RUMINNAT NUTRITION SYMPOSIUM: Tiny but mighty: the role of the rumen microbes in livestock production[1]

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ABSTRACT: The microbes inhabiting the rumen convert low-quality, fibrous, plant material into useable energy for the host ruminant. Consisting of bacteria, protozoa, fungi, archaea, and viruses, the rumen microbiome composes a sophisticated network of symbiosis essential to maintenance, immune function, and overall production efficiency of the host ruminant. Robert Hungate laid the foundation for rumen microbiome research. This area of research has expanded immensely with advances in methodology and technology that have not only improved the ability to describe microbes in taxonomic and density terms but also characterize populations of microbes, their functions, and their interactions with each other and the host. The interplay between the rumen microbiome and the host contributes to variation in many phenotypic traits expressed by the host animal. A better understanding of how the rumen microbiome influences host health and performance may lead to novel strategies and treatments for trait improvement. Furthermore, elucidation of maternal, genetic, and environmental factors that influence rumen microbiome establishment and development may provide novel insights into possible mechanisms for manipulating the rumen microbial composition to enhance long-term host health and performance. The potential for these tiny but mighty rumen microbes to play a role in improving livestock production is appreciated despite being relatively obscure.

Key words: metagenomics, microbes, performance, production, rumen

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INTRODUCTION

Ruminants were among the first animals to be domesticated and comprise a considerable component of modern livestock production with >3.5 billion domesticated individuals worldwide (http://faostat.fao.org). Ruminants are key to sustainable agriculture systems because they have a unique ability to convert low-quality forages into high-quality meat and milk products. They can make noncultivatable land productive through grazing and can utilize crop residues and by-products as feed sources, adding tremendous sustainable value to food animal production (Oltjen and Beckett, 1996). Meat and fiber products from ruminant animals are in high demand by a growing human population. There is a long history of research devoted to the improvement of ruminant livestock production, and the recent advent of high-throughput genetic technologies (e.g.,

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next-generation sequencing) has driven tremendous growth in rumen microbiome research.

Microbiome work in humans and rodents has revealed that microbes play essential roles in host health and function (Cho and Blaser, 2012; The Human Microbiome Project, 2012; Lloyd-Price et al., 2016). The full scale of microbial interplay in host function is not truly known but is becoming increasingly appreciated. Gastrointestinal tract (**GIT**) microbiome research has dominated much of the human microbiome work, given its large role in health and well-being (D'Argenio and Salvatore, 2015; Shreiner et al., 2015; Singh et al., 2017). Similar to human and rodent models, it is widely hypothesized that the composition of the rumen microbiome influences host animal health and performance (Bath et al., 2013; McCann et al., 2014a; Malmuthuge and Guan, 2017). Additionally, because ruminants lack the enzymes necessary for digestion and fermentation of the primary components of the feed they consume (structural biomass of forages), the rumen microbes are essential for host survival and performance. Greater understanding of rumen microbial function and dynamics may lead to novel improvements in ruminant well-being and performance.

HUMAN AND RODENT MODELS

Microbiome research in humans and rodent models has undergone explosive growth. In recent years, the role of microbes, especially GIT microbes, in human health has been widely publicized in both research and popular press media. One example of this is the use of "fecal transplantation" for patients afflicted with *Clostridium difficile*. Fecal transplantation involves the transfer of a healthy person's intestinal microbiota into an ailing person's GIT to resolve symptoms of *C. difficile* infection (Gough et al., 2011; Gianotti and Moss, 2017). The finding that *C. difficile* is curable via microbial transplantation is important because it suggests that the mature gut microbiome can be altered or changed. Fecal transplantation was associated with the recovery to a prior "normal" microbial composition, suggesting that microbiome manipulation may be a novel, long-term treatment for ailments and diseases associated with microbial disturbances. However, an alteration to a new microbial composition—versus a recovery to a previous composition—may not result in similar long-term success. This could prove to be a drawback to the use of microbiome intervention strategies to improve adult function or performance. Greater opportunities to influence or manipulate the microbiome over the long term or lifetime may exist during the colonization period.

Colonization of the GIT microbiota is widely thought to be primarily initiated at birth followed by consecutive waves of microorganism introduction until relative stabilization at weaning (Benson et al., 2010). It has been well documented that maternal vaginal, perineal, and fecal microbiota initiate the establishment of microbial populations in the neonate at birth (Neu and Rushing, 2011; Collado et al., 2015; Houghteling and Walker, 2015; Mueller et al., 2015). However, Aagaard et al. (2014) recently reported that the placenta harbors a unique microbiome, indicating yet another maternal influence on offspring microbial colonization, and that the colonization may initiate earlier than previously thought.

In humans, the maternal influence on microbial GIT colonization is of growing interest because of the upsurge in cesarean deliveries (**CD**) coupled with a parallel upward trend in autoimmune diseases and allergies (Neu and Rushing, 2011), perhaps because of improper establishment of GIT microbiota in cesarean infants. Biasucci et al. (2008) reported a less diverse intestinal microbiota in CD versus vaginally delivered infants, the latter who acquired microbial communities most resembling their mother's vaginal microbiota. In contrast, microbial communities of CD infants were more similar to those found on their mothers' skin (Dominguez-Bello et al., 2010), suggesting microbial species colonizing CD infants originate from skin, and possibly the hospital environment. These alterations in initial GIT colonization likely alter the microbiota in the long term and may lead to increased rates of asthma, allergic rhinitis, celiac disease, gastroenteritis, and type I diabetes (Neu and Rushing, 2011). However, other confounding factors, such as maternal diet and intrapartum antibiotic exposure, may contribute to these differences in GIT colonization along with mode of delivery (Aagaard et al., 2016).

Beyond colonization, GIT microbiota can be influenced by other factors including diet, life events (e.g., antibiotic treatments, stress), and lifestyle (level of activity; Spor et al., 2011). There is also evidence of a genetic component associated with microbiome variation. Family members tend to have microbiota profiles more similar than nonmembers, indicating a potential host genotype effect (Spor et al., 2011). Whereas composition and temporal patterns of microbial communities vary across infants, temporal patterns among fraternal twins can be remarkably parallel (Palmer et al., 2007) and even moreso in monozygotic twins (Goodrich et al., 2014). Studies in mice have also indicated a genetic component to gut microbial composition. Spor et al. (2011) reported substantially different

gut microbial composition in mice cross-fostered onto dams of a different strain, and Benson et al. (2010) reported quantitative trait locus associated with relative abundances of specific microbial taxa in an intercrossed mouse line.

