Animal (2020), **14:S1,** pp s78–s86 © The Animal Consortium 2020
doi:10.1017/S1751731119003252

animal

Review: Ruminal microbiome and microbial metabolome: effects of diet and ruminant host

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(Received 4 June 2019; Accepted 18 November 2019)

The rumen contains a great diversity of prokaryotic and eukaryotic microorganisms that allow the ruminant to utilize ligno-cellulose material and to convert non-protein nitrogen into microbial protein to obtain energy and amino acids. However, rumen fermentation also has potential deleterious consequences associated with the emissions of greenhouse gases, excessive nitrogen excreted in manure and may also adversely influence the nutritional value of ruminant products. While several strategies for optimizing the energy and nitrogen use by ruminants have been suggested, a better understanding of the key microorganisms involved and their activities is essential to manipulate rumen processes successfully. Diet is the most obvious factor influencing the rumen microbiome and fermentation. Among dietary interventions, the ban of antimicrobial growth promoters in animal production systems has led to an increasing interest in the use of plant extracts to manipulate the rumen. Plant extracts (e.g. saponins, polyphenol compounds, essential oils) have shown potential to decrease methane emissions and improve the efficiency of nitrogen utilization; however, there are limitations such as inconsistency, transient and adverse effects for their use as feed additives for ruminants. It has been proved that the host animal may also influence the rumen microbial population both as a heritable trait and through the effect of early-life nutrition on microbial population structure and function in adult ruminants. Recent developments have allowed phylogenetic information to be upscaled to metabolic information; however, research effort on cultivation of microorganisms for an in-depth study and characterization is needed. The introduction and integration of metagenomic, transcriptomic, proteomic and metabolomic techniques is offering the greatest potential of reaching a truly systems-level understanding of the rumen; studies have been focused on the prokaryotic population and a broader approach needs to be considered.

Keywords: diet, fermentation, genetics, rumen, ruminant

Implications

The microbial community in the rumen is one of the most diverse gut ecosystems yet described in the animal kingdom. An increased understanding of this complex microbiome, the dietary factors that affect it, the influence of the host on the rumen microbiome and the effect of rumen fermentation on the host should allow us to develop approaches that maximize the conversion of fibrous feedstuffs produced on land not suitable for primary cropping into human-edible food while minimizing the environmental consequences of ruminant agriculture.

Introduction

The anatomically distinct forestomaches of the rumen, reticulum and omasum sit before the true stomach (abomasum) in the digestive tract of ruminants. The presence of a symbiotic microbial population in the rumen and reticulum (hereafter referred to as the rumen) allows ruminants to utilize ligno-cellulose material and to convert non-protein nitrogen into microbial protein. Because of this, ruminants, when used to transform fibrous feedstuffs produced on land not suitable for primary cropping, can be net contributors to the global supply of human-edible food and make a major contribution to the sustainability of the global food system (Schader *et al.*, 2015). However, while microbial fermentation in the rumen plays a central role in the ability of ruminants to utilize fibrous substrates, rumen fermentation also has potential deleterious consequences in particular associated with the emissions of greenhouse gases, excessive nitrogen excreted in manure and may also adversely influence the nutritional value of ruminant products (Scollan *et al.*, 2011). Given the wide range of consequences of rumen fermentation on the nutrition and metabolism of ruminants, it is perhaps not surprising that significant research effort has been exerted both to

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understand the microbial population in the rumen and ultimately to manipulate it to maximize productivity while decreasing the environmental load of ruminant agriculture.

Rumen fermentation

As noted above, the ability of ruminants to utilize cellulolytic and hemicellulolytic feedstuff distinguishes them from monogastric farm animals. Degradation of plant material in the rumen requires colonization of ingested plant material by a complex microbial consortium and occurs in a time-dependent manner that is influenced by the nature of the substrate ingested (Elliott *et al.*, 2018). The resulting consortia functions synergistically to degrade the substrate, with cross feeding between microbes such that the rate and extent of degradation is greater than that could be accomplished by a microbial monoculture (Krause *et al.*, 2013). The anaerobic nature of the rumen dictates that degradation of substrates is incomplete and the end products of fermentation are volatile fatty acids (**VFAs**; predominantly acetate, propionate and butyrate), in addition to CO_2. On occasions, and depending on substrate, intermediate products of fermentation such as lactic acid may also accumulate.

