

Microbiome of the rumen

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Summary

The rumen plays a central role in the ability of ruminants to digest cellulose and thereby produce human-edible food from resources that would otherwise not be available for our consumption. Significant progress has been made to culture and characterize bacteria and archaea from the rumen, and molecular techniques allow quantitative and qualitative studies on microbial populations. Up to now, rumen protozoa cannot be maintained in axenic culture, but techniques to clone and express ciliate genes in phages have allowed genes from a range of rumen protozoa to be characterized. However, ultimately, we are still ignorant about the roles and activities of much of the rumen microbial ecosystem. The animal's diet is the most obvious factor influencing the rumen microbiome. Due to their antimicrobial activities, plant extracts and plant secondary compounds (e.g. saponins, essential oils and polyphenolic compounds) have shown potential to manipulate rumen fermentation in terms of decreasing greenhouse gas emissions and improving the efficiency of nitrogen utilization. In addition, the host itself might 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. Enhanced studies that consider not only which microbes are present in the rumen but also the functional genes present (metagenomics) and their expression in the rumen (metatranscriptomics) promise to provide new insights into rumen function and how it might be manipulated to the benefit of mankind.

Zusammenfassung

Das Mikrobiom des Pansens

Der Pansen spielt eine zentrale Rolle bei der Umwandlung von nicht direkt für den Menschen zur Ernährung nutzbarer pflanzlicher Ressourcen, speziell der Cellulose. In den letzten Jahren wurden erhebliche Fortschritte bei der Kultivierung und Charakterisierung von Bakterien und Archaeen aus dem Pansen erzielt, und molekulare Methoden ermöglichen sowohl quantitative als auch qualitative Untersuchungen des Pansenmikrobioms. Obwohl bis heute die Anzucht von Protozoen aus dem Pansen in Reinkultur nicht möglich ist, ist es über Klonierung und Genexpression in Phagen gelungen, die Gene einer Reihe ciliärer Protozoen zu charakterisieren. Dennoch ist unser Wissen über die Bedeutung und die Stoffwechselaktivität des Pansenmikrobioms nach wie vor rudimentär. Das Mikrobiom des Pansens wird in erster Linie durch die von Wiederkäuern aufgenommene Nahrung beeinflusst. Aufgrund ihrer antimikrobiellen Wirkung haben Pflanzenextrakte und sekundäre Pflanzenstoffe wie Saponine, ätherische Öle und Polyphenole das Potenzial, über geänderte Fermentationsprozesse im Pansen die Emission klimawirksamer Gase zu verringern oder die Effizienz der Stickstoffnutzung zu steigern. Darüber hinaus beeinflusst sehr wahrscheinlich das Tier selbst das Mikrobiom des Pansens, sowohl über Vererbung als auch über den Einfluss der Ernährung während der ersten Lebenszeit auf die Struktur und Funktion mikrobieller Populationen im Pansen der adulten Tiere. Erweiterte Studien, die nicht nur die Art der Mikroben, sondern auch die funktionellen Gene (Metagenomik) und ihre Expression (Metatranskriptomik) im Pansen berücksichtigen, sind vielversprechende Ansätze zur Gewinnung neuer Erkenntnisse über die Bedeutung des Pansens und die Möglichkeit, sein Mikrobiom zum Nutzen der Menschheit zu verändern.

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Introduction

The forestomachs of ruminant animals contain a great diversity of prokaryotic (bacteria, archaea and viruses) and eukaryotic (protozoa and fungi) microorganisms that together breakdown and ferment the feed into products such as volatile fatty acids (VFA) and synthesize microbial protein which represent the main supply of energy and protein for the host animal, respectively (Dehority 2003). The rumen represents a unique evolutionary advantage for ruminant animals. Symbiotic microbes allow ruminants to utilize ligno-cellulose material and to convert non-protein nitrogen into microbial protein. 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). Whilst microbial fermentation in the rumen plays a central role in the ability of ruminants to utilize fibrous substrates, rumen fermentation also has potential deleterious environmental consequences (increased greenhouse gas, mainly CO₂ and methane, and other pollutant emissions) and may influence the nutritional value of ruminant products (higher levels of saturated fat and potential pathogen transfer) (Scollan et al. 2011). Besides, the conversion of dietary protein is rather inefficiently with 71% of the nitrogen uptake excreted in manure instead of being retained in tissues or milk (fig. 1). Given the importance of the rumen fermentation, it is perhaps not surprising that a great deal of effort has been devoted to investigating methods for manipulating this complex ecosystem (Newbold 2017, Newbold et al. 2017).