Research in humans indicates that the GIT microbiome is influenced by a host genetic component and is initially established before, at, and/or shortly after birth and that the composition is subject to external influences despite relative stabilization after weaning (Rodriguez et al., 2015). There is also evidence that the GIT microbiota can be influenced to improve health (Singh et al., 2017), giving rise to the question of microbiome manipulation to improve human health and well-being. A similar question can be posed for the livestock industry—what are the long-term prospects associated with enhancing rumen microbial populations? First, however, we need to identify factors that influence rumen microbial colonization. There also needs to be continued efforts to characterize and quantify the populations by taxonomic and functional assignments and better understand the interplay between the host and the microbes, as well as among the microbes themselves.

RUMEN MICROBIOME

Ruminants have the ability to convert low-quality, fibrous, plant material into high-quality meat and milk (Ross et al., 2012). Consumed plant fibers are fermented by microbes into volatile fatty acid (VFA) (e.g., acetate and propionate), which serve as energy for the animal (Jami et al., 2013). This complex network of microbes leads to other end products such as formic acid, hydrogen (H_2), methane (CH_4), carbon dioxide (CO_2), vitamins, and other bioactive molecules necessary for downstream pathways. Microbial digestion can account for up to 70% of total dietary energy (Flint and Bayer, 2008). The end products synthesized by microbial fermentation are critical to other processes including development of the rumen epithelium and establishment of the immune system. These end products are necessary for host function, and balance of these fermentation patterns is critical. When out of balance, acidosis, nitrate toxicity, ammonia intoxication, and other metabolic disorders may arise, all of which are detrimental to host production and health (Baldwin, 1984; Church, 1988; Russell and Rychlik, 2001; Millen et al., 2016).

The symbiosis between the rumen microbiota and the host relies on a balance of the host environment (i.e., rumen environment) and microbial fermentation. Rumen microbiota provide enzymes necessary for fermentation of feedstuffs consumed by ruminants, as well as the synthesis of amino acids and vitamins that are absorbed in the small intestine for host health (Millen et al., 2016). In turn, the host environment must be favorable for microbial growth and survival in order for the microbes to produce these symbiotic end products. Specifically, the rumen environment 1) is moist because of host saliva and water consumption, 2) is maintained at 39°C, an optimal temperature for enzyme activity, 3) is primarily anaerobic, and 4) provides continuous substrate availability (Millen et al., 2016).

The microbes are predominantly strict anaerobes due to the physiological conditions within the rumen; however, there are a few facultative anaerobes, specifically those associated with adherence to feed particles (Millen et al., 2016). A significant proportion of rumen microorganisms remain unknown or unclassified. There are rumen microbes classified in each of the three domains of life: bacteria, archaea, and eukarya. The rumen also harbors viruses of prokaryotes, termed bacteriophages and archaeaphages (Puniya et al., 2015). The rumen viral communities have not been well studied, despite evidence of a potentially important role in biomass turnover in an ecosystem (Berg Miller et al., 2012; Puniya et al., 2015). A rumen virome study (Berg Miller et al., 2012) has enabled greater ability to study viromes. For example, Ross et al. (2013) used massively parallel sequencing of virus-like rumen particles to demonstrate large between-animal variation in the rumen virome of lactating dairy cattle.

Bacteria

The most predominant microbes in the rumen belong to the bacterial kingdom and are largely strict anaerobes. Bacteria number approximately 10^{10} to 10^{11} cell/g of rumen content (Church, 1988) and account for more than 95% of the population in the rumen (Puniya et al., 2015). These bacteria can be described as solid-associated, liquid-associated, rumen epithelium-associated, and eukaryote-associated. Solid-associated bacteria can have strong or weak attachment to feed particles. These microbes compose 70% to 80% of the entire microbial population in the rumen (McAllister et al., 1994). The liquid-based fraction are planktonic bacteria that are actively digesting the soluble components of feed or have detached from the feed particles (McAllister et al., 1994; Puniya et al., 2015) and comprise approximately 30% of the bacterial

population (Millen et al., 2016). Rumen epithelium-attached bacteria, known as epimural bacteria, are the most distinct from the other bacterial microbes and may be more influential to host metabolic activity (Liu et al., 2016). Epimural bacteria are often facultative anaerobes and can produce urease, suggesting that they help maintain rumen anaerobiosis, and yet do not contribute directly to ruminal digestion (Millen et al., 2016). Finally, the eukaryote-associated microbes are attached to eukaryotes present in the rumen, such as protozoa and fungi (Miron et al., 2001).

Further classification of rumen bacteria is based on substrate preference and end-product formation. Many bacteria have multiple substrate targets; the main substrates include cellulose, hemicellulose, pectin, starch, and amino acids (Puniya et al., 2015). Cellulose is highly prevalent in ruminant forage-based diets; the primary cellulolytic bacteria include *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* (Schroeder, 2013). The most common bacteria that degrade hemicellulose, also ubiquitous in forage diets, include *Butyrivibrio fibrisolvens*, *Prevotella ruminocola, Ruminococcus flavefaciens*, and *Ruminococcus albus* (Puniya et al., 2015). Although cellulose and hemicellulose are the most predominate substrates in a forage-based diet, pectin is also present and is fermented by *B. fibrisolvens, P. ruminocola, Lachnospira multiparus, Succinivibrio dextrinosolvens*, and *F. succinogenes* bacterial species (Schroeder, 2013).

Instead of a strictly forage diet, many ruminant livestock are fed a grain-based diet or provided with a concentrate-based supplement. Grain-based rations introduce larger amounts of starch as a substrate for rumen microbial fermentation. Rumen bacteria that play a prominent role in starch fermentation include *B. fibrisolvens, P. ruminocola, F. succinogenes, Clostridium* species, *Streptococcus bovis, Ruminobacter amylophilus, Succinimonas amylolytica*, and *Selenomonas ruminantium* (Puniya et al., 2015). Other substrates are also present in the rumen beyond the main components of fiber and starch. For example, amino acids are readily fermented by bacteria belonging to the genus *Prevotella* to produce adenosine triphosphate (ATP) (Puniya et al., 2015).