During rumen fermentation, the NAD+ reduced to NADH must be reoxidized to allow fermentation to continue. In the anaerobic conditions of the rumen, NAD+ must be regenerated by electron transfer to acceptors other than oxygen and the major sink is the reduction of CO_2 to CH_4 (other sinks include sulphate, nitrate and fumarate; Morgavi *et al.*, 2010). It has been suggested that the inhibition of methane production without the provision of alternative pathways for the disposal of hydrogen would disrupt rumen function (Morgavi *et al.*, 2010). However, some studies have suggested that in sheep, goats and cattle, methane production can be significantly decreased with little effect on rumen fibre degradation and diet digestibility (Martinez-Fernandez *et al.*, 2016). Clearly, as noted by Ungerfeld (2015), there is a need to more fully understand the effect of different hydrogen sinks on both methane production and rumen function.

Dietary protein entering the rumen is broken down rapidly via peptides and amino acids, resulting in ammonia formation and subsequent loss of N from the animal (Walker *et al.*, 2005). The resultant low efficiency of nitrogen retention represents a financial loss (as more dietary protein must be fed), and in extreme cases excess rumen ammonia concentrations can lead to metabolic stress in the animal, while excess N excretion in manure can cause environmental damage (Walker *et al.*, 2005).

The rumen microbiome

The microbial community in the rumen is one of the most diverse gut ecosystems yet described in the animal kingdom (Weimer, 2015), composed of not only bacteria (10^{10} to 10^{11} organisms/ml) but also archaea (10^8 to 10^9 organisms/ml), protozoa (10^5 to 10^6 organisms/ml), fungi (10^3 to 10^4 organisms/ml) and an as yet largely uncharacterized virome.

Bacteria

Traditionally, microbiologists relied on culture-based methods, largely based on the original work by Hungate and colleagues (Krause *et al.*, 2013) to isolate members of the rumen bacterial community. It was thought that these cultivation techniques had enabled researchers to describe the circa 200 most abundant and diverse bacteria in the rumen ecosystem. Indeed, the results obtained from amplicon sequencing of the 16s rRNA gene via next generation sequencing have largely agreed with this at phylum level and have allowed studies to be discussed based on the known activity of cultured bacteria (Wilkinson *et al.*, 2018).

However, Stewart *et al.* (2018) described 913 novel microbial genomes assembled from metagenomic sequencing of the rumen of 42 cattle, and the same authors have recently extended this work assembling over 4900 novel microbial genomes from the rumen of 282 cattle (Stewart *et al.*, 2019). As a result, several initiatives are underway to improve our ability to culture rumen microorganisms. A collaborative activity between a wide range of research organizations, the Hungate 1000 project, has produced 501 genomes (480 bacteria and 21 archaea) from rumen microbes (Seshadri *et al.*, 2018) with access to bacterial cultures available via the project website (http://www.rmgnetwork.org/hungate1000.html). The collection encompasses 75% of genus-level taxa reported from the rumen and has allowed the assignment of individual microbes to the major metabolic pathways involved in rumen function (Wilkinson *et al.*, 2018). However, according to Stewart *et al.* (2019), the Hungate collection represents only a fraction of the diversity present in their novel microbial genomes assembled from metagenomic sequencing. Clearly, it is vital that more of these strains are brought into culture, so we can study their function *in vitro* and *in vivo*, and gain mechanistic insight into the structure and function of the rumen microbiome.

Protozoa

With their striking appearance, rumen protozoa are assumed to be important for the welfare of their host. However, even though protozoa can contribute up to 50% of the biomass in the rumen, the role of protozoa in the rumen microbial ecosystem remains unclear (Newbold *et al.*, 2015). Most protozoa in the rumen are ciliates, with a few flagellate species; ruminants commonly harbour distinct protozoal populations from birth and typically, this does not change through life (Williams and Coleman, 1992). Protozoal identification and taxonomy have usually relied on morphologic identification by optical microscopy (Newbold *et al.*, 2015). Recently, sequencing of 18S rRNA genes has help to both clarify the phylogeny of the rumen ciliates and reveal an apparent higher diversity of ciliates than estimated by conventional morphological methods (Moon-van der Staay *et al.*, 2014; Kittelmann *et al.*, 2015). However, it has been suggested that copy

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number variation in ribosomal RNA genes across the different genera may have limited the use of 18S rRNA amplicon sequencing in ecological studies (Newbold *et al.*, 2015).

