联合国粮食及农业组织 | Food and Agriculture Organization of the United Nations | Organisation des Nations Unies pour l'alimentation et l'agriculture | Продовольственная и сельскохозяйственная организация Объединенных Наций | Organización de las Naciones Unidas para la Alimentación y la Agricultura | منظمة الأغذية والزراعة للأمم المتحدة

E

COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Item 9.2 of the Provisional Agenda

Nineteenth Regular Session

Rome, 17–21 July 2023

DRAFT STUDY ON THE SUSTAINABLE USE AND CONSERVATION OF MICROORGANISMS OF RELEVANCE TO RUMINANT DIGESTION

NOTE BY THE SECRETARIAT

1. The Commission on Genetic Resources for Food and Agriculture (Commission) at its Seventeenth Regular Session adopted its Work Plan for the Sustainable Use and Conservation of Micro-organism and Invertebrate Genetic Resources for Food and Agriculture (Work Plan).[1] The Work Plan addresses microorganisms and invertebrates as functional groups and foresees that two of these groups will be addressed at each forthcoming session of the Commission. For the current session, the Work Plan foresees addressing soil micro-organisms and invertebrates, with emphasis on bioremediation and nutrient cycling organisms and micro-organisms of relevance to ruminant digestion.[2]

2. In response to the Work Plan, FAO commissioned Queen's University Belfast, United Kingdom, to prepare a study on the sustainable use and conservation of microorganisms of relevance to ruminant digestion. A draft version of the study[3] was presented to the Twelfth Session of the Intergovernmental Technical Working Group on Animal Genetic Resources for Food and Agriculture. The Working Group invited Members and observers to submit concrete comments on and inputs to the draft study by 1 April 2023.[4] Two countries provided comments. These are made available to the Commission in the document *Submissions by Members on the draft study on the sustainable use and conservation of microorganisms of relevance to ruminant digestion*.[5] A revised version of the study is provided in this document. Following review of the draft study by the Commission, it will be published as a Background Study Paper.

[1] CGRFA-17/19/Report, paragraph 95.
[2] CGRFA-17/19/Report, Appendix E, paragraph 14.
[3] CGRFA/WG-AnGR-12/23/6/Inf.1.
[4] CGRFA/WG-AnGR-12/23/Report, paragraph 28.
[5] CGRFA-19/23/9.2/Inf.2.

DRAFT STUDY ON THE SUSTAINABLE USE AND CONSERVATION OF MICROORGANISMS OF RELEVANCE TO RUMEN DIGESTION

Sharon A. Huws, Linda B. Oyama and Christopher J. Creevey

School of Biological Sciences, Institute for Global Food Security, Queen's University Belfast, 19 Chlorine Gardens, Belfast BT9 5D, United Kingdom.

Table of Contents

Acknowledgements

The authors would like to thank members of the Global Research Alliance (GRA) Rumen Microbial Genomics network for filling in the survey and conveying their thoughts on the future of the sustainable use and conservation of microorganisms of relevance to rumen digestion. The support of officers from FAO, namely Julie Bélanger, Paul Boettcher, Madhur Dhingra, Fanette Fontaine, Irene Hoffmann, Anne Mottet and Dafydd Pilling, is also gratefully acknowledged. We are also indebted to Hayden Montgomery, (GRA and the Global Methane Hub) and Sinead Waters (Teagasc Ireland and Co-chair Large Animal Grouping of the GRA) and independent anonymous reviewers from the Netherlands and Canada for reviewing the document before finalization.

Abbreviations and acronyms

AMR: antimicrobial resistance

DFM: direct-fed microbials

DHP: 4-hydroxy-4(H)-pyridone

EPS: extracellular polymeric substances

FAO: Food and Agriculture Organization of the United Nations

FAOSTAT: Food and Agriculture Organization of the United Nations statistics

GH: glycosyl hydrolase

GHG: greenhouse gas

GRA: Global Research Alliance

GWP: global warming potential

ICSP: International Committee of Systematics of Prokaryotes

IPCC: Intergovernmental Panel on Climate Change

LAB: lactic acid bacteria

MAG: metagenomically assembled genome

3-NOP: 3-nitrooxypropanol

UN: United Nations

UNFCCC: United Nations Framework Convention on Climate Change

VFA: volatile fatty acid

WHO: World Health Organization

Executive summary

It is estimated that over 11.7 percent of humans do not have access to sufficient food and hence suffer from nutrient deficiencies and conditions such as anaemia and stunting. Moreover, it is predicted that the world's population will reach 10.4 billion in the 2080s. Ruminant products are high in protein and micronutrients, however, ruminant production is a major source of methane (CH_4), a greenhouse gas (GHG) that has between 27 and 30 times the global warming potential (GWP) of carbon dioxide (CO_2).

Ruminant productivity and methane emissions are mainly a consequence of the biochemical processes that occur within the rumen (the main compartment of the forestomach of ruminant animals) when dietary carbohydrates are broken down by rumen microbes. The process results in the production of volatile fatty acids (VFAs), which provide a source of energy for the animal, but also involves the generation of hydrogen that is used by methanogenic rumen microbes mainly to convert CO_2 into methane. The production of certain VFAs can result in more hydrogen being used up and thus directed away from methanogenesis. The need to understand the rumen microbiome has consequently never been greater.

The rumen is a complex, dynamic ecosystem composed of anaerobic bacteria, protozoa, anaerobic fungi, methanogenic archaea and the very understudied bacteriophages. The scientist Robert Hungate is considered the "father of rumen microbiology". His research resulted in many of the culture technologies for anaerobic bacteria and archaea used today and revealed that the rumen bacteria contain the most abundant and diverse group of rumen microorganisms. The rumen bacteria have a multitude of functions. For example, they can be amylolytic (have the capacity to break down starch), cellulolytic (have the capacity to break down cellulose), proteolytic (have the capacity to break down protein) and lipolytic (have the capacity to breakdown lipids/fats. Many of these microbes are considered to be generalists, i.e to have a broad range of functions, and others as more specialist.

While the rumen bacteria are the most numerous rumen microorganisms, the rumen protozoa occupy the most space within the rumen (up to 50 percent). However, they remain understudied. This is also the case for the rumen fungi, although they have received more attention in recent years, with a total of 18 genera now identified. Together, the rumen protozoa and fungi are termed the "eukaryotome" or "eukaryome". Certain rumen protozoa (e.g. *Entodinium* and *Epidinium* spp.) are fibrolytic, while others (e.g *Dastrychia* and *Isotrichia* spp.) utilize "simple carbohydrates", thus aiding forage breakdown and increasing the availability of nutrients to the host. Likewise, anaerobic rumen fungi are potent fibre degraders thanks to their extensive repertoire of carbohydrate-degrading enzymes. Rumen protozoa are, however, linked to methanogenesis. Defaunated ruminants (animals that have had the protozoa chemically removed from their rumens) have been found to have on average 11 percent lower methane emissions than their non-defaunated counterparts. Defaunated ruminants are also more productive in terms average daily weight gain or milk production, presumably because bacterial ingestion and digestion by the rumen protozoa is eliminated. This must, however, be balanced against the possible impact on fermentation products accounted for by rumen protozoa.

