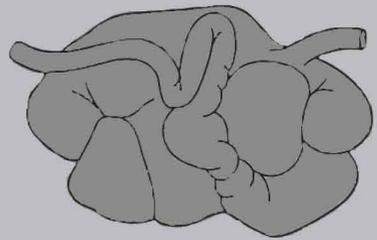


Improving rumen function

Edited by Dr C. S. McSweeney, CSIRO, Australia

Professor R. I. Mackie, University of Illinois, USA



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Preface

Most ruminant animal species were domesticated by humans by 2500 BC. Since then domesticated ruminants have co-migrated with people as they spread across the globe by providing a source of food for survival and reproduction, draft power for migration and later on for cultivation, as well as fibre from which clothing could be fashioned for protection from the environment. Their special relationship with mankind arises from their ability to convert human-indigestible carbohydrate and nitrogen into high quality protein in the form of meat and milk. The ability of both lifeforms to co-exist without competing for the same source of essential macro-nutrients has sustained their relationship over many thousands of years. The capacity of the ruminant to convert vegetation that is indigestible by humans into metabolizable protein and energy for the animal is due primarily to the microorganisms that inhabit the pre-gastric stomach (reticulum, rumen, and omasum) of these herbivores. Although humans realised the benefit of domesticating these animals long ago, it was only during the 20th century that the central role of 'rumen' microorganisms in this process was documented in detail. By mid-century some rumen microorganisms had been isolated and an understanding of the biochemical processes that defined the process of anaerobic fermentation were being published. The first book on rumen microbiology *'The Rumen and Its Microbes'* was written by R. E. Hungate and published in 1966. This classic book provided a description of the rumen microorganisms identified at that time, their physiological activities and requirements as well as the methods that had been developed to cultivate and isolate strict anaerobes. The publication also had a practical dimension in that it discussed this basic knowledge in terms of improving productivity of the ruminant animal and preventing abnormalities in rumen fermentation due to different feeding practises. Even then it was recognised that the discipline of rumen microbiology needed to be relevant to the agricultural sciences and not studied independently. Rumen microbiology continued to flourish following publication of Hungate's book and another book was published in 1988 titled *The Rumen Microbial Ecosystem* (ed. P. N. Hobson) and subsequently a 2nd edition in 1997 (eds P. N. Hobson & C. S. Stewart). These newer books followed the basic format of 'The Rumen and its Microbes' but called on the many experts who had entered the field to contribute specialist chapters on the new microorganisms and biochemical activities that had since been discovered as well as the evolving microbiology techniques.

In the last 25-30 years, microbiology has undergone a revolution in that the field has evolved from culture-based techniques to molecular approaches that

can interrogate complex ecosystems without the requirement for cultivation. Like the previous books focussing on rumen microbiology, this current publication seeks to present the new knowledge on the rumen microorganisms and biochemical pathways that have been discovered with the advent of molecular rumen ecology studies in the 21st century. However more than ever rumen microbiology is being challenged to make itself relevant to the major issues facing the ruminant industries. In particular, the public perception of negative impacts of ruminant production on the environment from methane and nitrous oxide emissions and nitrification of soil and water from urine and manure have been given special attention in the book. Although environmental issues are receiving greater emphasis in rumen research, there is still a demand from industry to increase efficiency of production while providing a satisfying, nutritious and healthy product, all of which can be directly impacted by the rumen ecosystem.

Unique anatomical and physiological adaptations have evolved in some domesticated ruminant species to enable a diverse rumen microbiome to function efficiently on relatively low protein forages high in lignocellulose so that their host can survive and reproduce. Much of the gains in efficiency of ruminant production during the 20th century were achieved with high quality diets and intensive feeding of formulated balanced rations. Ruminant production systems that compete with both humans and feed efficient monogastric livestock for dietary nutrients of high quality will come under greater scrutiny by environmental activists, regulators, as well as the consumer. In the future there needs to be increasing emphasis placed on the unique ability of ruminants to convert lignocellulosic feedstuffs into high quality protein for human consumption so that their rightful place in sustainable livestock production systems can be justified environmentally and economically. This book should therefore provide a reference source for students and researchers as foundational information on rumen microbiology and metabolism for the next 20-30 years of ruminant production given the pressures being faced by climate change.

