chain. High-grain, low-forage diets alone or supplemented with polyunsaturated fatty acids (e.g., 18:2, 18:3) or with ionophores (lipid-soluble compounds that eliminate gram-positive bacteria) often induce milk fat depression, also known as the “low-fat milk syndrome.” This condition is characterized by a reduction in milk fat yield by 50% or more during early lactation with no effect on milk yield or other milk components.

Knowledge about the metabolism of unsaturated fatty acids in the rumen dates to 1957 when Shorland et al. (1957) demonstrated the conversion of linoleic (18-carbons, 2 double bonds; 18:2) and linolenic (18-carbons, 3 double bonds; 18:3) acid to saturated fatty acids (18:0 primarily) using whole-rumen contents. Subsequent research during the 1960s and 1970s utilizing pure cultures of various species of microorganisms elucidated the microbial enzymes involved as well as the intermediates and end-products of the process (Harfoot, 1978). More recently (2000s), advances in chromatography to better identify biohydrogenation intermediates along with modern microbial genetics and molecular phylogenetic techniques for identifying and classifying microorganisms based on gene sequences (small-subunit rRNA) have substantially advanced knowledge of the role and contribution of specific microbial species in the process of biohydrogenation (Jenkins et al., 2008).

Recent work using molecular technologies has been useful in determining with greater certainty the changes in the microbial ecology that are associated with milk fat depression. For example, feeding high-starch diets not only increases amylolytic bacteria, but also the abundance of *Megasphaera elsdenii*, which ferments lactate and sugars (Table 1). Weimer et al. (2010) reported that high abundance of *Megasphaera elsdenii* in the rumen microbiota was strongly correlated with milk fat depression. More recently, Rico et al. (2015) confirmed the relationship between *Megasphaera elsdenii* and diet-induced milk fat depression as well as with *Selenomonas ruminantium* (a lactate utilizer) and *Streptococcus bovis* (starch and sugars fermenter). That study also detected a decrease in fungi and ciliate protozoa abundance with milk fat depression. From the standpoint of protozoa, the data support the hypothesis of Lima et al. (2015) that these microorganisms might play an important role in fiber degradation in the rumen.

## Dietary effects on rumen microbiota in beef production

Cattle diets vary more than other livestock species in their nutrient composition. They range from almost all low-quality forage diets to nearly all grain concentrates depending on the production system and location. These dietary changes completely alter the substrates available to the rumen microbiota and, in turn, may lead to effects on microbial community composition, function, and fermentation end-products (Petri et al., 2013). Dietary transition in growing beef cattle occurs when animals move from one segment of the industry to another with the typical pattern being from cow-calf to stocker and from stocker to feedlot.

The shift from a medium-quality forage to winter wheat pastures is often seen in the southern US after the calf is weaned. Dietary increases in energy and protein facilitate greater animal performance but require the rumen microbiome to adjust accordingly. These effects were first observed by evaluating rumen bacteria when transitioning from a bermudagrass hay diet to wheat pasture (Pitta et al., 2010). The 16S rRNA sequencing indicated *Prevotella* and *Rikenella* were the most abundant genera observed,



Beef cattle eating hay (source: ellisia/stock.adobe.com).

but the shift to wheat pasture increased relative abundance of *Prevotella* while it decreased *Rikenella*. The genus *Succiniclasicum* also increased on the wheat pasture diet and coincided with an expected greater propionate production. Bacterial community diversity decreased on wheat pasture as is generally observed with greater dietary energy. Among the rumen epimural microbes, Petri et al. (2013) reported that a high-grain diet increased the abundance of *Atopobium*, *Desulfocurvus*, *Fervidicola*, *Lactobacillus*, and *Olsenella*. Further work has revealed that increased phyla *Bacteroidetes* relative to *Firmicutes* in the liquid fraction may be linked to greater biofilm formation for cattle grazing wheat pasture, which causes frothy bloat (Pitta et al., 2014b).