Methane is produced mainly via the hydrogenotrophic pathway, which results in methane being produced from hydrogen and CO_2, although a small amount can be produced through utilization of methyl groups (methylotrophic pathway) or, even less commonly, from acetate (the acetoclastic pathway). Hydrogenotrophic methanogens include the genus *Methanobrevibacter* (*Mbb.*), which is subdivided into the SGMT clade (*Mbb. smithii*, *Mbb. gottschalki*, *Mbb. millerae* and *Mbb. thaurei*) and the RO clade (*Mbb. ruminantium* and *Mbb. olleyae*), which are the most abundant rumen methanogens. Specifically, the *Mbb. gottschalkii* and *Mbb. ruminantium* clades have been confirmed as the two largest groups and account for 74 percent of all rumen archaea globally. Methylotrophic methanogens are less abundant and include *Methanosarcinales, Methanosphaera*, and *Methanomassiliicoccaceae*. The *Methanosarcinales* can also produce methane via the acetoclastic pathway.

It has been shown that the rumen contains a core microbial community, the diversity of which is driven primarily by diet but is also influenced by the species and breed of host. Recent work has also demonstrated, based on hereditability estimations, the potential to breed for specific host-selected

microbiomes, especially in the context of reducing GHG emissions. Minor groups of rumen organisms also appear to be geographically specific, probably driven by climate-specific changes in the plant material consumed or linked to local breeds of ruminants.

There are indications, although based on a low number of publications, that increasing focus on efficiency and reduction of emissions in the livestock sector is linked to a reduction in rumen bacterial diversity. If this is true, then current practices may lead to the loss of entire groups of rumen microbes that, while peripheral to the core functions of fermentation, may represent biodiversity vital for resilience to changing climatic conditions. This underlines the need to capture and catalogue the extant natural communities of rumen bacteria, archaea, fungi, protozoa and viruses, as there is a danger they will be lost. Respondents to a survey of members of the Global Research's Alliance's (GRA's) Rumen Microbial Genomics (RMG) network conducted for the present study and covering individuals from academia, industry and government from Africa, Europe, North America and Latin America, among others, mentioned this risk of losing microbial diversity. However, they indicated that their expectations for the health of the diversity of the rumen microbiome over the next decade were positive, primarily because of advances in knowledge generation and the promise offered by initiatives in the field of culturing and cataloguing the microbiome.

Effective management of the rumen microbiome can result in mitigation of methane emissions from ruminants. An "optimum" rumen microbiome, in terms of productivity, animal and environmental health, can be achieved through breeding or dietary interventions. Efforts to breed ruminants with such an "optimum" microbiome are well underway globally, with a lot of data having been generated. Making this a commercially feasible option will require more data from more animals. However, early data suggest that breeding efforts could result in reductions of up to 30 percent in methane emissions based on residual methane emissions, although much more data are required.

Dietary interventions can be broadly grouped into the following categories: plant-based strategies (e.g. feeding plants that are high in secondary compounds, such as tannins), targeted methane inhibitors (such as 3-NOP, which is commercially known as Bovaer®), oils and oilseeds, and hydrogen sinks (e.g. chemicals or microbes that utilize hydrogen so that there is less available for methanogenesis). Forage-based strategies can decrease methane emissions by up to 18 percent based on emission intensity for milk produced (g CH_4/kg of milk), and forage management interventions (as opposed to using different forages) such as feeding less mature forages can increase average daily weight gain or milk production by up to 13 percent based on methane emission intensity for a given amount of milk produced (g CH_4/kg milk). Methane inhibitors, especially 3-NOP (Bovaer®), can achieve methane reductions of 35 percent based on methane intensity and daily methane measurements. However, their effects on production gains remain unclear.

More recently developed dietary interventions to reduce methane emissions include feeding macroalgae. The red seaweed macroalgae *Asparagopsis taxiformis* inhibits methanogens and consequently methanogenesis, and so can be considered a methane inhibitor. It has been shown to reduce methane intensity and daily methane emissions by up to 80 percent in both dairy and beef animals, which is the largest reduction achieved to date. The active ingredient in *A. taxiformis* is bromoform, which is carcinogenic, and therefore this strategy requires more animal and human health studies so that any potential trade-offs can be monitored in depth. Potential future strategies, supported by a growing wealth of data, include the use of hydrogen sinks and the of use of novel direct-fed microbes. The expert survey respondents noted the need to further enhance knowledge of rumen microbes and management strategies promoting livestock production efficiency.

The interconnectedness of microbes across the human–animal–environmental axis has been demonstrated by many researchers, and the implications of this need to be considered, especially with respect to One Health challenges such as the spread of antimicrobial resistance (AMR). It has been shown that rumen bacteria possess antimicrobial resistance genes on integrative elements that are easily transferred to other bacteria, illustrating the importance of rumen bacteria to the spread of AMR genes. Rumen microbes also offer novel bioactive compounds, which can be used to enhance planetary health (e.g. therapeutic development of novel antimicrobials).

Current policies affecting the management of microorganisms of relevance to ruminant nutrition include those related to climate change and those, such as the Nagoya Protocol, related to access and benefit-sharing. Climate policies are increasingly influencing the allocation of research funding, with many funders focusing calls on optimizing the rumen microbiome to achieve reductions in methane emissions. Climate policies have also influenced innovation, leading to more effort being put into the development of practical innovative solutions that improve understanding of "optimal" rumen microbiomes and how to achieve them. However, regulatory frameworks also act as a barrier to the use of such technologies because of the amount of time needed to obtain approval. For example, 3-NOP is currently approved as a dietary additive for dairy cows in many countries, including Australia, Brazil, Chile and the countries of the European Union. However, the approval process took approximately 10 years, and a large body of research was needed in order to provide the required evidence of the product's efficacy and safety.

Feeding dietary additives has a cost to the farmer, and uptake will be constrained unless these costs can be borne by the consumer, manufacturer or through a governmental payment scheme under an emissions-reduction policy. The cost implications of feeding dietary additives are also a barrier to their use in developing countries. The alternatives for these countries may be improving ruminant efficiency and/or land management-based ones such as utilizing more dietary legumes. However, the latter will not reduce methane emissions to the same extent as using additives such as 3-NOP. The expert survey respondents noted that there was insufficient funding to study and implement methane mitigation technologies, especially in low- and middle-income countries.

With regard to the sharing of rumen microbial genetic resources, it should be noted that the paperwork associated with the need to comply with the Nagoya Protocol acts as a barrier to exchange. While the ethos of the protocol is admirable, there is a need to simplify these requirements in order to ensure the conservation of rumen microbial genetic resources globally. Likewise, lack of open access to rumen microbial cultures is the major hurdle, with many cultures remaining in laboratory freezers across the world, and therefore in danger of being lost. Most funding agencies and journals have an open-access policy that requires that all data must be publicly available when articles are submitted for review. However, this is not the case for research on novel microbial isolates, and this results in poor access to such isolates for continued research and societal benefit. This is a major challenge and requires changes. However, it must also be noted that such changes will require enhanced infrastructure for existing culture collections to enable them to maintain and make available the increased number of isolates.