C. S. McSweeney
R. I. Mackie

Introduction

Major advances in analytical techniques and genomics have transformed our understanding of rumen microbiology. This understanding is of critical importance to livestock production since rumen function affects nutritional efficiency, waste emissions from ruminants (such as methane and nitrous oxide) as well as animal health. This collection reviews what we know about rumen microbiota and the role of nutritional strategies in optimising their function for more sustainable livestock production.

Chapter 1 sets the scene by providing the latest information in the colonisation and establishment of the rumen microbiota. The chapter reviews the establishment of gastrointestinal microbiota in young ruminants and how it can be modulated for promoting health and favouring desirable phenotypes. Case studies of early-life strategies for improving health and production and for reducing enteric methane emissions are also provided. The chapter concludes by providing potential areas for future research and gives examples of resources to use for further information on the subject.

Part 1 Tools to understand the ruminal microbiome

Chapters in the first part of the book summarise advanced methods for analysing the rumen microbiome. Chapter 2 reviews how the gut microbiome can be 'brought to life' in the omics era. Research on the mammalian gut microbiome has in recent years been principally defined by culture-independent analysis of the genetic potential inherent in these microbial communities. However, there has been a renewed interest in culture-based studies of the gut microbiome to increase both the breadth and depth of gut microbial isolates, as these are widely recognised to provide the clearest opportunity to link biological activities with specific microbes. The chapter begins by providing an overview of the methodological approaches widely used to culture fastidious gut microbes, and our evolving understanding of how macro- and micronutrients impact their growth. In addition, alternative culturing approaches are discussed including genomic, genetic and antibody-based isolation strategies that take advantage of the existing wealth of metagenomic data.

Moving on to Chapter 3, this chapter focuses on rumen metabolomics as a tool for discovering and understanding rumen functionality and health. The rumen is a complex ecosystem essential for the health and productivity of the animal. Rumen metabolomics research is generating important data about the metabolites present in the rumen, and the factors affecting the rumen microbiome and metabolome. The chapter begins by providing an overview

of the technologies and extraction techniques for rumen metabolome analysis. It also reviews the factors impacting the composition and functionality of the rumen metabolome. The chapter concludes by providing a section on future trends in this emerging field of research and a summary of the variety of technologies available for metabolomic analysis.

The final chapter of Part 1 reviews mathematical modelling of microbial functionality in the rumen. To benefit from all the data gathered, with new techniques and new data types being introduced continuously, mathematical models need to be constructed that can capture the biological evidence gathered and to predict functionality at the level of the whole rumen. Chapter 4 reviews ways of quantifying rumen microbial functionality at the whole rumen level as well as units and sampling techniques used during mathematical modelling. These can then be used to predict fermentative and digestive processes in the rumen.

Part 2 The rumen microbiota

The second part of the book reviews recent research on the role of different communities of rumen microbiota such as bacteria, archaea, protozoa, anaerobic fungi, viruses and the rumen wall microbial community. As Chapter 5 points out, microbial genome sequencing has had an enormous impact on our understanding of many biological systems, including the identity, relationships and functions of the resident bacteria of the rumen. Since the first rumen bacterial genome was sequenced in 2003, over 500 genomes from cultured isolates and over 5000 genomes from metagenomic data studies have revealed a complex picture of how their genomic repertoire are formed by their ecological interactions during the breakdown of the plant material consumed by the host. Yet this picture remains incomplete since many organisms known to be present are missing from the genomic database. A complete understanding of the rumen microbiome relies on these gaps being addressed. The chapter reviews the power of sequencing a single genome and the curation of a reference genome catalogue for the rumen microbiome. It looks at the application of metagenomic data in identifying novel genomes and key functions in the rumen, as well as the use of genome sequencing to reveal interactions across the rumen microbiome.

