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HOST MICROBE INTERACTIONS

Altering the Gut Microbiome of Cattle: Considerations of Host-Microbiome Interactions for Persistent Microbiome Manipulation

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Abstract
The beef cattle industry represents a significant portion of the USA's agricultural sect, with beef cattle accounting for the most red meat consumed in the USA. Feed represents the largest input cost in the beef industry, accounting for approximately 70% of total input cost. Given that, novel methods need to be employed to optimize feed efficiency in cattle to reduce monetary cost as well as environmental cost associated with livestock industries, such as methane production and nitrogen release into the environment. The rumen microbiome contributes to feed efficiency by breaking down low-quality feedstuffs into energy substrates that can subsequently be utilized by the host animal. Attempts to manipulate the rumen microbiome have been met with mixed success, though persistent changes have not yet been achieved beyond changing diet. Recent technological advances have made analyzing host-wide effects of the rumen microbiome possible, as well as provided finer resolution of those effects. This manuscript reviews contributing factors to the rumen microbiome establishment or re-establishment following rumen microbiome perturbation, as well as host-microbiome interactions that may be responsible for possible host specificity of the rumen microbiome. Understanding and accounting for the variety of factors contributing to rumen microbiome establishment or re-establishment in cattle will ultimately lead to identification of biomarkers of feed efficiency that will result in improved selection criteria, as well as aid to determine methods for persistent microbiome manipulation to optimize production phenotypes.

Keywords Cattle · Microbiome · Rumen · Manipulation · Host-microbiome interactions

Introduction

With feed costs representing greater than half of the total cost of production in the beef cattle industry in the USA, novel methods to improve feed efficiency and nutrient utilization in cattle are becoming increasingly critical [1]. Additionally, agriculture represents one of the largest anthropomorphic producers of greenhouse gas emissions globally, with cattle representing the largest portion of livestock methane emissions [2]. Methane production alone is estimated to reduce feed efficiency by 2 to 12%, shunting potential carbon sources for the host to methane emissions [3–5]. Increasing efficiency in the beef cattle industry will not only reduce greenhouse gas emissions and reduce natural resources required to meet expected animal protein needs but will also decrease input costs.

The rumen microbiome is responsible for the successful breakdown of low-quality feedstuffs into usable energy for ruminants, providing approximately 70% of energy to the host animal [6]. Spanning several kingdoms, including Bacteria, Archaea, Protozoa, and Fungi, the rumen microbial ecosystem fulfills several functional niches, including proteolytic, fibrolytic, and lipolytic functions. Following the degradation of forages or concentrates, a number of metabolites are produced and released, including volatile fatty acids (VFA), biohydrogenated lipids, and other metabolites. These microbes also provide microbial crude protein (MCP), an important source of protein in the ruminant. The metabolites produced are either absorbed across the rumen epithelium or in the lower gastrointestinal tract and can then enter the bloodstream to be available to the host [7–9].

Divergences in rumen microbial communities are associated with a number of host phenotypes, including feed efficiency [10], diseased states [11], and methane emissions [12].

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Rumen microbes produce glucogenic, lipogenic, and aminogenic precursors that partake in or regulate energy metabolism in cattle [13]. Differences in relative abundances of different microbial populations have been associated with varying levels of methane emissions in the rumen of goats and sheep [12], whereas other phenotypes, such as feed efficiency, have been associated with differences in composition of bacterial and archaeal communities [10, 14]. Differences in microbial communities can also affect the metabolic profile of ruminants as well [15–17]. The listed functions are just a small portion of the functional capacities possessed by microbes and illustrate the importance of these microbes within the context of ruminant physiology and health.

Although the importance of the rumen microbiome is well-established, attempts to manipulate the microbiome to improve cattle production have not been met with long-term success [18]. Antibiotics, probiotics, feed additives, and other methods of disrupting the microbial ecosystem of the rumen can provide targeted, immediate, and acute alterations to the rumen microbial profile, but no significant, persistent changes have been achieved to the microbial community composition [19–22]. These studies suggest that there is some degree of host specificity or regulation mechanism dictating the gut microbiome in ruminants. Understanding the mechanisms dictating establishment, or re-establishment, of the rumen microbiome will provide necessary information in order to effectively provide persistent, long-lasting changes that increase production and feed efficiency. Currently, much of the research has been conducted on the re-establishment of the rumen microbiome following perturbation; however, understanding the mechanisms dictating establishment (i.e., colonization) of the rumen microbiome will ultimately provide researchers and producers with novel selection criteria. The present review focuses on existing knowledge affecting microbiome establishment, host-microbiome interactions affecting community establishment and composition, as well as the physiological implications resulting from rumen microbiome activity, in an ultimate effort to identify necessary methods and advances to effectively manipulate the rumen microbiome long-term for lasting production outcomes. Figure 1 provides a brief outline of this review, as well as prospective areas of research.

The Average State of the Rumen Microbiome

The rumen microbiome is a diverse ecosystem, possessing many functional and phylogenetically differences. Bacteria in the rumen account for approximately half of the microbial genetic material, followed closely by protozoa, and fungi (~2%) and methanogenic Archaea (2–4%) account for the remaining microbial abundances [23]. Bacteria are the most well-studied of the rumen microbiota due to their diversity, both functional and phylogenetic, and ease of analysis. Rumen bacteria have had a rich history of culture-based research, even though the majority are still non-culturable [24], providing longer history of analysis and more information than other microbes. Robert Hungate pioneered the field of ruminant microbiology in the 1960s and characterized his findings [13] using culture-based methods. Since the Hungate era, new sequencing technologies have allowed expansion of Hungate's and his colleagues' research, starting first with bacteria, primarily due to the greater knowledgebase available from bacteria.