Describing the rumen microbiome

Traditional rumen microbiology studies have relied on our ability to culture and characterise microorganisms from the rumen (Hobson & Stewart 1997). Whilst significant progress has been made using these techniques over the years, it is recognised that only a relatively small proportion of the microbes within the rumen are recovered leaving us ignorant about the roles and activities of the clear majority of the rumen microbial ecosystem (Hungate 1966, Creevey et al. 2014). Molecular techniques allow both

quantitative and qualitative studies on microbial populations in the rumen to be carried out. Ribosomal genes have been used to describe how specific microbial groups respond quantitatively and qualitatively to changes induced by manipulation, through the characterization of 18/16S rRNA gene pools using massively parallel amplicon sequencing (Pinloche et al. 2013, de la Fuente et al. 2014, Belanche et al. 2016a,b, 2017). However, increasingly studies are expanding to consider not only which microbes are present in the rumen but also the functional genes present, their expression and how this might ultimately allow an increased understanding of the role of the rumen in the health and wellbeing of man and animals. Progress in this area has been rapid but is currently limited by our ability to culture and characterize major components of the rumen microbiome.

Bacteria and archaea

Significant efforts, including the Hungate 1000 project, are underway to improve our ability to culture rumen microbes including the use of metagenomic information to identify nutrient requirements of specific organisms. A collaborative activity between a wide range of research organisations, the Hungate 1000 project, has generated 501 genomes (480 bacteria and 21 archaea) from rumen microbes (Seshadri et al. 2018) with access to bacterial cultures available via the project web site (<http://www.rmgnetwork.org/hungate1000.html>). The Hungate 1000 collection encompasses 75% of genus-level taxa

Table 1. Bacterial and archaeal(*) phyla distribution of the 501 Hungate catalogue genomes; bacteria isolated from the rumens of a diverse group of animals worldwide. – Seshadri et al. (2018), CC BY 4.0.

Phylum	Number of cultures
Actinobacteria	33
Bacteroidetes	64
Euryarchaeota*	21
Fibrobacteres	2
Firmicutes	341
Fusobacteria	1
Proteobacteria	32
Spirochaetes	6
Synergistetes	2

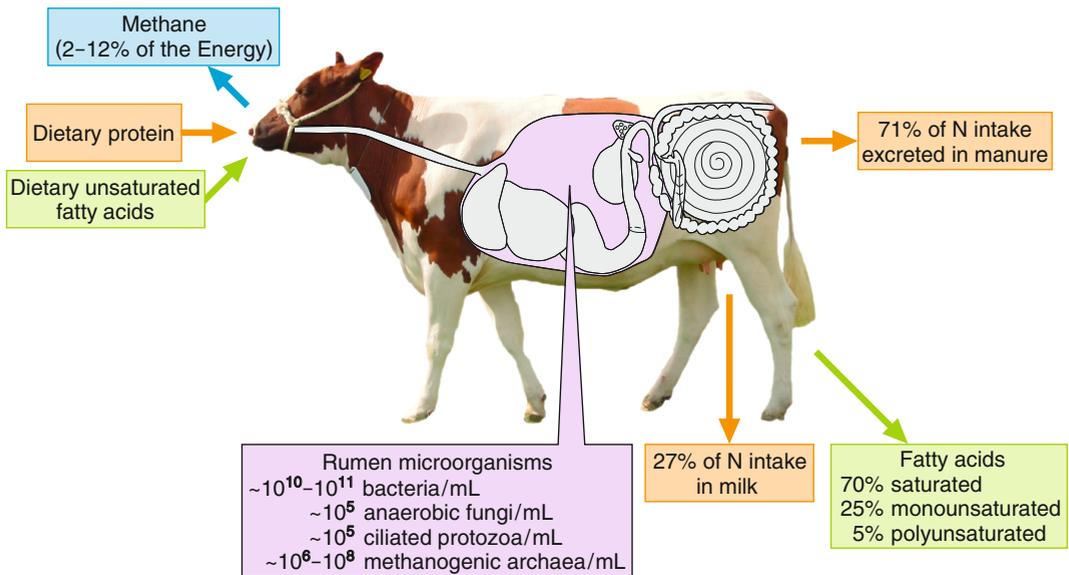


Fig. 1. Main metabolic processes associated with rumen microorganisms.

reported from the rumen and has allowed assignment of individual microbes to the major metabolic pathways involved in rumen function. Members of the Firmicutes and Bacteroidetes phyla contribute most of the Hungate genome sequences in the rumen (68% and 12.8%, respectively), with the Lachnospiraceae family making up the largest single group (32.3%). Archaea are mainly from the *Methanobrevibacter* genus or are in the Methanomassiliicoccales order (table 1). In addition, Stewart et al. (2018) described 913 novel microbial genomes assembled from metagenomic sequencing of the rumen of 42 cattle. The increase in both culturable microbes and the availability of annotated genomes has allowed the development of rumen specific tools for the functional interpretation of 16S rDNA based studies (Wilkinson et al. 2018) to complement the increasing number of true metagenomics studies in the literature (Mayorga et al. 2016, Huws et al. 2016, Kamke et al. 2016).