Chapter 6 reviews the types of methanogenic archaea found in the rumen. The chapter provides a summary of rumen cultivation studies and molecular surveys of rumen archaeal community composition. It also discusses the current understanding methanogen function via genomic information retrieved from pure cultures of methanogens, re-assemblies of genomes derived from metagenome data sets from mixed enrichment cultures, as well as directly from rumen samples. The chapter concludes by providing an understanding of the

importance of rumen methanogens in methane production and other aspects of rumen function.

Chapter 7 reviews what we know about the ruminal ciliated protozoa, including their taxonomy and population 'types'. It assesses ecological fluctuations in protozoa populations, protozoa interactions in the rumen as well as the effects of protozoa function on ruminant nutrition, health and emissions. The chapter also discusses the challenges of working with rumen protozoa in culture. The chapter includes a case study describing the manipulation of rumen ciliates. It concludes by highlighting current gaps in research and gives examples of key research material and conferences on the subject.

Chapter 8 discusses the life cycle, taxonomy and morphological features of anaerobic fungi. It goes on to provide an overview of all currently known monocentric, polycentric and bulbous genera of anaerobic rumen fungi. Sections on the genomics and meta-omics of anaerobic fungi are also included, followed by a review of the interactions between rumen fungi and other members of the rumen ecosystem. The chapter concludes by emphasising the importance of expanding the research available on anaerobic rumen fungi and directs readers to useful websites and other resources for further information.

As Chapter 9 indicates, despite the sustained research focus on rumen microbial ecology, there is still a relative lack of knowledge surrounding the rumen mobilome including the major factors that contribute to the mobilome (for example viruses and plasmids), and the extent to which the mobilome impacts on rumen function. Viral populations have been shown to co-exist with, and predate on, the rumen microbiota. Non-viral extrachromosomal elements, such as plasmids, are often intrinsically linked with rumen microbial populations. This chapter reviews current understanding of rumen viral populations and extrachromosomal elements, as well as describing carriers of mobile genetic elements, such as extracellular membrane vesicles. The chapter also explores the impact of the mobilome on rumen function.

Chapter 10, the final chapter of Part 2, discusses the rumen wall microbiota community. The rumen microbes are usually divided into three distinct groups based on their habitats: the planktonic microbiota, feed particle associated microbiota, and the epimural microbiota. Among these three groups of microorganisms, the epimural community is the least studied and understood. The members of epimural microbiota are key players in oxygen scavenging, tissue recycling, urea metabolism, and nutrient transportation. The recent development of nucleic acid sequencing techniques has enabled us to better explore the composition and functional of this community. The chapter summarizes the current knowledge on the rumen epimural microbial community including its diversity, ecology, functions, and effects on host physiology.

Part 3 Nutrient processing in the rumen and host interactions

Chapters in Part 3 address the way the rumen processes nutrients such as fibre and protein as well as outputs such as energy, lipids and methane emissions. The first chapter discusses ruminal fibre digestion. Since ruminants obtain most of their energy from their symbiotic microbiota, the efficiency of feed conversion and end-product meat and milk quality is tightly linked to the dynamics and function of the rumen microbiome. Chapter 11 provides an overview of the role of the microbiota in ruminal lignocellulose degradation and the mechanisms they utilize in the decomposition of biomass. It discusses findings from studies on well-known *Ruminococcus*, *Fibrobacter* and *Prevotella* isolates, as well as those from poorly understood and as-yet uncultured *Bacteroidetes* lineages. Collectively, these approaches have revealed new information related to the hydrolytic capacity of cellulosomes, free enzymes, outer membrane vesicles, polysaccharide utilization loci and large multi-modular enzymes, which are generating deeper insights into the intricate microbial networks that engage in ruminal fibre digestion.