Archaea

The rumen archaea are composed primarily of methane-producing microbes, known as methanogens (Janssen and Kirs, 2008; Puniya et al., 2015). Most methanogens utilize H_2 and sometimes formate to reduce CO_2 to CH_4, whereas others oxidize methyl groups to CO_2 and ultimately reduce the methyl group to CH_4 (Janssen and Kirs, 2008). These methanogens rely on the production of H_2 from other rumen microbes (often protozoa), but by utilizing and removing H_2 from the rumen environment, they reduce the inhibitory effects of H_2 accumulation on subsequent fermentation (Hungate, 1966; Puniya et al., 2015). Similar to bacteria, methanogens are present in rumen fluid or attached to solids, protozoa, or the rumen epithelium (Janssen and Kirs, 2008). These archaea do not make up a large portion of the rumen microbial population at 2% to 4% of the total microbial mass (Millen et al., 2016), yet contribute to proper fermentation patterns by acting as an electron sink for reducing powers (equivalents) (Puniya et al., 2015). Up to 37% of the methane produced in the rumen is a result of the symbiosis of methanogens with ciliates (Finlay et al., 1994). The methanogens utilize intermediates (H_2) of cellulolytic bacterial and anaerobic fungi fermentation to generate CH_4 and ATP (Stewart et al., 1997). Cultivated methanogens belong to one of five genera: *Methanobacterium*, *Methanobrevibacter*, *Methanomicrobium*, *Methanoculleus*, and *Methanosarcina* and to one of seven species: *Methanobacterium formicium*, *Methanobacterium bryantii*, *Methanobrevibacter olleyae*, *Methanobrevibacter millerae*, *Methanomicrobium mobile*, *Methanoculleus olentangyi*, and *Methanosarcina barkeri* (Nagaraja, 2016)). The majority of rumen archaea (92.3%) belong to the genera *Methanobrevibacter*, *Methanomicrobium*, and one genus cluster yet to be cultured (Janssen and Kirs, 2008).

Protozoa

Although the number of protozoa in the entire rumen microbial population is low, protozoa comprise nearly 50% of the biomass in the rumen (Williams, 1986). Protozoa consist of both ciliates and flagellates, with ciliates being the more abundant (Gruby and Delafond, 1843). Protozoa are strict anaerobes and are dependent on the host for nutrient supply. It has been reported that up to 62% of the cellulolytic activity in the rumen is attributed to protozoa (Coleman, 1985); however, other studies have reported much lower activity levels (Halliwell, 1957). Protozoa can engulf bacteria and feed particles and digest carbohydrates, proteins, and fats (Williams and Coleman, 1992). The population balance of the protozoa is critical to

maintain a favorable rumen environment for other microbes to work most efficiently. For example, protozoa digestion and fermentation of substrate prevent rapid fermentation by other microbes into lactic acid, thus avoiding an accumulation of lactate and subsequent detrimental drop in ruminal pH (Williams, 1986).

Fungi

Fungi may account for 5% to 20% of the microbial biomass in the rumen (Rezaeian et al., 2004) and harbor enzymes necessary for digestion of plant materials including cellulase, xylanase, and other hydrolases (Puniya et al., 2015). Because rumen fungi exist in the anoxic conditions of the rumen, they contain hydrogenosomes in place of mitochondria, separating them from other fungi (Yarlett et al., 1986) and enabling production of H_2 as a substrate for other rumen microbes (e.g., methanogens; Mountfort, 1987). Fungi play a major role in fiber digestion and due to their complex and substantial enzymatic activity may have competitive advantages over bacteria, specifically digesting plant structural material (Mountfort, 1987).

Ruminal Fermentation Pathways

Microorganisms can be grouped based on substrate preference (Millen et al., 2016). One group breaks down complex polymers like carbohydrates, proteins, and lipids. This group includes fermentative/hydrolytic bacteria, protozoa, and fungi that digest polymers into monomers that are then either absorbed or more frequently catabolized into VFA, gas, and alcohols. The second group consists of the archaeal methanogens that are capable of converting H_2 and CO_2 or acetate into methane. The third group is composed of the homoacetogens that convert H_2 and CO_2 to acetate; however, these are usually outcompeted by methanogens (Millen et al., 2016). For carbohydrate fermentation, monomers end up as pyruvate from which VFA production begins.

One glucose molecule can produce 1 mole of butyrate and 2 moles of both acetate and propionate. Acetate is produced from two different pathways, pyruvate-formate lyase or ferredoxin oxidoreductase, both of which result in formation of acetyl-CoA that is converted to ATP and acetate via phosphotransacetylase and acetokinase (Church, 1988). Propionate, the main glucose precursor for ruminants, is produced from either the dicarboxylic acid pathway or the acrylate pathway. The two major pathways for butyrate (and high fatty acid) synthesis are the reverse ß-oxidation and the malonyl-CoA pathways (Church, 1988). Production of acetate and butyrate generates reducing equivalents (reduced form of NAD [NADH]) and therefore excess H_2. Normally the H_2 is used for conversion of CO_2 to CH_4, but other hydrogen sinks, such as sulfur, nitrate, and unsaturated fatty acids, can also incorporate the excess H_2 (Millen et al., 2016). The amount of energy (ATP) generated from production of acetate, propionate, and butyrate, including ATP generated from conversion of CO_2 to CH_4 using NADH, is 2.5, 2.75, and 3.5 moles, respectively. When adjusted for production of gas, acetate and butyrate will generate 2.5 and 1.75 moles of ATP, respectively; because no gas is released during the formation of propionate, ATP yield per unit gas is technically infinite (Millen et al., 2016).

Ruminal fermentation is regulated and balanced based on nutrient balance, VFA production, and microbial competition. Interactions among microbes are critical to this balance. For example, proteolytic degradation of protein into branched chain fatty acids and ammonia is critical for fibrolytic microbe growth and in turn increased degradation of fiber (Millen et al., 2016). Another common interaction is between succinate-producing bacteria (fibrolytic and amylolytic) and succinate-utilizing bacteria (e.g., *Selenomonas ruminantium*); succinate-utilizing bacteria keep succinate from accumulating in the rumen by converting it to propionate (Millen et al., 2016). Similarly, regulation of lactic acid in the rumen is a result of the interactions among lactic acid-producing microbes and lactate-utilizing bacteria; however, when the rate of lactic acid production exceeds the rate of utilization, ruminal acidosis occurs (Millen et al., 2016). Another common interaction is among hydrogen-producers and hydrogen-utilizers (primarily methanogens). This interaction results in greater production of acetate and in turn greater ATP generation by utilizing the H_2 (Millen et al., 2016). However, acetate accumulation reduces microbial growth (Church, 1988).

Overall, there is a complex network of rumen microorganisms that interact and compete for substrates resulting in a critical balance of end products to provide energy for microbial growth, further fermentation, and beneficial end products for the host.

METHODS AND TECHNOLOGY FOR IDENTIFYING THE RUMEN MICROBES

The rumen microbial ecosystem is complex and is suggested to consist of upwards of 2,000 species

(Firkins, 2010). The interactions among microbes also add to the system complexity (Levy and Borenstein, 2013). Many microbes depend upon other microbes for nutrient supply; some relationships are synergistic, whereas others are antagonistic (Jami et al., 2013).