Firmicutes and *Bacteroidetes* are the dominant bacterial phyla in the rumen of cattle. *Firmicutes* are typically greatest in relative abundance in predominantly forage-based diet, whereas *Bacteroidetes* are usually more abundant in diets consisting primarily of concentrate [25, 26]. *Firmicutes* and *Bacteroidetes* are then typically succeeded in abundance by Proteobacteria, Tenericutes, and Actinobacteria [27]. At the genus level, *Prevotella* are most common, potentially due to the wide range of functional capacities of species within *Prevotella* [28, 29]. Currently, there is no known "normal" rumen microbiome, though core microbiota have been found across species, diet, and geographical locations [30]. Given this, the rumen microbiome is typically examined under specific conditions, such as diet, production stage, or illness [11, 27, 31–33]. What is normal on one diet or in one situation may not be normal in another. Therefore, discussed subsequently are some of the largely known factors affecting microbiome establishment in ruminants.

Factors Affecting Microbiome Establishment in Ruminants

Diet

Diet is the greatest known external influence on the composition of the rumen microbiome in ruminants [18]. Researchers in one study collected 742 samples of the rumen content of 32 ruminant species as well as gut content of species whose gastrointestinal systems were similar to that of a ruminant from 35 countries around the world and found that, though animal-to-animal rumen microbiome varied greatly, diet was the largest factor affecting microbial community composition [18]. Though inter-animal variation is high across most studies, diet had a significant effect on the rumen microbiota. However, these changes have been mainly conducted on bacterial communities and methanogenic archaeal communities.

During transitioning periods in which cattle are moved from a predominantly forage-based diet to a high-grain diet, large changes are seen in the relative abundances of the bacteriome. In a study conducted by Fernando et al. (2010),

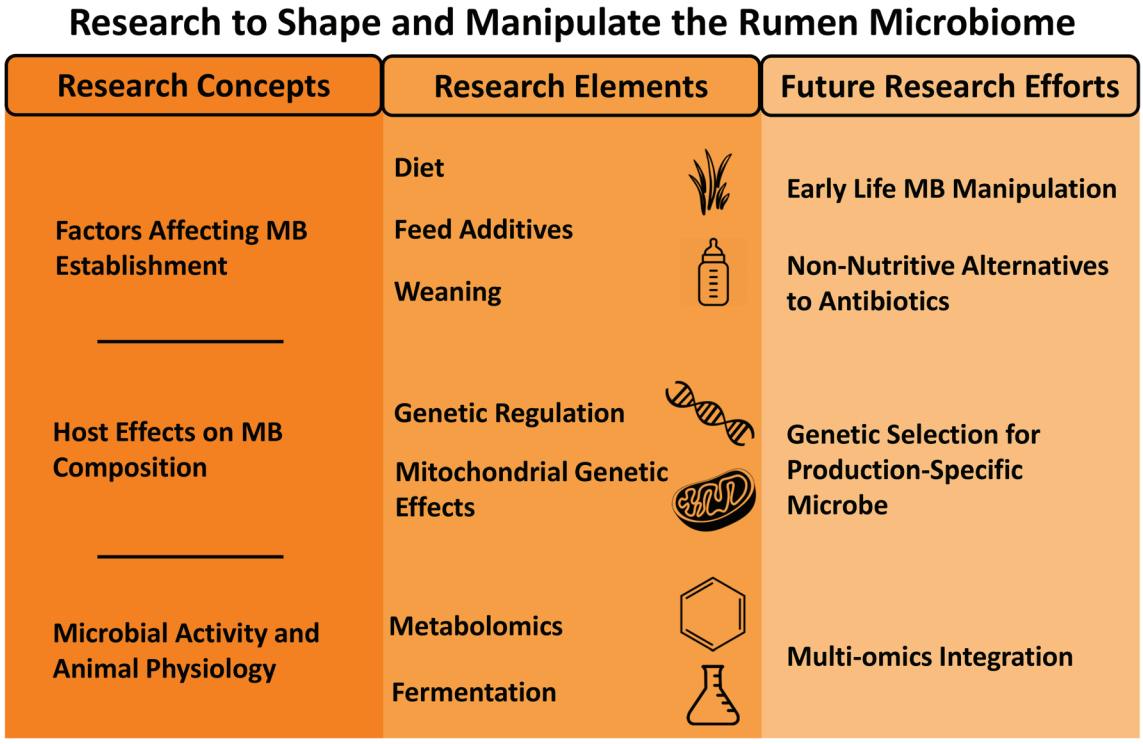

Fig. 1 Overview of research presented as well as potential areas of research to address contributing factors of rumen microbiome establishment

the transition from a hay-based diet to a high-grain diet (80% grain) resulted in several changes to the bacteriome, including changes in the *Firmicutes*-to-*Bacteroidetes* ratio, decreased fibrolytic bacteria such as *Fibrobacteres*, and increased species from *Prevotella*, among others. These changes have also been demonstrated in other studies [11]. The ratio of *Firmicutes* to *Bacteroidetes* in particular has been linked with other phenotypic traits, namely in adipose metabolism [31, 34, 35]. Abundances of *Bacteroidetes* and *Proteobacteria* species increased as grain became a greater percentage of the diet, which may be more effective at fermenting the readily digestible carbohydrates present in grain-based diets [25], and may be more tolerant of the lower pH that occurs when greater amounts of VFA and lactic acid are produced [36].

Certain species have been associated with grain-based diets, such as *Streptococcus bovis*. *S. bovis* is a lactate-producing bacterium that thrives at lower pH that often accompanies high-grain, rapidly fermentable diets. Enzymes produced by *S. bovis* function optimally at a pH ranging from 5 to 6, which characteristically occurs in the rumen of animals consuming high-grain diets [37]. During transition to high-grain diets, 67-fold increases in *S. bovis* have been observed [38]. Furthermore, other lactate-producing bacteria, such as *Lactobacillus* spp., also increase in abundance in the rumen on high-grain diets [39], further contributing to accumulation of lactic acid and reduction of ruminal pH.