Calves enter feedlots immediately after weaning or the stocker phase as calf-feds or yearlings, respectively. Although cattle in feedlots may be susceptible to stressful conditions aside from diet-related issues (e.g., mixing, regrouping, antibiotic treatments, or vaccination), which may alter the composition and function of the microbiome, to our knowledge, no attention has been placed in exploring the dynamics of the microbiome to such potentially adverse effects. The initial receiving diet is highly palatable and often contains high-quality forages such as alfalfa to stimulate intake. The receiving diet is then blended with a predominantly concentrate-based finishing diet at incremental steps until cattle are completely on the finishing diet. This progression allows cattle time to physiologically adjust to dietary changes in terms of rumen microbial and epithelial function while reducing occurrence of nutritional disorders such as acidosis. While many experiments have described differences between forage and concentrate diets on rumen microbiota, Fernando et al. (2010) was the first to describe this adaptation using a DNA-based evaluation of ruminal bacteria. Greater concentrate inclusion corresponded to increased *Selenomonas ruminantium* and *Megasphaera elsdenii*. While both species are capable of utilizing lactate (Table 1; Russell and Baldwin, 1978; Counotte et al., 1981) that can be produced by rapid starch fermentation, *S. ruminantium* also can produce propionate (Table 1), which increases with greater concentrate inclusion.

Ruminal production of propionate is an important fermentation end-product as it is the main gluconeogenic precursor in ruminants. Furthermore, two fibrolytic bacteria, *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens*, decreased 45 to 15-fold, respectively, in the final finishing diet compared with the pre-transition control (Fernando et al., 2010). More recent results suggest that the composition of the receiving diet also can affect the rate on transition by the rumen bacteria to the finishing diet (Anderson et al., 2016). Researchers compared the use of a complete commercial starter diet that is high in corn gluten feed and low in forage (RAMP) with an alfalfa- and corn-based control as both were blended with increasing amounts of a similar finishing diet. The rumen bacterial community for cattle on RAMP shifted after the second and third step diet to more readily adapt to the final finishing diet than cattle on the control (Anderson et al., 2016). Multiple operational taxonomic units (OTUs), a quantitative measure of species, classified at the family level as Prevotellaceae and Paraprevotellaceae, were important adaptations to dietary changes as some OTUs increased while others decreased. These findings reinforce the importance of the need for understanding the various roles of *Prevotella* in the rumen and its relevance to dietary changes (Stevenson and Weimer, 2007).

While the majority of rumen microbiota research focuses on growing beef cattle or high-producing dairy cattle, much less attention is given to moderate-to-low-quality forage diets consumed by the 30 million beef cows in the US. The diets are high in fibrous plant material that highlights the signature functions of rumen microbiota. A variety of nutritional strategies or supplements are commonly implemented to meet the cow's maintenance requirements and vary by region, season, and stocking intensity. Considering the dearth of research in this area, it is difficult to understand the importance of the ruminal microbiome composition beyond the main energetic contribution of fermentation end-products.

Protein is often supplemented in low amounts to cattle consuming low-quality forage to improve ruminal fermentation. Recent research compared a novel protein source, postextraction algal residue, with an isonitrogenous cottonseed meal control as well as an unsupplemented treatment (McCann et al., 2014a). The most significant impact on the rumen microbiome occurred in the liquid fraction as increasing algal coproduct inclusion resulted in greater phyla Firmicutes and corresponding families Ruminococcaceae, Lachnospiraceae, and Clostridiaceae. While the isonitrogenous cottonseed meal and algal coproduct diets led to similar levels of forage intake and utilization, the bacterial community for the cottonseed meal treatment was most similar to the unsupplemented control. These findings suggest bacterial community composition alone may not predict well the utilization of low-quality forages because ruminal fermentation is a complex process requiring interactions among different microbial groups.