The general lack of understanding of the rumen microbial ecosystem and the interplay among microbial species is due in part to a lack of sensitivity of past techniques. Classic microbiology methods have been limited because only a few species (approximately 10% to 11%) could be cultured (Jami et al., 2013; Wu and Lewis, 2013; Millen et al., 2016), and there is no single culture medium that can support the growth of the vast number of rumen bacteria (Kamra, 2005). Furthermore, quantification of specific species is difficult with conventional techniques because of the sheer number of biochemical tests required (Kamra, 2005). These early techniques made possible the simulation of an aerobic environment *in vitro* that allowed in-depth investigation into isolated cultures and responses to various substrates and products (McCann et al., 2014a).

Advanced genomic techniques provide accurate, precise quantification and characterization of rumen microbes. Although polymerase chain reaction (PCR)-based techniques facilitate better quantification than culture, they are limited to those species queried by species-specific probes. Similarly, next-generation sequencing techniques quantify each species or strain, but only those that exist in the database. DNA sequence-based techniques are used to first determine the taxonomic profile and then functional profile, or rather functional potential (Franzosa et al., 2015). Metagenomic sequencing of the rumen 16S rRNA subunit of the prokaryotic ribosome has been used to identify specific operational taxonomic units and phyla (Kim et al., 2011; Li et al., 2012) and determine a "core rumen microbiome" (Henderson et al., 2015). The 16S gene is considered universal, enabling the use of generic PCR primers for amplification from diverse taxa. Although this method avoids biases in microbial diversity associated with culturing techniques, there is still potential for PCR-based artifacts that accompany amplification of the 16S gene. Also, adequate primer design and verification can be limiting. Shotgun sequencing, in which long DNA sequences are fragmented into smaller segments and individually sequenced, can provide high-depth sequences and avoid the biases and limitations of past methods. Such technology has been used to more fully characterize the rumen microbiome. Although functional profiling can accompany both 16S and shotgun metagenomics approaches, 16S function analysis is inferred data whereas shotgun sequencing provides direct data for functional/metabolic aspects of the microbes present (Jovel et al., 2016). Although these methods provide valuable data, they are restricted to species-level taxonomic identification, where strain-level variation may provide more insight into biological questions (Franzosa et al., 2015).

To more accurately describe the functional activity, rather than potential, the use of multiomic data is required, including transcriptomics, proteomics, and metabolomics (Franzosa et al., 2015). One benefit of transcriptomic analysis is the option to simultaneously carry out metagenomic and metatranscriptomic sequencing (Giannoukos et al., 2012); transcriptomics can also provide insight into RNA viruses in the rumen (Culley et al., 2006; Zhang et al., 2006). An even more direct approach to determine functional activity is proteomic analysis, in which peptide mass and abundance are determined utilizing mass spectrometry-based methods; posttranslational modifications can also be identified (Altelaar et al., 2012). Metaproteomics can detect changes that occur despite no differences in microbiome profile (Franzosa et al., 2015), which may elucidate critical alterations associated with phenotypic changes. Detection of metabolites and small molecules within a microbial community is critical for various reasons, including the importance of these molecules in the mediation of microbial interactions and microbial-host interactions (Franzosa et al., 2015). Ultimately, there is no one method that can completely describe a microbial community in terms of both taxonomy and function. Integration of multiple techniques provides the most complete description of a microbial community (Franzosa et al., 2015). These advances in technology have enabled vast improvements in microbial identification and description, including taxonomy, abundance, and function. However, it is also worth noting that even such technological advancements have not enabled researchers to go beyond "correlative" interpretations of rumen microbiome data. Similar to the human gut microbiome, the complexity of the rumen microbiome will require an integrative systems approach that captures the multi'omics framework and constructs systems-level predictive models to determine "cause-and-effect" type relationships between rumen microbial dynamics and host performance (Waldor et al., 2015).

DEVELOPMENT OF THE RUMEN MICROBIOME

During the first few weeks of life, the rumen is not yet functional and the vast majority of suckled milk from the dam bypasses the rumen through the esophageal groove into the abomasum; the rumen wall villi responsible for nutrient absorption are also not yet developed, and the reticulo-rumen and omasum are rudimentary (Church, 1988). Early microbial establishment is critical for calf health and ultimately provides the necessary populations to ferment solid feed as the calf grows, which in turn provides energy for rapid growth after weaning. Development of the rumen microbiota is essential to rumen function, development, and immune response and enables efficient transition from the preruminant to ruminant stage. The VFA produced from microbial fermentation are critical to the development and function of the rumen papillae (Flatt, 1958; Suárez et al., 2006). Fiber ingestion and subsequent digestion lead to the expansion of the rumen, whereas carbohydrate digestion stimulates growth of the rumen wall and papillae needed for absorption of nutrients (Church, 1988).

The mucosal lining of the GIT is a critical barrier between the microbes and the host animal. Malmuthuge et al. (2012) reported downregulation of toll-like receptors in the GIT of calves that increased with age, indicating an inherent immune response to commensal microbes that shifts with age and GIT location. Alternately, levels of antimicrobial peptides (β-defensin) and peptidoglycan recognition proteins, similar to antimicrobial lectins, gradually increased after weaning. Rumen epithelial cells in cattle are capable of recognizing and responding to potentially harmful microbial fermentation through expression of stimulatory and inhibitory receptors common to the innate immune system. This may suggest a coordinated and detailed interaction between the innate immune system and the microbiome—as the microbiome develops and responds to host stage of development (availability of nutrients via consumption of solid feed, etc.), the innate immune response associated with the interaction between the mucosal epithelium and microbiome also shifts to establish and maintain health. Characterization of the immunological response associated with the mucosal epithelial cells and the microbial community will improve understanding of the role microbes play in the innate immune system.

COLONIZATION OF THE EARLY RUMEN MICROBIOME

Establishment of the rumen microbiome is critical to host metabolism, health, and immune development. Thought to be sterile at birth (Li et al., 2012), the neonate rumen is initially colonized by aerobic and facultative anaerobic microbial taxa, which are then gradually replaced by anaerobic taxa (Jami et al., 2013). Mode of inoculation is thought to primarily occur in the neonatal rumen via swallowing of saliva and digesta from the dam (Hungate, 1966) as well as other environmental sources such as bedding (Quigley, 2001). However, a small amount of milk does leak into the rumen during the first week of a calf's life despite the esophageal groove, indicating a potential effect of colostrum/milk on progeny rumen microbiome development.

A multitude of studies in mice (Ley et al., 2005), humans (Biasucci et al., 2008; Dominguez-Bello et al., 2010; Neu and Rushing, 2011; Thum et al., 2012), and ruminants (Hungate, 1966; Cannon et al., 2010) suggests a strong maternal influence on microbiome establishment. These maternal factors include the perinatal period and maternal milk. During vaginal delivery, maternal vaginal and intestinal microbiota serve as a source of microbiota for GIT colonization. Colostrum is another source of maternal microbial influence on the neonate. Not only does the first milk serve as a source of antimicrobial proteins, immunoglobulins, cytokines, growth factors, and leukocytes to initiate passive immunity, but it also contains bacteria that are some of the earliest species to be established in the preruminant calf (i.e., before rumination and/or chewing of the cud; Taschuk and Griebel, 2012). Because milk is the primary neonatal nutrient source, it may provide additional influence on microbiota development. It has been demonstrated that young ruminant animals separated from their dams establish microbial profiles generally associated with mature rumen microbial profiles, and milk replacers can influence these profiles (Cannon et al., 2010; Dias et al., 2017).