Although some microbes tend to be diet-specific with regard to great shifts in relative abundance, others are present, regardless of external factors. Members of *Prevotella* are among the most common microbes in the rumen, regardless of diet [30]. This persistence among diets is also supported by their abundance in the rumen, as *Prevotella* account for as much as 60% of the total bacterial populations in the rumen [28]. Examining this large and diverse genus of bacteria, Bekele et al. (2010) analyzed the effect of diet on the populations of ruminal *Prevotella* species using DGGE. Differences were observed between diets in targeted bacterial populations; however, unidentified species within *Prevotella* were similar between diets [40]. This may suggest that *Prevotella*, particularly uncultured members, possess great functional diversity. Indeed, species within *Prevotella* display a wide variety of functions, including cellulolytic, amylolytic, and fibrolytic activities [29, 40]. This great functional diversity in the rumen warrants further interrogation of those microbiota in order to further define the multi-faceted role of *Prevotella* in context of the rumen microbiome.

Diet can also influence the overall α- and β-diversity of the rumen microbiota. In a study conducted by Pitta et al. (2010), bacterial α-diversity decreased when animals were switched from a bermudagrass to a winter wheat diet, as measured by Shannon and Chao1 [41]. Principle component analyses also illustrated very distinct bacterial community composition based on diet [41]. Some of the same changes seen in shifting from one forage type to another were also some of the same trends observed in transition from forage to grain diets [25, 38, 41, 42] as well as other forage to forage diets [43]. In mice,

humans, and other monogastric species, differences in α-diversity have been associated with divergences in host phenotype, such as diseased states. Particularly, variation in bacterial diversity has been associated with energy utilization in humans and mice. In a study conducted by Turnbaugh and others (2009), decreased bacterial diversity of the lower gut was associated with obesity in human twins [34], a trend that has also been observed in mice [44]. While the relationship between energy utilization and the diversity of rumen microbial communities is still not entirely understood, research completed in other species suggest that microbial diversity may influence feed efficiency in ruminants as well.

Methanogenic archaea account for approximately 2–4% of the total microbial genetic material [23]. Methanogenic archaea are the primary cause of methane production in ruminants, and modulating their ruminal populations and/or functionality is of particular interest in the research community given that livestock contribute to 10–12% of anthropogenic greenhouse gas emissions [45]. Additionally, it is estimated that methane production results in a 2–12% reduction in feed efficiency in cattle [3]. Given the possible negative implications of methanogenic archaea in overall ruminant production, understanding their role in the rumen microbiome and contributions to ruminant physiology is fundamental to improving feed efficiency and reducing global methane production.

Changes occur in methanogenic archaeal communities as the result of differences in diet. Zhou and colleagues conducted a study in which animals were placed on low energy (LE) or high energy (HE) diets and stratified by residual feed intake (RFI) [46]. Several distinct differences were found between the LE and HE diets. Diversity of methanogenic species was greater in LE diet compared to the HE diet, which has been found in other studies in sheep [47]. Additionally, in a separate study, bulls fed fiber-based diet had greater α-diversity of methanogenic species than those fed a starch-based diet [48]. Fiber-based diets may present a wider range of substrates for methanogens, leading to greater diversity of archaeal communities. Furthermore, methane production is greater in animals on forage-based diets, which may be the result of the greater diversity of the rumen archaeal communities that occurs in predominantly forage diets. However, methane production on forage-based diets is also a function of the increased acetate/propionate ratio that is common on forage-based diets, as acetate is tightly linked to methanogenesis [3, 49, 50].

Certain species, such as *Methanobrevibacter gottschalkii*, were found only in LE diet samples, whereas certain strains of *Methanobrevibacter* sp. AbM4 and *Methanobrevibacter smithii* were only observed in the HE diet samples [46]. However, while different species were found to be associated with diet or the other, no differences were observed in total methanogens [46]. This conflicts with a study conducted by Wallace et al. (2015), in which archaea were present in greater abundance in a medium grain diet compared to a high-grain diet [51]. Diet may not be the greatest influence of methanogenic archaeal communities because they use secondary resources, such as hydrogen, as their main source of energy. This may cause them to be more closely dictated by the populations of other microbes in the rumen, which are themselves affected by feed [51]. Thus, archaeal communities may be indirectly affected by feed more so than directly affect by it, as is the case for bacteria and protozoa.

Protozoa and fungi are also important participators the rumen microbial ecosystem, though fewer studies have been conducted to interrogate the relationship between their populations and the diet. Protozoal populations may follow some of the same trends as are seen in bacterial populations when animals are moved to a grain-based diet from a diet primarily made up of forage. In a study conducted by Hristov and colleagues in 2001, increased grain resulted in decreased total protozoa [52]. Several protozoal populations were lower in the high concentrate (barley) diet and several genera were present in medium concentrate but not in high concentrate, including *Eudiplodinium*, *Dasytricha*, *Diplodinium*, *Metadinium*, *Ophryoscolex*, and *Ostracodinium* [52], which has been supported by other studies that have demonstrated that protozoal numbers peaked with diets consisting of 40 to 60% concentrate [53]. A study conducted by Franzolin and Dehority (1996) analyzed the long-term effects of a concentrate compared to forage-based diet. When the steers were fed a diet of 75% concentrate, total abundance of protozoa increased compared to the forage-based diet [54], but the 50% concentrate diet did not have any differences in protozoal numbers compared to either the forage-based or 75% concentrate diets. In that study, *Diplodiniinae* decreased when steers were transitioned from a 50% to a 75% concentrate diet [54], a similar trend as was found in the study conducted by Hristov et al. [52]. Although protozoal populations do change in response to diet changes, particularly in high-grain diets, they may be less flexible and resist change when compared to bacteria.