Protozoa

Whilst the rumen bacteria are the most numerate microorganism in the rumen, protozoa, due to their size, can contribute up to 50% of the biomass in the rumen (Williams & Coleman 1992). Most protozoa in the rumen are ciliates (fig. 2), with a few flagellate species (Williams & Cole-

man 1992). Ciliates survive by digesting rumen bacteria and thus play an important role in the inefficient use of dietary protein by ruminants. In addition, protozoa are indirectly involved in methane production as they harbour an active population of methanogenic archaea both on their external and internal surfaces (Finlay et al. 1994, Newbold et al. 1995).

Protozoa taxonomy has relied on morphologic identification by optical microscopy (Newbold et al. 2015). Recently sequencing of 18S rRNA genes has helped both to 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). Protozoa can be removed from the rumen, through a process known as defaunation, and the animal will still survive (Williams & Coleman 1992, Newbold et al. 2015). However, a recent meta-analysis suggested that absence of protozoa caused a decrease in organic matter degradation suggesting an important functional role in the rumen (Newbold et al. 2015). Despite repeated attempts, it has proven impossible to maintain rumen protozoa in axenic culture (Newbold et al. 2015). Thus, it has been difficult to establish conclusively a role of ciliate protozoa in the rumen and specifically fibre degradation.

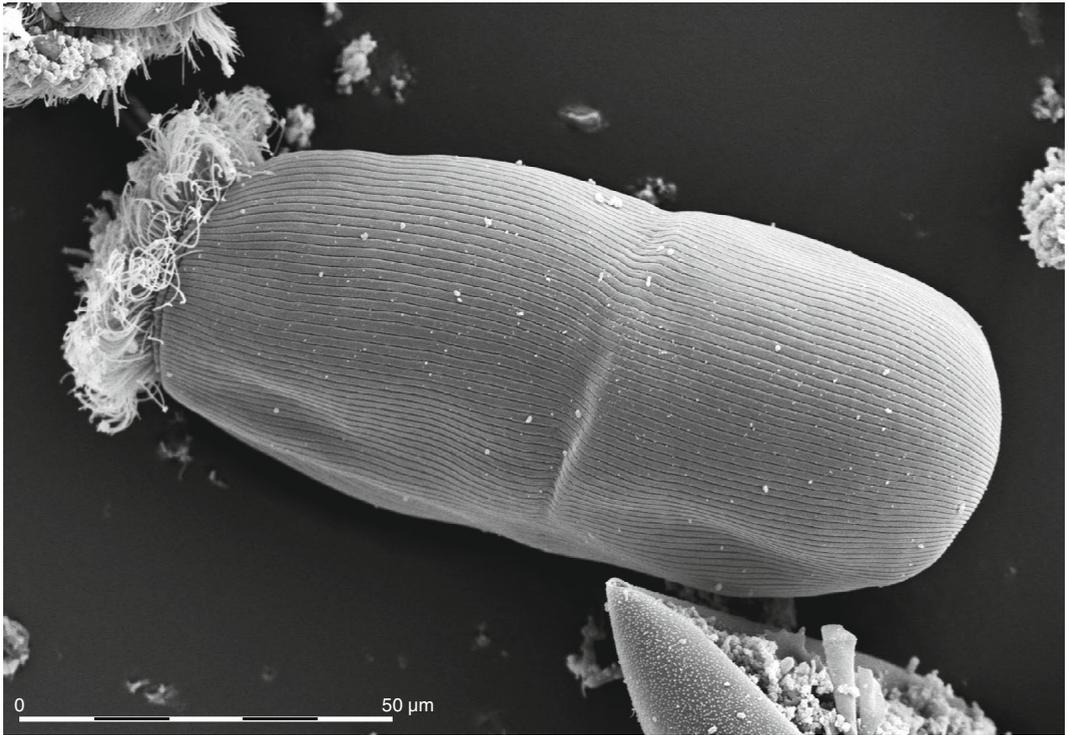


Fig. 2. *Entodinium* protozoan of the rumen, undergoing early stage division. – Photo by Sharon Franklin, USDA Agricultural Research Service.

