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Review: The compositional variation of the rumen microbiome and its effect on host performance and methane emission

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The rumen microbiome has the important task of supplying ruminants with most of their dietary requirements and is responsible for up to 90% of their metabolic needs. This tremendous feat is possible due to the large diversity of microorganisms in the rumen. The rumen is considered one of the most diverse ecosystems on the planet in terms of species diversity and functional richness. From the moment the feed is ingested, it enters a vast cascade in which specialized microorganisms degrade specific components of the feed turning them into molecules, which in turn are utilized as anabolic precursors and energy sources for the animal. The output of this degradation process not only affects the animal, but also has an extensive impact on the environment. Some of the byproducts that are emitted as waste from this process, such as methane, act as greenhouse gases which greatly contribute to global warming. Recent technological advances developed to study this community enabled a larger overview of its vast taxonomic and functional diversity, thus leading to a better understanding of its ecology and function. This deeper understanding of the forces affecting the microbiome includes the forces that shape composition, the variation among animals, the stability of its key components, the processes of succession on a short- and long-time scales such as primary colonization and diurnal oscillations. These collective understandings have helped to provide insights into the potential effects that these forces have on the outputs observed from the animal itself. Over the recent years, there has been a growing body of evidence demonstrating the link between the microbiome and its effect on productivity of the host animals and the environment, which has placed rumen microbiome studies in the forefront of animal agricultural research. In this review, we focus on the natural variations in community composition, which are not the results of different management or feed but rather intrinsic features of animals. We characterize the rumen microbiome, its potential impact on its host as well as the barriers in implementing the current knowledge to modulate the microbiome and point toward potential avenues to overcome these hurdles.

Keywords: rumen microbiome, feed efficiency, methanogens, acrylate pathway, microbial colonization

Implications

The improvement of feed efficiency and, through it, product yield in cattle remains one of the most coveted goals in animal agriculture. Until now, this feat was accomplished by selective breeding, improvements in management and feed composition. Although ruminants are completely dependent on their microbiome, only recently has a link been established between the animals' physiological traits, such as energy harvest efficiency and methane emission, and its resident microbial taxa composition. Increased understanding of the rumen microbiome and its effect on animal traits may lead to novel strategies aimed at steering this community toward an improved host phenotype for increased agricultural productivity and sustainability.

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Introduction

Ruminant herbivores have an evolved digestive system that allows absorption and digestion of large amounts of plant material as an energy source. This capability is of enormous significance to mankind, as ruminants essentially convert the energy stored in plant biomass to digestible food products (Bergman, 1990). Harnessing this process to mankind's benefit has been proposed as a driving force behind human social evolution and a decisive factor in human community fitness (Diamond, 1997).

This crucial ability to digest the plant material resides in the first compartment of the digestive tract of ruminants, termed the rumen, in which anaerobic degradation of the ingested feed occurs. Upon consumption, the feed is packed in the first two compartments of the digestive system of the cows, collectively called the reticulorumen. The feed is

partially digested and then regurgitated and chewed again in order to increase the surface area of the feed available for further degradation by the resident microorganisms (Van Soest, 1994).

The rumen is inhabited by a dense and highly diverse microbial community, consisting of bacteria, archaea, protozoa and fungi. They are responsible for the fermentation and degradation of plant fibers which result in the conversion of plant material into digestible compounds, such as volatile fatty acids (VFA), and serve as the main energy source for the animal (Bergman, 1990). In addition, the microbes themselves are subsequently digested in the abomasum and serve as a major protein source in ruminants (Kay, 1969). Hence, the rumen microbiome is an integral part of the ruminant digestive system, essential to the animals' early development, well-being and physiology.

Other gastrointestinal microbiomes, such as those found in humans, have only recently seen an increased interest in microbiome research, outside the context of illness and infection. In contrast, the rumen microbiome was recognized already during the early days of microbiology research as a central component of the animals' well-being and physiology and the environment. Until recently, the study of complex microbial communities relied mostly on cultivation dependent methods, which allowed for an appreciation of the wide array of functions performed by the microbial community in the rumen despite the limited ability to cultivate most of the rumen microbial diversity (Wallace *et al*., 2017). This is likely due to the observed functional redundancy of the key functions established in the rumen, in which similar metabolic tasks are performed by a large number of species (Taxis *et al*., 2015; Weimer, 2015).

Still, with novel molecular techniques arising, this paradigm has shifted toward a more general attempt at surveying the overall phylogenetic and functional diversity of the rumen microbiome, and characterizing its dynamics associated with time, host animal and environmental conditions.

Rumen microbiome composition

Cultivation-independent methods, such as the wide array of sequencing technologies and microbial fingerprinting methods available today, have been commonly used during recent years to study the dynamics of the rumen microbial communities (Brulc *et al*., 2009; Welkie *et al*., 2009; Hess *et al*., 2011; Henderson *et al*., 2015; Shabat *et al*., 2016; Tapio *et al*., 2017a; Wallace *et al*., 2017), greatly expanding the scope of observable phylogenetic and functional diversity within the rumen.