Microbial composition shifts with age and development of the host animal and tends to stabilize with weaning and maturity. Jami et al. (2013) elucidated rumen bacterial dynamics associated with aging in Holsteins by sequencing rumen fluid samples collected at ages of 1 d, 3 d, 2 mo, 6 mo, and 2 yr. A number of bacterial species essential for mature rumen function were detectable as early as 1 d of age before rumen activation or ingestion of dry

feeds. Bacterial communities fluctuated early on (day 1, day 3, and 2 mo) but achieved greater stability with maturity (6 mo, 2 yr). Bacterial communities also became more diverse with maturity, likely to accommodate a more complex diet. Overall, the entire mature rumen microbiome is more diverse and is relatively homogenous amongst animals of similar stage of development. Interestingly, rumen microbiome clustering occurred with each age (1 and 3 d; 2 and 6 mo; 2 yr), indicating a unique microbiome corresponding with each developmental stage, even after the rumen had become functional. It is important to note while microbial clustering coincided changes in developmental stage, dietary changes may also have been an influencing factor. Specifically, d 1 and 3 calves were fed exclusively colostrum, 2 mo calves fed milk and solid starter, and 6 mo and 2 yr calves were fed 70% concentrate and 30% roughage diet (Jami et al., 2013). Although bacteria identified in the d 1 and 3 calves were similar in taxonomy, abundance differences were quite distinct. There was a decrease in aerobic and facultative anaerobic bacteria accompanied by an increase in the obligatory anaerobic bacteria such as *Prevotella* and *Ruminococcus* (Jami et al., 2013). These bacteria are present in high abundance in mature ruminants; they are critical for fermentation of starch and fiber, yet also exist in the preruminant calf. This is supported by Li et al. (2012), who reported an extensive capacity for carbohydrate metabolism in preruminant calves even when restricted to a liquid milk replacer diet.

The mature rumen microbiome consists mostly of bacteria, as well as ciliate protozoa, anaerobic fungi, and bacteriophages (Bath et al., 2013). Although the dominant phyla remained the same, their relative ratios changed with maturation. Some shifts in microbial species were consistent with diet changes, whereas others were age-driven, such as an early shift from aerobic and facultative anaerobic taxa to obligate anaerobic taxa. Other data corroborate this clustering of microbiome according to host age (Jami et al., 2013). Although microbial inoculation of the rumen occurs very early in life and may indicate potential for metabolic and functional ability (even in absence of substrate availability), evidence strongly suggests that the rumen itself is essentially nonfunctional at birth and only begins to become functional as solid feed is introduced to the animal. Additionally, the mature rumen microbiome is distinct from that of the early ruminant, but research demonstrates that microbial manipulations early in life can persist into maturity, indicating a possibility of rumen microbiome programming (Yáñez-Ruiz et al., 2010; Abecia et al., 2013, 2014a, 2014b). There is evidence that initial "experiences" can influence later-life experiences, including early diet. Abecia et al. (2014b) reported that the microbial colonization and rumen fermentation were different in twin goat kids separated at birth, with one twin raised naturally by the dam and the other raised artificially using milk replacer. Additionally, other systems (e.g., neurological, morphological, and physiological) that set the stage early in life can be manipulated in the young animal with long-term consequences (Yáñez-Ruiz et al., 2015).

New Evidence in Microbiome Establishment

Although it is widely accepted that the neonate is microbiologically sterile at birth, there is evidence in humans that this establishment may actually be initiated prepartum through trans-placental transfer of maternal blood factors and microbial colonization from ingestion of amniotic fluid *in utero* (Thum et al., 2012; Guzman et al., 2015). The placenta has a unique microbiome niche composed of nonpathogenic commensal microbiota from Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla (Aagaard et al., 2014). The most abundant microbes in the placenta, *Escherichia*, were also in high abundance in meconium of neonates (Gosalbes et al., 2013), indicating that the placenta may be the source of *Escherichia* in newborns.

There is evidence of other prepartum, maternal microbial sources, including placental tissue, fetal membranes, and meconium. Guzman et al. (2015) reported the presence of several methanogens, fibrolytic bacteria, and proteobacteria in rumen fluid of vaginally birthed dairy calves collected 20 min within birth, despite the vagina, anus, tail, and legs of the dam being washed with sterile water when parturition was initiated. These species have not been identified in the placenta, suggesting other possible prepartum microbial sources.

FACTORS AFFECTING THE RUMEN MICROBIOME

Stabilization of a more homogenous rumen microbiome is associated with maturity in ruminants (Jami et al., 2013). However, many factors can affect and shift microbial profiles during the transition to the mature rumen environment, and further disruptions occur after maturity. Host genetics, age, diet, geographic location, and various maternal factors (previously described) are

known determinants in the establishment of the calf microbiome (Taschuk and Griebel, 2012). Understanding these factors and the effects on livestock production are key to developing strategies to optimize the rumen microbiome and subsequent host performance. Additional factors that may influence the rumen microbiome include location within the rumen (Fernando, 2008), diurnal variation (Li et al., 2009; Welkie et al., 2010; Mullins et al., 2013), ruminal content fraction (Larue et al., 2005; Mullins et al., 2013; McCann et al., 2014b), selective preferences, feed intake patterns, rumination time, drinking behavior (McCann et al., 2014a), heat stress (Uyeno et al., 2010), and many others. These factors extend beyond the scope of this article but should be considered when analyzing rumen microbiome profiles.

Host Effects

Interactions occur between the GIT microbiome and the host. Changes in the rumen microbiome coinciding with changes in age and development in the host animal have been widely recognized (Jami et al., 2013). Furthermore, human and mouse model research have demonstrated a host genetic relationship associated with the GIT microbiome. This relationship has also been reported in ruminants (Hernandez-Sanabria et al., 2013; Roehe et al., 2016).