## Feed efficiency

The ability of cattle to convert dietary nutrients to retail end-products such as milk and meat is the basis for various measures of efficiency. Animals that maintain similar levels of performance while consuming less feed are classified as efficient with a lower numerical residual feed intake (RFI). While many factors may contribute to efficiency differences within a population, some data do support a role for rumen microbiota in mediating observed phenotypical differences in efficiency (Jewell et al., 2015). Some of the earliest research in this area using fingerprinting techniques suggested more efficient cattle had greater levels of butyrate production and differences in bacterial communities compared with the least efficient animals (Guan et al., 2008). While sequencing of efficiency-associated denaturing



gel electrophoresis bands revealed multiple bacteria were associated with efficient and inefficient cattle, qPCR confirmed *Eubacterium rectale* had the strongest relationship with inefficient cattle on a high-energy diet (Hernandez-Sanabria et al., 2012). Others have observed *Prevotella* to be associated with inefficient (high RFI) cattle under various experimental conditions (Carberry et al., 2012; McCann et al., 2014c).

Greater sequencing depth per sample allowed a recent study to detect many associations with efficiency using different classifications based on average daily gain and feed intake (Myer et al., 2015). Most significantly affected bacteria such as *Dialister* and *Acidaminococcus* were linked with differences in average daily gain, which were greater on cattle with higher average daily gain. It was noteworthy that a *Prevotella*-identified OTU was much greater in animals with high average daily gain and low feed intake (Myer et al., 2015). Considering that some studies have detected *Prevotella* at more than 30% of all bacteria in the rumen, it is not surprising that *Prevotella*-identified OTUs have been positively and negatively associated with more efficient animals. Cattle classified as more efficient on a concentrate-based diet may not be similarly classified within the same contemporary group on a high-forage diet (Durunna et al., 2011; Carberry et al., 2012). Therefore, microbiota associated with efficiency differences are likely dependent on diet. Knowing that multiple factors contribute to efficiency differences observed with a population suggests that the relative importance of these factors will change depending on genetics, diet, and management of the evaluated contemporary group.