Techniques to clone and express ciliate genes in phages have allowed genes from a range of rumen protozoa to be characterised (McEwan et al. 1999, Newbold et al. 2005, Belzecki et al. 2007). As a result, a wide range of fibrolytic enzymes has been identified suggesting a highly evolved fibrolytic capacity in the rumen ciliates (Devillard et al. 1999, 2003, Bera-Maillet et al. 2005, Takenaka et al. 2004, Wereszka et al. 2004). Metagenomic screening of protozoal glucosidases and eukaryotic metatranscriptomes has confirmed these observations with a diverse range of glycoside hydrolases present in the rumen protozoa (Findley et al. 2011, Qi et al. 2011).

Factors that influence the rumen microbiome

Diet. Diet is possibly the most obvious factor influencing the rumen microbiome. We have shown that both gross 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 (Belanche et al. 2016b), influencing the rumen microbiome. The development of network-based models is allowing us to explore both the temporal and spatial development of microbial populations within the rumen, regarding the colonization and degradation of dietary fibre entering the rumen (Huws et al. 2016, Belanche et al. 2017).

Plant extracts and plant secondary compounds.

Due to their antimicrobial activities, plant extracts and plant secondary compounds (e.g. saponins, essential oils and polyphenolic compounds) have shown potential to manipulate rumen fermentation both in terms of decreasing methane emissions and improving efficiency of nitrogen utilization (Hart et al. 2008). However, inconsistency in the effects of plant extracts on the rumen microbiome has been reported which is mainly due to the lack of standardized methods to ensure homogeneity of the chemical composition of the extracts (Cieslak et al. 2013). 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 deglycosilation of saponins to sapogenins by rumen bacteria (Wallace et al. 2002). However, studies to evaluate the antiprotozoal effect of sapogenins in the rumen are yet to be conducted. 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). 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.

Polyphenolic compounds such as tannins and flavonoids have also been shown to reduce methane production in the rumen which has been associated to an effect on the methanogen and protozoa populations (Cieslak et al. 2013). We have recently published a detailed characterization of the changes in rumen microbial communities associated to the effect of an isoflavonoid rich extract on rumen fermentation (Ramos-Morales et al. 2018). We concluded that the decreases in ammonia and methane production were probably related to decreases in protozoa numbers, a less diverse bacteria population as well as changes in both bacteria and archaea communities.

Host. Evidence is mounting that the host itself might have an effect on the rumen microbial population (Pacheco et al. 2014). Within a flock sheep of the same breed on the same diet some animals will segregate into 'low' or 'high' methane producers (Pinares-Patiño et al. 2011). This appears to be heritable (Pinares-Patiño et al. 2013) and presumably relates to differences in the rumen microbiome. 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 (Penner et al. 2011) and possible variation in rumen outflow or volume (Pinares-Patiño et al. 2003, Goopy et al. 2014) have been suggested.

Early life. In addition to host factors, we have also investigated the possible role of early life

events on rumen function in adult ruminants. During rumen development, microorganisms establish in a defined and progressive sequence (Li et al. 2012, Jami et al. 2013, Rey et al. 2013). Methanogenic archaea and cellulolytic bacteria have been found in the undeveloped rumen of lambs well before the ingestion of solid feed begins (2–4 days) and reaches levels similar to those in adult animals around 10 days after birth (Fonty et al. 1987, Morvan et al. 1994, Guzman et al. 2015). The coexistence of the host and microbial gut communities is clearly immunologically driven, and we are only beginning to understand the complex ways in which they adapt to each other (Winkler et al. 2007, Hooper et al. 2012, Yanez-Ruiz et al. 2015).

Neonatal nutrition can have a profound and life-long effect on mammals (Desai & Hales 1997, Vickers 2014, Lucas 2005). Long-lasting changes in gut microbiota because of early life diet have been well documented in humans and there is growing consensus that the effects of early life events on the gut microflora are fundamental in shaping the microbial consortia in the gut over the rest of life (Castanys-Muñoz et al. 2016). We have reported that a simple nutritional regime (forage vs. concentrate) applied early in life modified the bacterial population colonizing the rumen in lambs and that the effect persists over 4 months after the end of the treatment (Yáñez-Ruiz et al. 2010). Abecia et al. (2014) working with goats found that dosing kids and their does with bromochloromethane during the weaning period modified the archaeal community composition colonizing the rumen, and although not all the effects persisted after weaning, some less abundant archaeal groups remained different in treated and control four months after the treatment stopped. Clearly, there is a need for more research in this area. However, if the concept that additives used in early life can affect rumen function in adult life can be confirmed, then it will fundamentally change our approach to rumen manipulation and potentially revolutionize ruminant production. Dietary additives/nutritional interventions might be applied during the weaning period (when animals are more commonly under more direct management and thus susceptible to dietary manipulation) with the effect measurable over the rest of animal's life.

