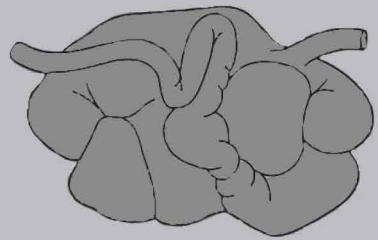


Improving rumen function

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Contents

Series list	xiv
Preface	xx
Introduction	xxii
1 Colonization and establishment of the rumen microbiota - opportunities to influence productivity and methane emissions <i>Diego P. Morgavi and Milka Popova, INRAE, France; David Yañez-Ruiz, CSIC, Spain; and Evelyne Forano, INRAE, France</i>	1
1 Introduction	1
2 Establishment of the rumen microbiota	2
3 Modulating the gastrointestinal microbiota in young ruminants for health and production	12
4 Case studies: early-life strategies for improving health and production	13
5 Case studies: early-life strategies for reducing enteric methane emissions	15
6 Conclusion and future trends	16
7 Where to look for further information	18
8 References	18
Part 1 Tools to understand the ruminal microbiome	
2 A question of culture: bringing the gut microbiome to life in the -omics era <i>Páraic Ó Cuív, Microba Life Sciences and Mater Research Institute - The University of Queensland, Australia</i>	29
1 Introduction	29
2 Culturing methods and nutrient effects on microbial growth: an overview	31
3 Genome-directed isolation of gut microbes	37
4 Molecular-based isolation of gut microbes	40
5 Antibody-based isolation of gut microbes	42
6 Conclusion and future trends	44
7 References	44

3	Rumen metabolomics – a powerful tool for discovery and understanding of rumen functionality and health <i>Tom F. O’Callaghan, Teagasc Moorepark Food Research, Ireland; and Eva Lewis, Devenish, UK</i>	55
	1 Introduction	55
	2 The rumen metabolome: technologies for analysis and extraction techniques	57
	3 Factors impacting the rumen metabolome	60
	4 Future trends in research	63
	5 Conclusion	64
	6 References	65
4	A conceptual approach to the mathematical modelling of microbial functionality in the rumen <i>André Bannink, Soumya Kar, Dirkjan Schokker and Jan Dijkstra, Wageningen University and Research, The Netherlands</i>	69
	1 Introduction	69
	2 Conceptual approaches in modelling whole rumen function	71
	3 Quantifying rumen microbial functionality	82
	4 Units and sampling techniques	85
	5 Conclusion and future trends	88
	6 Where to look for further information	89
	7 References	90

Part 2 The rumen microbiota

5	Genome sequencing and the rumen microbiome <i>Jessica C. A. Friedersdorff and Benjamin J. Thomas, Institute of Biological, Environmental and Rural Science (IBERS), Aberystwyth University and Institute of Global Food Security (IGFS), Queen’s University Belfast, UK; Sara E. Pidcock, Institute of Global Food Security (IGFS), Queen’s University Belfast, UK; Elizabeth H. Hart, Institute of Biological, Environmental and Rural Science (IBERS), Aberystwyth University, UK; Francesco Rubino and Christopher J. Creevey, Institute of Global Food Security (IGFS), Queen’s University Belfast, UK</i>	97
	1 Introduction	97
	2 The first rumen microbial genome	99
	3 The power of sequencing a single genome	99
	4 Curation of a reference genome catalogue for the rumen microbiome	101
	5 Application of metagenomic data for novel genome construction	102

6	Comparative genomics and key functions in the rumen	105
	7 The genome as a blueprint of the proteome	108
	8 Genome sequencing and interactions across the microbiome	109
	9 Conclusion	114
	10 Future trends	114
	11 Where to look for further information	120
	12 References	121
6	<i>The Rumen Archaea</i> <i>Graeme T. Attwood and Sinead C. Leahy, AgResearch Ltd and New Zealand Greenhouse Gas Research Centre, New Zealand; and William J. Kelly, Donvis Ltd, New Zealand</i>	133
	1 Introduction	133
	2 <i>Archaea</i> in the rumen	134
	3 Cultivation of methanogenic <i>Archaea</i> from the rumen	138
	4 The use of molecular techniques to identify and quantify rumen methanogens	148
	5 Hydrogenotrophic methanogens: <i>Methanobrevibacter ruminantium</i> M1	159
	6 Other hydrogenotrophic methanogens	165
	7 Methylophilic methanogens	167
	8 Acetoclastic methanogens: <i>Methanosarcina</i> sp. CM1	172
	9 Conclusions	173
	10 References	174
7	<i>Ruminal-ciliated protozoa</i> <i>Sharon A. Huws, Queen's University Belfast, UK; Cate L. Williams, Aberystwyth University, UK; and Neil R. McEwan, Robert Gordon University, UK</i>	191
	1 Introduction	191
	2 Discovery of rumen protozoa	192
	3 Rumen protozoal taxonomy and population 'types'	193
	4 Rumen protozoal genomics	197
	5 Ecological fluctuations in protozoa populations	198
	6 Protozoa interactions in the rumen	199
	7 Challenges of working with rumen protozoa	204
	8 The effects of protozoal function on ruminant nutrition, health and emissions	205
	9 Case study: manipulating the rumen ciliates	208
	10 Future trends and conclusions	210
	11 Where to look for further information	212
	12 References	212

8	The anaerobic rumen fungi	221
	<i>Matthias Hess, University of California-Davis, USA; Katerina Fliegerová, Czech Academy of Sciences, Institute of Animal Physiology and Genetics, Czech Republic; Shyam Paul, Indian Council of Agricultural Research, Directorate of Poultry Research, India; and Anil Kumar Puniya, Indian Council of Agricultural Research, National Dairy Research Institute, India</i>	
	1 Introduction	221
	2 The life cycle of anaerobic fungi	222
	3 Taxonomy and morphological features of anaerobic fungi	224
	4 Genera and species of anaerobic fungi	225
	5 Monocentric genera	226
	6 Polycentric genera	234
	7 Bulbous genera	237
	8 Genomics of anaerobic fungi	238
	9 Meta-omics of anaerobic fungi	253
	10 Interactions between rumen fungi and other components of the rumen ecosystem	256
	11 Conclusion	262
	12 Where to look for further information	262
	13 References	263
9	Ruminal viruses and extrachromosomal genetic elements	281
	<i>Rosalind Ann Gilbert and Diane Ouwerkerk, Department of Agriculture and Fisheries, Queensland Government and Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia</i>	
	1 Introduction	281
	2 Extrachromosomal elements	282
	3 Rumen viruses	287
	4 Role and impact of the mobilome on rumen function	299
	5 Conclusion and future trends	305
	6 Acknowledgements	306
	7 Where to look for further information	306
	8 References	306
10	The rumen wall microbiota community	321
	<i>Mi Zhou, University of Alberta, Canada; Junhua Liu, Nanjing Agricultural University, China; and Le Luo Guan, University of Alberta, Canada</i>	
	1 Introduction	321
	2 The rumen wall microbial community	322

3	The development of the rumen wall microbiome	325
4	Factors affecting the epimural microbiota	327
5	The impact of the epimural microbiota on ruminant production	329
6	Challenges and future trends	333
7	Conclusions	337
8	Acknowledgement	337
9	References	337
Part 3 Nutrient processing in the rumen and host interactions		
11	Ruminal fibre digestion <i>Adrian E. Naas and Phillip B. Pope, Norwegian University of Life Sciences, Norway</i>	349
1	Introduction	349
2	Lignocellulosic biomass	350
3	Carbohydrate-active enzymes	352
4	Prokaryotic strategies for fibre digestion in the rumen	355
5	Current gaps in knowledge	364
6	Improving the fibre digestion process	367
7	Summary and future trends	368
8	Where to look for further information	369
9	References	369
12	Ruminal protein breakdown and ammonia assimilation <i>Jeffrey L. Firkins, The Ohio State University, USA; and Roderick I. Mackie, University of Illinois, USA</i>	383
1	Introduction	383
2	Microbial nitrogen metabolism	384
3	Opportunities to improve efficiency of ruminal nitrogen (N) metabolism	404
4	Conclusion	407
5	Future trends	408
6	Where to look for further information	409
7	References	409
13	Factors influencing the efficiency of rumen energy metabolism <i>Emilio M. Ungerfeld, Instituto de Investigaciones Agropecuarias (INIA), Chile; and Timothy J. Hackmann, University of California-Davis, USA</i>	421
1	Introduction	421
2	Main pathways of rumen fermentation	422
3	Methane	435
4	Factors influencing the efficiency of microbial growth	442