Despite repeated attempts, it has proven impossible to maintain rumen protozoa in axenic culture (Newbold *et al.*, 2015). Thus, most studies have concentrated on describing the activity of mixed bacterial and protozoal co-cultures, maintained either in *in vitro* or in *in vivo* (Williams and Coleman, 1992). Thus, while much progress has been made in describing the role of protozoa in the rumen (Williams and Coleman, 1992), it has been difficult to establish conclusively that activity is due to protozoa as opposed to associated bacteria. Techniques to clone and express ciliate genes in phages have allowed genes from a range of rumen protozoa to be characterized (McEwan *et al.*, 1999; Newbold *et al.*, 2005; Belzecki *et al.*, 2007). As a result, a wide range of fibrolytic enzymes have been identified suggesting a highly evolved fibrolytic capacity in the rumen ciliates (Devillard *et al.*, 1999 and 2003; Takenaka *et al.*, 2004; Wereszka *et al.*, 2004; Bera-Maillet *et al.*, 2005). Recently, a draft macronuclear genome sequence from the rumen ciliate *Entodinium caudatum* has been released, promising a greater understanding of protozoal metabolism in the rumen (Park *et al.*, 2018).

Protozoa can be removed from the rumen through a process known as defaunation, and the animal will still survive (Williams and Coleman, 1992; Newbold *et al.*, 2015). A recent meta-analysis suggested that the absence of protozoa caused a decrease in organic matter degradation, suggesting an important functional role in the rumen (Newbold *et al.*, 2015). Furthermore, defaunation increased microbial protein outflow from the rumen and decreased methane production. These observations are consistent with the evidence that ciliates survive by digesting rumen bacteria, thus playing an important role in the inefficient use of dietary protein by ruminants and that protozoa are indirectly involved in methane production, as they harbour an active population of methanogenic archaea both on their external and internal surfaces (Morgavi *et al.*, 2010). A recent meta-analysis exploring time-dependent effects of removing protozoa (Li *et al.*, 2018) concluded that subsequent increases in methanogens, fungi and cellulolytic bacteria counteracted defaunation-induced effects on rumen fermentation, suggesting that defaunation might not always lead to lower levels of methane production. Protozoa also seem to stabilize rumen fermentation increasing rumen pH (Williams and Coleman, 1992), possibly because protozoa consume lactate more rapidly than bacteria (Newbold *et al.*, 1986). While clearly more long-term studies on the effects of defaunation on rumen microbiota and fermentation are needed, defaunation may not be an appropriate model to study the role of protozoa in the rumen.

Fungi

There is some debate about the contribution of the anaerobic fungi to the microbial biomass in the rumen. While the flagellated zoospores are clearly visible in rumen fluid, the vegetative growth of the rhizoids on and in plant material is less obvious. Chitin measurements and rRNA transcript abundance (Huws *et al.*, 2018) indicate that anaerobic fungi represent 10% to 20% of the rumen microbiome and they are thought to be crucial fibre degraders, especially when forages with poor quality are fed to ruminants (Krause *et al.*, 2013). Like the protozoal population, the close association of rumen fungi with methanogenic archaea (Edwards *et al.*, 2017) is thought to both enhance fungal activity and contribute to methane production. The taxonomy of the rumen fungi remains a subject of considerable debate; six genera are commonly recognized: the monocentric *Neocallimastix*, *Caecomyces* and *Piromyces* and the polycentric *Anaeromyces, Orpinomyces* and *Cyllamyces*. However, further genera are likely to exist and continue to be described (Edwards *et al.*, 2017). As with other areas, the use of molecular techniques, including the use of internal transcribed spacer 1 region and large subunit rRNA as taxonomic marker, and several genomes and transcriptomes have been reported from rumen fungi (Edwards *et al.*, 2017).

Archaea

Archaea make up 0.3% to 3% of the rumen microbiome (Janssen and Kirs, 2008) with most, although possibly not all being methanogenic. In most studies reported to date, the most abundant methanogens are *Methanobrevibacter*. *Methanobrevibacter* are hydrogenotrophic producing methane from H_2, CO_2 and formate produced by the protozoa, bacteria and fungi (Janssen and Kirs, 2008). Other significant hydrogenotrophic genera include *Methanosphaera, Methanimicrococcus* and *Methanobacterium* (Morgavi *et al.*, 2010). Less abundant are methylotrophs (*Methanosarcinales, Methanosphaera, Methanomassiliicoccaceae*), producing methane from methylamines, and methanol and aceticlastic archaea (*Methanosarcinales*), producing methane from acetate (Morgavi *et al.*, 2010). The diversity of archaea is less than that of the bacterial population but, as with bacteria, they are subject to significant effort to isolate and characterize new species, with 21 archaea from rumen recently becoming available via the Hungate 1000 (Seshadri *et al.*, 2018).

