Microbiomes in Ruminant Protein Production and Food Security

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Abstract

The global population is rapidly increasing and will surpass 10 billion people within the next 20 years. As diminishing resources continue to impact agriculture, and with the necessity to feed the world by 2050, the agricultural sector must be able to sustainably and efficiently produce high-quality sources of food that are both attainable to the global population and contribute to healthy, balanced nutrition. Ruminants are a unique contributor towards a sustainable and food secure world, as they are available and utilized across all economic and social demographics, and can produce high-quality protein from otherwise inedible plants from land that is typically unsuitable for crop production or cultivation. Thus, developing tools, methodologies, and systems for optimizing the production of protein from ruminants stands to make great impacts on food security. Breeding and genetics have played a role in this development, but cannot be a singular solution. Microbes are present at abundances that equal or exceed host cell counts, are ubiquitous throughout all mammalian systems and are required for regular host-physiological functions. Optimizing these host-microbe-symbioses in ruminants permits the opportunity to augment the utility and efficiency of microbiomes and their functions to produce production-specific phenotypes and outputs. This review, therefore, examines the role of microbiomes in ruminants to efficiently and sustainably produce high-quality protein for human consumption to aid in efforts to achieve global food security.

Keywords: Food Security, Microbiome, Protein, Ruminant, Sustainability

Review Methodology: We searched the following databases: CAB Direct, Agricola, Google Scholar, and Scopus. Keyword search terms used: food security, agriculture, sustainable protein, cattle microbiome, microbiomics, metagenomics, metabolomics, feed efficiency, bovine reproductive efficiency, methane production, beef cattle, agricultural sustainability, host-microbe symbioses, ruminant microbiology. In addition, we used the references from the articles obtained by this method to check for additional relevant material.

Introduction

The global population is expected to exceed 10 billion people by 2050 [1] and obtaining and maintaining the resources required to achieve a sustainable global food system will become more challenging as the global population rises. Attempting to undertake this global concern can be daunting, as there are many food systems to examine, variable issues to consider and defining the problem has been difficult. Over the past 50 years, government and global agencies have aimed to determine an adequate definition of sustainability, as past sustainability definitions tended to be either too vague or too complex to adequately address what the definition specifically sought to achieve [2]. The UN has developed a comprehensive sustainable development plan [3], and its extensive goals and campaigns make clear the complexity of the issue. When specifically examining global food sustainability, there are numerous attributes of food systems that should be considered. The Global Roundtable for Sustainable Beef provides a satisfactory definition which takes into account many facets of the sustainability development plan, including a global food system that is socially responsible, environmentally sound and economically viable, and production that prioritizes the planet, people, animals and progress [4]. Regardless of the many interpretations that have encompassed food systems sustainability [5–8], they all must include, to some degree, improving nutrition and food

security. Food security has been defined as a country's access to sufficiently meet dietary requirements, both from household food acquisition and allocation behaviour, as well as access to clean water and sanitation [9]. Thus, sustainably improving the nutrition available to individuals stands to make the greatest impact globally.

The agricultural sector's input in global food system sustainability and food security is critical, as a sustainable food system must efficiently network producers, land, environment, natural resources and finances. Importantly, these enterprises are responsible for efficiently supplying important nutrients to the population, specifically protein. However, over one billion people globally have insufficient protein intake, resulting in health and growth concerns [10, 11]. As a result of inadequate dietary protein, the growth of over 178 million children in developing countries under the age of 5 are predicted to be stunted [12], and globally, 90% of stunted children originate from only 36 countries [10, 12]. As populations increase, this greater demand for protein can be relieved by establishing sustainable and efficient systems to produce foods that help to meet dietary protein and amino acid requirements for healthy children and adults. Considering these issues, ruminants are well-positioned to meet this demand for increased protein. Ruminants constitute major protein sources globally, throughout all socioeconomic strata [13]. Within the USA, there are over 800 million acres of range and pasture, which amounts to roughly 35% of the country [14]. The majority of these areas are unsuitable for crop production or cultivation, are highly erodible if ploughed, provide habitats and critical food sources to wildlife and wild ungulates, and cultivation would increase the risk of erosion and runoff while also decreasing soil carbon sequestration [15–17]. The best land-use scenario, therefore, is to convert the energy from grass and forages produced on this otherwise non-arable land into edible food and protein for human use with ruminants (e.g. cattle, sheep and goats). Specifically, in a grain-fed production system, cattle generate 19% additional human-edible protein than they consume [18], upcycling these human-inedible plants into high-quality protein for human consumption. Alternatively, research has demonstrated that plant-based replacements can produce nutritionally similar food per unit cropland [19]. Although there are debates as to the considerations of livestock production and animal protein consumption [8, 20–23], with research supporting numerous stances, ultimately the nutritional requirements of the omnivorous human species cannot be met with solely plant-based food systems [21]. A sustainable food system for the human population requires, in part, animal-sourced nutrients in order to ensure adequate, balanced, dietary nutrition [20, 21, 24]. Consequently, optimizing ruminant production stands to make a pronounced impact on securing sustainable sources of food and protein for the human population.