Chapter 12 reviews ruminal protein breakdown and ammonia assimilation. Ruminal nitrogen (N) metabolism has long been associated with effective ruminant degradation of fibre, feed intake and productivity. The chapter focuses on the latest research on N metabolism as well as understanding the process of ammonia assimilation and its regulation in the rumen. An improved understanding of microbial proteolysis and capture of N as microbial protein can then be integrated into nutritional strategies to optimize ruminant animal productivity while minimizing its environmental impact.

Chapter 13 focuses on the factors influencing the efficiency of rumen energy metabolism. It begins by addressing the main pathways of rumen fermentation and how these pathways can be used to produce products such as volatile fatty acids and adenosine triphosphate (ATP) as well as the disposal of metabolic hydrogen. The chapter then reviews the production of methane in the rumen and how modulation of methanogenesis can be used to benefit animal productivity. The factors that can influence the efficiency of microbial growth are also discussed, followed by a review of the interactions between rumen energy and nitrogen metabolism. The chapter concludes by highlighting the importance of research on maximising the energy output of fermentation, controlling the profile of volatile fatty acids, and increasing the efficiency of microbial growth.

The subject of Chapter 14 is understanding rumen lipid metabolism to optimize dairy products and monitor animal health. Rumen lipid metabolism largely determines the fatty acid composition of dairy products. As such, milk fatty acids can be used as indicators of ruminal and hence animal health. Control

of rumen metabolism can also facilitate production of dairy products enriched in unsaturated fatty acids. To better understand the origin of fatty acids in dairy products, the chapter first addresses ruminal metabolism, intestinal digestion, transfer to and fatty acid metabolism in the mammary gland. The chapter then discusses the potential to improve the fatty acid composition of dairy products for enhanced human health, with a particular focus on technologies to protect unsaturated fatty acids from rumen biohydrogenation. Finally, the chapter examines the use of variation in the milk fatty acid profile, induced by changes in the ruminal lipid metabolism, as a monitor of rumen and animal health.

Chapter 15 examines the nutritional factors affecting greenhouse gas production from ruminants and its implications for enteric and manure emissions. Ruminants are significant contributors to global greenhouse gas (GHG) emissions. Mitigating enteric and manure methane (CH_4) production have been explored, but often in isolation of other GHG. Lowering enteric CH_4 emissions can cause unintended increases in GHG from manure. Considering the complexity of rumen and the impact that it can have on manure composition, a whole systems approach is required to assess the value of additives that mitigate enteric CH_4 emissions. The chapter summarises a range of nutritional strategies available for enteric- and manure- CH_4 abatement. Dietary additives including alternative electron acceptors, inhibitors, plant secondary compounds, and carbon (C) derived materials are reviewed for their efficacy as mitigants of overall GHG emissions and are evaluated for how they alter rumen and manure microbiomes.

The next chapter considers host-rumen microbiome interactions and influences on feed conversion efficiency, methane production and other productivity traits. Today, with our ability to assess the composition of the rumen microbial community as a whole, a new holistic view of the microbiome has emerged. This has led to an increased understanding of the role of the microbiome and its components on production efficiency, health, and waste emissions such as methane. Chapter 16 focuses on the recent discovery of the role of the ruminant microbiome on energy harvest, methane emission, and the potential host genetic factors determining its microbial composition and selection.

The final chapter of Part 3, Chapter 17, discusses the rumen as a modulator of immune function in cattle. The rumen and its microbiome play essential roles in supplying key nutrients, such as energy, protein, minerals and vitamins, to the host and also shape the cow's immune system. Rumen health disorders, such as subacute ruminal acidosis, cause ruminal dysbiosis provoking epithelial barrier dysfunction and inflammation. These conditions facilitate rumen- and hindgut-derived lipopolysaccharides (LPS) to translocate into the blood stream, thereby compromising systemic metabolism and immune response. The chapter summarises new research related to rumen health, LPS exposure, and their

role in modulating the systemic metabolome and liver health. It examines the effects of prolonged rumen acidosis episodes on udder health due to the long-term exposure of LPS and highlights the systemic role of LPS in impairing the blood-milk barrier and invading mammary gland tissue.