What remains unclear is the relationship between archaeal numbers and methane production. Wallace *et al.* (2014) suggested a direct correlation between archaeal abundance and methane production, while Danielsson *et al.* (2017) found that rumen methane production correlated with both rumen methanogenic and bacterial community structure. Most likely, rumen methanogenesis is a product of both rumen fermentation, and thus H_2 supply, and archaeal numbers (Belanche *et al.*, 2015). As noted above, protozoa harbour an active archaeal population on both their inner and outer surfaces. It is apparent that this archaeal population differs from the free-living population (Tymensen *et al.*, 2012) and may indeed vary between protozoal genera (Belanche *et al.*, 2014), with important consequences in terms of the relative role of different protozoal genera in overall methane production (Belanche *et al.*, 2015).

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Virome

The rumen virome remains by far the poorest characterized part of the rumen microbiome. Lytic phages have been isolated from the rumen and studies on their diversity have been reported (Gilbert and Klieve, 2015), including evidence to suggest that energy intake may be a major driver of the rumen virome (Anderson *et al.*, 2017). However, only recently has genome sequence of lytic phages been reported (Gilbert *et al.*, 2017), and metagenomic studies on the rumen virome are starting to appear (Namonyo *et al.*, 2018), suggesting that we may soon have a greater understanding of viral-mediated processes in the rumen. Evidence of the presence of RNA-based viruses that infect fungi (mycoviruses) has been recently published (Hitch *et al.*, 2019); however, their impact on rumen fungal populations and fibre degradation need to be further investigated.

Factors that influence the rumen microbiome

Diet

A recent global comparison study of the rumen microbiome in 742 samples from 32 animal species in 35 countries concluded that, while a common core of bacteria and archaea dominated in nearly all samples, differences in microbial community compositions were predominantly attributable to diet (Henderson *et al.*, 2015). Among dietary interventions, we can distinguish between those aimed at improving forage quality and changing the proportion of the diet, and those aimed at using feed additives to supplement the diet.

Molecular techniques based on either amplicon sequencing of ribosomal genes or whole metagenome sequencing (Huws *et al.*, 2018) are increasingly allowing us to explore both the temporal and spatial development of microbial populations within the rumen that are related to the colonization and degradation of dietary fibre entering the rumen (Elliott *et al.*, 2018). We have shown that shifts in the carbohydrate and protein content of diets consumed (Belanche *et al.*, 2012) and less obvious changes, such as the method of forage preservation and type of forage (Huws *et al.*, 2018), affect feed colonization by rumen microbes and subsequent digestion. However, there is a need to ensure both that studies consider the whole microbiome and not just the bacteriome and that changes in the composition of the microbiome are linked to changes in fermentation and host metabolism.

Nutritional strategies can affect the interactions between microbial groups and their effect on production and product quality. Perhaps the area that best illustrates this point is the quality of ruminant-derived products in terms of fatty acid profile. Fatty acid supplementation can affect microbial structure and fatty acid biohydrogenation in the rumen and thus influence the fatty acids available for absorption and appearance in meat and milk, with the effects apparently influenced by the source of rumen fluid, sheep *v.* cattle (Carreño *et al.*, 2019). It is known that bacteria are largely responsible for the biohydrogenation of fatty acids in the rumen while protozoa are not thought to be actively involved in biohydrogenation (Lourenço *et al.*, 2010). However, protozoa do affect the composition of the bacterial population in the rumen and thus potentially biohydrogenation (Newbold *et al.*, 2015). In addition, protozoa directly incorporate unsaturated fatty acids protecting them in the rumen from biohydrogenation and allowing direct transfer into milk and meat (Lourenço *et al.*, 2010) illustrating some of the differing levels at which microbial interactions might affect product quality.