Rumen fungi are the least characterized and understood rumen microbe. The fungal populations of the rumen are superior in their ability to break down more fibrous materials that may be difficult for other microbes to break down, possibly because they are more effective at penetrating tough plant walls [55, 56]. Grenet and colleagues demonstrated that fungi selectively chose "stemmy forages" more frequently than other substrates and were absent entirely from grains [57]. Rumen fungi are very sensitive to pH [58], which can drop rapidly due to digestion of readily fermentable carbohydrates in grain. Grain particles may also be too small for the fungi to adequately attach [57, 59]. Fungi have also been found to favor older plants with thicker cell walls compared to young, more readily digestible forages, likely due to difficulty attached to the thin

cell wall [57]. Overall, fungal populations seem to thrive on thicker-walled forages and survive poorly in high-grain diets.

Feed Additives

Feed additives are also used to manipulate host phenotypes as well as the rumen microbiome. Producers use feed additives to alter host phenotypes which can inadvertently alter the rumen microbial community profile. Moreover, additives can also be used to directly alter the rumen microbiota to manipulate the host phenotype. Commonly used feed additives including antimicrobials, supplements, and probiotics have been used to alter the rumen microbiota and/or host physiology. These different feed additives function in a variety of ways to improve ruminant feed efficiency or alter host phenotypes.

Antimicrobials have been used in animal agriculture for decades. Antimicrobials can be used to decrease infections, but typically when antimicrobials are used as feed additives, they target specific microbial populations in order to decrease a defined population of microbes that may contribute to adverse effects, such as sharp decreases in pH due to ruminal acidosis. One major antimicrobial used in cattle production is monensin. Monensin is an ionophore that disrupts ion flux across microbial membranes and causes cell death [60]. In ruminant systems, monensin, in part, is used to limit the effects of ruminal acidosis, namely decrease the negative effects of sharp decreases in pH following the introduction of a high-grain or concentrate diet with little time for microbes to adapt to the change. Monensin has also been demonstrated to improve feed efficiency and reduce methane emissions. Monensin interferes with intracellular protein transport, disrupting ion exchange across the cell membrane. When monensin is added to the ruminant diet, it disrupts production of VFA and lactate [60]. A previous study found that steers on a backgrounding diet that were also fed monensin gained weight at the same rate as steers not fed monensin but required significantly less feed than the control [61]. Another study also found that monensin decreased methanogenic archaea as well as methane production and dry matter intake [62]. These changes, which led to improvements in host phenotype, also altered the rumen microbiome. However, while use of antimicrobials can lead to improvements in host phenotype via alterations in the rumen microbiota, these changes are often short-lived and do not produce long-lasting modifications.

Non-nutritive supplements, such as essential oils, saponins, and tannins, can be added to the diet to modify not only the rumen microbial community structure but also host phenotype, such as feed efficiency. Some oils are used as an alternative to antibiotics because some long-chain fatty acids are toxic to the rumen microbes. Many of the oils act through disruption of the cell membrane, which allows for leakage of ions across the cell membrane [63–65]. Some of the non-nutritive supplements may also act as cation and proton transmembrane carriers, further disrupting the ion balance within the cell [66]. Additional potential modes of action are reviewed by Calsamiglia et al. [67]. Non-nutritive supplements that possess antimicrobial properties may be a desirable alternative to antibiotics given the negative public perception of antibiotics and of growing global antibiotic resistance. These non-nutritive supplements are of plant-based origin and thus may provide a more "natural" method for manipulating the rumen microbiome to improve desirable phenotypes and reduce negative aspects of livestock agriculture, such as methane production.

Essential oils have been used as alternatives to antibiotics, such as ionophores, to reduce methane production; however, success in reduction of methane production has been mixed. Macheboeuf et al. (2008) performed in vitro analysis of several essential oils on rumen fermentation and methanogenesis using increasing doses of essential oils to measure dose response [68]. Although essential oils reduced gas production up to 48% compared to control samples, many of the doses that resulted in decreased gas production also reduced in total VFA production, which may negate the positive results of gas production reduction [68]. However, in vivo, these results are often not observed. In a study examining the effects of supplementary oils on the microbial communities in the rumen compared to monensin, the addition of essential oils did not change methane production or archaeal communities compared to monensin but did result in decreased rumen fungal abundances [62], indicating that it may not provide significant enough changes to impact the rumen microbiome as well as production. Beauchemin and McGinn (2006) conducted a study to analyze the effect of essential oils on ruminal fermentation parameters and methane production [69]. Essential oils did not modify ruminal fermentation characteristics as measured by VFA concentrations, nor did essential oil supplementation affect methane production [69]. Utilization of essential oils as an alternative means of reducing methanogenesis may not be a viable option, but further analysis must be conducted.

Another supplement that has been of growing interest is the use of dried distillers grains (DDG). A previous study measured changes in bacterial communities following supplementation with different levels of DDG. The ratio of *Firmicutes* to *Bacteroidetes* changed with 25 and 50% DDG, with lower abundances of *Firmicutes* compared to *Bacteroidetes* [70]. These changes also resulted in decreased rumen pH [70]. These changes may have occurred due to the increased readily digestible starch content, which typically results in increased *Bacteroidetes*. Members of the phylum *Bacteroidetes* possess more amylolytic capabilities, whereas *Firmicutes* in the rumen possess predominantly fibrolytic abilities [71]. Changes to the rumen microbiome caused by supplementation are important as they can influence rate of digestion, available nutrients, and other physiological factors. However, the persistent effects of

supplementation on the rumen microbiome and subsequent physiological changes are still relatively unknown.