The first of such metagenomic studies were performed on the rumen of three steers, revealing that 95% of the microbiome is composed of bacteria, between 2% and 4% archaea, and around 1% Eukarya is composed of protozoa and fungi (Brulc *et al*., 2009). This study also emphasized for the first time, the vast differences observed in microbiome composition among different animals. In this study, one steer had a remarkably different microbiome composition compared with the other two steers analyzed, despite similar housing and dietary conditions and without any obvious differences in the animals' physiology (Brulc *et al*., 2009). Subsequent studies therefore, aimed to define the degree of variability in rumen microbiome composition and establish whether common features can still be found between all ruminants or within specific lineages, relevant to agriculture. In the most comprehensive analysis yet, the foregut microbiome of 32 ruminant and pseudoruminant species, yielding 742 samples from across the world, were analyzed. The results emphasized the shared and divergent traits in the rumen microbiome composition across a wide geographical range and animal lineages (Henderson *et al*., 2015). In this study, common microbial features were observed across most of the animals of different lineages sampled. The authors identified a core community of 30 bacterial taxa, with the dominant taxa being *Prevotella* (Bacteroidetes), *Butyrivibrio* and *Ruminococcus* (both Firmicutes), as well as unclassified Lachnospiraceae, Ruminococcaceae, Clostridiales (all Firmicutes) and Bacteroidales (Bacteroidetes) (Henderson *et al*., 2015). This shared presence of bacterial taxa across different foregut animal lineages suggests a longstanding relationship throughout evolution, hinting toward their key role in rumen metabolism and function (Shade and Handelsman, 2012). *Ruminococcus*, *Butyrivibrio* and *Fibrobacter* harbor the main known cellulolytic species, and the *Prevotella* genus, though non-cellulolytic, encompasses a wide array of species with different substrate degradation capabilities (Accetto and Avguštin, 2015). Within the cattle lineage, Jami and Mizrahi (2012a) identified 32 genera, recurrent in 16 cows, with *Prevotella* accounting for up to 72% of the bacterial population in the rumen, and hundreds of species-level operational taxonomic units (OTUs) belonging to this genus, strengthening the observed dominance of this genus in cattle (Stevenson and Weimer, 2007; Jami and Mizrahi, 2012b). *Prevotella* is most commonly associated with plant degradation and is not limited to ruminants. Studies on human societies living in rural areas, where the diet is mainly plant based, revealed a microbiome that is highly enriched in *Prevotella* (De Filippo *et al*., 2010). The full scope of functions within this genus is still relatively unknown because only a limited number of *Prevotella* species have so far been cultured. A recent study compared the genomes of 39 isolated species of *Prevotella* from different environments and concluded that carbohydrate-acting enzymes and polysaccharide-utilization loci are highly heterogeneous within this genus (Accetto and Avguštin, 2015). Genomes from rumen isolates were mainly oriented to plant cell wall and storage polysaccharides degradation (Accetto and Avguštin, 2015). However, this is only the tip of the iceberg, as the number of *Prevotella* species that reside solely in the rumen are thought to reach the hundreds (Kim *et al*., 2011; Jami and Mizrahi, 2012a).

Similarly to the bacterial population, a core community of rumen methanogens can also be found, with the

Methanobrevibacter consistently identified as the most abundant and ubiquitous genus across all animal species and geographical regions sampled (Morgavi *et al*., 2010; Henderson *et al*., 2015). Most of the methanogens in the rumen are hydrogenotrophic, using the hydrogen (H_2) produced through bacterial fermentation to reduce carbon dioxide (CO_2) into methane. This function is crucial for maintaining the directionality of the rumen metabolism, as the methanogens serve as an electron sink to decrease the partial pressure of H_2 in the rumen, which, at high concentrations, inhibits bacterial fermentation (Morgavi *et al*., 2010). Other methanogenesis pathways can be found in the rumen of adult animals such as the methylotrophic used by the newly defined Methanomassiliicoccaceae family (Paul *et al*., 2012; Iino *et al*., 2013; Oren and Garrity, 2015; Nkamga and Drancourt, 2016), and the less prevalent acetoclastic pathways used by members of the Methanosarcinales order (Lambie *et al*., 2015; Patra *et al*., 2017).

Despite their observed importance, the eukaryotic components of the rumen microbiome remain considerably underexplored. Researchers pointed out this knowledge gap, which also exists for most studied environments, where eukaryotic microorganisms are found to be ubiquitous yet are mostly overlooked (Caron *et al*., 2009).

The rumen eukaryotic community is composed of ciliate protozoa and fungi (Brulc *et al*., 2009). Protozoa are comparatively large single-celled eukaryotic microorganisms (10 to $>100\,\mu m$), and despite being smaller in richness compared with bacteria and archaea, estimated at 10^5 to 10^6 cells/ml rumen fluid (Sylvester *et al*., 2004; Abubakr *et al*., 2013), account for a large proportion of the microbial biomass in the rumen, estimated to reach up to 50% (Williams and Coleman, 2012). In addition, a recent study comparing the quantification of the protozoa 18S at the DNA and RNA level, revealed that while 18S accounts for $<1\%$ of the total microbial community at the DNA level, they account for 13% of the ribosomal RNA (rRNA) expressed, suggesting that this group is highly active in the rumen (Comtet-Marre *et al*., 2017).