It is difficult to separate the true host genetic relationship with the GIT microbiome because of confounding factors such as age/developmental stage of the host, diet, and environment (Malmuthuge and Guan, 2017). However, advances in metagenomic technology have improved the ability to analyze microbial genes associated with host phenotypes. Hernandez-Sanabria et al. (2013) reported sire breed influences on particular microbial phylotypes (both bacterial and methanogenic) in progeny. Variation in rumen microbial communities was associated with breed differences in Holsten versus Jersey dairy cows (Paz et al., 2016). Roehe et al. (2016) demonstrated a host genetic influence on rumen microbial activity and more specifically microbial metabolic activity related to methane production. Gonzalez-Recio et al. (2017) reported a strong association between the relative abundance of rumen microbes and the host genetic background, including both breed and single nucleotide polymorphism genotypes. In a near-total ruminal content exchange study in cattle, it was reported that ruminal pH and total VFA concentrations returned to pre-exchange values within 24 hr. Additionally, the rumen bacterial profiles were more similar to pre-exchange composition than donor animal composition (Weimer et al., 2010). Finally, Li et al. (2016) reported that the rumen microbiome of progeny of a sika deer × elk cross was different from either parental species, providing additional evidence of a host genetic association with the rumen microbiome. Taken together, these studies infer a host genetic association with microbiome characteristics and a potential for genetic selection based on these characteristics for a desired phenotype.

Furthermore, there is other evidence that host physiology is associated with the rumen microbiome. Goopy et al. (2014) reported that sheep with smaller rumens and shorter retention time had lower methane emissions than larger rumen contemporaries. Rumen volume and retention time are directly related, and particle retention time can impact the rumen microbiome as degradation and fermentation are prolonged with increased retention time. Additionally, retention time has been shown to be heritable (Ørskov et al., 1988). Other moderately heritable host traits, including milk protein, dry matter intake (DMI), residual feed intake (RFI), and milk fat, have also been associated with rumen microbial variation (Sasson et al., 2017), indicating it may be possible to indirectly select for such traits using rumen microbiome characteristics.

Diet

Ruminants are prone to many nutritional and dietary transitions throughout life. Even as preruminants, these animals shift from ingesting milk only to ingesting some solids, and depending on management practices, they could also be fed concentrate starter pellets. Once the rumen is functional, ruminants continue to experience dietary shifts; some shifts are imposed by design, whereas others are a result of environmental and seasonal shifts that alter the availability and quality of feedstuffs. Weaning and the transition to feedlot for finishing are prominent periods that require microbiome shifts to allow continued, feasible performance of the host (Fernando et al., 2010; Meale et al., 2016). Fernando et al. (2010) reported rumen microbial community changes associated with a shift in dietary forage:concentrate from 60:40 to 40:60 (i.e., from higher forage to higher concentrate) and even greater changes when the diet was further shifted to 20:80. The predominantly forage-based diet had greater number of bacteria from the Fibrobacteres phylum and fewer from the Firmicutes and

Bacteroidetes phyla. Also associated with this transition from a high forage to a high concentrate diet were increases in *Megasphaera elsdenii*, *Streptococcus bovis*, *S. ruminantium*, and *Prevotella bryantii*; these species have known roles in lactic acid utilization to stabilize ruminal pH and response to increased starch and decreased pH (Slyter, 1976; Counotte et al., 1981; Russell et al., 1981; Owens et al., 1998). Finally, fibrolytic bacteria, including *B. fibrisolvens* and *F. succinogenes*, tend to decrease with rising levels of dietary concentrate, further demonstrating bacterial differences in substrate preference. Ultimately, understanding how diet and dietary changes affect the rumen microbiome may lead to manipulation strategies to optimize performance and health during these inevitable transition periods in the ruminant livestock production cycle. Next-generation sequencing has been used to elucidate microbiome responses to dietary changes, such as shifts in diet (e.g., forage to concentrate), inclusion of dietary additives (e.g., antibiotics or growth promotants), or changing feed ingredients (McCann et al., 2014a). Dietary changes can cause shifts in rumen microbial abundance and composition, as well as the microbial metabolic networks (Wolff et al., 2017). Differences in response associated with phase association should also be noted. For example, the epimural microbiome appears to be more stable than the microbiomes associated with the solid or liquid fraction (Sadet et al., 2007). Yet, Roehe et al. (2016) suggested that any effect of diet could be considered a scaling effect, and thus other factors should be considered separately.

Geographic Location

Henderson et al. (2015) conducted an extensive survey of rumen microbiome data across 35 different countries. A core set of dominant microbes was observed across all ruminant species and across all locations. Specifically, a set of 30 bacterial groups were identified in >90% of samples and accounted for 89.4% of the total sequence data. These bacterial groups belong to the genera *Prevotella*, *Butyrivibrio*, *Ruminococcus*, *Lachnospiraceae*, *Ruminococcaceae*, *Bacteriodales*, and *Clostridiales*. However, although these "core" microbes were present in most samples, their taxonomic classification and functional roles remain unclear. Among the archaea, methanogens were the dominant group across all geographic regions with *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* accounting for nearly 75% of all archaea. The protozoa identified in the samples had more variability across region as well as within cohort at a similar location. Overall, most geographical differences in the microbial populations could be explained by differences in availability of certain substrates. Thus, when the effect of diet was accounted for, the microbiomes were relatively similar across the different geographic regions.

IMPACT ON LIVESTOCK PRODUCTION

Ruminants and the microbes that inhabit their rumen have an interdependent relationship. Ruminants do not harbor most of the enzymes necessary for breakdown of the feed they ingest (Puniya et al., 2015). The microbes in the rumen require the anoxic environment rich in substrate in order to replicate and survive. These two entities, when working at optimal levels, are able to convert a wide variety of low-quality feeds that cannot be used by nonruminants into high-quality end products including meat and milk. This interdependent relationship means that changes to either host or microbes can influence performance. There has been growing interest in determining relationships between host physiological traits (i.e., growth, milk yield and composition, feed efficiency, etc.) with the rumen microbiome (Jami et al., 2014; Myer et al., 2015). A better understanding of how the rumen microbes can influence such traits may help to determine if trait improvement can be achieved via microbial manipulation or genetic selection based on rumen microbiome composition.

Milk composition

Milk composition is critical to dairy production systems. However, it is also important in beef cattle systems because milk composition is critical to calf performance. The gut Firmicutes to Bacteroidetes ratio is a microbial parameter that has been associated with energy harvesting in humans and mice (Turnbaugh et al., 2006). In lactating dairy cows, Jami et al. (2014) reported that the Firmicutes to Bacteroidetes ratio was strongly correlated with daily milk-fat yield, and an increase in this ratio was correlated with an increase in milk-fat yield. Additionally, the genus *Prevotella* was negatively correlated with milk-fat yield, whereas Mitsuokella and Desulfovibrio were positively correlated with milk-lactose yield. Palmonari et al. (2010) reported a shift in the relative abundance of *M. elsdenii* in association with milk-fat depression. Weimer et al. (2010) also reported a depression in milk fat associated with a shift in the rumen microbial profile

stemming from a change in fermentable starch and the addition of monensin to the diet.