Probiotics are a growing area of interest of late. Probiotics, or direct-fed microbials (DFM), are live microbes that are added to the diet to modulate digestion, fermentation, or the microbial community composition. Yeast, particularly *Saccharomyces cerevisiae*, as well as other fungi have been one of the most widely used and studied probiotics. Supplementation with fungi, such as *Aspergillus oryzae*, resulted in increased bacterial concentrations, particularly those associated with fibrolytic activities [72]. In addition to changes to the bacterial populations, supplementation with *A. oryzae* also changed physiological parameters, including increases in ruminal volatile fatty acids and the animals weaned earlier [72]. Studies using *S. cerevisiae* also resulted in some of the same trends [73]. However, probiotic used has yielded mixed or inconsistent results [74]. Regardless of whether yeast use is successful or not, its use does not result in long-term changes to the rumen microbiome nor effects on efficiency. Other probiotics have also been used, such as inoculations from other ruminants. Repeated inoculation in beef heifers from bison rumen content resulted in changes to the rumen bacterial and protozoal abundances, which translated to changes of passage rate, chewing activity, and fermentation [75]. Bison inoculations resulted in increased α-diversity (Chao1, Shannon), as well as β-diversity [75]. However, in this study, effects were not measured long-term, and thus it has not been determined if repeated inoculation would result in permanent restructuring of the rumen microbiome. As is the case with antibiotic use, probiotics seem to have potentially desired effects on the rumen microbiota with regard to production as well as host physiology; however, results have also been mixed, and extended or permanent effects of probiotic use have not been observed.

Weaning and Weaning Age

Weaning is a time of significant change for beef cattle. In most beef cattle operations, calves are kept with the dam for approximately 7 to 8 months, whereas in dairy systems, calves are typically removed from the dam within 48 h. Even prior to weaning, and regardless of diet, there is a succession of bacterial communities over time in pre-ruminant calves [76], which has been supported by other studies [77–79]. However, at weaning, changes in microbial community composition, in part, are due to host physiological changes but also likely due to the introduction of solid feeds because diet is a large driver of microbial community composition and modulation [18, 76, 77]. Rey and colleagues (2014) analyzed the succession of bacteria in dairy calves. In the first several days of birth, the reticulorumen was dominated by anaerobic, lactose-consuming bacteria, primarily from the phylum Proteobacteria [77]. However, once solid feed was introduced at day 4 following birth, abundances of *Bacteroidetes* increased significantly [77]. At the genus level, many microbes of interest and importance in adults also changed in their relative abundance. Abundances of *Prevotella* quadrupled, rising from a mean abundance of approximately 11% to over 40% [77]. *Pasteurella*, however, decreased following introduction of a solid diet and was non-detectable following 3 weeks of age [77]. The shifts in bacterial abundances demonstrate the respective shift in bacterial functions in the rumen likely as a result of diet modification, rumen development, and overall fermentative environment.

Due to the importance of adequate rumen development, factors affecting the microbial success of the rumen are of interest in young ruminants. One such factor that affects the rumen microbiome is transport of the animals. Following weaning and during transport to a feedlot operation in beef calves, total amount of bacteria was not affected by transport, weaning, or changes in intake; however, protozoal populations were affected [80]. Relative abundance of *Entodinium* increased following weaning, whereas *Diplodinium* and *Epidinium* abundances tended to decrease over time and *Dasytricha* was completed eliminated by day 7 [80]. With regard to archaeal communities, establishment has been measured shortly after birth and has been demonstrated to stabilize soon thereafter [59]. As seen in bacterial populations, archaeal community establishment in the rumen at weaning is also affected by age and diet. For instance, one study found that methanogenic archaea abundances were greater in lambs fed forage-based diets compared to grain-based diets [81], which are trends also seen in adult animals [46].

Weaning age and method of weaning can also change rumen or gastrointestinal tract microbiome establishment and community structure. However, because weaning is a more labor-intensive process in the dairy industry compared to the beef industry, current understanding of the effects of weaning on the rumen microbiome is primarily from dairy cattle, with additional information coming from small ruminant systems. Weaning in dairy calves elicited an immune response in the lower gastrointestinal tract, but adding solid feed in addition to milk replacer resulted in changes to the immune response as well as gut bacteria [82]. Increased solid feed resulted in increased total amount of bacteria present in the gastrointestinal tract [82]. In 2016, one study examined the effects of weaning and weaning strategies on the rumen microbiome establishment. Pre-weaned calves had greater α-diversity compared to weaned calves, but the weaning strategy did not have any effect on microbial α-diversity [78]. When principle coordinate analysis (PCoA) was used, operational taxonomic units (OTU) clustered by pre- or post-weaning animals, but no differences were observed in weaning strategy [78]. In addition to these changes, abundances of specific bacteria also changed from pre-weaning to weaning, including decreased *Bacteroidetes*, increased *Proteobacteria*, and increased

Firmicutes populations [78]. Moreover, weaning age also impacts microbial community establishment. Early weaning of dairy calves resulted in a rapid change of the rumen microbiome compared to calves that were gradually adapted to a post-weaning diet; however, once the microbiome OTU were established, they remained stable following weaning [83]. Beyond this, not many differences were observed in the microbial community diversity metrics between early and gradually weaned calves [83]. Though changes in OTU abundances of historically dominant phyla changed at different rates in early-weaned compared to gradually weaned calves, no differences were observed in *Bacteroidetes*, *Firmicutes*, or the ratio of these two once calves were fully weaned [83]. Differences were observed, however, in less dominant phyla, including *Cyanobacteria*, *Spirochaetes*, *Synergistetes*, and *Verruccomicrobia*, which all decreased in early-weaned calves over time, whereas only *Elusimicrobia* and *Fibrobacteres* decreased by weaning in gradually weaned animals [83]. While many similarities between these weaning ages were observed in the rumen microbiome of calves, several differences were also observed at a finer resolution, indicating that this may be an important area of research in the future to optimize the rumen microbiota for production.