Part 4 Nutritional strategies to optimise ruminal function

The final group of chapters explore nutritional strategies to optimise rumen function, including the role of pasture, silage, cereal feed, plant secondary compounds and probiotics. Chapter 18 focuses on the role of rumen microbiome in pasture-fed ruminant production systems. Pasture has long been utilised as a feed source for ruminants but the fermentation of forage by ruminants results in the production of GHGs, which may negatively effect the environment and represents a loss of energy/N for animal performance. The chapter examines the relationship between the rumen microbiome, host feed efficiency and environmental outputs in pasture-based production systems. The impact of different forages and grassland management practices on rumen microbes are also reviewed.

Chapter 19 assesses the role of silage and concentrate in dairy cow nutrition to improve feed efficiency and reduce methane and nitrogen emissions. Sustainable dairy cow feeding strategies to mitigate GHG and N emissions should make the most of the unique ability of ruminants to convert local human-inedible biomass to high-quality dairy foods. The chapter reviews the potential of silage plant species (grass, forage legumes, maize) and stage of maturity of silage crops as well as forage-to-concentrate ratio to reduce the environmental footprint of dairy cows without compromising animal performance. Dairy cow performance is examined in terms of feed intake, milk yield, feed and N efficiency, and methane emission intensity. The role of concentrate composition (lipids, carbohydrates and protein) is also evaluated. As a case study, the potential of milled rapeseed to reduce environmental footprint of grass silage-based diet is evaluated in practical farm conditions.

The next chapter examines the use of feedlot/cereal grains in improving feed efficiency and reducing by-products such as methane in ruminants. Chapter 20 begins by highlighting the types of cereal grains fed to cattle such as barley, corn, wheat, oats and sorghum. It then addresses the dietary factors affecting methane production by ruminants and the role of starch and forage in methane production. A section on H_2 sinks in the rumen and methane production is also included, followed by a discussion on using cereal grains to improve feed efficiency and reduce methane production. The microbiology of cereal grain fermentation is examined, and the bacteria and archaea involved in this fermentation are also addressed. The chapter also highlights one of the

factors that contributes the most to the degradation of feedstuffs, feed retention time, which is then followed by a review of acidosis and other feed effects.

Chapter 21 considers plant secondary compounds and the beneficial roles they have in sustainable ruminant nutrition and productivity. Plant secondary compounds, also known as phytochemicals or phytochemicals, are secondary metabolites that, when extracted and concentrated, modulate the activity of gut microorganisms. The chapter describes the composition, activity, effects on rumen function and animal performance of three major groups of plant secondary compounds: essential oils, tannins and saponins. The positive effects of these compounds include more efficient use of dietary protein and energy and decreased methane emissions, which can also result in increased milk yield or liveweight gain. The chapter explores current limitations in the use of plant extracts as feed additives in ruminant livestock and how they can be overcome.

The final chapter of the book analyses the use of probiotics as supplements for ruminants. There is an increasing need to promote digestive efficiency and productivity while maintaining animal health and welfare. Probiotics are live microorganisms which confer a benefit for the host when administered in appropriate quantities. Chapter 22 begins by reviewing critical periods in the ruminant lifecycle as targets for probiotics. It then looks at types of probiotics, delivery mechanisms and regulation. The rest of the chapter summarizes and assesses the range of research on the benefits and modes of action of probiotics, starting with their potential in young ruminants. It then considers the role of probiotics in adult ruminants in the areas of feed efficiency, methane production, pathogen control and supporting the immune system.