Protozoa are not considered essential for animal survival, but are found to exert a large effect on the rumen ecosystem and host animal physiological characteristics (Yáñez-Ruiz *et al*., 2007; Mosoni *et al*., 2011; Newbold *et al*., 2015). A recent meta-analysis of multiple experiments in which ruminants underwent defaunation, a process of depleting the rumen of their protozoal components, revealed a broad effect on the ruminant physiology ranging from protein supply availability to methane emission, the latter decreasing by an average of 11% in defaunated animals (Newbold *et al*., 2015). The same group found that the holotrich protozoa contain a high density of endosymbiotic methanogens and may play a proportionally greater role in methanogenesis (Belanche *et al*., 2014 and 2015). These results emphasize the importance of studying the protozoal community and their relationship with the prokaryotic components of the rumen such as the methanogens (Belanche *et al*., 2014). When comparing the protozoal community with prokaryotic species in the rumen, Henderson *et al*. (2015) showed a higher degree of individuality both between and within foregut animal lineages, with a lower similarity both between geographically separated cohort and within specific cohorts than the ones seen in prokaryotic populations. De Menezes *et al*. (2011) made a similar observation which shows a strong individuality of the protozoa community between cows, overriding the effects of diet changes, despite being still observable in the bacterial and archaeal community. This lower similarity and individuality of the protozoa population suggests a more stochastic ecological mode of acquisition more dependent on random sampling from the environment and dispersal limitations, rather than strong selection within the rumen environment (Costello *et al*., 2012).

Anaerobic fungi also constitute an integral part of the microbiome and were shown to contribute to plant cell wall digestion. Specifically, fungi are thought to play a central role in the colonization and disruption of lignocellulolytic tissues which enable greater accessibility to fiber for bacteria (Akin and Borneman, 1990; Liggenstoffer *et al*., 2010; Mizrahi, 2013). All known fungi in the gut of herbivores belong to the Neocallimasticaceae family.

Like ciliates, anaerobic fungi remain largely underexplored, in terms of their diversity in ruminants and other mammalian guts. Currently, the largest study assessing the fungal diversity in the gut of herbivores (not limited to ruminant species) showed the limited knowledge in fungal diversity, with almost 40% of the sequences obtained from sequencing not belonging to any previously described genus and yielding eight newly identified, phylogenetically distinct anaerobic fungal lineages (Liggenstoffer *et al*., 2010). This emphasizes the limited knowledge of this important lineage and the ubiquitous nature of fungi in many gut environments. The difficulty in assessing taxonomic affiliation for fungi stems from the fact that the commonly used rRNA marker is limited in its phylogenetic information for fungi (Monard *et al*., 2013). Thus, the most commonly used marker to identify the phylogenetic affiliation of fungal species is the internal transcribed spacer 1 (ITS1) (Edwards *et al*., 2017). Phylogenetic identification is further complicated by the fact that this sequence is highly variable, making phylogenetic assignment difficult (Korabecna, 2007; Koetschan *et al*., 2014). To overcome this issue, the additional use of predicted secondary structure of the ITS1 region was shown to improve the ability to classify the fungal taxa using high-throughput sequencing (Koetschan *et al*., 2014). The improvement in classification of fungi in the rumen thus enabled the recent assessment of their composition and dynamics across different animals and diets (Ishaq *et al*., 2017; Tapio *et al*., 2017a).

Microbial composition across different rumen habitats

Adding to the complexity in the characterization of the rumen microbiome is the observation that different habitats exist within the rumen itself. Most research focuses on the comparative analysis between the liquid phase associated

microbiome and the fiber-adherent microbial community, with a recent increased focus on the epithelium-associated microbial community (Henderson *et al*., 2013; Jewell *et al*., 2015; Deusch *et al*., 2017). The recent studies differentiating between the different microenvironments agree that the microbial composition in each of these microenvironments differs from one another and has distinct responses to various treatments and diets can be observed (Jewell *et al*., 2015; Deusch *et al*., 2017). A recent study assessing the differences between the ruminal fractions showed that the fiber-adherent and liquid-associated fractions harbor similar taxa, but they differ in relative proportion; whereas, the epithelium-associated microbial community harbors taxa unique to this environment (Deusch *et al*., 2017). The fiber-adherent microbiome is characterized by a higher proportion of taxa associated with fiber degradation, such as Ruminococcaceae and *Fibrobacter* (Deusch *et al*., 2017; Vaidya *et al*., 2018), whereas the liquid phase is characterized by an enrichment of members of the Prevotellaceae family, capable to utilize a broad range of soluble substrates (Deusch *et al*., 2017; Vaidya *et al*., 2018). The epithelial community is characterized by taxa associated with urea hydrolysis and several aerobic taxa not found in the fiber-adherent and liquid-associated microbial community, the latter suggested to be involved in oxygen scavenging (Deusch *et al*., 2017). Similar to the bacterial population, the same study observed significant differences in the methanogenic population within the epithelial community harboring a higher methanogens/bacteria ratio and a higher proportion of species associated with the *Methanobrevibacter* genus (Deusch *et al*., 2017). These results emphasize the importance of differentiating between the different microbial subpopulations in the rumen in light of the scientific question being asked. Thus, exclusively relying on the liquid fraction of the rumen, as is commonly performed in these types of studies, might not capture the overall microbial diversity or the changes occurring following changes in management or diet.