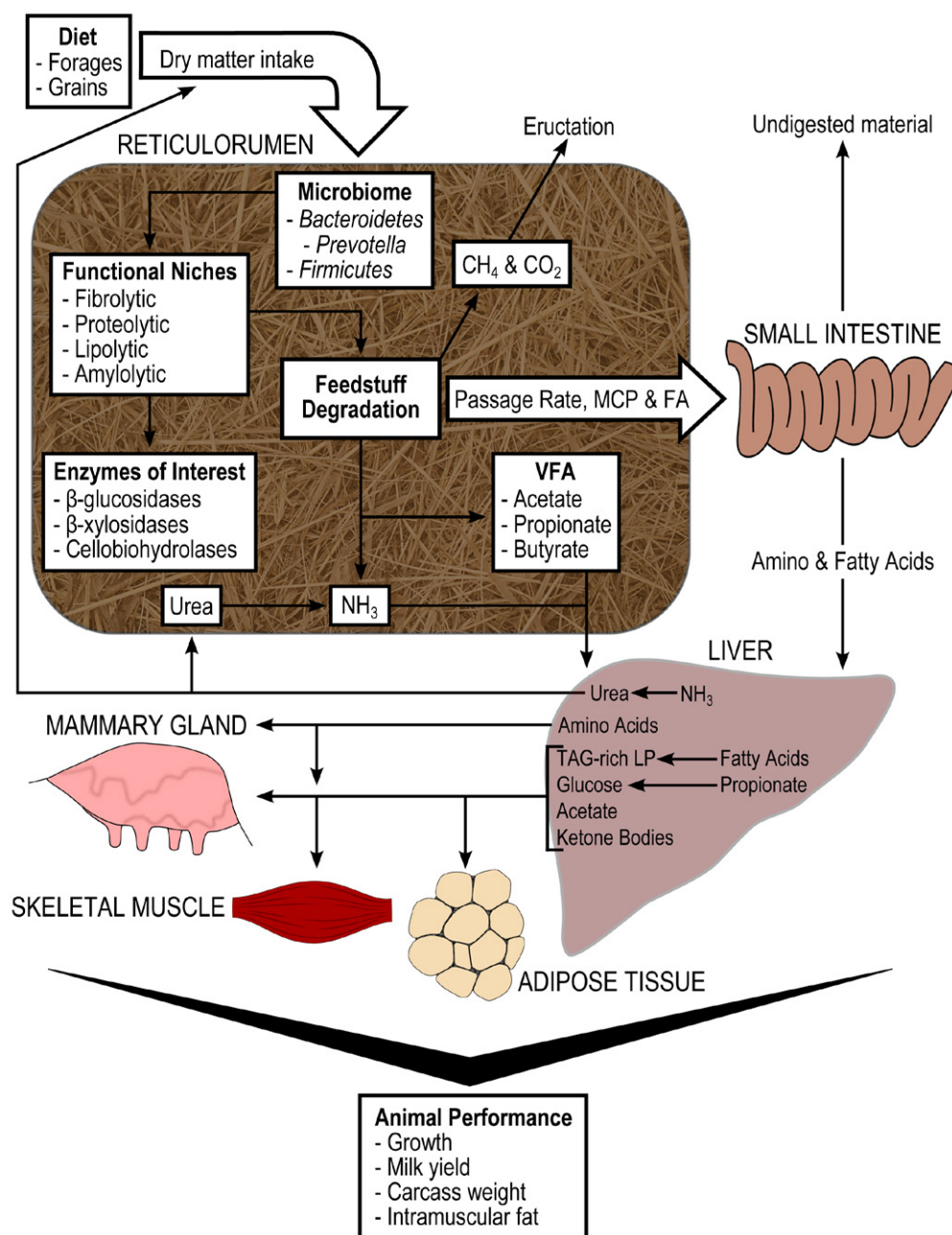


Figure 1. Diet-microbiota-host interactions in cattle (McCann et al., 2014b).

## Integration in Ruminant Nutrition: the Future Direction

The concept of integration in ruminant nutrition and physiology in an effort to apply “systems biology” concepts and tools has been the subject of increasing interest during the last few years. The review by Loor et al. (2013b) outlined and discussed the major milestones that have been accomplished by applying systems concepts in dairy cows. The main goal when applying systems biology concepts is to integrate information at various levels of biological regulation (e.g., gene to mRNA, mRNA to protein) as a means to arrive at a holistic view of how animals function. The applicability of high-throughput technologies (e.g., sequencing, proteomics, and metabolomics) to discern functional biological networks has been demonstrated through work in humans and model organisms. Ruminants are obviously complex

organisms, and ample evidence indicates that information gathering and integration are far better than continuing with the reductionist approach (Loor et al., 2013b). Both McNamara (2012) and Loor et al. (2013b, 2015) argue that another important goal of systems biology is to discover new properties that may arise from examining the interactions between components of a system, e.g., nutrition and the tissue genome. The same can be envisioned when the rumen microbiome is specifically placed as one of the key components of ruminant systems (see McCann et al., 2014b; also Figure 1).

Despite the abundance of knowledge about many specific rumen microorganisms and their function (e.g., Russell and Rychlik, 2001), our holistic understanding of the ruminal microbiome and how it cooperatively functions

to degrade a wide array of substrates is still limited in many regards. Despite high-throughput methods allowing researchers to further define microbial community occupants, their metabolic potential, and what they are actively doing, we are just beginning to learn how to use these data to better understand the ruminant system. A systems biology approach will facilitate integration of the data further defining the host–microbiome relationship and allowing researchers to address pertinent questions facing ruminants.

The knowledge generated to date in livestock nutrition by utilizing a more holistic approach to dietary management underscores the validity of molecular technologies in helping build diets tailored to create a desired outcome at the whole-animal level (Loor et al., 2015). Lack of available data still limit our ability to take into account when formulating diets any specific effects of nutrients on the microbiome and the tissue response. An important undertaking for the field of ruminant nutrition in the medium to long term will be the generation of such kind of data for inclusion into diet formulation systems.