Breed

Although breed can play a large part in physiological phenotypes of cattle, little is known about the differences in the rumen microbiota between breeds, particularly in beef cattle. Researchers conducted a study to delineate differences in the rumen bacterial and archaeal populations of beef cattle differing in sire breed and diet [84]. Several differences in bacterial populations were observed in the LE diet due to sire breed, including 24 bacterial phylotypes, with four Angus-associated phylotypes [84]. When steers were fed the HE diet, 37 bacterial phylotypes differed between sire breeds, with six Angus-associated phylotypes, one Charolais-associated phylotypes, and no Angus × Charolais (hybrid)-specific phylotypes [84]. Although differences were observed as a result of sire breed in bacterial phylotypes, no breed-associated differences were found in methanogenic archaea phylotypes [84]. This study was one of the first to analyze breed effects on the rumen microbiome, which indicated that there is possibly some host regulation or preferential selection of rumen microbiota establishment; however, these findings may have been breed-specific. Additionally, breed variation may contribute to host regulation of the gut microbiome establishment, but individual variation in host genetic regulation may play a greater role than breed variations.

Many external and physiological factors contribute to microbiome establishment and composition in ruminants. However, data support that diet is likely the largest external dictator of the rumen microbial community structure [18]. The change from predominantly liquid to solid feed may also account for the changes in the rumen microbiota following weaning. Breed may also impact the rumen microbiome and presents an interesting future area of research. However, beyond these factors, there appears to be some degree of host regulation of microbiome establishment and composition.

Host Effects on Rumen Microbiome Establishment

The rumen microbiome is a complex ecosystem with many confounding factors that lead to its establishment. Certain traits, such as feed efficiency, have been associated with differences in bacterial communities in the rumen and lower gastrointestinal tract of cattle, providing some indication that these important host phenotypes are related to the microbiome. Although some external factors, such as diet, contribute to fluctuations in the microbiome, the question remains as to what drives changes when controlling for such factors. Some mechanisms seem to be in place from the host that dictate the microbial community composition; however, those mechanisms are still widely unknown.

Host Genetic Regulation

Many traits, such as carcass quality and milk yield, are associated with quantitative trait loci (QTL) or single nucleotide polymorphisms (SNP), typically indicating at least a moderate ability to genetically select for those traits. Attempts have been made to associate gut microbiota with genetic factors for which producers, researchers, or others can select. In one study, researchers attempted to correlate mice gut microbiomes with genetic traits. Several taxonomic groups (26) were associated with 13 QTL, with each of the four dominant bacterial phyla corresponding with a QTL [85]. The QTL associated with the bacteria were distributed across eight different chromosomes, which indicated that the gut microbiome is a heritable trait [85]. Similar trends have been observed in humans as well. Monozygotic twins had more similar gut bacteriomes than did dizygotic twins [86]. Another study revealed that different taxa exhibited varying heritability [87]. In addition, the researchers also found 37 potential SNP that may be involved in gut microbiome establishment [87]. These studies indicate that microbiome establishment may be a complex, phenotypic trait of which can be selected.

One important production trait that has been associated with ruminant host genotype is that of methane emissions. Methane contributes approximately 2 to 12% decrease in feed efficiency in ruminants, and ruminants produce approximately 80% of global livestock emissions [3]. Genotypic associations have been identified between methane yield and methane

production rate, and these traits were only loosely associated genetically with dry matter intake [88]. The genetic relationship between different methane production and yield traits with little or no genetic connection to dry matter intake is ideal for selection, in which low methane production can be selected for without negatively impacting dry matter intake [88]. Because methane production is thought to derive primarily from methanogenic archaea in the rumen, this study indicated that the host genetics may play a strong role in selecting for or against methanogenic archaeal communities or other microbial populations [88]. However, methane production had a strong genetic correlation with weaning weight and body weight, so selecting against methane production could result in decreased weaning weight [88].

While several studies have found genetic correlations between the gut microbiome and the host in mice and humans [34, 35, 87], almost no studies have been conducted to determine these same correlations in ruminants. Certain SNP have been associated with *Prevotella* abundance in the rumen of dairy cows [89]. In particular, *Prevotella* abundance in the rumen was associated with SNP on the DGAT1 gene, which is associated with milk fat composition [90, 91]. Other microbial populations were also associated with SNP on other host genes associated with fatty acid or cellular metabolism, including ACSF3, AGPAT3, and STC2 [92]. This is the first study in cattle that has attempted to find SNP associated with the rumen microbiome and how those may be correlated with important phenotypic traits. This study presented evidence that the microbiome is indeed associated with, to some degree, the host genome, which could have large implications for the cattle industry. Additional studies should be conducted in beef cattle to determine if production parameters important to that industry are also genetically correlated with the rumen microbiome.

Mitochondrial Associations

Mitochondrial DNA (mtDNA) is an often forgotten or excluded source of genetic variation when searching for SNP-associated traits. Mitochondrial DNA is inherited solely from the dam and is known as a source of SNP. In humans, mtDNA haplogroups in stool were associated with certain bacterial taxa [93]. Different haplogroups were associated with different genera in stool after further analysis, and these were not affected by gender, body mass index (BMI), or age [93]. Different haplogroups were also correlated with differences in relative abundance ratios of phenotypically important taxa, such as the *Bacteroides*/*Prevotella* [93, 94]. The researchers also speculated that the gut microbiome may be impacted by the mutations identified in the mtDNA, potentially mediated by the inflammatory response to reactive oxygen species and variation in key redox pathways. Although the mechanisms relating mtDNA to the gut microbiome are not yet defined, this study provides novel and foundational information about the role that the mtDNA may play in dictating the host gut microbiome and should be considered when assessing host influence in microbiome establishment and manipulation.