Compositional variation across individual animals

Although common compositional features can be observed across different foregut animal lineages, and even more so within specific lineages such as cattle, large differences still exist between different animals of similar lineages and under similar management conditions. Li *et al*. (2009), using denaturing gradient gel electrophoresis (DGGE) fingerprinting analysis, revealed that there was a much lower similarity between samples taken from different host animals compared with the high similarity observed between samples taken from different regions within the rumen of the same animal (cranial, caudal, dorsal, ventral and central), and the stability across different sampling time points within the same cow. In a study using automated ribosomal intergenic spacer analysis to examine the changes in ruminal bacterial communities during the feeding cycle, similar observations were made which emphasize both the stability of the rumen microbial community when established within a cow and the large differences in composition between different cows (Welkie *et al*., 2009). Similarly, Jami and Mizrahi (2012a), using the Bray–Curtis index, which takes into account both identity and abundance of the bacterial taxa, demonstrated that the average pairwise similarity between cows was 0.51 in 16 lactating cows under similar housing conditions and diet (Jami and Mizrahi, 2012a). This observation is not limited to bacteria and similar inter variation within the same host species can be seen in the other microbial domains inhabiting the rumen as well (Zhou *et al*., 2010; Henderson *et al*., 2015). Thus, there is ample evidence of inter-animal variations, even under similar conditions, and a high degree of individuality in terms of microbiome composition in the rumen.

Microbiome impact on animal physiology

Feed efficiency and production

In ruminants, fermentation products of rumen microbial activity – mainly VFA – serve as a major source of energy for the animal (Bergman, 1990). It is thus tempting to speculate that the observed inter-animal variations in microbial composition might be linked to the animals' physiological parameters of efficiency and energy loss of their individual hosts.

The potential effects of microbiome composition on animal physiology, with emphasis on performance and methane emission, have been investigated in a number of studies (Table 1). By examining microbial differences between efficient and inefficient cows, using DGGE, a connection between VFA composition, rumen bacteria and production efficiency was suggested (Hernandez-Sanabria *et al*., 2010). This study identified several taxa associated with parameters related to feed composition and production efficiency.

In a study assessing the potential correlation between taxa abundance of the microbiome components and physiological parameters of 15 cows, a high correlation was observed between the ratio of Firmicutes/Bacteroidetes and daily fat production in the milk (Jami *et al*., 2014). The difference in ratio was almost exclusively driven by the vast difference in abundance of the *Prevotella* genus, belonging to the Bacteroidetes phylum, which negatively correlated with milk fat yield. Additional evidence of a connection between production and the microbiome was shown in a study investigating the dynamics of the microbial population in the cow's rumen throughout two lactation periods (Jewell *et al*., 2015). This study also found a negative correlation between specific OTUs associated with the *Prevotella* genus and production efficiency. Surprisingly, these findings mirror observations from other gastrointestinal systems, in which a decreased amount of Bacteroidetes correlated with increased adiposity in the blood and tissue of mice. The authors suggest that the 'obese' microbiome has an increased capacity to harvest energy from the diet (Turnbaugh *et al*., 2006). However, in contrast to these findings, the same study also identified various *Prevotella* species associated with higher feed efficiency (Jewell *et al*., 2015), and an additional recent study showed that *Prevotella* might play a role in increasing milk production yield (Indugu *et al*., 2017). The large diversity mentioned within the *Prevotella* genus might be the cause

Table 1 *Summary of experiments assessing the link between the rumen microbial community and animal physiology*

Animals used	Methods	Main findings	References
Beef cattle	PCR-DGGE	Bacterial and SCFA profile linked to feed efficiency	Guan *et al.* (2008)
Beef cattle	PCR-DGGE	Higher methanogens diversity in inefficient animals. Differential prevalence of *Methanosphaera stadtmanae* and specific *Methanobrevibacter* strains between high and low RFI	Zhou *et al.* (2009)
Beef cattle	PCR-DGGE and qPCR	Identification of specific bacterial and archaeal OTUs associated specific VFA and different RFI phenotypes in cattle under low energy diet	Hernandez-Sanabria *et al.* (2010)
Beef cattle	PCR-DGGE and qPCR	Link between bacterial profile and feed efficiency is inconsistent across different diets	Carberry *et al.* (2012)
Beef cattle	Clone library and 16S sequencing	Differential abundance of a *Methanobrevibacter smithii* genotypes between high and low RFI animals	Carberry *et al.* (2014b)
Sheep	Metagenomic and metatranscriptomic	Higher methanogenesis-related genes expressed in high methane emitting sheep	Shi *et al.* (2014)
Dairy cattle	16S amplicon sequencing	Correlations between bacterial genera and production parameters. *Prevotella* negatively correlated with milk fat yield	Jami *et al.* (2014)
Beef cattle	16S amplicon sequencing	Higher relative abundance of *Prevotella* in inefficient animals	McCann *et al.* (2014)
Dairy cattle	16S amplicon sequencing	Core OTUs associated with either efficient (*Prevotella* spp.) or inefficient cows (*Prevotella, Butyrivibrio*) over the course of two lactation cycles	Jewell *et al.* (2015)
Steer	Metagenomics	*Methanobrevibacter* and Succinivibrionaceae more abundant in high methane emitting steers	Wallace *et al.* (2015)
Dairy cattle	16S amplicon sequencing, metagenomics and metabolomic	Lower bacterial diversity in efficient cows. Identification of *Megasphaera elsdenii* and the acrylate pathway as linked to high efficiency in dairy cattle	Shabat *et al.* (2016)
Sheep	Metatranscriptomics	Putative involvement of *Sharpea azabuensis* and *Megasphaera* spp. and the acrylate pathway in low methane emission phenotype	Kamke *et al.* (2016)
Dairy cattle	16S amplicon sequencing	*Prevotella*, S24-7 and Succinivibrionaceae lineages positively correlated with milk yield	Indugu *et al.* (2017)
Dairy cattle	16S amplicon sequencing	*Methanobrevibacter ruminantium* and *Methanobrevibacter gottschalkii* associated with low methane emission	Danielsson *et al.* (2017)
Beef cattle	Metatranscriptomics	Higher diversity of expressed metabolic pathways in inefficient cows	Li and Guan (2017)