Methane production

Methane gas has a greenhouse potential 25 times that of CO_2 (IPCC, 2014). It is an end product of ruminal fermentation by methanogens and results in a 2% to 12% energy loss to the host (Johnson and Ward, 1996; Lozano et al., 2017). Methane is estimated to account for 10% of total U.S. greenhouse gas emissions, and enteric fermentation accounts for 25% of the total methane emissions in the United States (EPA, 2016). Considering the animal efficiency and environmental impacts of methane production, methane mitigation strategies are warranted.

Methane is an end product produced by hydrogen utilizing methanogenic archaea in the rumen. The methanogenic microbes are established early in life, even in preruminant stages (Skillman et al., 2004; Guzman et al., 2015). Mitigation strategies include dietary changes (e.g., shift away from high-forage diets), use of methanogenic inhibitors (e.g., analogous of coenzyme M, inhibitors of methanopterin biosynthesis, nitrocompounds, and halogenated compounds), and lipid or plant compound supplementation. Such strategies mostly target the methanogenic archaea by directly or indirectly reducing substrate availability (Lozano et al., 2017). However, methanogenesis inhibition also results in H_2 accumulation because of electrons not used in CH_4 formation. Identification of ruminal electron sinks that divert excess H_2 to alternative pathways favorable for host production is important for methane mitigation strategies (Ungerfeld, 2013). Finally, genetic selection may provide an alternative means of reducing methane emissions by ruminants.

Diet can affect the amount of methane produced; forage-based diets are associated with an increase in methane production compared with concentrate-based diets because of the increase in hydrogen availability (Kumar et al., 2013). Sauvant and Giger-Reverdin (2007) reported that an 80% to 90% concentrate diet can reduce gross energy loss contributed by methane by 2% to 3% when compared with a 30% to 40% concentrate diet. As starch increases in the diet, digestion and fermentation are shifted toward more amylolytic bacteria that thrive and continue to reduce pH, not only limiting substrate for methanogens but also providing a less desirable environment for the methanogens (Van Kessel and Russell, 1996). Johnson and Johnson (1995) reported that methane production can also be lowered when forage is ground before feeding. Although dietary changes toward less forage may reduce methane production, they may result in other physiological issues, such as a rapid decrease in pH and shift in balance of microbes that could lead to subacute ruminal acidosis (Plaizier et al., 2008).

Ionophores have been used for years to improve feed efficiency in the livestock industry. Monensin, a popular ionophore, has also been proposed as a possible means of reducing methane production. The mode of action is primarily through inhibiting the microbes essential for the formation of substrates that methanogens require to produce methane (Russell and Strobel, 1989). Hook et al. (2009) indicated that the largest effect of monensin on rumen microbiome is not a change in quantity or diversity of the methanogens but rather a shift from Gram-positive to Gram-negative organisms, ultimately shifting fermentation from acetate to propionate. It has been reported that methane production is reduced with the use of monensin, the results vary across diets and host animal (Goodrich et al., 1984; Johnson and Johnson, 1995; Guan et al., 2006). Additionally, there is a possibility of adaptation to this treatment, decreasing its efficacy (Johnson and Johnson, 1995; Guan et al., 2006).

Host genetic selection for lower methane production may also be an effective mitigation strategy. Both gross methane and methane yield are repeatable and heritable traits (Pinares-Patiño et al., 2013); variation in methane yield can be attributed to direct host genetic influence independent of feed intake (Roehe et al., 2016). Additionally, Pinares-Patiño et al. (2011) reported that divergence in methane production phenotypes persisted across various diets and ages, indicating genetic selection potential. Other heritable host physiological measures (e.g., rumen size, retention time) that influence the rumen microbiome may be potential indicator traits that could be used for indirect selection for lower methane production and yield.

Investigation of the functional aspects of the microbiome associated with divergence in methane production may provide insights regarding the interaction between rumen microbes and methane production and also reveal potential methane mitigation strategies. For example, Kamke et al. (2017) reported a positive correlation between transcription of bacterial type III secretion system (***T3SS***) genes with methane yield in sheep, indicating microbial gene expression could be indicative of methane production. In cattle divergent

for methane emissions, 170% and 173% increases in relative abundance of the *methyl-coenzyme M reductase alphas subunit* (***mcrA***) and *formylmethanofuran dehydrogenase subunit B* (***fmdB***) genes, respectively, were observed in the high emission group (Roehe et al., 2016). Genes associated with the methanogenic pathways are often up-regulated in relation to methane yield. Shi et al. (2014) indicated that transcriptional differences in methanogenesis genes may contribute to host phenotypic variation in methane yield despite no differences in actual gene abundance. This further supports the use of technologies to assess the functional role of the microbes in these pathways.

Feed Efficiency

In response to a rapidly growing human population and the economic burden of feed costs, a major goal of the livestock industry is to improve feed efficiency. Feed efficiency describes the efficacy at which the conversion of feed to useable product occurs. Because feed efficiency is influenced by many factors (that also influence the rumen microbiome [e.g., diet, stage of development, energy availability]), it is likely that there is also a link between feed efficiency and the rumen microbiome (Hill, 2012). Furthermore, the conversion of feedstuffs to usable energy depends on assimilation of nutrients, which is contingent upon fermentation by the rumen microbes.

Both microbial species diversity and richness (Shabat et al., 2016) have been associated with divergence in feed efficiency, based on RFI estimates, in cattle and sheep. More efficient animals have been associated with a less rich, less diverse, and more dominant rumen microbiome in both species (18 differentiated species) and gene (34,166 differentiated genes) content (Shabat et al., 2016). In cattle divergent for feed efficiency, there was greater similarity of microbial profiles among the more efficient (low RFI) animals (Guan et al., 2008). Myer et al. (2015) observed abundance differences in various microbes at the phyla (Firmicutes and Lentisphaerae), genera (*Dialister, Lactobacillus, Acidaminococcus, Anaerovibrio, Lysobacter, Janibacter*, and *Leucobacter*), and family (Lachnospiraceae, Veillonellaceae, and Helicobacteraceae) levels, between feed efficiency groups as defined by average daily gain, average daily feed intake, and their interactions.