Gains in ruminant production have historically been made through selection-based programs focused on host production optimization. Yet, microorganisms are equally as critical for the normal function of numerous body systems [25–28]. Studies have continued to demonstrate the mutual, commensal and parasitic potential microorganisms impart on these ruminant systems [29–31], and until the turn of the century, little knowledge had been gained regarding the microbial impact on ruminant production. Through the advent of modern nucleotide sequencing technologies, novel microbial methods and tools have emerged that have enabled researchers and producers to investigate biological systems with further resolution, specifically with regard to the components contributing to the variation guiding such production efficiencies. Characterizing these microbiomes (the combined genetic material of all microorganisms in a specific environment) sets the groundwork for further research to determine the importance, function and complex networks of specific microbiota, core microbiomes, keystone species and/or microbial profiles within specific niches. As microbiomes in ruminant systems have the potential to greatly impact ruminant production, this review focuses on the examination and use of microbiomes in ruminants as a means to responsibly and sustainably improve ruminant production to ultimately secure high-quality sources of food and protein for human consumption.

Ruminant Microbiomes and Feed Efficiency

Microbiome research in ruminant production characteristically concentrates on nutrition. To improve food security through the availability of animal-based protein, nutritional efficiencies are commonly targeted by researchers to optimize the nutrient, dietary and metabolic needs of the ruminant host. Given the importance of the rumen and lower gastrointestinal tract microbiomes to host nutrient utilization, the implications of these microbiomes on ruminant production have been explored. Bacteria make up the largest portion of the rumen microbiome, in terms of abundance [32]. Because of these large populations and their connection to the overall metabolic potential of the rumen [32], variation in the populations of bacteria, including variation in abundance, diversity and individual taxa, can provide insight into the contributions of those bacteria to differences observed in cattle feed efficiency (FE). Early studies analysed the dissimilarities in bacterial community profiles in divergent FE steers on a finishing diet based on polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE) banding patterns [33]. The rumen bacterial signatures clustered by low- and high-FE, and rumen bacterial communities in steers with greater FE were more closely related to each other (91%) compared with steers with low-FE (73%). A later study conducted by Hernandez-Sanabria et al. found similar results using analogous methods of bacterial community analysis in animals differing in FE [34]. This study found too, that steers fed a finishing diet had rumen bacterial

communities that phylogenetically clustered by FE phenotype [35]. Similar differences have been observed in bacterial taxa and communities in recent studies using next-generation DNA sequencing techniques for microbial interrogation of extremes in FE phenotypes [36]. However, in contrast to these previous studies, the authors did not identify any phylogenetic clustering of bacterial communities as a function of FE phenotype, rather smaller taxonomic shifts in species and genera. Specifically, numerous bacterial genera were identified, such as *Succiniclasticum, Lactobacillus, Ruminococcus* and *Prevotella* [36]. These microbes are key contributors to ruminal function. For example, *Ruminococci* are cellulolytic, and are known to produce acetate, formate and hydrogen; all important metabolites of ruminal metabolism [37]. Rather than large changes in bacterial populations, these finer shifts in organisms have also been identified in other research [38, 39]. Organisms, including *Succinivibrio* spp., *Eubacterium* spp., and *Robinsoniella* spp. [34] as well as *Methanobrevibacter* sp. strain AbM4 and *Methanosphaera stadtmanae* [35], have been associated with differences in FE, to name a few. The putative functions of these organisms, such as succinate production in *Succinivibrio* spp., again indicate their metabolic contribution to the rumen [40]. The microbial taxa that vary as a result of FE likely also exhibit different metabolisms, in turn altering host FE phenotypes, which has been observed in sheep [40, 41] and cattle [42]. This suggests that the metabolism of the microbiota may also be important for dictating host phenotype, rather than the differences in relative abundance alone. Global changes to the microbial population are not often identified or implicated in FE divergences. Collectively, the aforementioned studies suggest that functionally significant microbes in the rumen, such as *Prevotella* or *Ruminococcus*, may greatly enhance the functional capability of the rumen to utilize nutrients, impacting fiber digestibility and/or host FE.