5	Interactions between rumen energy and nitrogen metabolism	449
6	Conclusion and future trends	452
7	Where to look for further information	453
8	References	453
14	Understanding rumen lipid metabolism to optimize dairy products for enhanced human health and to monitor animal health	467
	<i>Veerle Fievez, Nympha De Neve and Lore Dewanckele, Ghent University, Belgium</i>	
1	Introduction	467
2	Ruminal metabolism of dietary lipids and <i>de novo</i> fatty acid synthesis	470
3	Digestion and transfer of dietary and rumen fatty acids to the mammary gland and fatty acid metabolism in the mammary gland	473
4	Endogenous fatty acid metabolism in the mammary gland	475
5	Impact of ruminant fatty acids on human health	476
6	Rumen bypass polyunsaturated fatty acid (PUFA) products to improve the milk fatty acid profile	479
7	Rumen lipid protection technologies	480
8	Milk fatty acids originating from the rumen as biomarkers to monitor animal health	485
9	Conclusion	490
10	Where to look for further information	491
11	References	492
15	Nutritional factors affecting greenhouse gas production from ruminants: implications for enteric and manure emissions	505
	<i>Stephanie A. Terry, Agriculture and Agri-Food Canada, Canada and University of Sydney, Australia; Carlos M. Romero, Agriculture and Agri-Food Canada and University of Lethbridge, Canada; and Alex V. Chaves and Tim A. McAllister, Agriculture and Agri-Food Canada, Canada</i>	
1	Introduction	505
2	Case study: Dried distillers' grains plus solubles (DDGS)	514
3	Nitro-based compounds	516
4	Plant secondary compounds	521
5	Carbon-derived materials	524
6	Microbial hydrogen utilisation	528
7	Future trends and conclusion	530
8	Where to look for further information	531
9	References	532

16	Host-rumen microbiome interactions and influences on feed conversion efficiency (FCE), methane production and other productivity traits	547
	<i>Elie Jami, Agricultural Research Organization - Volcani Center, Israel; and Itzhak Mizrahi, Ben-Gurion University of the Negev, Israel</i>	
	1 Introduction	547
	2 Core community, resilience and natural variation in rumen microbiome composition	548
	3 Microbiome-dependent traits	550
	4 Methane production	554
	5 Nitrogen compounds: utilization and emission	557
	6 Microbiome and host genetics	558
	7 References	560
17	The rumen as a modulator of immune function in cattle	567
	<i>S. Aditya, University of Veterinary Medicine Vienna, Austria and Brawijaya University, Indonesia; and E. Humer and Q. Zebeli, University of Veterinary Medicine Vienna, Austria</i>	
	1 Introduction	567
	2 Prevalence of subacute ruminal acidosis (SARA) in dairy herds	569
	3 Rumen health, metabolic activity and disorders	570
	4 Rumen health and the mammary immune system	574
	5 Conclusions	578
	6 Acknowledgements	578
	7 Where to look for further information	579
	8 References	579
Part 4 Nutritional strategies to optimise ruminal function		
18	Role of the rumen microbiome in pasture-fed ruminant production systems	591
	<i>Sinéad M. Waters, David A. Kenny, Teagasc Animal and Bioscience Research Department, Ireland; and Paul E. Smith, Teagasc Animal and Bioscience Research Department and UCD College of Health and Agricultural Sciences, University College Dublin, Ireland</i>	
	1 Introduction	591
	2 Diet and rumen microbiome	594
	3 Cellulose degradation in the rumen	595
	4 The rumen microbiome and feed efficiency	603

5	The rumen microbiome and methane production	612
6	Methane production and residual feed intake	615
7	The impact of forage plants on animal performance	619
8	Conclusion	632
9	Where to look for further information	633
10	References	634
19	Optimising ruminal function: the role of silage and concentrate in dairy cow nutrition to improve feed efficiency and reduce methane and nitrogen emissions	651
	<i>Aila Vanhatalo and Anni Halmemies-Beauchet-Filleau, University of Helsinki, Finland</i>	
1	Introduction	651
2	Role of silage: grass, forage legumes and maize	653
3	Role of concentrates: lipids, carbohydrates and protein	662
4	Case study: Effects of milled rapeseed on milk production, milk fat composition and ruminal CH ₄ emissions of dairy cows in practical farm conditions	677
5	Summary and future trends	680
6	Where to look for further information	683
7	References	683
20	The use of feedlot/cereal grains in improving feed efficiency and reducing by-products such as methane in ruminants	693
	<i>Kristin Hales, US Meat Animal Research Center - USDA-ARS, USA; Jeferson Lourenco, Darren S. Seidel, Osman Yasir Koyun, Dylan Davis and Christina Welch, University of Georgia, USA; James E. Wells, US Meat Animal Research Center - USDA-ARS, USA; and Todd R. Callaway, University of Georgia, USA</i>	
1	Introduction	693
2	Types of cereal grains fed to cattle	695
3	Cereal grain production	698
4	Dietary factors affecting methane production by ruminants	699
5	The role of starch and forage in methane formation	701
6	H ₂ sinks in the rumen and methane production	704
7	Using cereal grains to improve feed efficiency and reduce methane production	706
8	Microbiology of cereal grain fermentation	708
9	Bacteria and archaea involved in fermentation	710
10	Feed retention time	712
11	Acidosis and other negative feed effects	714
12	Summary	715

13	Where to look for further information	716
14	References	717
21	Plant secondary compounds: beneficial roles in sustainable ruminant nutrition and productivity <i>David R. Yáñez-Ruiz and Alejandro Belanche, Estación Experimental del Zaidín, CSIC, Spain</i>	727
1	Introduction	727
2	Essential oils (EO)	731
3	Tannins	740
4	Saponins	751
5	Future trends and conclusion	757
6	References	758
22	The use of probiotics as supplements for ruminants <i>Frédérique Chaucheyras-Durand and Lysiane Dunière, Lallemand Animal Nutrition and Université Clermont Auvergne, INRAE, UMR 454 MEDIS, France</i>	775
1	Introduction	775
2	Critical periods in the ruminant lifecycle as targets for probiotics	776
3	Definitions, delivery mechanisms and regulation	781
4	Benefits and modes of action of probiotics: young ruminants	785
5	Benefits and modes of action of probiotics: feed efficiency in adult ruminants	788
6	Benefits and modes of action of probiotics: methane production	793
7	Benefits and modes of action of probiotics: pathogen control	794
8	Benefits and modes of action of probiotics: effects on the immune system	797
9	Conclusions and future trends	800
10	Acknowledgements	801
11	Where to look for further information section	801
12	References	802
	Index	819

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.

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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

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- 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
- 7 Where to look for further information
- 8 References

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).

Index

- α -linolenic acid 667
- β -defensin 777
- γ -aminobutyric acid (GABA) 35

- 3-mercapto-1-propanesulfonic acid (MPS) 261
- 3-nitrooxypropanol (3-NOP) 64, 450, 516, 518
- 16S rDNA gene clone technology 323, 325
- 16S rRNA gene sequencing 9, 10, 62, 99, 103-105, 109, 147-151, 606

- AA. *see* Amino acids (AA); Auxiliary activity (AA); Auxiliary enzymes (AA)
- ACACA. *see* Acetyl-CoA carboxylase (ACACA)
- Acacia mearnsii* 521, 522
- Accessory genome 107
- Acetoclastic methanogens 137
- Acetoclastic pathway 135
- Acetogenesis 9, 138
- Acetogens 9, 34
- Acetyl-CoA 425, 426, 475
- Acetyl-CoA carboxylase (ACACA) 475
- Acid detergent fibre (ADF) 520, 601
- Acidosis 790, 791, 793
- Actinobacteria 5
- Active dry yeasts (ADY) 782, 783
- Acute mastitis 576
- Acute metritis 797
- Acute phase proteins (APP) 568
- Acute phase response (APR) 568, 570, 571
- Acyl fatty acids 427
- Adenosine triphosphate (ATP) 428-429, 431, 448
- ADF. *see* Acid detergent fibre (ADF)
- adh1*, 2, 3 genes. *see* NADP-dependent alcohol dehydrogenase (*adh1*, 2, 3) genes
- ADY. *see* Active dry yeasts (ADY)

- Aeromonas veronii* 38
- Agolin® 739-740
- AGORA models 39
- Agriosomyces longus* 232
- Akkermansia muciniphila* 39
- Aklioshbomyces papillarum* 232
- Amino acids (AA) 32, 106, 450-451, 573
- Ammonia (NH₃) 32, 106, 506-507, 516, 609
- Ammonium transporter (AmtB) 388, 389
- AMPs. *see* Antimicrobial peptides (AMPs)
- AMR. *see* Antimicrobial resistance (AMR)
- AmtB. *see* Ammonium transporter (AmtB)
- Amylolytic activity 73, 74, 77
- Amylopectin 701, 702
- Amylose 701, 702
- Anaerobic agar shake method 138
- Anaerobic bacteria 2, 35
- Anaerobic fungi 4, 263
 - bulbous genera 237-238
 - genera and species 225-226
 - genomics 238
 - single-cell 239-240
 - transcriptomics 240-241, 251-252
 - interactions with rumen ecosystem components
 - feed composition effect 257-258
 - lignocelluloses degradation 260-262
 - metabolic intermediates for host utilization 258-260
 - methanogens 256-257
 - life cycle 222-223
 - meta-omics
 - metagenomics 253-254
 - metatranscriptomics 254-256
 - monocentric genera 226-234
 - overview 221-222
 - polycentric genera 234-235
 - taxonomy and morphological features 224-225
- Anaerobic microbes 31, 138