## Literature Cited

- Anderson, C. L., C. J. Schneider, G. E. Erickson, J. C. MacDonald, and S. C. Fernando. 2016. Rumen bacterial communities can be acclimated faster to high concentrate diets than currently implemented feedlot programs. *J. Appl. Microbiol.* 120(3):588–599. doi: 10.1111/jam.13039
- Bach, A., S. Calsamiglia, and M.D. Stern. 2005. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 88(Suppl 1):E9–E21. doi:10.3168/jds.S0022-0302(05)73133-7
- Bionaz, M., and J.J. Loor. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics* 9:366. doi:10.1186/1471-2164-9-366
- Bobé, G., J.W. Young, and D.C. Beitz. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *J. Dairy Sci.* 87:3105–3124. doi:10.3168/jds.S0022-0302(04)73446-3
- Carberry, C.A., D.A. Kenny, S. Han, M.S. McCabe, and S.M. Waters. 2012. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. *Appl. Environ. Microbiol.* 78:4949–4958. doi:10.1128/AEM.07759-11
- Counotte, G.H.M., R.A. Prins, R.H.A.M. Janssen, and M.J.A. deBie. 1981. Role of *Megasphaera elsdenii* in the fermentation of DL-[2-<sup>13</sup>C]lactate in the rumen of dairy cattle. *Appl. Environ. Microbiol.* 42:649–655.
- Creevey, C.J., W.J. Kelly, G. Henderson, and S.C. Leahy. 2014. Determining the cultivability of the rumen bacterial microbiome. *Microb. Biotechnol.* 7:467–479. doi:10.1111/1751-7915.12141
- Davis, C.L., and R.E. Brown. 1970. Low-fat milk syndrome. In: A.T. Phillipson, editor, *Physiology of digestion and metabolism in the ruminant*. Oriel Press, Newcastle upon Tyne, UK. p. 545–565.
- DePeters, E.J., and L.W. George. 2014. Rumen transfaunation. *Immunol. Lett.* 162:69–76. doi:10.1016/j.imlet.2014.05.009
- Durunna, O. N., F. D. N. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. *J. Anim. Sci.* 89: 158–167. doi:10.2527/jas.2009-2514
- Fernando, S. C., H. T. Purvis, 2nd, F. Z. Najjar, L. O. Sukharnikov, C. R. Krehbiel, T. G. Nagaraja, B. A. Roe et al. 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. *Appl Environ Microbiol* 76: 7482–7490. doi:10.1128/AEM.00388-10
- Foster, D.M., and G.W. Smith. 2009. Pathophysiology of diarrhea in calves. *Vet. Clin. North Am. Food Anim. Pract.* 25:13–36 xi. doi:10.1016/j.cvfa.2008.10.013
- Fukuda, S., H. Toh, K. Hase, K. Oshima, Y. Nakanishi, K. Yoshimura, T. Tobe et al. 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 469: 543–547. doi:10.1038/nature09646
- Garrett, E. F., M. N. Pereira, K. V. Nordlund, L. E. Armentano, W. J. Goodger, and G. R. Oetzel. 1999. Diagnostic Methods for the Detection of Subacute Ruminant Acidosis in Dairy Cows. *Journal of Dairy Science* 82: 1170–1178. doi:10.3168/jds.S0022-0302(99)75340-3
- Guan, L. L., J. D. Nkrumah, J. A. Basubacate ruminal acidosisb, 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. doi:10.1111/j.1574-6968.2008.01343.x
- Harfoot, C.G. 1978. Lipid metabolism in the rumen. *Prog. Lipid Res.* 17:21–54. doi:10.1016/0079-6832(78)90004-6
- Hernandez-Sanabria, E., L. A. Goonewardene, Z. Wang, O. N. Durunna, S. S. Moore, and L. L. Guan. 2012. Impact of feed efficiency and diet on adaptive variations in the bacterial community in the rumen fluid of cattle. *Appl. Environ. Microbiol.* 78: 1203–1214. doi:10.1128/AEM.05114-11
- Jami, E., A. Israel, A. Kotser, and I. Mizrahi. 2013. Exploring the bovine rumen bacterial community from birth to adulthood. *ISME J.* 7:1069–1079. doi:10.1038/ismej.2013.2
- Jenkins, T.C., R.J. Wallace, P.J. Moate, and E.E. Mosley. 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *J. Anim. Sci.* 86:397–412. doi:10.2527/jas.2007-0588