Recent research has supported the supposition that dramatic shifts in the abundance of bacterial populations among animals differing in FE may not be the underlying cause of variation in FE, but rather the result of lower abundant, keystone species that are functionally superior or fill a specific niche. A study conducted by Shabat et al. found that greater-FE cows contained greater abundances of *Megasphaera elsdenii* in the rumen [43]. *M. elsdenii* are lactate-consuming bacterial species that are often found in association with high-grain diets due to the production of lactate by other bacteria, such as *Streptococcus bovis* [44]. The major byproducts of *M. elsdenii* include butyrate and propionate, of which greater concentrations or abundances have been associated with increased FE in ruminants [33, 34]. In the same study by Shabat et al., it was observed that less-FE animals did not have any taxa that dominated in phylogenetic annotations of genes, suggesting that greater diversity or lack of dominant functionality results in decreased FE [43]. Microbial phylogenetic diversity variations have often been implicated in deleterious phenotypes, such as health outcomes [45–48]. As the power of FE microbiome studies in ruminants beings to increase with the reduction in sequencing costs and availability of larger study populations, microbial phylogenetic diversity may further prove to be an important indicator in FE and animal health.

Another genus of interest in relation to FE in cattle is *Prevotella*. *Prevotella* is one of the most diverse genera in the rumen and is often the most abundant genus in the rumen [36, 49–51]. Species within *Prevotella* perform a diverse range of functions, including fibrolytic, amylolytic and proteolytic functions, and exhibit great variation at the genetic level [52–54]. Greater *Prevotella* abundances in the rumen have been associated with lower FE in cattle [36, 55]. The dominance of relative abundances compared with other genera and the great functional diversity of *Prevotella* may lead to decreased FE in cattle. It has been demonstrated that the increased diversity within species can lead to deleterious health outcomes [45–48]. However, little is still known about the contributions of *Prevotella* or its intra-species diversity towards divergences in FE in cattle, and the studies presented provide only correlation, not causation, of taxa-level associations with FE.

At the phylum level, Bacteroidetes and Firmicutes are the predominant bacteria identified in the rumen, often accounting for greater than 70% of the total relative bacterial abundance in the rumen [36, 51]. Members of Bacteroidetes tend to dominate the bacterial community composition when the host ruminant is fed a diet consisting of greater concentrate proportions [36], whereas Firmicutes are often more abundant when the ruminant diet consists primarily of forages [51]. The differences in abundance under these two conditions provide insight as to the functional relationship between these two phyla. The relationship between Bacteroidetes and Firmicutes is often quantified as a ratio between the two phyla. The Firmicutes:Bacteroidetes ratio has been used to identify differences in energy utilization in humans [56], mice [57] and ruminants [50], and are commonly examined and implicated in obesity and diabetes in animals and humans [58, 59]. As these phyla constitute large, functionally significant members of the rumen microbiome, and impact the capacity to ferment polysaccharides, the quantity of Bacteroidetes and Firmicutes are of great interest in energy utilization and FE in ruminants.