Chapter 1

Colonization and establishment of the rumen microbiota - opportunities to influence productivity and methane emissions

Diego P. Morgavi and Milka Popova, INRAE, France; David Yañez-Ruiz, CSIC, Spain; and Evelyne Forano, INRAE, France

- 1 Introduction
- 2 Establishment of the rumen microbiota
- 3 Modulating the gastrointestinal microbiota in young ruminants for health and production
- 4 Case studies: early-life strategies for improving health and production
- 5 Case studies: early-life strategies for reducing enteric methane emissions
- 6 Conclusion and future trends
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1 Introduction

The gastrointestinal (GIT) microbiota of ruminants cannot be dissociated from the host animal. Gut symbiotic microbes have a critical role in the interaction of the host animal with the surrounding environment, providing fundamental nutritional, immunological and protection services. As for other essential body 'organs', the GIT microbiota undergoes a series of development stages from early stages of formation until maturity. Differently from the embryogenesis process; however, the development of the GIT microbiota starts in earnest at birth and it is characterized by a succession of dynamic communities in the early stages of life (Savage, 1977; Jami et al., 2013; Rey et al., 2014). This process of acquisition of various microbial populations and their evolution within the ecosystem is essential for the correct functioning and interaction of the microbiota with the host (Costello et al., 2012).

Determinism is a strong driver dictating the microbial community structure of the GIT of animals as there is a strong selection by the diet, anatomy and

gut physico-chemical conditions (Ley et al., 2008). Yet, stochastic and historical events also influence the assemblage of the GIT microbiota that may have lasting effects in ruminants (Yáñez-Ruiz et al., 2010; Morgavi et al., 2015; Morais and Mizrahi, 2019). In this chapter, we review current information in the establishment of the microbiota in the rumen and posterior intestinal tract in young ruminants and its modulation for promoting health and favouring desirable phenotypes.

2 Establishment of the rumen microbiota

As the composition of the rumen microbiota directly influences the digestive and metabolic performance of the host animal, many studies have explored the microbial colonization of the rumen from birth to adulthood. These include early work using cultural methods (Fonty et al., 1983, 1988) to more recent studies using high-throughput sequencing methods in calves, lambs and goat kids (Jami et al., 2013; Rey et al., 2014; Guzman et al., 2015; Wang et al., 2017b; Abecia et al., 2018; Dias et al., 2018). The developing rumen in the newborn ruminant may provide a unique opportunity to manipulate the symbiotic microbiota for a long-lasting impact in the adult ruminant (Yanez-Ruiz et al., 2015).

2.1 Colonization: from birth (pre-ruminant) to a fully functional rumen

Recent reviews describe the microbial community successions that occur in the rumen from birth to weaning and after, when animals feed exclusively on solid feeds (Malmuthuge et al., 2015; Yanez-Ruiz et al., 2015; Meale et al., 2017a). Functional populations, as well as taxa present in adult rumens, appear very early after birth, in a progressive way and in a defined sequence. Several studies monitored the establishment of the rumen bacterial community in calves from birth to weaning using high-throughput sequencing and qPCR approaches (Jami et al., 2013; Rey et al., 2014; Guzman et al., 2015). They show that rapid changes occur in the composition of the rumen bacterial community during the first days of life. Proteobacteria and *Streptococcus*-related sequences are proportionally abundant in 1-3-day old calves and are rapidly replaced by strictly anaerobic bacterial taxa (Jami et al., 2013). Proteobacteria are then gradually replaced by Bacteroidetes as the animal grows, Firmicutes being present from early age to adulthood (Table 1). These results are in accordance with early studies using culture techniques reporting that aerobic and facultative anaerobic bacteria establish first (Fonty et al., 1987). Notwithstanding, strict anaerobes that are important for function in the mature rumen, such as cellulolytic bacteria and methanogenic archaea are already present in the rumen at 1 or 2 days after birth (Fonty et al., 1987; Gagen et al., 2012; Jami et al., 2013; Guzman et al., 2015). Methanogenic archaea

Table 1 Time line for colonization of the major bacteria phyla from birth to adulthood (values expressed as range of mean percentages)