The expert survey respondents indicated that they believed that there was currently no activity on the development of policies, legislation and institutional arrangements for the management of microorganisms of relevance to ruminant digestion in their respective jurisdictions and that they believed that progress in this area is essential if the sector is to move forward in terms of addressing challenges it currently faces.

Based on a review of the available scientific data, current policies and regulations and the opinions expressed by experts, the authors recommend the following potential ways in which the Commission and its members could contribute to addressing gaps and weaknesses in the sustainable use and conservation of microorganisms of relevance to ruminant digestion:

- establishing a global expert group to work on the prioritization of activities related to the management of micro-organisms of relevance to ruminant digestion and on the identification of threats to the sustainable use and conservation of these organisms;
- ensuring adequate resourcing global research initiatives for the culture, cataloguing and management of rumen microbes;
- promoting open-access policies ensuring that all pure culture microbial isolates must be deposited in culture collections before publication of any data related the respective organism(s);
- enhancing the capacity of global culture collections to deal with the increased demand that having an open policy requiring isolate deposition in a culture collection would bring;
- promoting the funding of research on innovations in the management of the rumen microbiome, particularly with respect to ruminant breeding and dietary innovations;

- improving funding opportunities for database development for isolate genomes and phenotypes while also enhancing the computational expertise available to translate these underpinning data into improved metagenomic annotations and ultimately enable inference of fermentative capacity and nutrient availability to the host;
- instigating a change to the Nagoya Protocol to enable ease of sample/microbial exchange globally, and
  providing stimulus to encourage global collaboration, especially collaboration involving low- and middle-income countries; and
- providing stimulus to encourage global collaboration, especially collaboration involving low- and middle-income countries.

1. Scope of the study

This study has been prepared at the request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture. It aims to provide policymakers, researchers and livestock nutritionists and producers with:

1. an introduction to microorganisms of relevance to ruminant digestion and their roles;

2. an overview of trends in the diversity of microorganisms of relevance to ruminant digestion, the significance of these trends, and the factors driving them;

3. an overview of the current status of the sustainable use and conservation of microorganisms of relevance to ruminant digestion worldwide (including their significance in fields such as climate change adaptation and mitigation and human health), covering the status of relevant research, constraints to the advancement of work in this field, and potential means of addressing these constraints;

4. an overview of the current status of policies, legislation and institutional arrangements relevant to the management of microorganisms of relevance to ruminant digestion worldwide, including those in the field of access and benefit-sharing, covering gaps and weaknesses in such frameworks and potential means of addressing them;

5. an overview of organizations relevant the sustainable use and conservation of microorganisms of relevance to ruminant digestion worldwide, covering gaps and weakness in terms of collaboration in this field, potential means of addressing these gaps and weakness, and potential strategic areas of collaboration between organizations working in this field and the Commission and its members; and

6. an overview of potential ways in which the Commission and its members could contribute to addressing gaps and weaknesses in the sustainable use and conservation of microorganisms of relevance to ruminant digestion.

The study is based on a review recent literature and on the results of a survey sent to members of the Rumen Microbial Genomics network of the Global Research Alliance[6] in order to solicit their opinions on the sustainable use and conservation of microorganisms of relevance to ruminant digestion.[7] Twenty responses were received from experts from diverse geographical locations and sectors (see Annex 1).

2. The state of ruminant agriculture

Ruminants have a forestomach composed of four compartments – the reticulum, the rumen, the omasum and the abomasum. The rumen, the main fermentative compartment of the forestomach, is a complex, dynamic ecosystem composed of anaerobic bacteria, protozoa, anaerobic fungi, methanogenic archaea and bacteriophages. These microbes interact with each other in a multitude of ways and establish a symbiotic relationship with the host, providing it with energy from the breakdown of plant material that has a high fibre content. This enables ruminants to convert human-inedible feeds into nutritious human-edible foods (meat and milk) and to live on marginal land that is unsuitable for growing food crops (Kingston-Smith *et al*., 2010). Given that an estimated 29.3 percent of the global population – 2.3 billion people – were moderately or severely food insecure in 2021, that 11.7 percent (923.7 million people) faced severe food insecurity (FAO, IFAD, UNICEF, WFP and WHO, 2022) and that the world population is projected to reach 10.4 billion in the 2080s (United Nations Department of Economic and Social Affairs, Population Division, 2022), the need to understand the rumen microbiome in order to sustainably improve production and ensure food security has never been greater.

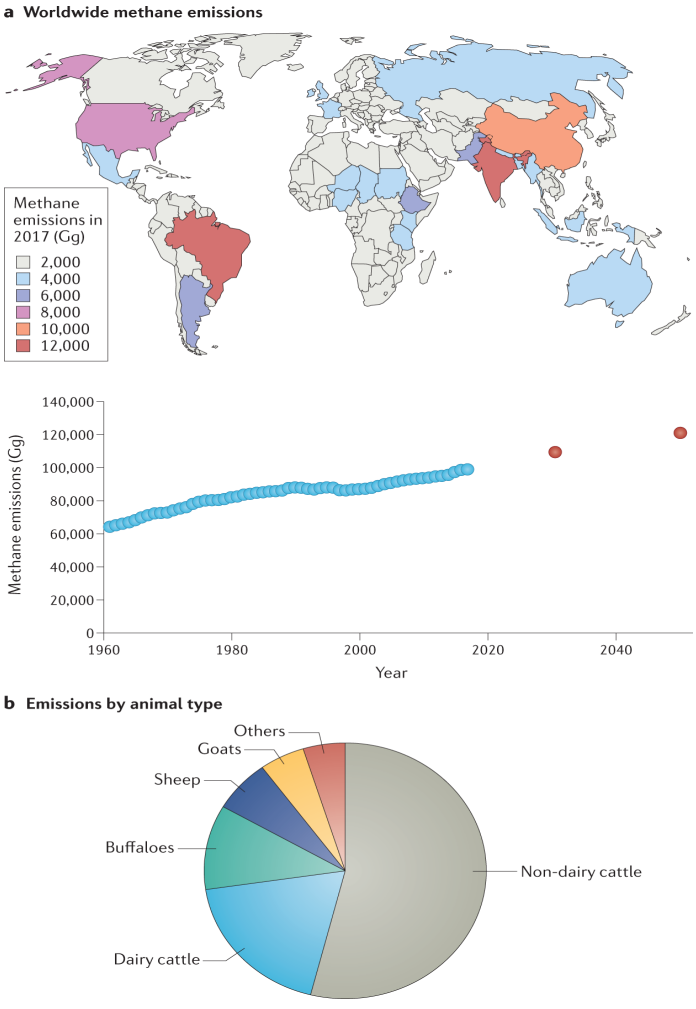
The rumen archaea cause the production of methane (CH_4), a greenhouse gas (GHG) that has between 27 and 30 times the global warming potential (GWP) of carbon dioxide (CO_2) (IPCC, 2021). Global methane emissions from ruminants, most of which come from enteric fermentation, have continuously

[6] https://globalresearchalliance.org/research/livestock/ rumen-microbial-genomics-network/
[7] The survey can be found at the following address: https://forms.gle/PF9mkyyu1XLR3Brj8

increased over the past decades (Figure 1), and are currently estimated to contribute 30 percent of global anthropogenic methane emissions, 17 percent of the global food system GHG emissions and 5 percent of global GHG emissions (FAO, 2020; Arndt *et al*., 2022; Figure 1). Production of methane has also been shown to divert 6 to12 percent of energy away from productivity (Johnson and Johnson, 1995), and therefore decreasing methane emissions may contribute to sustainable livestock production through enhanced growth or milk production. However, it should be noted that data elaborating on the 6–12 percent values in different feeding systems are scarce and may differ substantially based on breed and diet.