DGGE = denaturing gradient gel electrophoresis; SCFA = short-chain fatty acids; RFI = residual feed intake; qPCR = quantitative PCR; OTUs = operational taxonomic units; VFA = volatile fatty acids.

for these conflicting results and its internal composition might dictate the effect of this population on its host physiology (Jami and Mizrahi, 2012a; Accetto and Avguštin, 2015; Ley, 2016). In a large cohort study, which assessed the efficiency of 146 cows along with their microbial composition, gene content and metabolic output of the rumen, Shabat *et al*. (2016) observed direct evidence of different microbiome composition and gene abundance between cows with differing energy harvest capabilities. Low residual feed intake (low RFI; efficient) was shown to harbor a less diverse microbiome, but also directed toward production of molecules serving as energy for the animal compared with the microbiome of the less efficient, high RFI cows. In addition, this study revealed a differential enrichment of specific taxonomic and genomic components of the microbiome between efficient and inefficient cows (Shabat *et al*., 2016). These results are the product of several converging observations obtained from different types of analyses. The use of 16S rRNA analysis allowed to identify several taxa enriched in the rumen of efficient cows, including the lactate utilizing *Megasphaera elsdenii* and *Coprococcus catus* significantly enriched in efficient cows. The *M. elsdenii*, although found in relatively low proportion in both efficient and inefficient cows compared with other species in the rumen, could also be seen significantly enriched in the rumen of efficient cows using shotgun metagenome sequencing and read alignment to its genome. The metagenome also identified the acrylate pathway, encoded among others by the *M. elsdenii* and *C. catus*, as the only propionic acid pathway significantly enriched in the rumen of efficient cows. The taxonomic and genomic results were further strengthened by the metabolomic analysis, assessing the overall metabolic outputs of fermentation, in which lactate was found to be in lower abundance in the rumen of efficient cows, suggesting it to be more efficiently utilized by the microbiome. The congruence between the different methods used highlights lactate as a crucial intermediate and the acrylate pathway, significantly enriched in the rumen of efficient cows, as a contributing factor in steering metabolism toward production of VFA. On the other hand, inefficient cows exhibiting a lower abundance of this species and the mentioned genes had their metabolism directed toward increased methane production (Shabat *et al*., 2016). The authors proposed a model in which the microbiome composition affects the ratio of end products during fermentation. The efficient cows' microbiome was shown to steer production toward VFA through the

intermediate product lactate, driven by the enrichment of lactate utilizing bacteria via the acrylate pathway such as *M. elsdenii* and *C. catus* (Shabat *et al*., 2016). In contrast, within inefficient cows, rumen metabolism was shown to be steered toward unusable end products such as methane, evidenced by the specific enrichment of the *Methanobrevibacter ruminantium* species and the increase in methane production.

These findings were further strengthened by another study showing the connection between the microbiome composition and the methane emission, which also observed that lactate utilization, directed toward butyrate production by, among others, *Megasphaera* spp., results in low methane yield in sheep (Kamke *et al*., 2016).

Methane emission

In parallel to the efforts carried out at understanding the role of the microbiome in energy harvest and feed efficiency, an increasing interest at linking the microbiome to the observed energy loss through methane emission has developed (Tapio *et al*., 2017b). Methanogenic archaea are a driving force for the rumen microbiome metabolism and serve as an electron sink for the entire ecosystem driving the directionality of the fermentation process (van Lingen *et al*., 2016). However, methanogenesis carries drawbacks related to both the animal's energy-harvesting efficiency and the vast impact it exerts on the environment. Methane cannot be absorbed by the host and is therefore emitted to the environment and constitutes a substantial energy loss for the animal (Johnson and Ward, 1996). Methane is also a potent greenhouse gas greatly contributing to the greenhouse effect. These drawbacks led to intensified research efforts to improve performance along with mitigating energy losses through methane.

Evidence of a connection between the methanogenic population and production efficiency was observed in a study assessing the methanogen identity and composition in efficient *v.* inefficient cows (Zhou *et al*., 2009). This study revealed that, while similar methanogen abundance was observed between the groups, their composition was significantly different and a higher proportion of *Methanosphaera stadtmanae* and *Methanobrevibacter* spp. strain abM4 could be observed in inefficient cows (Zhou *et al*., 2009). Using metatranscriptomics analysis to assess the expression levels of microbial genes in sheep classified as low and high methane emitters, Shi *et al*. (2014) showed increased expression of CO_2/H_2 methanogenesis pathway genes in the rumen of high methane emitting sheep.

In a recent study, metagenomics analysis showed a significantly higher prevalence of *Methanobrevibacter* in the rumen of high methane emitting steers (Wallace *et al*., 2015). This study, however, also noted differences in the microbiome beyond the methanogens themselves, with both bacterial taxa and genes differentially expressed in the rumen of high emitters compared with low emitters. The authors emphasized the higher prevalence of the Succinivibrionaceae family and related genes within the low emitting sheep. Succinivibrionaceae was also found in high abundance in studies characterizing the microbiome of the Tammar Wallaby, and was speculated to be related to their observed naturally low methane emission (Pope *et al*., 2011). Interestingly, a prominent Succinivibrionaceae species, *Succinivibrio dextrinosolvens*, is a known minor lactate producer in the rumen (O'Herrin and Kenealy, 1993). Thus, methanogenesis is a complex process that may involve not only the methanogens but also other components of the microbiome. Network analysis approaches using patterns of co-occurrence and abundance-based correlations may help uncover these connections (Layeghifard *et al*., 2017), and a recent study applying these approaches revealed several putative interactions between the different microbial domains in the rumen (Tapio *et al*., 2017a). The authors, however, pointed out that no mechanistic explanation is yet available and requires further investigation (Tapio *et al*., 2017a).