- Jewell, K.A., C.A. McCormick, C.L. Odt, P.J. Weimer, and G. Suen. 2015. Ruminant bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. *Appl. Environ. Microbiol.* 81:4697–4710. doi:10.1128/AEM.00720-15
- Jeyanathan, J., C. Martin, and D.P. Morgavi. 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: A review. *Animal* 8:250–261. doi:10.1017/S1751731113002085
- Khafipour, E., S. Li, J.C. Plaizier, and D.O. Krause. 2009. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. *Appl. Environ. Microbiol.* 75:7115–7124. doi:10.1128/AEM.00739-09
- Kumar, S., N. Indugu, B. Vecchiarelli, and D.W. Pitta. 2015. Associative patterns among anaerobic fungi, methanogenic archaea, and bacterial communities in response to changes in diet and age in the rumen of dairy cows. *Front. Microbiol.* 6:781.
- Laarman, A.H., A.L. Ruiz-Sanchez, T. Sugino, L.L. Guan, and M. Oba. 2012. Effects of feeding a calf starter on molecular adaptations in the ruminal epithelium and liver of Holstein dairy calves. *J. Dairy Sci.* 95:2585–2594. doi:10.3168/jds.2011-4788
- Lane, M.A., and B.W. Jesse. 1997. Effect of volatile fatty acid infusion on development of the rumen epithelium in neonatal sheep. *J. Dairy Sci.* 80:740–746. doi:10.3168/jds.S0022-0302(97)75993-9
- Li, R.W., E.E. Connor, C. Li, R.L. Baldwin Vi, and M.E. Sparks. 2012. Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environ. Microbiol.* 14:129–139. doi:10.1111/j.1462-2920.2011.02543.x
- Lima, F. S., G. Oikonomou, S. F. Lima, M. L. Bicalho, E. K. Ganda, J. C. Filho, G. Lorenzo et al. 2015. Prepartum and postpartum rumen fluid microbiomes: characterization and correlation with production traits in dairy cows. *Appl Environ Microbiol* 81: 1327–1337. doi:10.1128/AEM.03138-14
- LoCascio, R. G., M. R. Ninonuevo, S. L. Freeman, D. A. Sela, R. Grimm, C. B. Lebrilla, D. A. Mills et al. 2007. Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates strain specific, preferential consumption of small chain glycans secreted in early human lactation. *J Agric Food Chem* 55: 8914–8919. doi:10.1021/jf0710480
- Loor, J.J., G. Berton, A. Hosseini, J.R. Roche, and E. Trevisi. 2013a. Functional welfare—using biochemical and molecular technologies to understand better the welfare state of periparturient dairy cattle. *Anim. Prod. Sci.* 53:931–953.
- Loor, J.J., M. Bionaz, and J.K. Drackley. 2013b. Systems physiology in dairy cattle: Nutritional genomics and beyond. *Ann. Rev. Anim. Biosci.* 1:365–392. doi:10.1146/annurev-animal-031412-103728
- Loor, J.J., M. Vailati-Riboni, J.C. McCann, Z. Zhou, and M. Bionaz. 2015. Triennial Lactation Symposium: Nutrigenomics in livestock: Systems biology meets nutrition. *J. Anim. Sci.* 93:5554–5574. doi:10.2527/jas.2015-9225
- Malmuthuge, N., Y. Chen, G. Liang, L. A. Goonewardene, and L. Guan le. 2015. Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. *J. Dairy Sci.* 98:8044–8053. doi:10.3168/jds.2015-9607
- Malmuthuge, N., M. Li, L.A. Goonewardene, M. Oba, and L.L. Guan. 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. *J. Dairy Sci.* 96:3189–3200. doi:10.3168/jds.2012-6200
- Mao, S.Y., R.Y. Zhang, D.S. Wang, and W.Y. Zhu. 2013. Impact of subacute ruminal acidosis (subacute ruminal acidosis) adaptation on rumen microbiota in dairy cattle using pyrosequencing. *Anaerobe* 24:12–19. doi:10.1016/j.anaerobe.2013.08.003
- McCann, J.C., M.L. Drewery, J.E. Sawyer, W.E. Pinchak, and T.A. Wickersham. 2014a. Effect of postextraction algal residue supplementation on the ruminal microbiome of steers consuming low-quality forage. *J. Anim. Sci.* 92:5063–5075. doi:10.2527/jas.2014-7811