It has been shown that the half-life of methane in the atmosphere is approximately 12 years, and that therefore its GWP (a measure of how much energy the emission of 1 tonne of a gas will absorb over a given time, relative to the emission of 1 tonne of CO_2) is much less than that of CO_2, which stays in the atmosphere longer (Cain *et al*., 2019). This has resulted in the generation of a new measure, known as GWP*, to estimate the global warming potential of methane (Cain *et al*., 2019; Lynch *et al*., 2020), which differs from GWP in that it accounts for the half-life of methane in the atmosphere. IPCC employs both standard GWP and GWP* calculations in its reporting.

Figure 1. Global methane emissions



Note: Methane emission estimates calculated from Food and Agriculture Organization of the United Nations (FAOSTAT) enteric fermentation data, shown by **a.** region (red dots on graph show predicted emissions) and **b.** animal type (others mainly include non-ruminant emissions).

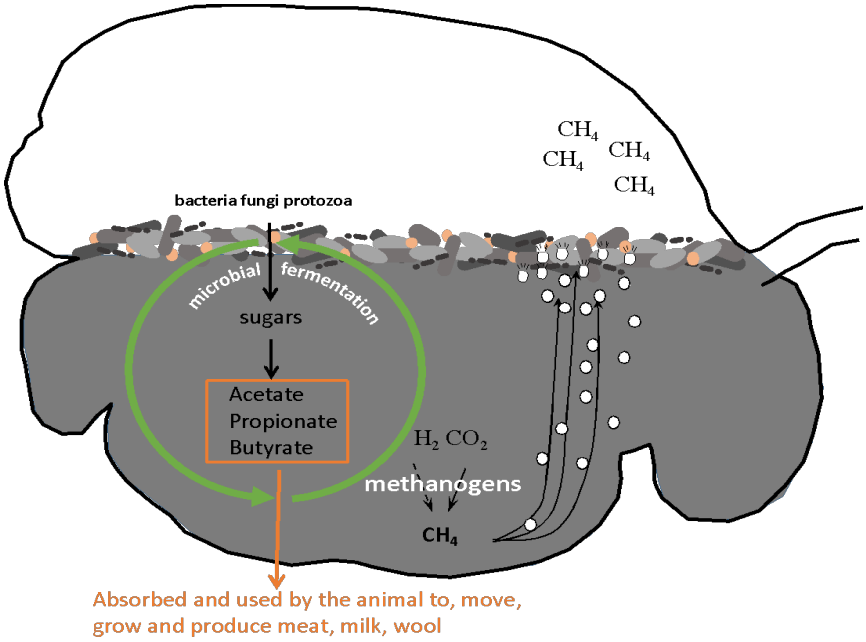
Source: Mizrahi *et al*., 2021. Reproduced with permission.

Nitrogen use by ruminants can also result in excess nitrogen being released to the environment, and this can be converted into nitrous oxide (N_2O) by environmental microbes (e.g. in manure and soil). N_2O has approximately 273 times the GWP of CO_2 as compared over a 100-year period (IPCC, 2021).

There has been a tendency for at least the last 50 years to move towards intensive farming practices, with productivity as the main goal. Enhanced productivity has been achieved through effective breeding and over-provision of protein, the latter being a strategy that is detrimental to the environment as only about 25 percent of the protein is utilized by the ruminant, with the rest being excreted, mainly in urine (Huws *et al*., 2018). The large amounts of nitrogen accumulating in manure and deposited on the land then get converted into N_2O, creating a much larger GHG challenge. Ensuring nitrogen-use efficiency in ruminants through provision of appropriate sources and levels of protein is thus another way of reducing global warming. Emphasis is now being placed on evaluating local sources of feed protein obtained in a sustainable manner, feeding ruminants at levels that are optimum (which may be lower than average levels fed in the last few years under a drive for high production) for their health and production, and minimizing unnecessary soil nitrogen deposition.

The productivity and environmental impact of the animal are mainly consequences of the biochemical process that occurs as feed enters the rumen and the process of digestion begins (Huws *et al*., 2018; Ungerfeld, 2020; Mizrahi *et al*., 2021). Essentially, dietary carbohydrates are broken down by the rumen microbes (Figures 2 and 3) and go through biochemical processes that result in the production of volatile fatty acids (VFAs), which serve as a source of energy for the animal. This process results in the generation of hydrogen, and this is used by methanogens mainly to convert CO_2 into methane. Production of certain VFAs, notably propionate, utilizes more hydrogen than others and can therefore help to redirect hydrogen away from methanogenesis. VFAs have also been shown to trigger rumen epithelial immune responses, therefore aiding ruminant health (Zhan *et al*., 2019).

Figure 2. Carbohydrate fermentation and methanogenesis in the rumen



Source: Mottet (FAO), from various sources. Reproduced with permission.

While constraints remain, for example taxonomic complexity and difficulties with the culturing of microorganisms, the last decade has seen innovations with respect to "omic" technologies for studying complex microbial ecosystems and with respect to our ability to culture microorganisms from such ecosystems. An explosion of knowledge in the field of microbial ecology has occurred since the development of "omic" technologies and related computational tools, but moving from an understanding of correlations to an understanding of causation through a functional understanding has been challenging, especially on a whole rumen microbiome basis, i.e. including all the microbial groups, and this needs to be a focus of future research efforts. Our ability to obtain genomes from metagenomes (metagenomically assembled genomes – MAGs which are single-taxon DNA assemblies arising from metagenomic DNA sequences), alongside our enhanced ability to culture pure isolates, including potentially MAGs, allows us to move towards an understanding of causation while

providing genetic and biological resources for biotechnological exploitation. The current state of the art is discussed below, alongside facilitators, barriers and opportunities.