Individual animal variation also appears to contribute to variation in FE and the rumen microbiome. Henderson et al. analysed rumen microbiomes and other species with rumen-like gastrointestinal systems [60]. Henderson et al. found that, besides diet, individual animal variation contributed the greatest variation in the rumen or gut microbiomes of these animals, although, there was a 'core' microbiome across most of the samples [60]. These data confirmed previous studies that observed, when accounting for diet, individual animal variation still

contributed to variation in the bacterial community compositions, which may be partly responsible for differences in FE [33, 61, 62]. Although a core rumen microbiome appears to exist [60, 62, 63], the variation in microbiota that represent a low relative abundance may be responsible for the greater divergence in host FE phenotypes. Researchers in other microbiome fields have suggested that rather than global shifts or variation in microbial community composition being responsible for differences in observed phenotypes, keystone species that are present at low relative abundances may be responsible for great variations in phenotypes [64]. If keystone species are driving variation in host FE phenotypes, this could potentially account for some of the individual variations in FE and the rumen microbiome, though more analyses are needed to confirm. Ultimately, when examining the recent advancements in elucidating the variation in FE, and determining the rumen microbiome impact on FE, current meta-analyses have been key in determining the status of the field. A meta-analysis conducted by Gleason and White in 2018 examined the relationship between various measures of FE and the rumen microbiome [65]. In beef cattle, the diet and microbiome appeared to have the greatest influence on FE and dry matter intake [65]. However, the authors reported that, due to lack of sufficient available datasets, further examination of the relationship between FE and the rumen microbiome was not possible [65].

Ruminant Microbiomes and Methane Production

Beyond individual species or genera of microbes, domains and kingdoms of microbes can impact FE and nutrient utilization in ruminants. Ruminal archaea are the primary producers of methane. Methanogenesis in ruminants is a highly debated topic, predominantly due to the negative impact of methane as a greenhouse gas on the environment and the deliberation of its impact on FE. Methanogenesis from livestock contributes an estimated 28% to anthropomorphic greenhouse gas emissions [66]. In addition, it is estimated that methanogenesis in cattle results in a 2–12% reduction in FE [67]. Due to the contribution of methane to reductions in FE in ruminants, methane mitigation strategies have been assessed with regard to populations of methanogenic archaea (commonly referred to as methanogens), including how they relate to the rumen microbiome. Several studies have identified relationships between methane production, the rumen microbiome and FE. A study conducted by Zhou et al. examined the effect of low- or high-energy diets on methanogen abundance in steers [35]. This study found that total methanogen populations did not differ between diets, nor between low- and high-FE steers, although differences were observed at the genus level between both diet and FE ranking [68]. A study later conducted by Wallace et al. measured methane production and methanogen populations in steers fed two different diets, one predominantly concentrate-based and the other forage-based, and likewise found similar results with regard to limited differences observed in methanogen populations as a function of diet [69]. In contrast to results from Zhou et al., Wallace et al. did note that archaeal abundances were greater in steers with greater methane emissions [35, 69]. Cattle with the same level of dry matter intake, but differing extremes in residual body weight gain exhibited no differences in enteric methane production, *in vitro* methane production and methanogen abundances in the rumen and cecum [70]. These findings were in contrast to the idea that variation in residual weight gain on high-grain diets was a function of reduced methane production. The authors concluded that the differences in residual weight gain under similar dry matter intakes may be more related to metabolic differences than that of digestion-related FE variance.

Host-microbial symbioses have also been implicated in rumen microbial methane production. Using rumen metagenomic profiling, researchers identified links between microbial genes and methane emissions [71]. Interestingly, when comparing breed differences with methane emissions and archaeal abundances, the rankings were consistent within the diet, suggesting that archaea abundances and subsequently methane emissions, may be under host genetic control. Indeed, of the 3970 microbial genes identified from metagenomic analyses, 20 genes were associated with methane emissions and explained 81% of the variation. These genes primarily identified as methane metabolism genes. For example, the methyl-coenzyme M reductase alpha subunit gene (mcrA) was included in a cluster of genes to be associated with methane emissions. Methyl-coenzyme M reductase catalyses the final methanogenesis reaction [72]. The transcript association of this gene with methane production within other ruminants such as dairy cattle and sheep has also been identified [73, 74]. In cattle, researchers have also identified that methane emissions were heritable, and have subsequently derived genomic expected breeding values for methane traits based on 747 head of Angus cattle phenotyped for methane traits and genotyped for 630 000 single nucleotide polymorphisms [75]. Overall, data support genetic cross-talk with the ruminal microbiome and the potential to genetically select for microbial profiles resulting in environmentally-significant production phenotypes.