Wallace *et al*. (2015) also identified the *Megasphaera* genus as being significantly enriched in the rumen of low emitting cows. As mentioned, *M. elsdenii* was subsequently identified twice in the context of both increased efficiency and low methane emission (Kamke *et al*., 2016; Shabat *et al*., 2016). The consistency of these results suggest that lactate, and lactate-related bacterial species and genes, may represent a central intermediate pathway to the production of VFA, naturally enriched in a subset of animals, serving as an alternative to methanogenesis. This is supported by previous meta-analyses and theoretical studies suggesting VFA as alternative electron sinks to methane in the rumen (Ungerfeld, 2013 and 2015).

Interestingly, previous studies attempted to enrich the rumen of cows with *M. elsdenii* isolated from the rumen, in order to stabilize ruminal pH by decreasing lactate concentration and alter VFA profile toward increasing acetate and butyrate production (Zebeli *et al*., 2012; Weimer *et al*., 2015). Despite being unsuccessful in stably transplanting the bacteria and altering rumen composition, focusing on this species or other lactate utilizers may represent an interesting avenue worth pursuing for the purpose of both increased feed efficiency and methane mitigation.

Potential modulation of the rumen microbiome

Modulation of the microbiome toward a more optimized phenotype remains one of the main challenges in the field and has been attempted in numerous studies (Table 2). Prior attempts resulted in transient or no success in obtaining a stable optimized population in the mature rumen (Weimer, 2015). The rumen microbiome, although quite dissimilar between different animals, exhibits a remarkable specificity and resilience within its host (Weimer, 2015). Although these features serve as an anchor for studying the effect of different microbial makeups on the host over time (Li *et al*., 2009; Jewell *et al*., 2015), they also hinder the possibility of microbial manipulation and to follow the long-term effect of such changes. Most attempts at introducing bacteria, indigenous to the rumen, resulted in only a temporary

Table 2 *List of rumen exogenous inoculation experiments and the main effects on the microbiome and animal*

Animals	Type of inoculum	Main findings	References
Gnotobiotic lambs	Designed minimal bacterial consortium	Consortium partly established until the end of the experiment. Minimal microbiome contributes to the health of lambs	Mann and Stewart (1974)
Gnotobiotic lambs	Combinations of mixed defined bacterial consortium	Consortium partly established	Lysons *et al.* (1976)
Gnotobiotic lambs	Simplified undefined microbial community + *Fibrobacter succinogenes* and protozoa	VFA composition and animal health affected by the complexity and type of inoculate	Fonty *et al.* (1983)
Adult sheep	Genetically modified *Prevotella ruminicola*	Inoculum below detectable levels after 3 h. Suspected bacteriocin activity responsible for the extinction	Attwood *et al.* (1988)
Adult cows	Antibiotic resistant *Selenomonas ruminantium* and *Mitsuokella multiacidus*	*S. ruminantium* persisted in the rumen after 30 days. Rapid extinction of *M. multiacidus*	Flint *et al.* (1989)
Adult sheep	Multiple dosage of antibiotic resistant isolated *S. ruminantium*	Transient increase of the inoculum. Undetectable after 24 h	Wallace and Walker (1993)
Adult goat	Antibiotic resistant *Ruminococcus albus*	Detectable after 14 days (end of experiment)	Miyagi *et al.* (1995)
Gnotobiotic lambs	Twice weekly dosing of multiple cellulolytic *Ruminococcus* strains	Gradual extinction 3 weeks after final dosing	Krause *et al.* (1999)
Adult sheep	Recombinant *Butrivibrio fibrisolvens* expressing *Ehrlichia ruminantium* xylanase gene	Rapid decline to extinction after 144 h *in vivo*	Kobayashi *et al.* (2001)
Adult sheep	Dosing of multiple *Ruminococcus* Spp.	Not stably established. Changes in the overall microbiome and physiological parameters	Krause *et al.* (2001a)
Sheep, cattle	Recombinant *B. fibrisolvens* with *Neocallimastix patriciarum* xylanase	Decline to extinction after 22 days	Krause *et al.* (2001b)
Dairy cattle	Repeated dosing of *Ruminococcus flavefaciens* + probiotic	Rapid decline of inoculated species regardless of added probiotic	Chiquette *et al.* (2007)
Dairy cattle	Total exchange of rumen fluid	Transient change in pH and VFA profile. Rapid recovery of the bacterial community to pre-exchanged composition	Weimer *et al.* (2010)
Reindeer	*R. flavefaciens* strain 8/94-32	Strain undetected post-dosing. Modulation of the overall microbial composition	Præsteng *et al.* (2013)
Dairy cattle	Multiple *M. elsdenii* strains	*M. elsdenii* population reverted to baseline levels after 24 h	Weimer *et al.* (2015)
Beef cattle	Repeated inoculation of bison rumen fluid	Alteration of the rumen microbiome Increased protein digestibility	Ribeiro *et al.* (2017)
Dairy cattle	Total exchange of rumen fluid	Transient change in milk production and bacterial community. Solid-associated community reverted to pre-exchanges composition	Weimer *et al.* (2017)
Beef cattle	Total exchange of rumen fluid	Strong host individuality in response to inoculation during microbiome re-establishment	Zhou *et al.* (2018)