Phyla	Age							
	3 days	7 days	14 days	28 days	42 days	6 months	2 years	
Bacteroidetes	13.9-42.6	56.3-56.9	46.0-61.3	49.9-56.3	56.3-74	38.5-55.2	38.5-50.2	
Firmicutes	5.1-13.9	13.9-17.5	13.9-34.0	13.9-42.1	10-43.9	36.8-48.9	34.5-56.7	
Actinobacteria	0.05-4.9	0.6-4.9	0.9-4.9	0.3-4.9	0.3-4.1	3	3	
Fusobacteria	4.7-5.5	4.7-5.3	0.2-0.6	0.2-0.3	0.2-0.4	0.1	0.1	
Spirochaetes	0-0.4	0.1-0.4	0.4-2.6	0.4-0.9	0.4	0.7-1.2	0.9-2.5	
Fibrobacteres	0-0.3	0-0.3	0.2-0.3	0.3-1.5	0.3-1.6	0.2-1.7	0.5-2.1	
Tenericutes	0	0.8	0.2	0.9	1.0	1.0-1.6	1.3-2.3	

Data collected from Li et al. (2012), Jami et al. (2013), Rey et al. (2014), Yáñez-Ruzi et al. (2015), Abecia et al. (2018).

can be enumerated in the immature rumen of lambs at 2–4 days, well before the consumption of solid feeds, and after two weeks, their concentration is equivalent to that found in adult animals (Fonty et al., 1987; Morvan et al., 1994). Although not detected by culture, a low-abundant but diverse population of methanogens (predominantly *Methanobrevibacter* spp.) was identified using molecular methods in lambs placed into sterile isolators 17 h after birth (Gagen et al., 2012). A recent study in goat kids also indicated that active methanogens colonized the rumen at one day of life, *Methanobrevibacter*, *Methanosphaera* (both Methanobacteriales order) and Candidatus *Methanomethylophilus* (Methanomassiliicoccales order) being the top three genera (Wang et al., 2017b). There are four major methanogenic orders usually found in the rumen: Methanobacteriales, Methanomicrobiales, Methanosarcinales and Methanomassiliicoccales (Janssen and Kirs, 2008). All these are abundantly present in calves from day 1 to 2 weeks of age, whereas only Methanobacteriales and Methanomassiliicoccales could be qPCR-detected in the mature rumen (Friedman et al., 2017). Based on substrate utilization for methanogenesis, the authors suggest that the early methanogenic community may be characterized by a high activity of methylotrophic methanogenesis, likely performed by members of the order Methanosarcinales. Eukaryotic microorganisms also establish sequentially. Anaerobic fungi can be enumerated in the rumen of lambs by 8–10 days after birth (Fonty et al., 1987). Anaerobic fungi, which are cellulolytic, are thus present in the rumen long before the animal ingests solid feeds regularly. Ciliates are detected from 2 to 3 weeks of age, with *Entodinium* establishing first (15–20 days), then *Polyplastron*, *Eudiplodinium* and *Epidinium* (20–25 days) and finally *Isotricha* (50 days) (Fonty et al., 1988). In contrast to bacteria and archaea, protozoa do not establish when newborns are isolated from their dams shortly after birth (Fonty et al., 1988; Chaucheyras-Durand et al., 2019). In addition, ciliate protozoa require the presence of a complex microbiota to establish (Fonty et al., 1983, 1988). Figure 1 shows the main colonization events by groups of microbes in lamb's rumen throughout the suckling period and up to the end of weaning.

Colonization of the rumen wall by epimural bacteria is also age-related, with sequential diversification of bacterial morphotypes (Rieu et al., 1990). The phylum Proteobacteria is dominant on the rumen epithelium with an important contribution of the genus *Escherichia* (Jiao et al., 2015; Wang et al., 2017a). As for the lumen, the abundance of Proteobacteria associated with rumen epithelium decreases, and that of Firmicutes and Bacteroidetes increases with age (Jiao et al., 2015).

Large differences between digesta and epimural bacterial communities have been observed in the rumen of pre-weaned calves, with higher abundances of Prevotella and lower abundances of Bacteroidetes in digesta compared with epimural bacteria (Malmuthuge et al., 2014).

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