- McCann, J.C., T.A. Wickersham, and J.J. Loor. 2014b. High-throughput methods re-define the rumen microbiome and its relationship with nutrition and metabolism. *Bioinform. Biol. Insights* 8:109–125. doi:10.4137/BBI.S15389
- McCann, J.C., L.M. Wiley, T.D. Forbes, F.M. Rouquette, Jr., and L.O. Tedeschi. 2014c. Relationship between the rumen microbiome and residual feed intake-efficiency of Brahman bulls stocked on bermudagrass pastures. *PLoS ONE* 9:E91864. doi:10.1371/journal.pone.0091864
- McNamara, J.P. 2012. Ruminant nutrition symposium: A systems approach to integrating genetics, nutrition, and metabolic efficiency in dairy cattle. *J. Anim. Sci.* 90:1846–1854. doi:10.2527/jas.2011-4609
- Minuti, A., A. Palladino, M. J. Khan, S. Alqarni, A. Agrawal, F. Piccioli-Capelli, F. Hidalgo et al. 2015. Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the periparturient period in dairy cows. *J. Dairy Sci.* 98: 8940–8951. doi:10.3168/jds.2015-9722
- Morgavi, D.P., W.J. Kelly, P.H. Janssen, and G.T. Attwood. 2013. Rumen microbial (meta)genomics and its application to ruminant production. *Animal* 7(Suppl 1):184–201. doi:10.1017/S1751731112000419
- Myer, P.R., T.P.L. Smith, J.E. Wells, L.A. Kuehn, and H.C. Freely. 2015. Rumen microbiome from steers differing in feed efficiency. *PLoS ONE* 10:E0129174. doi:10.1371/journal.pone.0129174
- Nagaraja, T.G., C.J. Newbold, C.J. Van Nevel, and D.I. Demeyer. 1997. Manipulation of ruminal fermentation. In: P.N. Hobson and C.S. Stewart, editors, *The rumen microbial ecosystem*. Springer Netherlands. p. 523–632.
- Nagaraja, T.G., and E.C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. *J. Dairy Sci.* 90(Suppl 1):E17–E38. doi:10.3168/jds.2006-478
- Petri, R. M., T. Schwaiger, G. B. Penner, K. A. Beauchemin, R. J. Forster, J. J. McKinnon, and T. A. McAllister. 2013. Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. *Appl Environ Microbiol* 79: 3744–3755. doi:10.1128/AEM.03983-12
- Pitta, D., W. Pinchak, S. Dowd, J. Osterstock, V. Gontcharova, E. Youn, K. Dorton et al. 2010. Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. *Microb. Ecol.* 59: 511–522. doi:10.1007/s00248-009-9609-6
- Pitta, D. W., S. Kumar, B. Vecchiarelli, D. J. Shirley, K. Bittinger, L. D. Baker, J. D. Ferguson et al. 2014a. Temporal dynamics in the ruminal microbiome of dairy cows during the transition period. *J. Anim. Sci.* 92: 4014–4022. doi:10.2527/jas.2014-7621
- Pitta, D. W., W. E. Pinchak, S. Dowd, K. Dorton, I. Yoon, B. R. Min, J. D. Fulford et al. 2014b. Longitudinal shifts in bacterial diversity and fermentation pattern in the rumen of steers grazing wheat pasture. *Anaerobe* 30: 11–17. doi:10.1016/j.anaerobe.2014.07.008
- Rey, M., F. Enjalbert, S. Combes, L. Cauquil, O. Bouchez, and V. Monteils. 2014. Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. *Journal of Applied Microbiology* 116: 245–257. doi:10.1111/jam.12405
- Rico, D.E., S.H. Preston, J.M. Risser, and K.J. Harvatine. 2015. Rapid changes in key ruminal microbial populations during the induction of and recovery from diet-induced milk fat depression in dairy cows. *Br. J. Nutr.* 114:358–367. doi:10.1017/S0007114515001865
- Roche, J.R., A.W. Bell, T.R. Overton, and J.J. Loor. 2013. Nutritional management of the transition cow in the 21st century— a paradigm shift in thinking. *Anim. Prod. Sci.* 53:1000–1023.
- Russell, J.B., and R.L. Baldwin. 1978. Substrate preferences in rumen bacteria: Evidence of catabolite regulatory mechanisms. *Appl. Environ. Microbiol.* 36:319–329.
- Russell, J.B., and J.L. Rychlik. 2001. Factors that alter rumen microbial ecology. *Science* 292:1119–1122. doi:10.1126/science.1058830
- Shorland, F.B., R.O. Weenink, A.T. Johns, and D.I. Mc. 1957. The effect of sheep-rumen contents on unsaturated fatty acids. *Biochem. J.* 67:328–333. doi:10.1042/bj0670328
- Stevenson, D.M., and P.J. Weimer. 2007. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. *Appl. Microbiol. Biotechnol.* 75:165–174. doi:10.1007/s00253-006-0802-y
- Storm, E., and E.R. Orskov. 1983. The nutritive value of rumen micro-organisms in ruminants. I. Large-scale isolation and chemical composition of rumen micro-organisms. *Br. J. Nutr.* 50:463–470. doi:10.1079/BJN19830114