- Anaeromyces* sp. 234-235
A. contortus 235
A. mucronatus 234, 235
A. polycephalus 235
A. robustus 235
- Anoxic microbiological media 31-32
- Antibiotic resistance genes (ARGs) 113
- Antimicrobial defense proteins 576
- Antimicrobial peptides (AMPs) 112
- Antimicrobial resistance (AMR) 784
- Antisense-based expression regulation 252
- APP. *see* Acute phase proteins (APP)
- APR. *see* Acute phase response (APR)
- Arch915 149
- Archaea 174
acetoclastic methanogens 172-173
cultivation 138-139, 145-148
hydrogenotrophic methanogens 159-167
methylophilic methanogens 167
overview 133-134
in rumen 134-138
use of molecular techniques 148-153, 159
- ARGs. *see* Antibiotic resistance genes (ARGs)
- ARISA. *see* Automated ribosomal intergenic spacer analysis (ARISA)
- Artificial milk feeding 11
- Asaccharolytic microbes 34
- Aspartate metabolism 61
- 'Assembling the Fungal Tree of Life' project 224
- ATP. *see* Adenosine triphosphate
- Automated ribosomal intergenic spacer analysis (ARISA) 549
- Auxiliary activity (AA) 353, 354
- Auxiliary enzymes (AA) 241
- Avena sativa* 697, 699
- BA. *see* Biogenic amines (BA)
- Bacillus* sp.
B. pumilus 203
B. subtilis 41, 389
- Bacitracin 38
- Bacterial genomes 107
- Bacteriocins 112
- Bacteriological agar 32
- Bacteriophage adherence to mucus (BAM) model 304
- Bacteriophages 110
- Bacteroides* sp. 11, 41, 323
B. ruminicola 32, 285, 287, 292
- Bacteroidetes* sp. 2, 4-6, 8, 323, 328, 510-511, 513, 515
B. thetaiotaomicron 358
- BAM model. *see* Bacteriophage adherence to mucus (BAM) model
- Barley. *see* *Hordeum vulgare* L.
- Bathyarchaeota* 134
- Beef Industry Food Safety Council (BIFSCO) 796
- BEO. *see* Blend of essential oils (BEO)
- Beta-hydroxybutyrate (BHBA) 571
- BHBA. *see* Beta-hydroxybutyrate (BHBA)
- Bifidobacterium* sp. 6, 12-14, 787, 796
B. choerinum 285
- Biflagellate zoospores 233
- BIFSCO. *see* Beef Industry Food Safety Council (BIFSCO)
- Binning process 103
- Bioconductor 59
- Biogenic amines (BA) 62, 63, 568, 571
- Biohydrogenation 107, 407, 470
- Biomarkers 56, 57, 61
- Biophytum petersianum* 757
- Blend of essential oils (BEO) 738-740
- BMCS. *see* Brown midrib maize silage (BMCS)
- bMEC. *see* Bovine mammary epithelial cells (bMEC)
- Bovicin HC5 112
- Bovine mammary epithelial cells (bMEC) 796
- Bovine reproductive diseases 797
- Bovine respiratory disease (BRD) 797
- Bovine Rumen Metabolome Database 60
- Branched-chain FA 472
- BRD. *see* Bovine respiratory disease (BRD)
- Bromochloromethane 15
- Brown midrib maize silage (BMCS) 660
- Bryant, Marvin 281, 709
- Butyrylvibriocin OR79A 112
- Butyrivibrio* sp. 323, 326, 327
B. fibrisolvens 107, 108, 199, 284, 332, 486, 595, 790
B. hungatei 285
B. proteoclasticus 163, 285
- Buwchfawromyces* sp. 230-231
B. eastonii 231
- Caecomyces* sp. 237
C. churrovius 237
C. communis 237, 256
C. equi 237
C. sympodialis 237

- Callimastix frontalis* 226
Campylobacter sp. 326–328
C. jejuni 99
Candidatus Methanofastidiosus 134
Candidatus Methanomethylophilus 169, 170
Capellomyces sp. 232
C. elongatus 232
C. foraminis 232
 Capillary electrophoresis with mass spectrometry (CE-MS) 57
 Carbohydrate-active enzymes (CAZymes) 105, 153, 241, 251, 349, 350, 352–353, 360, 601
 Carbohydrate-binding modules (CBMs) 241, 251, 353, 354
 Carbohydrate esterases (CEs) 353, 355
 Carbohydrates 32
 Carbon dioxide (CO₂) 34, 505, 506, 516, 517, 527, 694
 Carboxylesterase (CE) 241
 Cas proteins. *see* CRISPR-associated (*Cas*) proteins
Castanea sativa 741
 CatchAll 297
Catenibacillus scindens 34
 CAZymes. *see* Carbohydrate-active enzymes (CAZymes)
 CBH. *see* Cellobiohydrolases (CBH)
 CBMs. *see* Carbohydrate-binding modules (CBMs)
 CE. *see* Carboxylesterase (CE); Cholesterol esters (CE)
 Cellobiohydrolases (CBH) 358
 Cellulolytic bacteria 2, 9
Cellulomonas fini 354
 Cellulose degradation 100, 350
 CE-MS. *see* Capillary electrophoresis with mass spectrometry (CE-MS)
 Cereal grains and feed efficiency 716
 acidosis and negative feed effects 714–715
 archaea in fermentation 712
Butyrivibrio fibrisolvens 710
 feed retention time 712–714
 methane production
 dietary factors affecting 699–701
 H₂ sinks 704–706
 reducing 706–708
 role of starch and forage 701–704
 microbiology of fermentation 708–709
 overview 693–695
Prevotella sp. 711
Ruminobacter amylophilus 710
Selenomonas ruminantium 711
Streptococcus bovis 711–712
Succinomonas amyolytica 711
 types and production 695
 barley 696, 699
 corn 696–697, 698–699
 oats 697, 699
 sorghum 697–698
 wheat 697, 699
 CEs. *see* Carbohydrate esterases (CEs)
 Chenomx 59
 Chestnut. *see* *Castanea sativa*
 Chitin utilization locus (ChiUL) 363
 Chloroform 64, 450
 Cholesterol 467, 571, 572
 Cholesterol esters (CE) 467
Christensenella minuta 38
 Chytridiomycetes 224
 Ciliate Entodiniomorphid protozoa 791
 CLA. *see* Conjugated linoleic acid (CLA)
 Class II bacteriocins 112
Clostridium cluster XIVa 40, 41
Clostridium sp.
C. aminophilum 32, 738
C. botulinum 301
C. cellulovorans 596
C. sticklandii 733, 738
C. thermocellum 356
 Clustered regularly interspaced short palindromic repeats (CRISPR) 111, 294, 298
 CNCM I-1079 strain 786–788
 Coenzyme M (CoM) 145, 162–163, 165, 170
 Colostrum 12
 CoM. *see* Coenzyme M (CoM)
 Commercial thermal cyclers 149
 Condensed Tannins (CT) 740–741, 743, 746, 748
 Conjugated linoleic acid (CLA) 207, 471, 472, 478, 484
 Cool-season forage grasses 654
Coprococcus catus 554
 Core genome 107
 Corn. *see* *Zea mays* L.
 Cornell Net Carbohydrate and Protein System model 629
Coxiella burnetii 38
 CP. *see* Crude protein (CP)
 CRISPR. *see* Clustered regularly interspaced short palindromic repeats (CRISPR)