Because feed efficiency is affected by diet, many of the rumen microbial differences associated with feed efficiency may be driven, in part, by diet. However, Hernandez-Sanabria et al. (2012) identified bacterial phylotypes associated with feed efficiency traits independent of diet (i.e., RFI). Furthermore, Ellison et al. (2017) determined rumen microbial abundance differences in lambs divergent for RFI across two different diets (forage versus concentrate). The abundance of 27 microbial species differed within the interaction of diet and feed efficiency status, indicating that some microbial species important to feed efficiency may differ with type of diet. Although the abundances of another 44 microbial species differed by diet type only, the abundance of another 11 microbes varied according to feed efficiency status alone, indicating the potential for a core group of microbes associated with feed efficiency variation that could be used to identify or select for feed efficient animals independent of diet type. Eight microbial species were in greater abundance in high RFI lambs: *Clostridium phytofermentans, Sharpea azabuenis, Ruminococcus flavefaciens, Dialister invisus, Prevotella bivia, P. paludivivens, P. timonensis*, and an unknown species, and three microbial species were in greater abundance in the low RFI lambs: *Prevotella nanceiensis*, *Methanobrevibacter smithii*, and *Mannheimia haemolytica*. In a follow-up forage-fed lamb study, Ellison et al. (2015) reported similar rumen microbial differences with respect to RFI classification. *Prevotella ruminocola, P. bryantii, P. marshii, Ruminococcus albus, R. bromii, Selemonas ruminantium, Dialister siccinatiphilus, Schwartzia succinivorans*, unknown *Neisseria* species, and an unknown *Alysiella* species were of greater abundance in high RFI lambs, and *Oscillibacter valericigenes, Butyrivibrio fibrisolvens, Treponema maltophilum, Methanobrevibacter smithii, R. callidus, Clostridium leptum, P. oris, P. pleuritidis*, and an unknown *Prevotella* species were in greater abundance low RFI lambs. Finally, Carberry et al. (2012) determined an association between rumen bacterial profiles and feed efficiency status (high or low RFI) in beef cattle; this association was more pronounced in cattle fed a high-forage diet. Carberry et al. (2012) reported a 1.7-fold greater abundance of *Ruminococcus albus* in low RFI versus high RFI cattle fed a forage-based diet. Taken together, these studies indicate that there are diet-independent differences in the rumen ecosystem grounded in a common core of microbial individuals found in the rumen; there also appear to be additional species differences within diet that may be indicators of feed efficiency potential.

In addition to rumen microbiome population dynamics associated with feed efficiency, the

functional aspects of these populations may also help describe the variation in energy harvesting. Significant differences in rumen metabolic activity associated with divergence in feed efficiency have been reported where concentrations of propionate, butyrate, valerate, and isovalerate were higher in more efficient animals as was total concentration of the short chain fatty acids (Shabat et al., 2016). Additionally, functional analysis of microbes associated with feed conversion ratio uncovered 49 genes that explained 85.5% of the variation. These genes were specific to enzymes involved in host-microbe interactions, synthesis of amino acids and vitamins, degradation of amino acids and proteins, enzymes associated with genetic information processing, and membrane processes (Roehe et al., 2016). The identification of specific microbial populations, microbial metabolic pathways, and microbial gene abundances may provide opportunities select for improved feed efficiency based on the rumen microbiome.

Because feed efficiency is a complex trait of economic importance, strategies for trait improvement are in demand. Aspects of the rumen microbiome are associated with host phenotypic variation in feed efficiency, indicating there may be potential for rumen microbes to serve as indicators for feed efficiency. Differences in the microbiome associated with variation in feed efficiency may be due to contributing factors associated with the feed efficiency measure of choice. Various feed efficiency measures have different confounding factors such as growth, body weight, age, DMI, and others that could be driving shifts in the rumen microbiome rather than the feed efficiency trait itself (Archer et al., 1999; Arthur et al., 2001), thereby warranting critical assessment of the feed efficiency measure used in each study. Regardless, Shabat et al. (2016) reported successful prediction (91% accuracy) of animal's feed efficiency status based on rumen microbiome characteristics. Selection for better feed efficiency may also result in lower methane production. Shabat et al. (2016) reported high enrichment of the methanogenesis pathway in lowly feed efficient cattle (high RFI) and hence greater methane production. Basarab et al. (2013) described a selection model in which the rate of genetic change when selecting improved feed efficiency, based on RFI, in turn reduced methane emissions in cattle. It does appear that selection for more feed efficient animals may be possible through rumen microbiome characteristics and that selection may also have the benefit of lowering methane production.

There are three major hypotheses regarding reasons for low RFI animals having lower CH_4 production (Basarab et al., 2013). The first two hypotheses are intake driven, in which differences in methane yield associated with low RFI animals are primarily driven by the effect of reduced DMI and retention time (Herd et al., 2002; IPCC, 2006; Nkrumah et al., 2006; Hegarty et al., 2007; Gomes et al., 2013). However, the third hypothesis is microbial driven, where both reduced DMI and retention time affect the rumen microbiome and lead to increased digestibility of DM and N (D'Mello, 2000; Nkrumah et al., 2006). Ultimately, higher rates of rumen fermentation favor a shift from acetate production to propionate production, decrease H_2 availability for methanogens since propionate production competes with CH_4 production for H_2 (Ungerfeld, 2015; Millen et al., 2016), and provide more energy per mole for the host (Church, 1988; Millen et al., 2016). Data are not consistent when comparing RFI phenotypes with CH_4 yield (Cruz et al., 2010; Gomes et al., 2013), yet the underlying factors affecting RFI, such as feed intake and feeding behaviors, alter the microbiome in terms of composition and fermentation patterns and warrant further investigation (Basarab et al., 2013).

CONCLUSION

The symbiotic relationship between ruminants and rumen microorganisms is paramount to the conversion of low-quality feed to high-quality end products. As the livestock industry faces a challenge to produce more pounds of meat and milk to meet the demands of the growing human population, continued improvements in production efficiency are essential. It has long been recognized that rumen microbes play an essential role in host health and performance. The advent of new, large-scale genetic technologies has helped bring rumen microbiome research to a new frontier. It is becoming increasingly possible to understand the complexities of the rumen microbiome and subsequent effects on the host animal. Furthermore, new research is aimed at determining the dynamics of rumen microbiome colonization, establishment, and development. The microbial composition in the rumen changes with host growth and development before stabilization at weaning. The composition becomes relatively stable with maturity, indicating that the potential to influence the composition of the rumen microbiome, and ultimately host animal performance, occurs earlier in life (e.g., before birth, at/near birth, before maturity).

The body of research suggests that the rumen microbiome provides a new avenue for improving host production efficiency; however, it is also clear that the many factors influencing the microbiome make this a challenging arena. With advances in technology and integration of multiple techniques, it may be possible to elucidate the establishment of the early microbiome and determine its potential for manipulation to improve long-term host efficiency and health. However, difficulty in establishing and maintaining desirable shifts in the microbiome presents challenges that are confounded by contributing factors such as breed, sex, age, diet, and environment. Yet human and rodent model research indicates that by elucidating the relative contributions of maternal, genetic, and environmental factors on rumen microbiome colonization and establishment, it may be possible to influence the early microbiome to favor improved host lifetime health and performance.

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