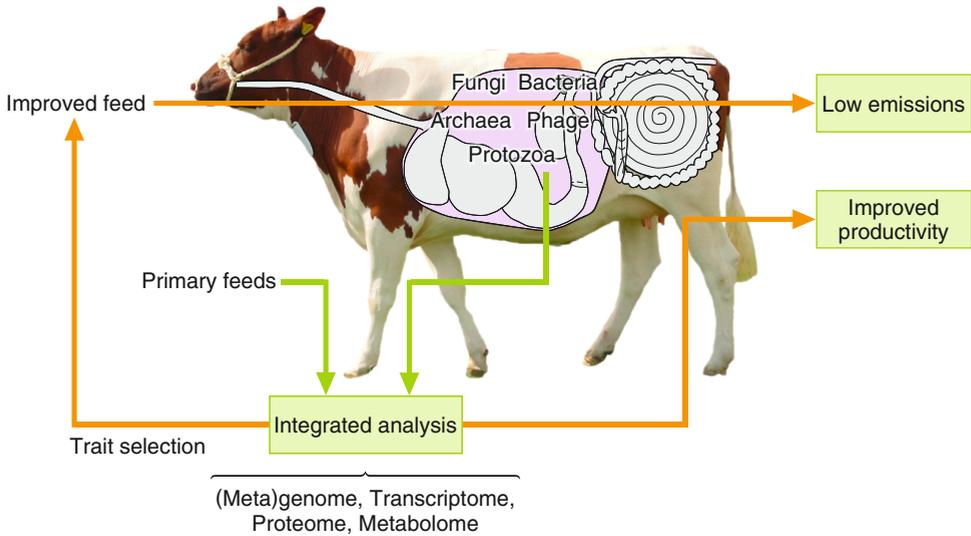


Fig. 3. A systems approach for manipulation of the rumen to improve productivity and reduce emissions.

Conclusions

The rumen plays a central role not only in the welfare and productivity of ruminants but also in their role in terms of environmental sustainability. Studies of the rumen microbiome have traditionally relied on our ability to culture and characterise microorganisms. It has been recognised that only a relatively small proportion of the microbes within the rumen are recovered by such techniques leaving us ignorant about the roles and activities of the clear majority of the rumen microbial ecosystem. Increasingly studies are expanding to consider not only which microbes are present in the rumen but also the functional genes present (metagenomics) and their expression in the rumen (metatranscriptomics). Coupled with a renewed focus on the application of molecular techniques to enhance our understanding of the activity of individual microbial groups in the rumen such studies promise to provide new insights into rumen function and how it might be manipulated to the benefit of mankind (fig. 3).

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Discussion

M. Merrow: I have a slightly detailed question on the protocol of feeding the grass hay ad libitum in comparison with the grass hay plus concentrate, because I am interested in how the circadian clock (“innere Uhr”) controls the microbiome in the gut. In doing the experiment, you were giving on one hand no daily signal to the microbes in the gut and on the other hand possibly a very strong, timed signal – a “Zeitgeber”. Did you give the concentrated feed ad libitum also?

C. J. Newbold: Yes we did give the concentrate ad libitum, but actually what you are indicating did occur. The young animals with concentrate spent significantly less time feeding as you might imagine and significantly more time resting, so there was a behavioral effect of the way we fed.

M. Merrow: That is really interesting. There are lots of feedbacks on the system, not just in terms of the nutrients that are getting in, but also on the core body temperature, hormone rhythms and so on. Those will all be amplified probably in the concentrated feed. So I think it is actually a really interesting experiment.

R. Amann: The idea of breeding not only the animal, but also the rumen microflora and optimizing it, is interesting. What steps towards an optimized rumen microflora are actually taken right now?

C. J. Newbold: There are various stages. A lot of commercial animals are fed large amounts of concentrate in order to get large amounts of production. There is a lot of rumen manipulation going on to modify the fermentation to stop the deleterious effects of excessive feeding. In addition, there is an increasing amount of rumen manipulation going on specifically to reduce methane, because of the greenhouse gas aspect. Unfortunately, the one that would be the most societally useful, increasing forage utilization, tends to be the one that is done least. So commercially, feeding additives as yeast for instance to modify high concentrate feeding would be the main part of the market, to decrease methane the next part, and to increase fiber digestion would be a very small part of the market – which is not the way it should be.