- CRISPR-associated (Cas) proteins 294
CRISPR-Cas systems 110, 174, 296
Crude protein (CP) 512, 620-622, 624, 632, 654, 675-676
CT. *see* Condensed Tannins (CT)
C-terminal domain (CTD) 363-364
Culture-independent metagenomic approaches 29, 30
Culture-independent techniques 350
Culturing techniques 29, 30
Culturomics 36, 102
Cyllumyces sp. 237, 238
 C. aberensis 237
 C. icaris 237
Cysteine-HCl 35
Cytophaga hutchinsonii 363
- Dairy cow milk production
 carbohydrates 682
 concentrate level 669, 671
 source 671, 675
 lipids 682
 medium-chain saturated fatty acids 663, 667
 supplements 662-663
 unsaturated fatty acids 667-668
 milled rapeseed effects and ruminal CH₄ emissions 677-680
 overview 651-653
 protein 682-683
 concentrate level 675-676
 source 676-677
 role of silage
 forage legume 658-659, 681
 grass 654, 658, 681
 maize 659-660, 681
 maize vs. grass and forage legume 660-662
 plant species 653-654
Dasytricha 398, 399
DDGS. *see* Dried distillers' grains plus solubles
Deadenylation activity 392
Deamination 387
Defaunation 206, 207, 447, 605, 756
Degrees of polymerization (DP) 351
Denaturing Gradient Gel Electrophoresis (DGGE) 150
Denitrobacterium detoxificans 138
Desulfobulbus 327
Desulfovibrio strain G11 145
DFI-MS. *see* Direct flow injection mass spectrometry (DFI-MS)
- DFM. *see* Direct-fed microbials (DFM)
DGGE. *see* Denaturing Gradient Gel Electrophoresis (DGGE)
Dhurrin 698
Diarrhoea 14
Dicarboxylic acids 705
Digitaria pentzii 260-261
Diglycerides 467
Dipeptidyl peptidases 32
Direct-fed microbials (DFM) 13-15, 782, 783, 787, 788, 796
Direct flow injection mass spectrometry (DFI-MS) 57, 59
Directive 2001/82/EC 784
DM. *see* Dry matter (DM)
DMI. *see* Dry matter intake (DMI)
DNA-based methods 290
DNA-sequencing technologies 29, 789
Docosahexaenoic acid 668
DP. *see* Degrees of polymerization (DP)
Dried distillers' grains plus solubles (DDGS) 514-516
Dry matter (DM) 520, 620
Dry matter intake (DMI) 550, 615, 616, 622, 624, 652, 654, 658, 660, 676, 700, 791
Dysosmobacter welbionis 35
- EBV. *see* Estimated breeding value (EBV)
ECM. *see* Energy corrected milk (ECM)
E. coli K88 pilus antigen 42
Eicosapentaenoic acid 668
Electron-transport phosphorylation (ETP) 428-429
Embden-Meyerhof-Parnas (EMP) pathway 422
Endoisopeptidase PeiR 163
Energy corrected milk (ECM) 550, 654, 668, 675, 676, 678, 679, 682
Energy metabolism
 adenosine triphosphate (ATP) generation 428-429, 431
 factors influencing microbial growth efficiency 442-449
 cell composition 446-447
 cell lysis 447-448
 energy sinks 443-446
 energy sources utilization for cell growth 448
 strategies for increasing 448-449
 hydrogen disposal 431-434
 interactions with nitrogen metabolism effect on energy spilling 450-451

- synchrony 451–452
 synthesis of amino acids 449–450
 methane
 methanogenesis-inhibited 438–439, 441–442
 production and VFA 436–438
 significance 435–436
 overview 421
 volatile fatty acids (VFA)
 production 422–423, 425–428
 Energy sinks
 comparison 445–446
 glycogen accumulation 444–445
 maintenance 443–444
 spilling 445
Enterococcus spp. 41
 E. faecalis 797
 E. faecium 785, 799
 Entodiniomorpha 192, 193
 Entodiniomorphida 193–195, 203
 Entodiniomorphids 199, 200
Entodinium sp.
 E. bursa 196
 E. caudatum 196, 198, 200, 202, 211, 402, 404, 602
 EO. see Essential oils
Epidinium spp. 4, 201
 Epimural bacteria 4
 Epimural microbiota 321–322, 325, 327–329, 332, 335, 336, 779
 Epithelial cells 714–715, 777
Eremoplastron bovis 202
Escherichia coli 4, 5, 12, 40, 200, 326, 332, 446, 447, 577, 795
Escherichia coli O157-specific IgG
 antibody 42–43
 Essential oils (EO) 406, 731–740
 Estimated breeding value (EBV) 616
 ESTs. see Expressed Sequence Tags (ESTs)
 ETP. see Electron-transport phosphorylation (ETP)
Eubacterium sp.
 E. limosum 110, 111
 E. ruminantium 607
Eudiplodinium sp. 4
 E. maggii 199, 203
 Eukaryotic proteases 385–386
 EukRep software 254, 369
 European Economic Area 784
Euryarchaeota 134
 EVs. see Extracellular vesicles (EVs)
 Exposome-Explorer 60
 Expressed Sequence Tags (ESTs) 203
 Extracellular vesicles (EVs) 287
 FA. see Feed additives (FA)
 FAD. see Flavin adenine dinucleotide (FAD)
Faecalibacterium sp. 35
 F. prausnitzii 14, 39
 Faecal N excretion (FNE) 750
 FAO. see Food and Agriculture Organization (FAO)
 FASN. see FA synthase (FASN)
 FA synthase (FASN) 472, 475
 FCR. see Feed conversion ratio (FCR)
 FDA. see Food and Drug Administration (FDA)
 FE. see Feed efficiency (FE)
 Federal Food, Drug, and Cosmetic Act (FFDCA) 784
 Feed additives (FA) 782–784, 786, 787, 789
 Feed conversion ratio (FCR) 550, 603, 604
 Feed efficiency (FE) 653, 654, 780, 788–793
Feramyces austinii 231
 Ferredoxins 432
 FFDCA. see Federal Food, Drug, and Cosmetic Act (FFDCA)
 Fibre digestion 369
 carbohydrate-active enzymes 352
 carbohydrate-binding modules (CBMs) 354
 glycoside hydrolases 353–354
 lytic polysaccharide monoxygenases (LPMOs) 354–355
 CAZyme and glycan diversity 365–366
 eukaryotes 364–365
 functional omic studies 366–367
 improving 367–368
 lignocellulosic biomass 350
 cellulose 351
 hemicellulose 351–352
 overview 349–350
 prokaryotic strategies
 bacteroidetes, the type IX secretion system and multi-modular CAZymes 362–364
 cellulosomes 355–357
 fibrobacter succinogenes and outer-membrane vesicles (OMVs) 361–362
 polysaccharide utilization loci (PUL) 358–360
 PULs in gram positives 360–361
 secreted enzymes 357–358

- Fibrobacteres* 1, 515
Fibrobacter spp. 32, 514, 631
 F. succinogenes 11, 100, 361, 362, 427, 517, 595, 596, 598, 607, 779, 788
Fibrolytic activity 73, 74
Fibrolytic bacteria 11
'Finger-printing' techniques 150
Firmicutes 2, 4-6, 8, 323, 328, 329, 511
FISH. *see* Fluorescence in situ hybridization (FISH)
Flavin adenine dinucleotide (FAD) 432
Flavin mononucleotide (FMN) 432
Flavobacterium johnsoniae 363
Flavonoids 740
Fluorescence in situ hybridization (FISH) 148
FMN. *see* Flavin mononucleotide (FMN)
FNE. *see* Faecal N excretion (FNE)
Food and Agriculture Organization (FAO) 782
Food and Drug Administration (FDA) 784
Food DB 60
Formyl transferase (*ptr*) 160
ptr. *see* Formyl transferase (*ptr*)
Fuller, R. 781
Functional redundancy and resilience 78, 81-82
Fusobacterium necrophorum 35, 335, 715
GABA. *see* γ -aminobutyric acid (GABA)
Gastrointestinal epithelium 6, 8, 14
Gastrointestinal tract (GIT) 1-2, 12, 16, 17, 701, 776
GDH. *see* Glutamate dehydrogenase (GDH)
GEI. *see* Gross energy intake (GEI)
Gellan gum 32
Gelling agents 32
GEMs. *see* Genome-scale metabolic models (GEMs)
Gene expression 252
Generally Recognized As Safe (GRAS) 784
Genome-scale metabolic models (GEMs) 80, 82
Genome sequencing and rumen microbiome 114-120
 comparative genomics and key functions 105-108
 curation of reference genome catalogue 101-102
 first genome 99
 interactions 109
 antimicrobials effect 113
 competition resources 112-113
 using CRISPR sequences 111
 viral sequences and prophage infections 110-111
metagenomic data application 102-105
overview 97-98
proteome 108-109
single genome 99-101
Genome-wide association studies (GWAS) 367, 559
Genotype-phenotype algorithms 39
'Genotype-to-phenotype' analyses 38
Ghazallomyces constrictus 232
GHG production and nutritional factors 531-532
 carbon-derived materials 524
 biochar 526-528
 humic substances 524
 dried distillers' grains plus solubles (DDGS) 514-516
enteric methane production and manure emissions 507-508
 dietary fat 513-514
 diet digestibility and fermentable carbohydrates 509-511
 nitrogen content 511-513
microbial hydrogen utilization 528-530
nitro-based compounds
 3-nitrooxypropanol (3-NOP) 518
 nitrate 516
overview 505-507
plant secondary compounds
 tannins 521
GHGs. *see* Greenhouse gases (GHGs)
GHs. *see* Glycoside hydrolases (GHs)
GIT. *see* Gastrointestinal tract (GIT)
Global Infectious Disease and Epidemiology Online Network 38
Global Natural Product Social Molecular Networking database 59
Global Rumen Census 152
Global warming 63, 133
Global warming potentials (GWP) 694
Glucomannans 352
Gluconeogenesis 61, 99
Glucuronoxylans 352
Glutamate dehydrogenase (GDH) 389
Glutamate synthase (GOGAT) 389
Glutamine synthetase (GS) 389
Glycerolipid metabolism 61
Glycolysis 422, 425
Glycoside hydrolases (GHs) 353-354