Given the effect of diet on the rumen microbiome, it is perhaps not surprising that a wide range of dietary additives have been used to manipulate rumen fermentation (Figure 1). The main targets for rumen manipulation can be summarized as:

- Increased microbial degradation of fibre: increasing the yield of VFAs that can be absorbed by the host and increasing intake in forage fed animals.
- Decreased protein degradation and ammonia production in the rumen: reducing the financial and environmental cost of inefficient dietary protein utilization in ruminants.
- Optimizing VFAs production: ensuring that the pattern of VFAs production matches the production requirements of the host.
- Improved animal health: preventing the accumulation of harmful intermediates of fermentation in the rumen and maximizing the degradation of dietary toxins.
- Decreased greenhouse gas production: decreasing the production of greenhouse gases from ruminant agriculture has been and remains a major challenge to the ruminant sector.
- Improved human health: improving the nutritional composition of ruminant products, predominantly lipid and fatty acid content/composition and preventing pathogen transfer in the food supply chain.

Newbold (2017) summarized the potential benefits and limitations of a range of dietary additives. However, in response to the EU legislation to the ban of antimicrobial growth promoters in animal production systems, we have become increasingly interested in the use of plant extracts to manipulate rumen fermentation, boost animal production and decrease greenhouse gas emissions.

Saponins have shown potential as antiprotozoal agents to ultimately increase microbial supply to the host and decrease methane production (Newbold *et al.*, 2015). This effect has been reported to be transitory due to the deglycosylation of saponins to sapogenins by rumen bacteria (Wallace *et al.*, 2002). We have recently shown that the antiprotozoal effect of derivatives from hederoside B, the major saponin in ivy fruit, differed depending on the composition and linkage of the substituent to the sapogenin (Ramos-Morales *et al.*, 2017). Furthermore, our most recent results show that antiprotozoal activity is not an inherent feature of all saponins and that small variations in the structure of a compound can have a significant influence on their biological activity (Ramos-Morales *et al.*, 2019a).

Polyphenolic compounds such as tannins and flavonoids have also been shown to reduce methane production in the rumen. We have recently shown that an isoflavonoid-rich extract from liquorice decreased ammonia production and methane, effects that were attributed to decreases in protozoa numbers and bacteria diversity, as well as changes in the

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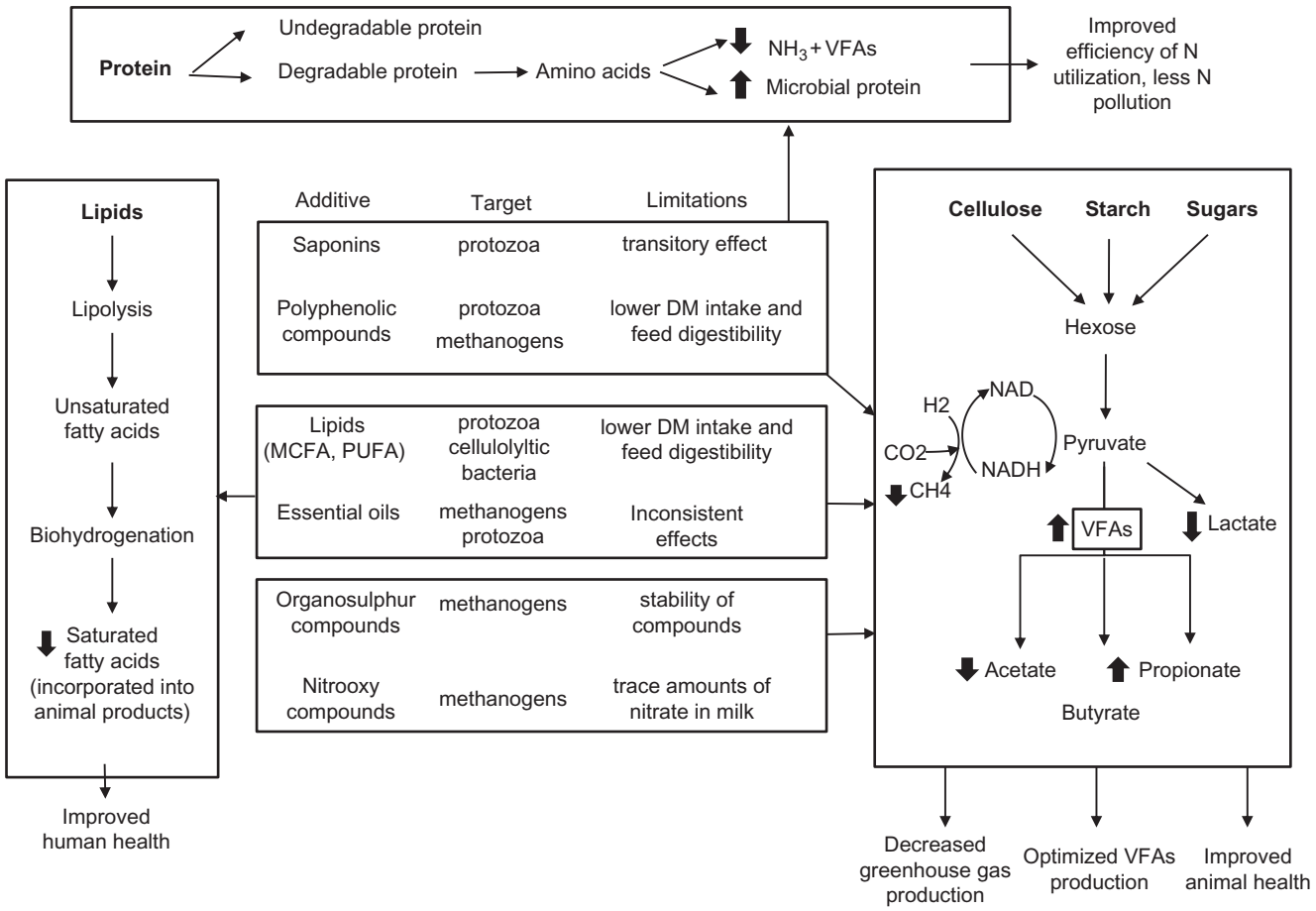