3. State of knowledge on rumen microorganisms involved in ruminant digestion

3.1 Bacteria

Robert Hungate, an American scientist who was based initially at the University of Texas, then later at Washington State University before a final career move to University of California, Davis in 1956, is widely considered to be the "father of rumen microbiology". Many of the culture technologies for anaerobic bacteria he developed (Hungate, 1966) are still widely used throughout the world to this day. These cultivation techniques have illustrated that bacteria are the most abundant and diverse group of rumen microorganisms and that they have a multitude of functions. For example, they can be amylolytic – breaking down amylase, cellulolytic – breaking down cellulose, proteolytic – breaking down protein, or lipolytic – breaking down lipids/fat, with many described as generalists, i.e. as having a broad range of functions, and others as more specialist (Figure 3). While there have been significant technological advances during the last decade, the functions of the rumen bacteria and their interactions with the host and other members of the rumen microbiome are still poorly understood. This lack of understanding has constrained attempts to beneficially manipulate the rumen microbiome for enhanced ruminant phenotype (e.g. enhanced production) and reduced environmental impact. A major globally consolidated effort to close this gap is needed.

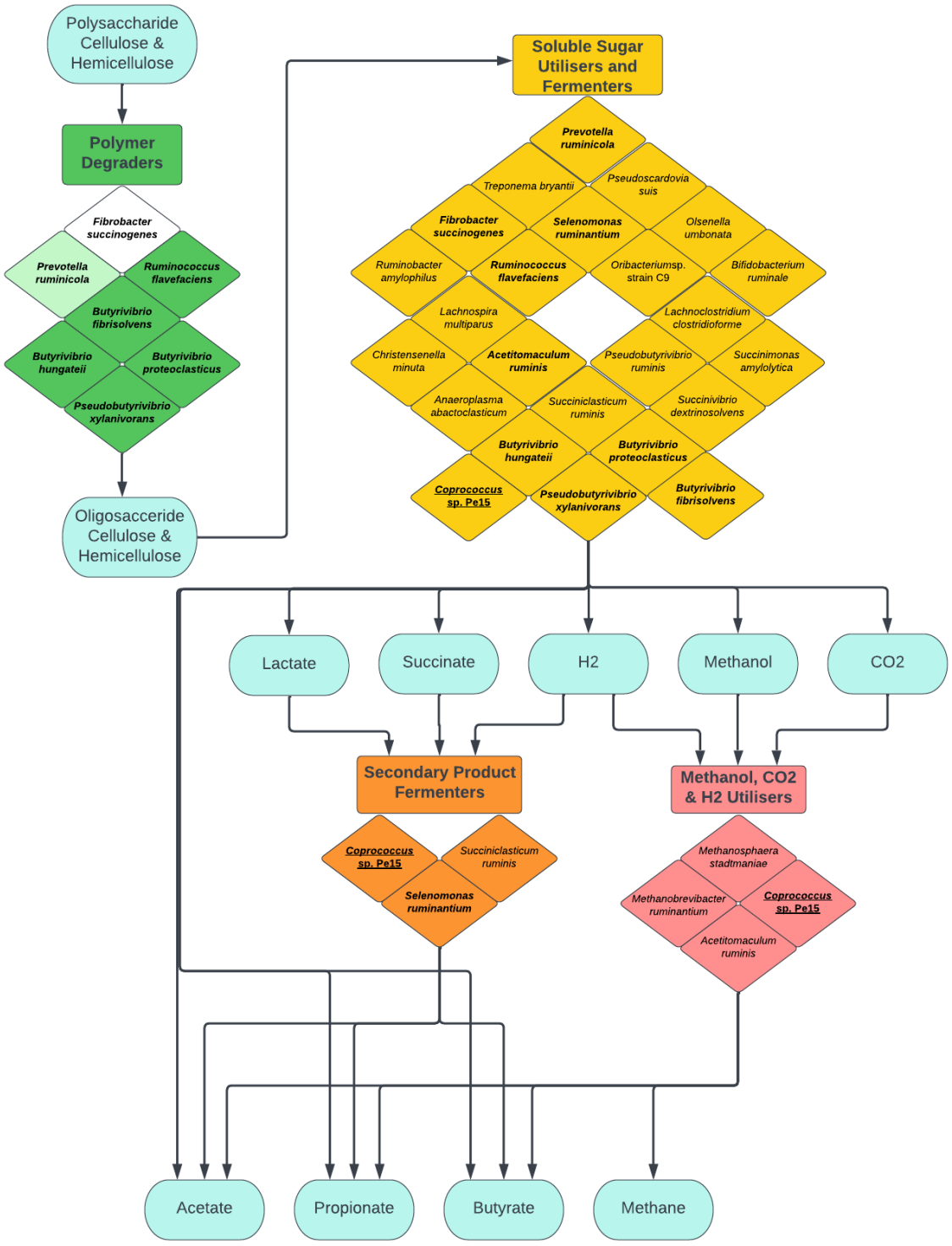
One major challenge that impedes our ability to understand rumen bacterial function relates to accurately defining the taxonomy of rumen bacteria. Classically, the rumen bacteria have been placed into the phyla Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria (listed in descending order of abundance) (Henderson *et al*., 2015). However, recent attempts to streamline bacterial taxonomy in general via the International Committee of Systematics of Prokaryotes (ICSP) have resulted in the names Bacteroidetes and Actinobacteria being changed to Bacteroidota and Actinobiota. We use the new names in this document.

The dominant families and genera within phylum Firmicutes are family *Lachnospiraceae* and genera *Butyrivibrio* and *Pseudobutyrivibrio*, while phylum Bacteroidota, is dominated by family *Prevotellaceae* and genus *Prevotella* (Henderson *et al*., 2015). *Prevotella* are classically described as one of the most proteolytic genera in the rumen microbiome (Griswold, White and Mackie, 1999). *Butyrivibrio* and *Pseudobutyrivibrio* are known to produce the VFA butyrate and to play a major role in plant degradation thanks to a cascade of carbohydrate-active enzymes (CAZYmes), also referred to as glycosyl hydrolases (GHs) (Palevich *et al*., 2019; Pidcock *et al*., 2021).

Classically, genera *Butyrivibrio* and *Pseudobutyrivibrio* have been classified as six species, namely *B. hungatei*, *B. fibrisolvens, B. proteoclasticus*, *Butyrivibrio* sp., *P. ruminis* and *P. xylanovorans* (Palevich *et al*., 2019; Pidcock *et al*., 2021). However, a recent study of 71 genomes from the genera *Butyrivibrio* and *Pseudobutyrivibrio* using pangenomics[8] and average nucleotide identity showed that these two genera are probably composed of 32 genera and 42 species (Pidcock *et al*., 2021). Although *Butyrivibrio, Pseudobutyrivibrio* and *Prevotella* dominate numerically within the rumen ecosystem, the rumen also contains many other, less-dominant bacteria, in whose absence the ecosystem would be dramatically different or cease to exist altogether (Berry and Widder, 2014). For example, a recent study showed that the rumen families *Flammevirigaceae* and *Eubacteriaceae*, although low in density, were likely to be keystone families in terms of providing the "glue" to keep the communities together, aiding the degradation of fresh perennial ryegrass (Huws *et al*., 2021).

[8] A pangenome is the entire set of genes from all strains within a clade.