- Glycosyl hydrolases 10, 106, 192
 Glycosyltransferases (GTs) 353, 355
 GNB. *see* Gram-negative bacteria (GNB)
 GOGAT. *see* Glutamate synthase (GOGAT)
 GPB. *see* Gram-positive bacteria (GPB)
 Gram-negative bacteria (GNB) 335, 568
 Gram-positive bacteria (GPB) 576
 GRAS. *see* Generally Recognized As Safe (GRAS)
 Greenhouse gases (GHGs) 133, 593, 651, 662, 694
 Gross energy intake (GEI) 593
 GS. *see* Glutamine synthetase (GS)
 GS-1 activity 392
 GS-GOGAT pathway 394-396
 GTs. *see* Glycosyltransferases (GTs)
 Gut microbiome
 antibody-based isolation 42-44
 culturing methods and nutrient effects on microbial growth 31
 carbon sources 33-34
 micronutrients 34-35
 nitrogen sources 32-33
 reductants 35-37
 genome-directed isolation 37-40
 molecular-based isolation 40-41
 overview 29-30
 GWAS. *see* Genome-wide association studies (GWAS)
 GWP. *see* Global warming potentials (GWP)
- H₂-independent methylotrophic pathway 135
 HAB. *see* Hyperammonia-producing bacteria (HAB)
 Habitat-simulating media 32, 37
Haemophilus influenzae 37, 99
 Haptoglobin 568
hdrABC. *see* Heterodisulfide reductase (*hdrABC*)
 Heatmap clustering 59
Helicobacter pylori 99
 Hemicellulose 426
 Heterodisulfide reductase (*hdrABC*) 165, 170
 HGT. *see* Horizontal gene transfer (HGT)
 High-quality hay (HQH) 328
 High-throughput sequencing approach 2
Hirudo verbena 38
 Homoacetogens 137
Hordeum vulgare L. 696, 699
 Horizontal gene transfer (HGT) 282, 284
 Host-rumen microbiome interactions and feed conversion efficiency (FCE)
 core community 548-549
 genetics 558-560
 methane production 554-557
 microbiome-dependent traits 550, 553-554
 nitrogen compounds 557-558
 overview 547-548
 resilience 549-550
 variation 549
 HQH. *see* High-quality hay (HQH)
 HS. *see* Humic substances (HS)
 HS-CoM. *see* N⁵-methyl-H₄MPT to coenzyme M (HS-CoM)
 HT. *see* Hydrolysable tannins (HT)
 Human Serum Metabolome 60
 Human Urine Metabolome 60
 Humic substances (HS) 524, 528
 Hungate, Robert 709
 Hungate 1000 project 101, 102, 112, 173, 709
 Hybridization 148
 Hybridization-based approaches 240
 Hydrogenotrophic activity 9
 Hydrogenotrophic archaea 528
 Hydrogenotrophic methanogenesis 9, 554, 557
 Hydrogenotrophic methanogens 137, 152, 556
 Hydrogenotrophic pathway 135
 Hydrolysable tannins (HT) 740, 741, 743, 746, 748
 Hyperammonia-producing bacteria (HAB) 387, 558, 733, 738
- ICEs. *see* Integrative and conjugative elements (ICEs)
 ICP-MS. *see* Inductively coupled plasma mass-spectroscopy (ICP-MS)
 IEC. *see* Intestinal epithelial cell (IEC)
 IgA production 6-8
 IgG2 monoclonal antibodies 42
 Illumina MiSeq sequencing technique 101, 323, 324, 789
 Immunobiotics 798
 Immunoglobulins 6, 7, 776
 Immunomagnetic-based cell separation 42
 Inductively coupled plasma mass-spectroscopy (ICP-MS) 57
 Inner-outer membrane vesicles (I-OMVs) 287

- Integrative and conjugative elements (ICEs) 283, 284
- Integrative biology 82
- Internal transcribed spacer (ITS) sequences analysis 226
- Intestinal epithelial cell (IEC) 798
- Intracellular viral genomes 283, 284, 292, 294
- Intra-ruminal recycling 400
- In vitro* gas production (IVGP) systems 730
- I-OMVs. *see* Inner-outer membrane vesicles (I-OMVs)
- Ionophores 209, 406–407
- Ion Torrent sequencing technology 153
- ISO4-G1 enrichment culture 171
- ISO4-H5 genes 170, 171
- Isoprenoids 732
- Isothermal DNA amplification approaches 239
- Isotricha* 4, 398, 399
- Isotrichidae 193
- ITS. *see* Internal transcribed spacer (ITS) sequences analysis
- IVGP. *see* *In vitro* gas production (IVGP) systems
- JGI GOLD database 159
- Joblinomyces apicalis* 232–233
- Johne's disease 12
- KEGG. *see* Kyoto Encyclopaedia of Genes and Genomes (KEGG)
- Keratinization 714
- Khoyollomyces ramosus* 233
- Klebsiella aerogenes* 200
- Kyoto Encyclopaedia of Genes and Genomes (KEGG) 59–60
- Lachnospira multiparus* 622
- Lactic acid bacteria. *see* *Bifidobacterium* sp.
- Lactobacillus* spp. 11, 15, 43
 - L. acidophilus* 43, 795
 - L. amylovorus* 795
 - L. brevis* 796
 - L. plantarum* 167, 796
 - L. rhamnosus* 796
 - L. salivarius* 795
- Lactococcus lactis* 796–797
- Lantibiotics 112
- Large subunit (LSU) sequences 226
- Lateral gene transfer (LGT) 286, 287
- LBP. *see* Lipopolysaccharide-binding protein (LBP)
- LCFA. *see* Long-chain FA (LCFA)
- LC/GC. *see* Liquid/gas chromatography (LC/GC)
- LEE. *see* Locus of enterocyte effacement (LEE)
- Leptospirillum ferrodiazotrophum* 37
- LGT. *see* Lateral gene transfer (LGT)
- Liebetanzomyces polymorphus* 231
- Lignin 620, 621
- Linear programming model 77
- Linoleic acid 667
- Lipid metabolism 491
 - as biomarkers to monitor animal health accumulation of *trans*-10 intermediates 485–486 identifying subacute ruminal acidosis 489–490 odd- and branched-chain fatty acids 486–487 poor in iso-fatty acids 487 de novo fatty acid synthesis 472–473 endogenous mammary metabolism of odd and branched-chain fatty acids 475–476 of saturated and mono-unsaturated fatty acids 476 fatty acids digestion and transfer intestinal digestion 473–474 transport in blood and transfer to mammary gland 474–475 impact on human health 476 conjugated linoleic acids and *n*-3 fatty acids 478–479 *trans* fatty acids 477–478 lipolysis and biohydrogenation 470–472 overview 467, 469 polyunsaturated fatty acid (PUFA) products 479–480 protection technologies aldehyde treatment 483 calcium salts 480 encapsulation within lipids 483–484 fatty acyl amides 483 lipid composite gels 484 tyrosinase cross-linking of emulsions 484
- Lipidomics 57
- Lipolysis 407
- Lipopolysaccharide-binding protein (LBP) 568