Figure 1 Potential effects of dietary additives on the rumen microbiome and fermentation and limitations of their use in animal feeding. MCFA=medium chain fatty acids; PUFA=polyunsaturated fatty acids; VFA=volatile fatty acids.

structure of bacteria and archaea (Ramos-Morales *et al.*, 2018). When nine compounds were synthetized from the natural alkaloid haemanthamine and tested *in vitro* for their effects on rumen protozoa and fermentation parameters, results showed that simple esterifications of haemanthamine or its derivative dihydrohaemanthamine with acetate, butyrate, pivalate or hexanoate led to compounds that differed in their effects on rumen fermentation (Ramos-Morales *et al.*, 2019b). It is clear then that understanding the degree to which structural features in a compound may affect the biological activity of a plant extract is essential. The effect of plant extracts on rumen fermentation has been reported to be highly variable (Newbold, 2017) but given that growth stage, harvest and storage conditions can all alter the structure of bioactive molecules in plants, it is questionable if studies are comparing like with like.

Host effects

Experiments involving near total exchange of the rumen contents between animals have shown that the individual animal has strong effect on the re-establishment of the rumen microbial community (Weimer, 2015; Zhou *et al.*, 2018), suggesting that the host animal has a strong effect on the rumen microbial population.

Evidence that the host might influence the rumen microbial population is mounting (Huws *et al.*, 2018). Sasson *et al.* (2017) suggested that several bacterial operational taxonomic units were highly heritable in dairy cattle. Roehe *et al.* (2016) ranked beef cattle based on relative archaeal abundance and reported this remained consistent, suggesting that archaeal abundance in ruminal digesta is under host genetic control. However, Difford *et al.* (2018) suggested that while the abundance of some bacteria and archaea taxa were influenced by the host's genotype, host genetics influencing the rumen microbiome and methane production were largely independent. The mechanisms by which the host might control the rumen microbial population remain unknown, but factors such as modifying the gene expression of the rumen epithelium and possible variation in rumen outflow or volume have been suggested (Huws *et al.*, 2018).

With evidence of the apparent heritability of host effects on the rumen microbiome, there has been an explosion in studies relating the rumen microbial population to animal phenotype and production effects (Huws *et al.*, 2018). Such studies have considered both microbial abundance and gene abundance and/or expression (Huws *et al.*, 2018). However, the extent to which such relationships are causal rather than casual remains undetermined.