Although many strategies have been utilized for the reduction of methane emissions in cattle, their efficacy is typically circumstantial, as many dietary and management factors influence their use and effectiveness. The addition of ionophores, such as monensin, to diets has been common practice in the beef industry, as supplementation has been shown to increase average daily weight gain and FE [76]. The method of action has been theorized to be primarily due to the perceived selective lethal targeting of monensin on Gram-positive bacteria, which produce important

volatile fatty acids for growth and maintenance, such as propionate [77]. The reduction in methane production has also typically been attributed to monensin use, as the reduced ruminal viability of Gram-positive bacteria impacts the Gram-positive production of substrates available for methanogen growth [77]. However, as technologies have permitted deeper investigation into microbial species, studies have demonstrated that monensin supplementation may not follow the Gram-positive theory, and that rather than suppressing classical Gram-positive bacterial populations, monensin influenced finer shifts in key microbial species important to rumen function [78, 79]. In the same studies, methane production was not reduced long-term when heifers were fed monensin in confinement [79]. These mixed results provide further evidence that additional research is needed regarding methane mitigation, specifically from a dietary or dietary supplementation approach.

Ruminant Microbiomes and Reproductive Efficiency

Another challenge to the livestock industry is the prevalence of reproductive losses which has been estimated to cost the beef and dairy industry over 1 billion dollars annually [80]. For example, in beef cattle, optimal reproductive efficiency is often defined as a calving interval of 365 days [81]. Every additional day the cow does not produce a calf results in delayed profit for the producer. Failure of a cow to produce a calf may result in the cow being culled from the herd and little to no return on investment. The development of reproductive technologies and management methods, such as estrus synchronization, artificial insemination and *in vitro* fertilization have contributed to improvements in reproductive efficiency [82]. Current research is examining the reproductive tract microbiomes and their potential to further improve ruminant reproductive efficiency.

It was previously thought that the uterus and vagina were sterile environments except in the case of pathogenicity [83, 84]. However, within the last decade, research on microbiomes of the reproductive tract has been widely explored in humans, antithetical to the previous dogma of sterility [85, 86]. In healthy women, the vagina is dominated by *Lactobacillus*, which may contribute to the low pH of the vagina [87]. The low pH and dominance of *Lactobacillus* likely reduce pathogen presence and vaginal microbial dysbiosis [87]. Additionally, studies have indicated increased diversity along with decreases in *Lactobacillus* dominance of the reproductive tract is associated with reproductive issues, such as reduced fertility or pre-term birth [88, 89]. As it is now widely accepted in the human scientific community that reproductive tracts contain unique, native microbiomes capable of affecting reproductive health and fertility, this knowledge can be translated into livestock reproductive microbiomics.

Lactobacillus appears to be important for human reproductive health, however, *Lactobacilli* are present in very low abundances in the vagina of cattle and other ruminants, suggesting that other vaginal microbiota may fulfill the function of protecting the host from pathogenic microbiota [90]. Similar to humans, ruminants possess unique uterine and vaginal microbiomes [46, 90, 91]. In cattle, vaginal bacterial communities are dominated by Firmicutes, Bacteroidetes and Proteobacteria, not unlike the rumen and lower gastrointestinal tract microbiomes [46, 90, 91]. The role of the uterine microbiome is much less explored or understood. Until the last several years, it was widely accepted that the uterus was a sterile environment, with the exception of dysbiosis [85]. In the uterus, the dominating phyla include Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes [90, 92]. Although it is very similar in bacterial composition to that of the vagina, the uterus typically has less microbial diversity than the vagina and greater abundance of unassigned or yet-to-be-defined taxa [91]. Further research is needed to understand the entirety of the reproductive tract microbiome, especially the uterus, in bovine and at different stages of growth and production.