- Lipopolysaccharides (LPS) 304, 335, 487, 568-570, 574-578, 779, 792
- Liquid chromatography-high resolution MS 59
- Liquid/gas chromatography (LC/GC) 56, 57, 59
- Liquid-liquid extraction 59
- Liver abscesses 715
- Liver enzymes 571
- Live yeast additives 790-792, 798
- Locus of enterocyte effacement (LEE) 301
- Long-chain FA (LCFA) 478
- LPMOs. *see* Lytic polysaccharide monoxygenases
- LPS. *see* Lipopolysaccharides (LPS)
- LSU. *see* Large subunit (LSU) sequences
- Lucerne 628
- Lysogenic cycle 110
- Lysolecithin 474
- Lysophosphatidylcholines (lysoPC) 572, 573
- Lytic cycle 110
- Lytic polysaccharide monoxygenases (LPMOs) 354-355, 358
- MAA. *see* Milk amyloid A (MAA)
- Machine-learning methods 59
- Macrophages 776
- MAG. *see* Metagenome re-assembled genomes (MAG); Metagenomically Assembled Genome (MAG)
- MAGIC. *see* Metagenomic Alteration of Gut microbiome by in situ Conjugation
- Maize. *see* *Zea mays* L.
- Malondialdehyde (MDA) 577
- Mannheimia haemolytica* 797
- Manual of Systematics of Archaea and Bacteria* 38
- MAPK. *see* Mitogen-activated protein kinase (MAPK)
- Mass spectrometry (MS) 56, 57, 59
- Mathematical prediction models 83, 84, 88
- mcr*. *see* Methyl coenzyme M reductase (*mcr*)
- mcrA*. *see* Methyl coenzyme-M reductase subunit A (*mcrA*)
- mcrA* gene sequencing 149
- mcrII/mrt*. *see* Methyl coenzyme reductase II (*mcrII/mrt*)
- MD-2. *see* Myeloid differentiation factor 2 (MD-2)
- MDA. *see* Malondialdehyde (MDA)
- Mechanistic rumen models 77
- Megasphaera elsdenii* 14, 77, 433, 554, 556, 594, 779, 793
- Membrane vesicles (MVs) 282, 287
- Mercapto-1-propionic acid (MPA) 261
- MetaboAnalyst 59
- Metabolic hydrogen 433
- Metabolite Reference Libraries 59
- Metabolomic technologies 572
- 'Metab' package 59
- Metagenome re-assembled genomes (MAG) 153, 159
- Metagenomes 109
- Metagenomically Assembled Genome (MAG) 103-105, 120, 286, 334
- Metagenomic Alteration of Gut microbiome by in situ Conjugation (MAGIC) 41
- Metagenomic analyses 40
- Meta-omic approaches 30, 211
- Metaparental mating (MPM) 40-41
- Meta-proteomics technique 109
- Metatranscriptomic approach 38
- Methane (CH₄) 63-64, 133, 505, 506, 508, 509, 516, 517, 528, 531, 651-653, 658, 659, 661, 667, 675, 681, 694, 700, 704
- Methanimicrococcus* 517
- Methanobacillus omelienskii* 139
- Methanobacteriaceae* 148, 149, 159
- Methanobacteriales* 4, 135, 172, 173
- Methanobacterium* sp.
- M. bryantii* 139
- M. formicicum* 110, 111, 139, 167
- M. mobilis* 145
- M. ruminantium* 139, 159
- M. sohngeni* 139
- M. thermoautotrophicum* 173
- Methanobrevibacter* sp. 2, 4, 8, 16, 139, 145-147, 149, 153, 159, 165-166, 200, 256, 325, 519, 557, 604, 605
- M. gottschalkii* 152, 548, 555, 613
- M. millerae* 110, 111, 166-167
- M. olleyae* 165, 256
- M. ruminantium* 100, 111, 139, 151-152, 159-160, 162-165, 513, 548, 594, 605, 613, 712
- M. smithii* 594
- M. wolinii* 165-166
- Methanococcales* 149
- Methanococcus maripaludis* 397
- Methanogenesis 4, 16, 34, 64, 74, 101, 134, 135, 137, 138, 153, 256, 432,

- 437–439, 450, 529, 530, 554,
556, 705
- Methanogens 9, 16, 34, 64, 73, 74,
134–136, 138, 139, 149, 437, 555,
557, 612
- Methanogen symbionts 149
- Methanol 135, 137, 428
- Methanomassilicoccales* 4, 16, 146, 147,
149, 151, 159, 168–171, 325
- Methanomassilicoccaceae* 152, 169,
450, 519
- Methanomassiliococcus* 256
- Methanomethylophilus* 2, 4
- Methanomicrobiales* 4, 148, 517
- Methanomicrobium* sp. 150, 510
- M. mobile* 145
- Methanomicrococcus* 510
- Methanoplasma gallocaecorum* 169
- Methanosarcinaceae* 149
- Methanosarcinales* 4, 135, 148, 159, 517
- Methanosarcina* sp. 137, 139, 172
- M. barkeri* 145, 173, 556
- M. mazei* 397
- Methanosphaera* sp. 2, 4, 8, 16, 34, 135, 146,
147, 149, 152, 159, 171–172, 517
- M. stadmanae* 594, 604, 712
- Methanospirillum hungatei* 145
- Methenyl-H₄MPT 162
- Methoxymirabilis oxyfera* 530
- Methylamines 135, 137, 428, 450
- Methylated compounds 16, 135
- Methylated sulfur compounds 428
- Methyl coenzyme M reductase (*mcr*) 149,
159, 613
- Methyl coenzyme-M reductase subunit A
(*mcrA*) 756
- Methyl coenzyme reductase II (*mcrII*/
mrt) 159
- Methylotrophic methanogens 135, 152,
325, 428, 528
- Methyl-viologen hydrogenase
(*mvhADG*) 170
- MFD. see Milk fat depression (MFD)
- MGEs. see Mobile genetic elements (MGEs)
- Microbial dysbiosis 779
- Microbial metabolism 73
- Microbial protein breakdown 408–409
and ammonia assimilation 387–399
anaerobic rumen fungi 397–398
archaeal nitrogen metabolism 397
ciliate protozoa 398–399
enzymatic pathways 389–390
- mechanisms of regulation 390–392
in rumen bacteria 393–396
transport 388–389
- efficiency of nitrogen metabolism
dietary factors 405–407
rumen-degraded protein
measurement 404–405
- intra-ruminal recycling 400–402
overview 383–384
proteolysis and deamination 384–387
protozoa-mediated proteolysis and intra-
ruminal recycling 402–404
sequencing approaches with *in vitro/ex*
 vivo cultures 404
synthesis 399–400
- Micromanipulator method 205
- Milk amyloid A (MAA) 574, 575
- Milk Composition Database 60
- Milk fat depression (MFD) 668
- Mini-ICEBs1 41
- Mitogen-activated protein kinase
(MAPK) 577
- Mitomycin C 292
- Mobile genetic elements (MGEs) 282, 301
- Molecular fingerprinting technique 747
- Monensin 705
- Monoglycerides 467
- Monoterpenes 732
- Mono-unsaturated FA (MUFA) 476
- Morphology
of bulbous genera 237–238
of monocentric genera 233–234
of polycentric genera 235
- MPA. see Mercapto-1-propionic acid (MPA)
- MPM. see Metaparental mating
- MPS. see 3-mercapto-1-propanesulfonic acid
(MPS)
- MS. see Mass spectrometry (MS)
- MUFA. see Mono-unsaturated FA (MUFA)
- Multi-omics techniques 70, 78, 83, 85, 88,
333, 337, 800
- mvhADG*. see Methyl-viologen hydrogenase
(*mvhADG*)
- MVs. see Membrane vesicles (MVs)
- Mycobacterium avium* 12
- Mycoplasma genitalium* 37, 99
- MyD88. see Myeloid differentiation primary
response gene 88 (MyD88)
- Myeloid differentiation factor
2 (MD-2) 568
- Myeloid differentiation primary response
gene 88 (MyD88) 568

- N⁵-methyl-H₄MPT to coenzyme M (HS-CoM) 162
 NADP-dependent alcohol dehydrogenase (*adh1, 2, 3*) genes 162
 NADPH-dependent F₄₂₀ dehydrogenase (*npIG1,2*) genes 162
Nanopusillus acidilobi 37
 Natural antisense transcripts (NATs) 252
 Natural milk feeding 11
 NCDDs. see Non-catalytic dockerin domains (NCDDs)
 NDF. see Neutral detergent fibre (NDF)
 NEFA. see Non-esterified fatty acids (NEFA)
Neocallimastigomycota 221, 224, 599
Neocallimastix sp. 13, 226, 229–230, 258, 259, 600, 601
 N. californiae 229, 230
 N. cameroonii 229, 230
 N. frontalis 145, 147, 226, 229, 230, 398
 N. hurleyensis 229
 N. joyonii 229, 234
 N. patriciarum 226, 229, 241, 259–260, 398, 789
 N. variabilis 229
 Neutral detergent fibre (NDF) 519, 520, 601, 620, 621, 624, 632, 659, 662, 703, 790
 Neutrophils 568, 577, 776, 795
 Next-generation sequencing (NGS) technologies 88, 152, 153, 159, 174, 211, 238, 622, 709
 NFκ-B. see Nuclear factor κ-B (NFκ-B)
 NGS. see Next-generation sequencing (NGS) technologies
 Nitrification 517
 Nitrogen (N) recycling 72
 Nitrogen retention 628
 Nitrogen-use efficiency (NUE) 653, 654, 658, 660, 669, 675, 676, 681
 Nitrous oxide (N₂O) 505, 507, 527, 651, 694
 NMR. see Nuclear magnetic resonance (NMR)
 Non-catalytic dockerin domains (NCDDs) 241, 251
 Non-esterified fatty acids (NEFA) 467, 571
 Non-ribosomal peptide synthetases (NRPSs) 163–164, 167
 Non-structural carbohydrates (NSC) 654
 Non-structural polysaccharides 135
 Non-volatile fatty acids 34
npIG1,2. see NADPH-dependent F₄₂₀ dehydrogenase (*npIG1,2*) genes
 Nrf2. see Nuclear factor erythroid 2-related factor 2 (Nrf2)
 NRPSs. see Non-ribosomal peptide synthetases (NRPSs)
 NSC. see Non-structural carbohydrates (NSC)
 Nuclear factor erythroid 2-related factor 2 (Nrf2) 577
 Nuclear factor κ-B (NFκ-B) 568
 Nuclear magnetic resonance (NMR) 56, 57, 59
 NUE. see Nitrogen-use efficiency (NUE)
 Oats. see *Avena sativa*
 OBCFA. see Odd- and branched-chain FA (OBCFA)
 Odd- and branched-chain FA (OBCFA) 469, 473, 474, 486
 Oleic acid 667
 OM. see Organic matter (OM)
 Omics techniques 78
 OMVs. see Outer-membrane vesicles
Oontomyces anksri 230
 Operational taxonomic units (OTU) 557, 559, 605, 606
 Organic matter (OM) 506, 509, 601, 621, 630, 663, 667
 Orpin 226
Orpinomyces sp.
 O. bovis 234
 O. intercalaris 234
 O. joyonii. see *Neocallimastix joyonii*
Ostertagia circumcincta 146
 OTU. see Operational taxonomic units (OTU)
 Outer-membrane vesicles (OMVs) 287, 361–362, 368
Oxytricha trifallax 198
 PacBio sequencing technologies 101
 Pangenomes 107
 Papilla epithelium surface 567
 Parakeratosis 714
 Partial genome sequences 39
 Partial least squares discriminant analysis (PLSDA) 59
 Pasture-based diets 61
 Pasture-fed ruminant production systems 633–634
 cellulose degradation 595
 bacterial degradation 596, 598–599
 fungi and 599–601
 protozoa and 601–602
 diet and rumen microbiome 594–595