Microbiome Re-Establishment Following Disturbances and Associated Parameters

In order to measure the effect of the host on microbiome re-establishment following disturbance, several studies have been conducted. However, one of the most extreme disturbances to the rumen microbiome is that of rumen content evacuation or exchange. A previous study was conducted in which the researchers performed rumen content exchanges (RCE) between Holstein cows that were chosen based on their differences in rumen pH and total VFA concentrations. Immediately following RCE, the rumen pH and VFA most resembled that of the donated rumen content [19]. However, the rumen pH and VFA concentrations returned to their original values and concentrations within 48 h, except in one of the four animals [19]. The bacterial communities were then analyzed from each of the four animals from several time points throughout the 9 weeks of study. In the first experiment of two animals, the bacterial communities returned completely to their native bacteriome by the end of the 9 weeks. However, in the second experiment, the bacterial communities in one of the two cows did not completely return, though the communities most resembled the native bacteriome rather than the donated bacteriome [19]. In the cow that did not experience complete return of its bacterial community composition, the VFA concentrations and pH values also did not return completely, suggesting a host effect in the microbiome establishment, at least in adult animals, as well as the wide-reaching physiological impact of the rumen microbiome [19]. However, because there was not a complete re-establishment in one of the animals, this may indicate that host control over gut microbiome composition is variable, which in turn may allow for persistent manipulation of the gut microbiome.

A later study conducted used RCE to measure how microbiome re-establishment was associated with a major production parameter: milk yield. Cows used in the study were chosen based on differences in milk production and were designated low producing (LP) or high producing (HP). When LP cows received rumen content from HP cows, milk yield increased significantly [95]. Interestingly, when the bacterial communities returned to the original composition, so did the milk production [95]. Although not all cows experienced complete return of their original bacterial community composition, all bacteriomes most resembled that of the native bacteriomes than the donated bacteriomes [95]. This study provided insight as to the relationship between the host and the stability of its microbiome and the effects that changes to

the rumen microbiome can have on host physiology and important production parameters.

While these studies have illustrated compelling evidence that ruminant host genetic regulation may impact microbiome establishment and community composition, and these relationships may be responsible for important production traits in both beef and dairy cattle industries, much is still unknown about the mechanisms governing these influences. The rumen and lower gastrointestinal tract microbiomes contribute significantly to the effective breakdown of feedstuffs and have been associated with feed efficiency in beef cattle [10]. It may be possible in future studies to genetically associate the rumen microbiome with the host as well as other important production parameters to improve the overall efficiency and sustainability of the beef cattle industry.

Animal Physiology Resulting from Rumen Microbiome Activity

The rumen microbiome is critical for host physiology as it produces energy substrates and other nutrients that the animal needs to perform adequately. Nutrients that are generated and released in the rumen can then be absorbed across the rumen epithelium or in the lower gastrointestinal tract and typically enter the circulatory system of the ruminant. While these metabolites, nutrients, and substrates are critical to the animal, most rumen microbiome research fails to incorporate the multitude of fermentative and physiological factors resulting from microbial activity. Unfortunately, only several products are routinely measured, such as VFA, nitrogen (several forms), and glucose. These shortcomings must be addressed in future research. Employing several additional techniques when assessing the ruminal microbiome activity can lead to better interrogation of the relationships among host physiology and the rumen microbiome, such as proteomics to assess microbial crude protein, trace mineral analyses, and transcriptomics, to name a few. Although it requires researchers to analyze great amounts of data, utilizing a more systems-based approach, in tandem with gut microbial metagenomics, and host-microbial interactions will aid in the effort to fully understand the mechanisms behind gut microbiome stability, resiliency, and establishment.

Fermentation Products

Volatile fatty acids are important by-products of microbial fermentation in the rumen as they are the main glucogenic and fat precursors, particularly propionate and acetate. Production of VFA also contributes to the rumen pH, which is a critical measurement that contributes to overall health of the rumen and, in turn, can select for or against certain microbial populations. As with the rumen microbiome, VFA production is intimately linked to diet, predominantly due to the changes in substrate, as well as rumen microbial activities. Different supplements, including corn, beet pulp, and barley, can result in varying total VFA concentrations and differences in rumen pH, without affecting dry matter intake [96]. Many other studies have found associations among types of feed, VFA concentrations, and pH as well [97–99]. The relationship among the host, VFA, and the rumen microbiota has been extensively explored and previously reviewed [100–102]. Although VFA production and pH have been well-studied in cattle, there is still much knowledge to gain regarding the manipulation of the microbiome and its effects on these factors or vice versa and how that will, in turn, modify energy substrate and nutrient production.

Serum Metabolome

Since their inception, metabolomic techniques have provided researchers with a multitude of information [103]. The serum metabolome can provide a detailed perspective about the physiology of the animal at the time of collection, providing far more information than has ever been generated, with an average of over 100 known metabolites being identified in single sample and hundreds more unidentified [104]. Serum metabolomics has been used widely to understand diseased states in particular [105]. Thus, serum metabolomics is a useful tool for researchers to be able to understand effects of a wide range of conditions on the overall host physiology.

Serum metabolomic techniques have started to be applied to ruminant systems because they can capture information about what has been absorbed from the environment (e.g., rumen) as well as endogenous production of metabolites. Animals differing in RFI also exhibited different metabolic profiles in plasma using nuclear magnetic resonance imaging (NMR) [106]. During the second period of the trial, ten metabolites were identified that differed between low and high RFI, which accounted for more than half of the differences in RFI [106]. Several metabolites accounted for the majority of differences observed in RFI, including glutamate, citrate, acetate, and carnitine, all of which are directly or indirectly involved in intermediary metabolism [106]. This study alone provided a wealth of information with regard to phenotypic differences in beef cattle, but more studies need to be conducted to determine how these can be manipulated to optimize beef cattle production and how the microbiome relates to the serum metabolic profiles of the animals.