Although becoming increasingly prevalent, few studies have examined the relationships among the uterine microbiome, dysbiosis and reproductive efficiency. Santos and Bicalho used PCR-DGGE and 454 pyrosequencing to interrogate the uterine bacterial community composition of dairy cattle of varying diseased states, including healthy, metritic and endometritic cows [93]. The study revealed that bacterial communities clustered by health state, regardless of days postpartum [93]. It was also observed that healthy cows were greater in bacterial phylogenetic diversity than unhealthy animals [93], which has been supported by additional studies in cattle [94]. Greater diversity may indicate, in part, the role of the uterine microbiome for reducing and preventing infection [93, 94]. Recently, research has begun to evaluate the use of probiotics and their effects on reproductive health in cattle. Genís et al. administered lactic acid bacteria (LAB), such as *Lactobacillus* spp., to cows prior to calving and assessed the occurrence of postpartum metritis among treatment groups [95]. Results indicated a decrease in the prevalence of metritis among cows treated with vaginal LAB, as well as reduction in neutrophil gene expression [95]. Although, *Lactobacillus* is not a dominant organism in the reproductive tract of cows as determined by previous studies [90, 91], this study suggests the addition of *Lactobacillus* spp. may still provide protection against pathogen colonization as similar to human vaginal microbiomes. Further research is needed, however, on the use of reproductive tract probiotics to reduce the incidence of postpartum diseases and may be a suitable replacement to minimize the need for antibiotics. In addition, as postpartum diseases may delay a cow's time to subsequent conception, probiotics must be studied for their effect on fertility and improving reproductive efficiency.

Ruminant Microbiomes in Health and Disease

Beef cattle health and food-safety are important issues that the industry has faced for decades. Not only does a sustainable food system need to address diseases affecting the food-producing animals that may reduce productivity but also zoonoses that impact food safety and human health.

One of the most common and economically important health problems to the US beef industry is bovine respiratory disease (BRD) [96]. Respiratory-related illness is the leading cause of mortality in all cattle and calves in the USA [97], and collectively BRD costs the beef industry over US$1 billion annually due to loss of production, treatment costs, increased labor costs and mortality [98]. The most common bacterial agents associated with BRD are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somnus*, *Mycoplasma bovis* and less frequently *Trueperella pyogenes* [98], and they are opportunistic pathogens [99]. As BRD is a multifactorial disease, many factors play a role in causing sufficient disease. Co-infection with viral pathogens, stress caused by transportation, commingling of multi-origin cattle and changes in weather have all been associated with the development of BRD [100, 101]. Additionally, there are host factors, such as the animal's commensal bacterial populations, that may increase or reduce the risk for BRD. In both humans and cattle alike, commensal organisms in the nasopharynx likely inhibit opportunistic bacterial infections, and when dysbiosis occurs, this protection is voided [101–103].

Studies utilizing 16S metataxonomics have shown that *Proteobacteria* and *Firmicutes* comprise the majority of nasopharyngeal phyla in all cattle followed by lesser proportions of *Actinobacter*, *Bacteriodetes* and *Tenericutes* [104, 105]. Additionally, culture-based works have shown that the largest fraction of genera within the upper respiratory microbiota include *Moraxella*, *Pasteurella, Manheimmia*, *Acinetobacter* and *Staphylococcus* [106–108]. There is evidence that feedlot cattle that were never treated for respiratory disease during the first few weeks after arrival had increased bacterial diversity and richness of their nasopharyngeal microbiome compared with cattle that were treated [104]. In that study, there was a significantly greater number of species at day 0 and 60 in healthy cattle compared with cattle that had BRD [104]. Interestingly, at entry to the feedlot there were significantly greater relative abundances of *Lactobacillus* and *Pediococcus* in healthy cattle, and all cattle that would later be treated for BRD had detectable taxa associated with either *Mannheimia haemolytica* or *Pasteurella multocida* [104]. In a study by Zeineldin et al. calves with BRD were more likely to harbor *Proteobacteria, Firmicutes and Tenericutes* phyla than healthy calves, and at the genus-level *Acinetobacter, Solibacillus and Pasteurella* were more common in BRD affected calves [109]. Furthermore, there was a relatively greater abundance of *Acinetobacter* species in BRD calves, while there was no difference in relative abundance of *Mannheimia* between healthy and diseased calves [109].