- feed efficiency of ruminants 603–607
 digestibility and 609, 612
 volatile fatty acids (VFAs) 608–609
 forage plants impact on animal performance 619–620
 grasses 630–632
 grassland management 621–622
 legumes 622–629
 methane-producing microbes 612–615
 methane production and residual feed intake 615–616, 619
 overview 591–594
 Pattern recognition receptors (PRRs) 6
 PC. *see* Phosphatidylcholines (PC)
 PCA. *see* Principal component analysis (PCA)
 PCR. *see* Polymerase chain reaction (PCR)
 PCR-based approach 239, 253
 PCR-denaturing gradient gel electrophoresis (PCR-DGGE) 323, 553
 P-cresol 61
Pecoramyces ruminantium 231, 239, 241, 251
 Pectin methyl esterases (PMEs) 168
 Pectin polysaccharides 620
 Pectins 426, 428
 pEHR vector system 40
 Peptide degraders 106
 Peptidoglycan recognition protein (PGLYRP1) 777
Peptostreptococcus anaerobius 32, 733
 Perennial ryegrass (PRG) 623–625, 629, 630
 Pfam protein families 10
 PGLYRP1. *see* Peptidoglycan recognition protein (PGLYRP1)
 Phage Communities from Contig Spectrum (PHACCS) 297
Phascolarctobacterium spp. 34
 Phenol-Explorer 60
 Phenylalanine 732
 Phenylpropanoids 732
 Phenylpropene secondary metabolites 731, 732
 Phloroglucinol 529
 Phosphate salts 32
 Phosphatidylcholines (PC) 572, 573
 Phosphoenolpyruvate 426
 Phospholipids (PL) 467
 Phosphorus recycling 72
 Phytochemicals/phytochemicals. *see* Plant secondary compounds
Piromonas sp. 258, 259
 P. communis 230
Piromyces sp. 147, 230, 238, 600, 601
 P. communis 398
 P. rhizinflata 601
 PL. *see* Phospholipids (PL)
 Plant secondary compounds 758
 essential oils (EO)
 activity 732–733
 composition 731–732
 effects on animal performance 739–740
 effects on rumen microbiome and function 733–734, 738
 overview 727–731
 saponins
 activity 754–755
 composition 751, 754
 effects on animal performance 756–757
 effects on rumen function 755–756
 tannins
 activity 742–743
 composition 740–741
 effects on animal performance 749–750
 effects on rumen function 743, 746–749
 PLs. *see* Polysaccharide lyases (PLs)
 PLSDA. *see* Partial least squares discriminant analysis (PLSDA)
 PMEs. *see* Pectin methyl esterases (PMEs)
 Polyflagellate zoospores 234
 Polymerase chain reaction (PCR) 149–151
 Polyphenol oxidase (PPO) 627
Polyplastron sp. 4
 P. multivesiculatum 197, 203, 211, 556
 Polysaccharide hydrolysis 100
 Polysaccharide lyases (PLs) 353
 Polysaccharide utilization loci (PULs) 350, 358–360
 Polyunsaturated fatty acid (PUFA) 207, 469, 470, 476, 478–480
 PPO. *see* Polyphenol oxidase (PPO)
Prevotella sp. 510, 512, 513, 553, 598–599, 605, 606
 P. brevis 711
 P. bryantii 32, 359, 594, 711
 P. ruminicola 32, 106, 393, 394, 396, 427, 711, 733, 738
 PRG. *see* Perennial ryegrass (PRG)
 Principal component analysis (PCA) 59
 Proanthocyanidins. *see* Condensed Tannins (CT)

- Probiotics 800
 benefits and modes of action
 effects on immune system 797-799
 feed efficiency in adult ruminants 788-793
 methane production 793-794
 pathogen control 794-797
 young ruminants 785-788
 critical periods in ruminant lifecycle 776-781
 definitions 781-782
 delivery mechanisms 782-784
 overview 775-776
 regulation 784-785
- Pro-inflammatory cytokines 568
- Prophages. *see* Intracellular viral genomes
- Propionate production 436, 438
- Propionibacterium* sp. 793, 794
P. acnes 486
P. freudenreichii 486
- Proteobacteria* 2, 4-6, 8, 105, 323, 328, 389, 390
- Proteomics 108-109
- Protozoa 73, 74, 77, 78, 81, 322, 400-401, 557
- PRRs. *see* Pattern recognition receptors
- Pseudomonas* sp.
P. citronellolis 523
P. plecoglossicida 523
- Pseudobutyribrio* 627
- PUFA. *see* Polyunsaturated fatty acid
- PULs. *see* Polysaccharide utilization loci (PULs)
- Pyruvate 61, 425, 426
- QIIME. *see* Quantitative Insights Into Microbial Ecology (QIIME)
- qRT-PCR. *see* Quantitative real-time PCR (qRT-PCR)
- Quantitative Insights Into Microbial Ecology (QIIME) 152
- Quantitative metatranscriptomic methods 366
- Quantitative real-time PCR (qRT-PCR) 2, 4, 151, 323, 605, 789
- Quebracho. *see* *Schinopsis lorentzii*
- QuEChERS method 59
- RCC. *see* Rumen Cluster C (RCC)
- RDP. *see* Rumen-degraded protein (RDP)
- Reactive oxygen species (ROS) 577
- Red clover (RC). *see* *Trifolium pratense*
- Regulation EC 1831/2003 784
- Residual feed intake (RFI) 332, 550, 553, 593, 604, 606, 608, 609, 615, 616, 626, 788
- Residual intake and gain (RGI) 550
- Restriction modification (RM) systems 294
- RFI. *see* Residual feed intake (RFI)
- RGI. *see* Residual intake and gain (RGI)
- Rikenella-like bacterium 38
- RIM-DB. *see* Rumen and Intestinal Methanogen-DB (RIM-DB)
- RM systems. *see* Restriction modification (RM) systems
- RO. *see* *Ruminantium-olleyae* (RO)
- ROS. *see* Reactive oxygen species (ROS)
- RP4 (RK2)-mediated conjugative transfer 40
- RUGs. *see* Rumen Uncultured Genomes (RUGs)
- Rumen and Intestinal Methanogen-DB (RIM-DB) 152
- Rumen Cluster C (RCC) 147, 150, 168, 256
- Rumen-degraded protein (RDP) 383
- Rumen health
 and mammary immune system 574-578
 metabolic activity and disorders 570-574
 overview 567-569
 subacute ruminal acidosis (SARA) 569-570
- Rumen metabolomics 55, 64-65
 factors impacting 60-63
 targeted vs. untargeted metabolomics 56-57
 technologies for analysis and extraction techniques 57
 data interpretation 59-60
- Rumen microbial functionality 89
 conceptual approaches in modelling community dynamics 78-82
 interactions 74-75, 77-78
 interactions between ruminant host and rumen content 71-72
 intra-ruminal conditions 72-73
 types 73-74
 overview 69-71
 quantifying 82-85
 units and sampling techniques 85, 87-88
- Rumen microbiota 17
 establishment
 colonization 2, 4-6
 interaction host-microbiota 6-8