Figure 3. Overview of the stages of degradation of plant material in the rumen and examples of the bacteria involved



Notes: The figure shows examples of bacterial species that act as polymer degraders, soluble sugar utilisers and fermenters, secondary product fermenters, methanol, CO_2 and H_2 utilizers in the rumen. Many rumen organisms are specialists in specific stages of this process, but some have been identified as being active in two phases (names highlighted in bold) or even three phases (names highlighted in bold and underlined). Polymer degraders consist of primary colonizers (light green), secondary colonisers (dark green) and those with no early-phase temporal pattern (white).

Source: Huws *et al.,* 2021. Reproduced with permission.

More recently, sequencing-based research has moved beyond taxonomy to attempt also to define function through use of shotgun metagenomics, which results in the sequencing of all the DNA in an environment. Generally, this approach is used with the aim capturing the DNA of all living cells in an environmental sample, but it will also capture DNA from dead cells and naked DNA. This untargeted approach has the disadvantage that it is highly complex to analyse the acquired data and that it risks under-representing the DNA from organisms with very low abundances. However, it has the advantage of being agnostic with respect to microbial groups and species and not restricted to specific types of genes. As such, it can capture whole and fragmented genomes for the whole microbiome, allowing prediction of genes, profiling of enzymes and reconstruction of entire metabolic pathways from the organisms in the environment. This can lead to the discovery of new enzymes and pathways and allows the abundance of functional genomic elements (such as genes) to be quantified across and between samples. However, this is only possible if genes can be annotated, and at present many genes remain unknown.

Shotgun metagenomics was first applied to the rumen in order to discover novel biomass-degrading enzymes from switchgrass-associated microbes (Hess *et al*., 2011). Subsequently, metagenomics has been used to study many aspects of rumen microbiology, including methane emissions from cattle (Wallace *et al*., 2015) and sheep (Shi *et al*., 2014), biomarkers to predict ruminal methanogenesis (Auffret *et al*., 2018), the effect of feed conversion ratio, breed and host genetics on the composition of the rumen microbiome (Roehe *et al*., 2016), nutrient acquisition (Mayorga *et al*., 2016; Rubino *et al*., 2017), the effects of diet (Auffret *et al*., 2017), and the impact of feed additives on the abundance of antimicrobial-resistance (AMR) genes (Thomas *et al*., 2017). The rumen also remains a source of valuable bioactives for the biotechnology industry, and metagenomics is a key tool for such bioprospecting (Oyama *et al*., 2017; Roumpeka *et al*., 2017).

Another major advance in understanding of the capacity of rumen bacteria has been our increased ability to obtain single-strain bacterial genomes from MAGs. This approach was first used by Tyson *et al*. (2004) for an acidophilic microbiome, and Hess *et al*. (2011) were the first to use it in ruminants, assembling 15 draft MAGs from the switchgrass-associated microbiome of cattle. Subsequently, Svartstrom *et al*. (2017) assembled 99 microbial MAGs from the moose rumen, Stewart *et al*. (2018) assembled 913 MAGs from the rumen of cattle, and Parks *et al*. (2017) assembled over 8 000 novel MAGs from 1 500 public datasets, some of which originated from the rumen. The ability to obtain MAGs has helped immensely in terms of enhancing our understanding of the rumen microbiome, but there is a need to obtain these MAGs in culture in order to test hypotheses and exploit the microbes in biotechnological applications, for example for use as direct-fed microbials (DFMs) or for use of their enzymes. Also, as with most 16S rDNA or shotgun metagenomic sequencing based studies, these involve rumen samples taken at a certain point in time. The diversity and function will change temporally, and therefore these data are also somewhat biased.

The recent Hungate Collection Joint Genome Initiative[9] also represents a major step change in our understanding of the rumen microbiome. The Hungate Collection was a flagship project of the Global Research Alliance on Agricultural Greenhouse Gases (GRA),[10] whose mission is to bring countries together to find ways of growing more food without increasing GHG emissions. The project provided 501 rumen bacterial and archaeal genomes (Seshadri *et al*., 2018). This is an immense achievement, not only greatly enhancing our ability to understand the rumen microbiome but also resulting in a lot of scientific impact in terms of follow-on publications. This will probably soon generate societal impact through the commercialization of innovations. The project has now ended – because of a lack of funding rather than because there are no more rumen microbial cultures available – and many rumen microbe genomes therefore remain unavailable. The phylum Bacteroidetes and family Bacteroidota are underrepresented in the Hungate Collection as compared with their representation in the Global Rumen Census dataset (previous GRA flagship project) and genomes from so-called unculturable rumen bacteria and MAGs are also hugely underrepresented.

[9] https://genome.jgi.doe.gov/portal/HungateCollection/HungateCollection.info.html
[10] https://globalresearchalliance.org/

Where advances in rumen culturomics are concerned, the ability to culture rumen bacteria has notably improved in recent years through the development of new culture media that provide more bespoke nutritional components for the microbes, coupled with use of dilution-to-extinction methods, which together allow the culture of less-fastidious microbes and hence a larger number of novel isolates. For example, development of the enhanced rumen bacterial media RMO2 (Kenters *et al*., 2011) and use of the dilution-to-extinction technique allowed the isolation of 54 novel bacteria. Partners at Queen's University Belfast, United Kingdom, and Ben-Gurion University, Israel, have also recently placed substantial emphasis on rumen bacterial culturomics, with over 400 novel isolates (according to 16S rDNA) in pure culture (as yet unpublished), including those that are most closely related to so-called unculturable bacteria, illustrating the potential power of such applications. While such efforts will undoubtedly provide a basis for further understanding and biotechnological exploitation of the rumen microbiome, i.e use of their enzymes for industrial processes, the potential to use some of these bacteria as DFMs in the diets of ruminants in order to redirect hydrogen away from the methane biochemical pathways is immense. In particular, in combination with additives that reduce methane emissions, these potential DFMs could not only further reduce methane output but also increase production in a sustainable manner, through re-direction of hydrogen through to beneficial VFA formation, thus providing energy for the host. Therefore, there is clearly a need for stakeholders in future to prioritize efforts to culture, genotype and phenotype novel rumen microbes globally, including those associated with a range of species, breeds and diets, as that will provide a major step change in our ability to innovate.

3.2 Archaea

Methanogenic archaea, often called methanogens, are important electron sinks and are responsible for the production of methane in the rumen, which is then eructed and released to the environment (Mizrahi *et al*., 2021; Leahy *et al*., 2022). Methanogens are members of the phylum Euryarcheota and sit within the orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanocellales*, *Methanopyrales*, *Methanomassiliicoccales* and *Methanosarcinales.* The archaea are an ancient lineage of microbes that although they look phenotypically like bacteria are phylogenetically distinct.