There is evidence that bacterial genera change over time immediately after weaning differently among calves that are diagnosed with BRD and their healthy cohorts [110]. The majority of clinical cases of BRD in feeder cattle occur within the first few weeks of arrival at the feedyard. In a study by Holman et al. 14 Angus × Hereford heifers of single farm origin were transported to a feedyard and nasopharyngeal swabs were collected at days 0, 2, 7 and 14 [111]. Within 2 days of transport to the feedyard, nasopharyngeal microbiota changed significantly with regard to phylogenetic diversity and richness, and continued to shift throughout the study period as determined by UniFrac distances [111]. Although relative abundance of BRD-associated bacteria did not significantly change over time, it is likely that the instability caused by entry into the feedlot may contribute to increased risk for BRD soon after feedlot arrival [111].

In addition to respiratory tract microbiota, another important microbial community of food security interest is that of the lower gastrointestinal tract and feces. Foodborne pathogens like *Escherichia coli* O157:H7 may be shed in the manure of cattle and cause direct or indirect gastrointestinal infection in humans [112]. *E. coli* O157:H7 is a pathogenic bacterium capable of causing severe illness or even death when ingested by humans [113]. Cattle populations are known reservoirs for this pathogen, and are typically asymptomatic carriers of the organism [114–116]. The terminal rectum mucosa, also known as the recto-anal junction (RAJ), is the primary site of colonization by *E. coli* O157:H7 [117, 118]. Not only can cattle become colonized by the organism, but they also regularly shed the pathogen in their feces [119]. Additionally, their hides may become contaminated, and at slaughter, hide-to-carcass transfer can lead to food safety concerns [120].

Cattle typically shed small quantities of the bacteria in their manure, but there have been instances where individual animals may shed up to 6.5×10^7 CFU per gram of feces [121]. Cattle shedding $>10^4$ CFU per gram have been termed 'supershedders' [122]. Supershedders (SS) only represent a small proportion of the EHEC O157:H7 positive animals, but contribute the majority of environmental contamination [123, 124]. Recent studies have aimed to identify differences in SS compared with cattle that were not shedding (NS) the organism [121, 125]. Wang et al. determined that although there were no differences in alpha diversity measurements between SS and NS, there were microbiota composition differences and large animal-to-animal variation in taxonomic and beta diversity measurements [125]. The core microbes of the terminal rectum were *Firmicutes, Bacteroidetes and Proteobacteria* [125]. These findings were consistent with other studies looking at rectum content in dairy cows [126] and fecal microbes in beef and dairy cattle [127–130]. Additionally, Wang et al. determined that there were unique microbes associated with NS that may be also be

associated with propionate and butyrate production [125]. The increased production of these short-chain fatty acids may create a gut environment that is unfavorable for colonization and lead to reductions in *E. coli* O157:H7 shedding [131]. Supershedding cattle may harbour a more diverse fecal microbiome and specific differences in species in these animals compared NS cattle may play an important role in supershedding [121]. It remains unclear if *E. coli* O157:H7 overgrowth is caused by intestinal dysbiosis.

Conclusions

Microbiomes are, in part, responsible for the normal function of mammalian systems. These complex networks of microbes aid in the function and health of the host and its microbial niche. In ruminants, maintaining efficient and healthy systems such as the gut, reproductive tract and respiratory tract are important for production, as microbial dysbioses can lead to inefficiencies in feed, reproduction losses, disease and health issues. As research continues to develop past the characterization of microbiomes with regard to production in ruminants, researchers can begin to connect and define the complex network dictating these host-microbe symbioses. Researchers have begun to link microbiomes through genomics to their host [132] or link microbiome function to production phenotypes, such as FE [133]. Building upon the knowledge gained from microbiomes throughout ruminant production will ultimately permit strategies to select for or manipulate microbiomes to obtain desirable, healthy and efficient microbiomes in adult ruminants. These advances have the potential to greatly impact the livestock sector in producing greater amounts of high-quality protein for human consumption. Such progress also takes into account the promotion and support for animal health, as commensal microbial technologies are natural solutions. Sustainably and efficiently improving these sources of high-quality protein through novel tools and technologies will be a key process for global food production and food security.

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