- management practices impact 11-12
- microbial activities and functions
 - 9-10
 - transmission modes 10-11
 - weaning and stabilization 8-9
- improving health and production 13-15
- modulating in young ruminants 12-13
- overview 1-2
- reducing enteric methane
 - emissions 15-16
- Rumen papillary development 15
- Rumen protozoa 211
 - challenges 204-205
 - discovery 192-193
 - ecological fluctuations 198-199
 - effects of function on ruminant nutrition,
 - health and emissions 205
 - fibre digestion 206-207
 - methane emissions 208
 - nitrogen-use efficiency 206
 - products 207
 - genomics 197-198
 - interactions
 - with rumen bacteria 199-200
 - with rumen fungi 200
 - with rumen methanogen 200-201
 - with rumen plant 201-204
 - manipulating 208-210
 - overview 191-192
 - taxonomy and population types
 - 193-197
- Rumen simulation technique (RUSITEC) 525, 712
- Rumen Uncultured Genomes (RUGs) 153
- Rumen-undegraded protein (RUP) 385
- Rumen wall microbiota community
 - archaea composition and function
 - 324-325
 - bacteria composition and function
 - 323-324
 - development 325-327
 - factors affecting 327-329
 - host-microbial interaction 335-336
 - impact on ruminant production
 - feed efficiency 332-333
 - methane emissions 333
 - milk composition 329, 332
 - manipulation strategies 336
 - microbial functions 333-335
 - overview 321-322
 - sample collection and data
 - handling 336-337
- Ruminal viruses 287-294, 296-299, 305-306
 - and extrachromosomal elements 282
 - membrane vesicles 286-287
 - plasmids 284-286
 - viral genomes 282-284
- mobilome role and impact 299
 - cell lysis 300
 - horizontal gene transfer 300-302
 - influence on ruminant host 304
 - microbial populations
 - modulation 302-303
 - phenotypic traits and growth habits 303-304
 - overview 281-282
- Ruminantium-olleyae* (RO) 555
- Ruminobacter amylophilus* 487
- Ruminococcaceae* 510, 514
- Ruminococcus* sp. 514, 606-607, 779
 - R. albus* 14, 34, 112, 285, 356, 393, 395, 486, 517, 596, 619
 - R. flavefaciens* 112, 203, 356, 486, 517, 596
 - R. gnavus* 35
- Ruminomyces elegans* 234
- RUP. *see* Rumen-undegraded protein (RUP)
- RUSITEC. *see* Rumen simulation technique (RUSITEC)
- SAA. *see* Serum amyloid A (SAA)
- Saccharomyces cerevisiae* yeast (SCY) 14, 15
- Saccharomyces* sp.
 - S. boulardii* 783, 787
 - S. cerevisiae* 794, 795
- Salmonella enterica* 795
- Salmonellosis 795
- Samanea saman* 757
- Sapindus saponaria* 209
- Saponins 209, 406
- SARA. *see* Sub-acute ruminal acidosis (SARA)
- Saturated FA (SFA) 469
- ScaA. *see* Scaffoldin protein (ScaA)
- Scaffoldin protein (ScaA) 356, 357, 596
- Scanning electron microscopy
 - technique 326
- SCD. *see* Stearoyl-CoA desaturase (SCD)
- Schinopsis lorentzii* 741
- SCY. *see* *Saccharomyces cerevisiae* yeast
- Selenomonas* spp. 32
 - S. ruminantium* 199, 427, 487, 517, 594, 595, 733, 779

- Serratia marcescens* Cbp21 enzyme 355
 Serum amyloid A (SAA) 568, 792
 SFA. *see* Saturated FA (SFA); Short-chain fatty acids (SFA)
Shigella 12
 Short-chain fatty acids (SFA) 473
 Shotgun proteomics 109
 SILVA database 152
 SIMCA tools 59
 Single molecule real time (SMRT) sequencing 238
 Small Molecular Pathway Database (SMPDB) 60
 Small subunit ribosomal RNAs (SSU rRNAs) 148
Smithii-gottschalkii-millerae-thaurei (SMGT) 555, 557
 SMPDB. *see* Small Molecular Pathway Database (SMPDB)
 SMRT sequencing. *see* Single molecule real time (SMRT) sequencing
 Solid phase extraction 59
Sorghum bicolor 697, 699
Sphaeromonas sp. 237
 S. communis 287
 Sphingomyelins 573
Spiribacter salinus 37
 Spizellomycetales 224
 Squamous multilayer epithelium 567
 SSU rRNAs. *see* Small subunit ribosomal RNAs (SSU rRNAs)
Staphylococcus aureus 577
 Stearoyl-CoA desaturase (SCD) 476
 Steroidal saponins 751
 Straight-chain FA 472
Streptococcus spp. 5, 32, 112
 S. bovis 77, 112, 289, 487, 517, 594, 733
 S. equinus 289, 290, 292, 303
 Structural polysaccharides 134-135
 Sub-acute ruminal acidosis (SARA) 62-63, 324, 328, 485, 489-490, 567, 569-570, 575, 577, 578, 671, 701, 779, 790
Succiniclasticum 1
Succinivibrio dextrinosolvens 146, 554, 607
Succinivibrionaceae 37, 514, 554
Succinomonas amyolytica 487
Sutterella sp. 35
Syntrophomonas wolfei 139, 145

 T9SS. *see* Type IX secretion system
 TAG. *see* Triacylglycerides (TAG)
Tahromyces munnarensis 233
 TALC. *see* *Thermoplasmatales*-associated lineage C (TALC)
 Tannins 209-210, 406
 Taxonomic binning 103
 TCA. *see* Tricarboxylic acid cycle (TCA)
 TEM. *see* Transmission electron microscope (TEM)
 Temperature Gradient Gel Electrophoresis (TGGE) 150
 Terminal Fragment Length Polymorphism (TRFLP) 150
 Terpenes 731
 Terpenoids 732
Tetrahymena thermophila 198
 TGGE. *see* Temperature Gradient Gel Electrophoresis (TGGE)
Thermoplasmatales 168, 169
Thermoplasmatales-associated lineage C (TALC) 146, 147, 150
 Thiamine supplementation 62, 63
 TLRs. *see* Toll-like receptors (TLRs)
 TMA. *see* Trimethylamine (TMA)
 TMAO. *see* Trimethylamine-*N*-oxide (TMAO)
 TMR. *see* Total mixed ratio (TMR)
 Toll-like receptors (TLRs) 6-7, 335, 568, 576, 777, 779
 Total mixed ratio (TMR) 60, 61, 667, 678
 Toxic Exposome Database 60
 Traitair 38, 39
 Transducing phage 283
 Transmission electron microscope (TEM) 288, 290
 TRFLP. *see* Terminal Fragment Length Polymorphism (TRFLP)
 Triacylglycerides (TAG) 467
 Triacylglycerols 572
 Tricarboxylic acid cycle (TCA) 61
Trichoderma reesei 354, 357-358
Trifolium sp.
 T. pratense 626-629
 T. repens 623-629
 Trimethylamine (TMA) 168
 Trimethylamine-*N*-oxide (TMAO) 168
 Triterpenoid saponins 751
Triticum aestivum L. 697, 699
 Type II methanotrophs 530
 Type I methanotrophs 530
 Type IX secretion system (T9SS) 362-364, 368

 Ubiquitin-aided proteolysis 386
 Unsaturated FA (UFA) 407, 469

- UTase uridylylation/deuridylylation activity 392
- van Leeuwenhoek, Antonie 192
- Veillonellaceae* 514
- Verstraetearchaeota* 134
- Very-low-density lipoproteins (VLDL) 474
- Vestibuliferida 192, 193, 195, 199, 203, 210
- VFA. *see* Volatile fatty acids (VFA)
- VirSorter software 369
- VLDL. *see* Very-low-density lipoproteins (VLDL)
- Volatile fatty acids (VFA) 34, 72, 73, 135, 332, 421–423, 425–428, 436–438, 441, 442, 506, 567, 592, 608–609, 624, 627, 652, 671, 694, 701, 779
- Water-soluble carbohydrates (WSC) 631, 632, 654
- Weaning stage 8–9, 777
- Wheat. *see* *Triticum aestivum* L.
- White clover (WC). *see* *Trifolium repens*
- WHO. *see* World Health Organization (WHO)
- Wolinella succinogenes* 99, 138
- World Health Organization (WHO) 782
- WSC. *see* Water-soluble carbohydrates (WSC)
- XPORT system 41
- Xylans 352, 620
- Xyloglucan 352
- Xylose 426
- Yeast FA 791
- Yeast Metabolome Database 60
- Zea mays* L. 653–654, 696–699