Methanogens typically reside in carbon-rich environments, acting as terminal reducers of carbon in the process of dietary carbon metabolism (Attwood and Leahy, 2020). As noted above, ruminant diets are mainly composed of complex carbohydrates, which are metabolized by bacteria to produce simple sugars, which are then converted to produce VFAs (Figure 2). Many of these VFAs and other products produced during complex carbohydrate degradation serve as energy sources for methanogens (Zinder, 1993), and as noted above some utilize hydrogen in their production, leaving less hydrogen available for methanogenesis. This hydrogen redirection away from methanogenesis is likely to be a reason why ruminants that naturally emit less methane often have improved production parameters. Irrespective, methane itself is produced mainly via the hydrogenotrophic pathway, which results in methane being produced from hydrogen and CO_2 (Figure 2), a small amount can be produced through utilization of methyl groups (methylotrophic pathway) or, even less commonly, using acetate as a substrate (acetoclastic pathway) (Morgavi *et al*., 2010; Tapio *et al*., 2017). However, more recently, it was shown in an *in vitro* experiment that 18 percent of rumen methane was produced from formate, and little is known regarding formate production in the rumen (Leahy *et al*., 2022).

Hydrogenotrophic methanogens include the genus *Methanobrevibacter (Mbb.)*, which is subdivided into the SMT clade (*Mbb. smithii*, *Mbb. gottschalki*, *Mbb. millerae* and *Mbb. thaurei*) and the RO clade (*Mbb. ruminantium* and *Mbb. olleyae*), which are the most abundant rumen methanogens (Tapio *et al*., 2017). Specifically, the *Mbb. gottschalkii* and *Mbb. ruminantium* clades have been confirmed as the two largest groups, accounting for 74 percent of all archaea globally (Henderson *et al*., 2015). Methylotrophic methanogens are less abundant and include *Methanosarcinales, Methanosphaera* and *Methanomassiliicoccaceae* (Attwood and Leahy, 2020). The *Methanosarcinales* can also produce methane via the acetoclastic pathway (Morgavi *et al*., 2010). These methanogens are strict anaerobes and very challenging to grow outside the rumen environment, requiring an exceptionally low redox potential (-340 mV). Nonetheless they can be grown in a laboratory, and much of the understanding regarding their functional capacity stems from studies with isolated pure cultures. Indeed, the ability to

grow them in the laboratory has led to 14 strains being genome sequenced, namely three unknown rumen methanogens, *Methanobacterium formicium*, two strains of *Mbb. ruminantium*, *Mbb. boviskoreani*, *Mbb. gottschalkii*, *Mbb. millerae*, *Mbb. olleyae*, *Mbb. thaueri*, *Mbb. woesei*, *Methanosarcina barkeri* and a *Mbb*. sp. The ability to grow these *in vitro* has also enabled it to be shown that many of the rumen methanogens associate closely with hydrogen-producing protozoa and fungi, thus enhancing their ability to produce methane (Vogels, Hopper and Stumm, 1980; Bauchop and Mountfort, 1981; Cheng *et al*., 2009; Belanche, de la Fuente, and Newbold, 2014).

3.3 Protozoa

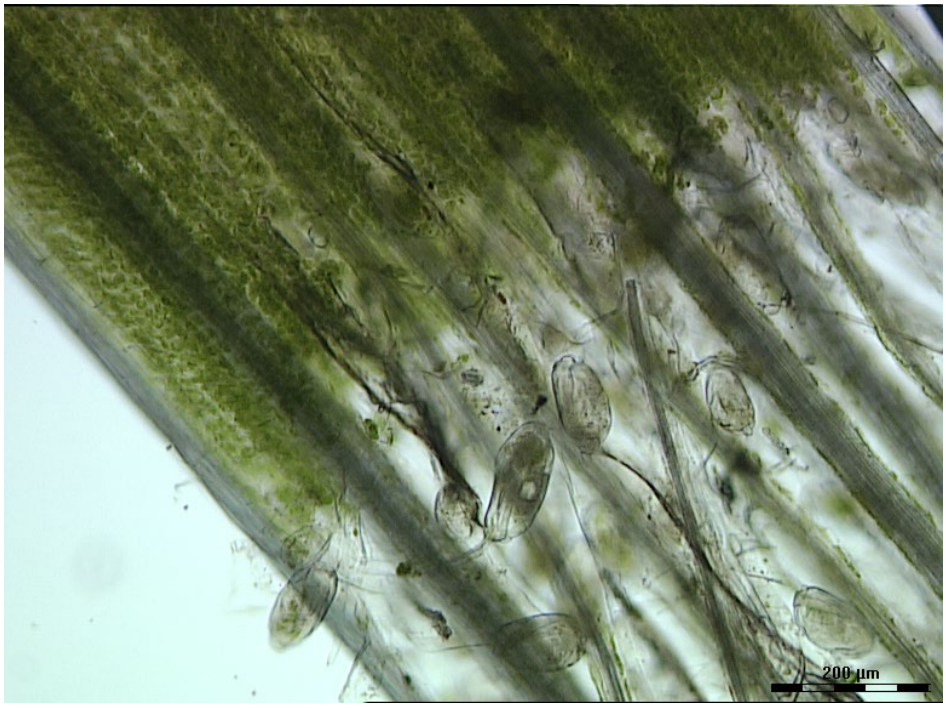
While the rumen bacteria are the most numerous rumen microorganisms, the rumen protozoa occupy the most space within the rumen (up to 50 percent), and yet they are understudied (Williams and Coleman, 1997; Williams, McEwan and Huws, 2020). The rumen protozoa were first reported by Gruby and Delafond in 1843 and, along with fungi, make up the rumen "eukaryotome" (sometimes called the "eukaryome") (Williams and Coleman, 1997; Newbold *et al*., 2015). Most of the protozoa in the rumen are ciliates, with some flagellate species also present. Only a few genuine flagellates have been identified in the rumen, for example *Trichomonas* sp., *Monocecromonas* sp. and *Chilomastix* sp. (Williams and Coleman, 1997). Ruminants commonly harbour distinct protozoal populations from birth, and the diversity of these populations does not change through life, although abundances fluctuate. For example, protozoal populations can be A-type (characterized by an abundance of *Polyplastron multivesiculatum*), B-type (characterized by an abundance of *Epidinium caudatum* or *Eudiplodinium maggii*), O-type (characterized by an abundance of *Entodinum*, *Dastrychia* and *Isotrichia*), or K-type (characterized by an abundance of *Elytroplastron bubali*) (Kittlemann and Janssen, 2011).

Because of the difficulties involved in culturing them and subsequently performing genomic studies, the latter due to their complex genetic structure, the rumen protozoa are largely understudied (Williams, McEwan and Huws, 2020). Because of this complex genetic structure, to date only one rumen protozoan has been genome sequenced, namely *E. caudatum* (Park *et al*., 2021). Also, protozoa cannot currently be grown axenically (in the absence of other microbes) in the laboratory, or even monoaxenically (in the presence of one bacterium), and this causes challenges in assigning function specifically to rumen protozoa (Williams, McEwan and Huws, 2020). The function of the rumen protozoa therefore remains somewhat controversial. It is known that animals can survive the removal of protozoa the rumen, a process known as defaunation (Williams and Coleman, 1992; Newbold *et al*., 2015).