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Early life

In addition to heritable host factors, we have also investigated the possible role of early-life factors on the establishment of the rumen microbiome in adult animals. The rumen microbial population establishes in a defined and progressive sequence (Yáñez-Ruiz *et al.*, 2015). Bacteria and archaea have been reported as being present in the underdeveloped rumen of lambs prior to the ingestion of solid feed, with counts like those recorded in adult animals seen around 10 days after birth (Yáñez-Ruiz *et al.*, 2015). The rumen eukaryotes seem to establish later with anaerobic fungi appearing by day 8 to 10 (Fonty *et al.*, 1987), while under farm conditions ciliate protozoa appeared by 15 days of age in sheep and 29 to 46 days in cattle (Naga *et al.*, 1969; Fonty *et al.*, 1988). In both sheep and cattle, small protozoa established first, and Holotrich protozoa last, with mixed population of ciliate protozoa typical of the adult animals apparent by 80 to 150 days of age in cattle (Naga *et al.*, 1969). In non-ruminant species, it is accepted that the coexistence of the host and microbial gut communities is immunologically driven (Yáñez-Ruiz *et al.*, 2015). In general, the immune response in the mucosal areas of the gut is orchestrated by mucosal-associated lymphoid tissue and gut-associated lymphoid tissue in the gut. However, in the rumen no organized lymphoid tissue exists in the epithelium, and it has been suggested that saliva seems to be the main vehicle of introducing immunoglobulins into the rumen (Yáñez-Ruiz *et al.*, 2015).

Weaning conditions have a major effect on colonization of the rumen, with the presence of the dam promoting inoculation of microbes in the digestive tract of the naturally raised newborns as compared to those fed milk replacers and kept isolated from adult animals (Abecia *et al.*, 2017). Another distinctive feature between natural and artificial systems is the near absence of protozoa in the rumen of artificially reared animals, as protozoa can only be inoculated in the rumen by direct contact with adult animals through saliva (Williams and Coleman, 1992). Inoculation of lambs after birth with rumen fluid from adult sheep improved rumen fermentation parameters and increased protozoal numbers (De Barbieri *et al.*, 2015). Similarly, lambs kept in isolation from birth had positive changes in rumen fermentation following inoculation with rumen fluid from adult sheep when the rumen was functional at 15 weeks of life (Morgavi *et al.*, 2015). Recently, we investigated how maternal *v.* artificial rearing shapes the rumen microbiota in lambs (Belanche A., unpublished data). Differences in the rumen bacterial and methanogens communities disappear later in life when all lambs were grouped on the same pasture up to 23 weeks of age. However, lambs naturally reared on the ewe retained several long-lasting microbiological features in the eukaryotic community such as higher fungal diversity and differences in the protozoal population as well as higher feed digestibility during the grazing period.

Yáñez-Ruiz *et al.* (2010) found that feeding a hay concentrate diet compared to hay alone to lambs led to a difference in both the bacterial and archaeal population at weaning and that the effect persisted over 4 months after the end of the treatment. Abecia *et al.* (2014) reported that dosing kids and their does with bromochloromethane during the weaning period modified the archaeal community and, although not all the effects persisted after weaning, some less abundant archaeal groups remained different in treated and control groups 4 months after the treatment stopped. We have recently found that in dairy cattle, yeast fed from day 0 to 60 influenced the evenness and the diversity of the rumen bacterial but not archaeal population at weaning (Newbold C.J., unpublished data). Proteobacteria numbers were also lower in yeast fed animals at weaning but the treatment had no effect on the archaeal population. These effects seemed to persist over the length of the trial (32 months) with a more complex population developing in yeast-supplemented animals (Newbold C.J., unpublished data, Figure 2), while proteobacteria numbers remained lower in yeast-supplemented