## About the Authors



**Juan J. Loor** is Associate Professor in Animal and Nutritional Sciences at the University of Illinois. He received a B.S. from the University of California–Davis and earned an M.S. and Ph.D. studying lipid nutrition, biohydrogenation, and metabolism in ruminants at Virginia Tech. Since joining the University of Illinois in 2005, his research program has centered on nutritional and physiological genomics during the neonatal period, lactation and rapid growth in beef, and dairy cattle. A central goal of his research program is to uncover physiologic regulatory mechanisms induced by nutritional management through integration of rumen microbiome, circulating metabolome, and tissue transcriptome data. **Correspondence:** jloor@illinois.edu.



**Ahmed Elolimy** is a Ph.D. student at the University of Illinois and received an M.S. in animal nutrition from Cairo University, Egypt. Subsequently, he was a visiting scholar for 6 mo at the Molecular Nutrition Unit at the Technical University of Munich, Germany. His research interests focus on using the Systems Biology concept to explore the role of the rumen microbiota in modulating neonatal and periparturient physiological adaptations to nutritional management. He has contributed to two peer-reviewed papers and one book chapter.



**Josh McCann** is a Ph.D. candidate at the University of Illinois and received an M.S. from Texas A&M University. His research interests center on improving nutrient utilization by understanding the effects of nutritional strategies on the rumen microbiome and epithelium. Additional research focuses on gestational nutrition in beef cattle and the link to muscle development. He has contributed to seven peer-reviewed papers and more than 20 scientific abstracts presented at national conferences.

- Tajima, K., R. I. Aminov, T. Nagamine, H. Matsui, M. Nakamura, and Y. Benno. 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl. Environ. Microbiol.* 67: 2766–2774. doi:10.1128/AEM.67.6.2766-2774.2001
- Wang, X., X. Li, C. Zhao, P. Hu, H. Chen, Z. Liu, G. Liu et al. 2012. Correlation between composition of the bacterial community and concentration of volatile fatty acids in the rumen during the transition period and ketosis in dairy cows. *Appl. Environ. Microbiol.* 78: 2386–2392. doi:10.1128/AEM.07545-11
- Weimer, P.J. 1998. Manipulating ruminal fermentation: A microbial ecological perspective. *J. Anim. Sci.* 76:3114–3122.
- Weimer, P.J., D.M. Stevenson, and D.R. Mertens. 2010. Shifts in bacterial community composition in the rumen of lactating dairy cows under milk fat-depressing conditions. *J. Dairy Sci.* 93:265–278. doi:10.3168/jds.2009-2206