VFA = volatile fatty acids.

increase of the inoculum, followed by its decrease to extinction in a matter of days to a few weeks (Wallace and Walker, 1993; Krause *et al.*, 2001a; Chiquette *et al.*, 2008; Zebeli *et al.*, 2012; Weimer *et al.*, 2015). The microbiome is resilient to such an extent, that a recent study, which almost completely replaced the rumen fluid of one cow with the rumen fluid from another cow, showed that within just a few weeks, rumen microbiome content reverted to a content more closely resembling its original composition (Weimer *et al.*, 2017). This stresses the fact that even the most extreme perturbation of the microbiome could not change the rumen microbial composition in a stable manner and suggests a high host specificity of the rumen microbiome composition once established. Moreover, recent studies show a connection between the individual animals' genetics and its respective microbiome, as well as heritability of some rumen microbiome components (Roehe *et al.*, 2016; Li *et al.*, 2016; Sasson *et al.*, 2017). These factors might hinder successful inoculation of previously isolated microbial strains.

This challenge is not exclusive to rumen microbiome studies and can be observed in microbiome studies of other gastrointestinal systems (Kristensen *et al.*, 2016). However, a recent study succeeded in engrafting a known endogenous species within the already established human gut. In this study, the researchers administered orally *Bifidobacterium longum*, a naturally residing member of the human

microbiome. The inoculated bacterium could stably colonize the gut in a subset of inoculated humans, which exhibited an initially low abundance of this bacterium along with low levels of genes related to carbohydrate utilization (Maldonado-Gómez *et al*., 2016). Hence, long-term microbial modulation is possible but may require a consideration of the ecological niches taken up by the initial microbiome to assess the receptivity of the resident microbiome to the inoculated species.

The challenges in modulating the adult microbiome led scientists to assess the possibility of early intervention, at a time when the microbiome is still developing, and a higher success was achieved through dietary modulation (Abecia *et al*., 2013 and 2014) or direct microbial consortia inoculation of germ-free animals (Lysons *et al*., 1976; Fonty *et al*., 1983), resulting in observable long-term effects. Thus, the dynamics of initial colonization and its long-term effects on the adult microbiome may provide a potential avenue in future attempts at microbiome modulation.

Rumen development and microbial colonization

The digestive system of ruminants is unique in that it switches from a monogastric functionality (similar to non-ruminant mammals) to becoming fully functional post weaning (Van Soest, 1994).

During the first weeks of life, when the animals are still suckling milk, the rumen is not functional: the ingested milk bypasses the rumen due to a closure of the esophageal groove by reflex action (Van Soest, 1994). The proportional weight of the rumen within the digestive tract is considerably smaller, on average 25%, compared with the mature rumen reaching 60% to 80% of the total digestive system (Krishnamoorthy and Moran, 2012). In addition, many of its functional attributes, such as the rumen wall villi which are involved in the absorption of nutritional components, are not yet developed (Van Soest, 1994).

Developmental changes of the rumen structure and physiology with age have been shown to be linked to the development of the rumen microbial population. The extensive host-bacteria crosstalk through their fermentation products in the form of VFA was shown to be instrumental to the development of the rumen papillae (Sander *et al*., 1959). This emphasizes the crucial role of the rumen microbiome has on its host development. Early research in rumen microbial colonization in calves and lambs revealed that initial colonization is done by aerobic and facultative anaerobic microbial taxa immediately after birth, which gradually decrease in abundance while being replaced by mostly anaerobic taxa (Fonty *et al*., 1987; Minato *et al*., 1992). These studies showed that some bacterial species, whose functions are associated with the mature functionality of the rumen such as cellulolytic bacteria, begin to appear immediately after birth and before weaning or exposure to plant-based feed (Fonty *et al*., 1987; Minato *et al*., 1992). More recently, culture-independent methods observed similar dynamic changes occurring in the rumen over time (Li *et al*., 2012; Jami *et al*., 2013; Rey *et al*., 2013; Kumar *et al*., 2015; Dill-McFarland *et al*., 2017). These studies revealed rapid bacterial population turnover with the community found in 1-day-old calves being vastly different from that found in 3-day-old calves (Jami *et al*., 2013). These changes are suggested to be influenced partly by the oxygen availability during this period, as the initial community is mainly composed of aerobic and facultative anaerobes, which are quickly being supplanted by anaerobic and facultative bacteria (Fonty *et al*., 1987; Minato *et al*., 1992; Jami *et al*., 2013; Rey *et al*., 2013). These results suggest that the oxidative state of the rumen is a major driver of change in the newborn rumen community. The redox potential effect on the microbiome is not limited to the early stages of development and has also been shown to be a factor affecting the adult methanogenic community as well, with different redox potential affecting the growth of specific methanogenic species isolated from the rumen (Friedman *et al*., 2016). The studies on rumen colonization further revealed that bacteria associated with mature rumen function, such as cellulose and hemicellulose degradation, can already be found 1 day after birth (Fonty *et al*., 1987; Minato *et al*., 1992; Jami *et al*., 2013). Those include the cellulolytic bacteria *Ruminococcus albus* and *Ruminococcus flavefaciens* and members of the *Prevotella* genus (Jami *et al*., 2013). Thus, the rumen is primed immediately after birth, with functional bacteria relevant to the mature rumen. The extent of initial priming with plant degrading bacteria during colonization and how they are able to thrive before the ingestion of plant material remains unclear and warrants further investigation.