A recent study used LC-MS to perform untargeted metabolomics on serum from steers differing in feed efficiency [107]. Steers with low RFI had greater serum abundances of several metabolites, including pantothenate and carnitine, which are directly involved in intermediary metabolism, particularly fat and carbohydrate metabolism [107]. The results from this study support that of a previous study by Karisa et al.

(2014) but also expanded upon the work of Karisa et al. by using LC-MS rather than NMR to analyze the serum metabolome [106]. However, the few untargeted serum metabolomic studies that exist in ruminants are still associative and have not moved beyond to provide explanations for variations in host phenotypes. Incorporating serum metabolomic techniques with other methods, including genomics, transcriptomics, and trace mineral analysis, may provide greater insight as to the relationship between the host and rumen microbiome.

Rumen Metabolome

The rumen metabolome, like the serum metabolome, can provide information for researchers about the state of the animal at the time of collection [103]. Rumen fluid metabolomics has been almost exclusively applied to dairy cattle, with little research conducted to assess the use of rumen metabolomics, particularly untargeted metabolomics, in beef cattle. One study that analyzed four biofluids from dairy cows (milk, urine, rumen fluid, and serum) using NMR and gas chromatography (GC) found several metabolites present in all four fluids, while many metabolites were found solely in rumen fluid [108]. Differences in metabolites were also found between biofluids from cows on different diets as well [108]. This study highlighted the importance of a multi-systems approach to metabolomics and how changes in metabolites at different levels (systems) can be associated with host phenotype.

In beef cattle, protein is the main product desired, requiring effective conversion of feed to mass. Thus, understanding the relationship between the microbiome and this conversion, as well as the host physiology, will ultimately lead to the ability to optimize these various aspects of ruminant production to maximize production with minimal input. Steers differing in feed efficiency varied in their rumen fluid metabolome [109]. Ninety metabolites differed between high- and low-efficiency animals, with most of them involved in fatty acid and amino acid metabolism [109]. Differences in plasma metabolites, particularly fatty acids, were also observed between animals differing in feed efficiency [109]. However, concentrations of fatty acids differed between the rumen and plasma metabolomes [109], indicating that some other factors are involved in the transport of metabolites from the rumen to the blood. This study was one of the first uses of untargeted metabolomic techniques to examine the beef cattle rumen metabolome in relation to host phenotypes, such as feed efficiency. This application of metabolomics has contributed to our understanding of the mechanisms underlying divergences in phenotype of beef cattle, but much more information is needed to not only fully comprehend the contributing factors to differences in phenotype, but how those different factors can be manipulated in order to optimize production.

Summary and Future Directions

Many factors contribute to ruminant efficiency phenotypes, including the rumen microbiome, physical and genetic differences in the animal, host physiology, as well as external factors such as diet and management. The rumen microbiome is a key mediator of nutrient production in cattle, but much is still unknown about the ability to manipulate the microbiome, factors affecting its establishment, and the physiological effects on the host as a result of those manipulations. Host genetics appears to play a strong role in microbiome establishment, though this can be overcome by other factors such as diet or antibiotics, at least short-term. However, it is still unknown how all of these factors can be used and manipulated in conjunction to optimize beef cattle production, including improved feed efficiency, reduced methane emissions, and other efficiency metrics.

The ruminant metabolome has revealed associations between different metabolites and important production-related phenotypes in beef cattle, particularly metabolites associated with intermediary metabolism. However, few studies have attempted to relate the rumen fluid metabolome with the serum metabolome, in order to further define and understand the relationship between rumen dynamics and the animal's endogenous metabolism. Those studies that have been conducted have primarily been conducted in dairy cattle or other species, as is the case with host-microbiome interactions [16, 17, 110]. To advance this field of research in cattle, these types of systems approaches must be undertaken. Further, these studies should also mirror that of other fields, in which multinational groups perform analyses to interrogate many aspects of the same experiment to add additional layers of robustness and analytical overlap once systemic methods of data analysis are attempted.

Beyond the relationship between the rumen and serum metabolome, no studies have performed metabolomic analyses of rumen and serum in conjunction with the beef cattle rumen microbiome, particularly following a rumen disturbance (such as RCE). In other environments and disciplines, such as environmental toxicology or cancer biology, "-omics" technologies have provided deeper information as to the effects microbes have on their environment because metabolomic techniques can analyze over 100 metabolites at once, compared to traditional assay techniques in which only one or few molecules can be measured at a time [111–113]. In addition, many of the metabolites that have been found using untargeted metabolomics in animals of varying phenotypes, such as differences in RFI, have been those known to be involved in intermediary metabolism and may help explain differences observed in the physiology of the animal [109]. Applying multiple -omics techniques as well as across multiple physiological systems (e.g., blood, rumen fluid, urine) will provide more details regarding the critical relationships between the host and its

microbiome. A more detailed and inclusive approach will provide more in-depth associations among the various factors contributing to divergences in feed efficiency, such as ssNMR, epigenetics, and other "-omics" techniques, in conjunction with microbiomics, and would yield a systemic data set amenable for concerted multi-omics approach. Such approaches will allow researchers to make connections that have not been previously not been available to researchers. Utilizing the entirety of these tools in cattle to acquire a comprehensive picture of host-microbe interactions will ultimately lead to a better understanding of these complex relationships, enable to researchers to better elicit production outcomes through persistent microbiome manipulation, and provide better selection criteria to improve livestock systems.

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