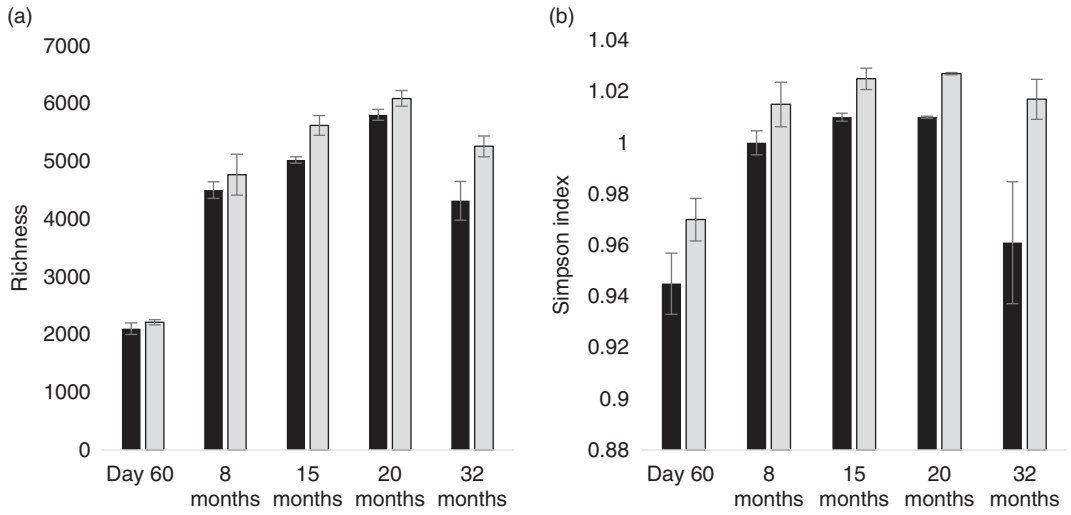

Figure 2 Effect of the addition of live yeast to the diet of cattle from birth to 60 days after birth (weaning) on bacterial diversity ((a) richness and (b) Simpson index) in the rumen at 60 days and 8, 15, 20 and 32 months after birth. Black and grey bars represent the control and yeast treatment, respectively.

https://doi.org/10.1017/S1751731119003252 Published online by Cambridge University Press

animals. However, the effects were small, and no effects were observed on rumen fermentation parameters, blood chemistry, weight gain or the eventual milk production of the cattle.

These findings suggest that the early-life intervention determines initial microbial community and thus fermentation parameters, but the persistency of these effects later in life is weak suggesting that post-weaning factors have a greater influence on adult communities and production outcomes.

The rumen metabolome

With the increasing ability to describe the rumen microbiome through both amplicon sequencing of ribosomal genes and metagenomic sequencing, there has been a growing interest in linking changes in the rumen microbiome to changes in the fermentation and metabolites in the rumen. We, and many other authors, have used coordination plots to link changes in the rumen microbiome to changes in common rumen metabolites (Belanche *et al.*, 2016). We have provided correlations between the abundance of microbial phyla and genera and specific rumen metabolites (Belanche *et al.*, 2019), in an attempt to provide a functional context to changes in the rumen microbial population; however, in general, these approaches have been limited to a small range of well-defined rumen metabolites. Metabolomic techniques to describe a potentially wider range of metabolites have been used to study the link between gut microbiomes and the metabolome in several gut ecosystems (Dougal *et al.*, 2012; Yan *et al.*, 2017). In rumen-based studies, metabolomics has been used to investigate the effect of diet (O'Callaghan *et al.*, 2018; Yang *et al.*, 2018) to link the host genotype to efficient phenotypes in growing cattle (Artegoitia *et al.*, 2017); the main aim has been to help to elucidate the effects of early-life nutritional interventions on rumen function (Abecia *et al.*, 2018) and to understand the effect of plant extracts on rumen function (Wang *et al.*, 2019). While metabolomics provides a route to achieving a link between taxonomic-based studies and metabolic function, the current techniques are difficult to compare between studies with both extraction technique (Ribeiro de Almeida *et al.*, 2018) and analysis technique (Goldansaz *et al.*, 2017) contributing to differences between studies.

Future look

While the introduction of molecular techniques and next generation amplicon sequencing has undoubtedly increased our knowledge of the rumen microbiome, there is a danger that it has encouraged the cataloguing of rumen microbial populations rather than an understanding of their function. Recent developments that allow phylogenetic information to be upscaled to metabolic information (Wilkinson *et al.*, 2018) are clearly an important development in this area and will require an increased focus and revival in culture-based techniques to allow rumen microbes to be isolated and characterized. However, it is perhaps the introduction and integration of metagenomic, transcriptomic, proteomic and metabolomic techniques that offer the greatest potential of reaching a truly systems-level understanding of the rumen (Huws *et al.*, 2018). Recent studies in which amplicon sequencing has been combined with metaproteomic and metabolomic analysis have established that combining techniques allows a deeper insight than previously possible into the complex network of microbial adaptation in the rumen (Deusch *et al.*, 2017). However, in applying these techniques, it will be important to consider a whole microbiome approach, as many of the current studies focus only on the bacteriome and archaeal population and largely ignore the eukaryote population. True understanding of the rumen ecosystem will only be achieved by considering all aspects of the microbiome.

Acknowledgements

An abstract of the current review has been published in Advances in Animal Biosciences (Newbold CJ and Ramos-Morales E 2019. Ruminal microbiome and microbial metabolome: effects of diet and host. Advances in Animal Biosciences 10, 387).

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Declaration of interest

None.

Ethics statement

None.

Software and data repository resources

None.

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