One of the major changes observed throughout rumen development consists of a shift in composition within the Bacteroidetes phylum. In the mature rumen this phylum is dominated by the genus *Prevotella*, which is the overall dominant genus across many ruminants (Stevenson and Weimer, 2007; Jami and Mizrahi, 2012a; Henderson *et al*., 2015). However, during the initial stages of development the *Bacteroides* is the dominant genus within Bacteroidetes and is quickly overtaken by the *Prevotella* within the first 2 months (Li *et al*., 2012; Rey *et al*., 2013). This drastic shift in dominance was proposed to coincide with the introduction of plant-based feed, and has been observed to occur in monogastric animals, including humans (De Filippo *et al*., 2010).

The rapid changes in community composition are not limited to the bacteria and can also be found in the methanogenic archaea community (Friedman *et al*., 2017). The establishment of methanogenic communities in the rumen of calves and lambs was shown to occur close to birth (Fonty *et al*., 1987; Minato *et al*., 1992; Skillman *et al*., 2004; Friedman *et al*., 2017), with a recent study observing that methanogens could be detected as early as 20 min after birth (Guzman *et al*., 2015).

Similarly to the bacterial population, the initial methanogenic population differs greatly compared with the one in mature animals (Skillman *et al*., 2004; Friedman *et al*., 2017). Comparative analysis of the order level composition of the methanogenic community of pre-weaning calves during the first 2 months after birth and mature animals showed that

the mature rumen is composed of mostly the Methanobacteriales order and a low proportion of the newly characterized Thermoplasmata class. In contrast, within the rumen of pre-weaning calves, two additional orders could be detected: the Methanosarcinales and Methanomicrobiales (Friedman *et al.*, 2017). These compositional differences were shown to affect the nature of methanogenesis in terms of substrate utilization prevalence. Although the mature rumen is mainly composed of hydrogenotrophic methanogens, whose metabolism is driven by the reduction of CO_2 with H_2 to produce methane, the young rumen seems to have a higher prevalence of other pathways to produce methane. This include the methylotrophic pathway in which several species of methanogens metabolize methanol and methylamine compounds as a substrate for methanogenesis (Thauer *et al.*, 2008; Poulsen *et al.*, 2013). When Friedman *et al.* (2017) incubated the rumen fluid of young calves and mature cows with different methanogenesis substrate, methane production in the fluid derived from young calves increased 5- to 10-fold following the addition of methylamine or methanol; whereas, H_2 addition only slightly increased methane production. Although no information about methylamines' presence can be found regarding the developing rumen environment, choline, their main precursor, can be found in high abundance in milk-fed newborn calves; the availability of which may be responsible for the different methanogenesis characteristics in the young rumen (Hill and Mangan, 1964; Neill *et al.*, 1978; Wallace, 1979; Zeisel *et al.*, 2003; Artegoitia *et al.*, 2014). In contrast, the mature rumen fluid responded the strongest to the addition of H_2 during incubation. This suggests that environmental conditions during early stages of colonization favor alternative pathway to methane production (Friedman *et al.*, 2017).

Conclusions

Although the main goals in animal agriculture have remained the same over the years, our approach to the major challenges revolving around them has evolved along with the deeper understanding of the tripartite relationship between the environment, the host animal and its residing microbes. The recognized role of microbes in ruminant physiology predates the modern interest of what is called today 'microbiome'; however with the advent of high-throughput technologies, new data emerge at a rapid rate, each with its own methodological setup. The aim of this review is to consolidate those results and describe the recent observations that were reproduced to some degree across different studies and conducted under different setups. The recurrence of these observations has the power to steer research more accurately and successfully toward broader agricultural applications.

Such is the case for the remarkable congruence observed in several recent studies, in which similar microbial taxa and genes linked to feed efficiency or methane emission, in cattle and sheep. Although not always converging, both phenotypes relate to energy conversion from the feed. The acrylate pathway, which converts lactate to propionate, was highly enriched in both efficient cows and low methane emitting sheep and cows. These results highlight the central role of specific microbial pathway and the associated taxa could have on improving yield and developing a more environmental friendly livestock farming.

To this day, long-term, stable attempts at modulating the rumen microbiome have proven unsuccessful due mostly to the resilience of the rumen microbiome once established. However, the recent success in stably introducing a specific bacterial species in the gut of humans suggests that targeted introduction and modulation of the microbiome is possible. Whether stable, targeted microbial inoculation can be applied in the rumen environment remains to be tested, but considering that similar ecological principles apply to the microbiome of different environment such as microbial colonization (Costello *et al.*, 2012; Yatsunenko *et al.*, 2012; Jami *et al.*, 2013), this latest development may open a pathway to a more targeted approach by considering the ecological features of the resident microbiome.

Understanding the ecology of the rumen unique microbial system may open new avenues for the optimization of livestock agriculture, toward increased agricultural productivity, together with mitigation of its negative impact on the environment.

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Declaration of interest

None.

Ethics statement

All ethical standards have been met.

Software and data repository resources

None.

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