A meta-analysis using 23 *in vivo* studies conducted by Newbold *et al*. (2015) to elucidate the effect of defaunation and infer the function of the rumen protozoa found evidence that the absence of protozoa caused a decrease in organic matter degradation, especially of neutral and acid detergent fibre, confirming the data of Williams and Coleman (1992), which showed that some of the rumen protozoa (i.e. *Epidinium*, *Polyplastron* and *Entodinium* spp.) possess fibrolytic capacity. Indeed, light microscopy of rumen contents clearly shows that *Epidinium* spp. are strongly associated with plant material and can be seen to scavenge plant chloroplasts, which are rich in protein and lipids (Huws *et al*., 2009; Huws *et al*., 2012; Figure 4). Several protozoal carbohydrate-active enzymes have been identified using metatranscriptomics, with the most highly expressed being GHs 5 and 11, polysaccharide lyases and deacetylases, xylanases and enzymes active against pectin, mannan and chitin; the latter are probably used to digest rumen fungi, which have a chitin-rich cell membrane (Williams *et al*., 2020b). Defaunation can therefore decrease dietary ruminal fermentation, which in turn can decrease hydrogen generation and result in reduced methanogenesis. In recent times, a step change in our understanding of the rumen protozoa has been achieved by integrating single-cell sequencing and an assembly-and-identification pipeline, an approach that allowed 52 high-quality ciliate genomes to be obtained from 22 rumen morphospecies (Li *et al*., 2022). These genomes allowed resolution of the taxonomic and phylogenetic framework, resulting in 22 of the morphospecies being grouped into 19 species spanning 13 genera and enabling reassignment of the genera *Dasytricha* from the family *Isotrichidae* to a new family, *Dasytrichidae* (ibid.).

Protists have been linked to methanogenesis based on the finding that defaunation reduces methane output by approximately 11 percent (Hegarty, 1999; Morgavi *et al*., 2010; Newbold *et al*., 2015). This presumably occurs because protozoa are colonized by methanogenic archaea and thus have an indirect role in methane production (Belanche, de la Fuente and Newbold, 2014). Methanogens probably colonize rumen protozoa because the protozoa possess hydrogenosomes, which release an abundance of hydrogen as a consequence of anaerobic fermentation, and this hydrogen is used by the methanogens to produce methane via the hydrogenotrophic pathway (Vogels, Hopper and Stumm, 1980). This suggests that removal of protozoa may be a way to decrease enteric methane emissions. However, it should be noted that the rumen protozoa vary substantially in their contributions to plant degradation and methane production. For example, as noted above, *Epidinium* spp. contribute substantially to plant degradation (Huws *et al*., 2009; Figure 5), whereas the main methanogens and methanogenesis are supported mainly by the holotrich protozoa (Belanche, de la Fuente and Newbold, 2014). Therefore, a strategy that eliminates all protozoa may not be optimal. However, elimination of subgroups of protozoa is technologically challenging at present. Available data suggest that milk production or average daily weight gain increases in defaunated animals, probably because rumen bacteria evade digestion by the protozoa, thus allowing them to increase microbial protein synthesis and the supply of protein to the host and also probably allowing the bacteria to degrade the plant material more efficiently (Newbold *et al*., 2015).

Figure 4. Light microscopy image of rumen contents taken from a ruminant possessing B-type protozoal diversity and showing close interactions of *Epidinium* spp. with plant material



Note: Scale bar: 200µm.
Source: Huws *et al*., 2018. Reproduced with permission.

3.4 Fungi

The flagellated zoospores of anaerobic fungi (Neocallimastigomycetes) were first observed in the early 1900s. However, it was not until the 1960s that their true identity was confirmed (Orpin, 1974, 1977a). They were initially incorrectly classified as protozoa and later reclassified as belonging to the fungal phylum Chytridiomycetes (Barr, 1980, 1988). In 2007, they were acknowledged as a distinct phylum, the Neocallimastigomycota (Hibbett *et al*., 2007). Neocallimastigomycota contains only one order (*Neocallimastigales*) and one family (*Neocallimastigaceae*), with 18 genera described to date, namely the monocentric rhizoidal *Neocallimastix, Piromyces*, *Oontomyces*, *Buwchfawromyces*, *Pecoramyces*, *Liebetanzomyces*, *Feramyces*, *Agriosomyces*, *Aklioshbomyces*, *Capellomyces*,

Ghazallomyces, *Joblinomyces*, *Khoyollomyces* and *Tahromyces*, the polycentric rhizoidal *Anaeromyces* and *Orpinomyces*, and the bulbous *Caecomyces* and *Cyllamyces* (Hess *et al*., 2020). The establishment of 18 genera is recent development – only nine genera were known in 2018 (Huws *et al*., 2018) – and involved major input from scientists globally.

Anaerobic fungi are the most potent fibre-degrading organisms in the known biological world, primarily because of their efficient and extensive set of enzymes for degrading plant structural polymers (Solomon *et al*., 2016) and their ability to physically penetrate plant structural barriers (Orpin, 1977a,b). The latter action benefits other rumen microbes by increasing the surface area available for colonization. These fungi also show amylolytic activity (Gordon and Phillips, 1998). The activity of anaerobic fungi is enhanced by methanogenic archaea (Cheng *et al*., 2009), which are now known to physically attach to anaerobic fungal biomass and through enhanced hydrogen removal. Anaerobic fungi are clearly beneficial rumen microbes and have been shown to improve feed intake, feed digestibility, feed efficiency, daily weight gain and milk production (Lee *et al*., 2000; Dey *et al*., 2004; Paul *et al*., 2004; Tripathi *et al*., 2007; Saxena *et al.*, 2010; Puniya *et al*., 2015). Chitin measurements (Rezaeian, Beakes and Parker, 2004) and rRNA transcript abundance (Elekwachi, 2017) indicate that anaerobic fungi represent 10 to 20 percent of the rumen microbiome in terms of abundance. However, like protozoa, they are not routinely studied, despite the availability of suitable cultivation-independent tools (Edwards *et al*., 2017).

Despite the known importance of the rumen eukaryotome (fungi and protozoa), our understanding of it is far less complete than our understanding of the rumen bacteria. Beyond what has been learned through study of their fibre degrading enzymes, much of the activity and metabolism of anaerobic fungi remains unknown, particularly because of the limited annotation of the multiple genome sequences and transcriptomes that are now available (Edwards *et al*., 2017). Thus, there are still many challenges that need to be overcome to enable the study of the rumen microbiome, as a whole, in relation to key livestock production challenges, including ensuring food security and reducing environmental impact.

3.5 Viruses

Viruses are infectious microbes consisting of a segment of nucleic acid (either DNA or RNA) surrounded by a protein coat. A *virus* cannot replicate alone and uses the host to replicate and survive. Phages are a subset of viruses that utilize bacteria as their hosts. Phages can survive in a dormant (lysogenic) or lytic (replicating and causing the death of the bacterium) phase, with some alternating their state frequently and others preferring to remain in either a lysogenic or a lytic phase.

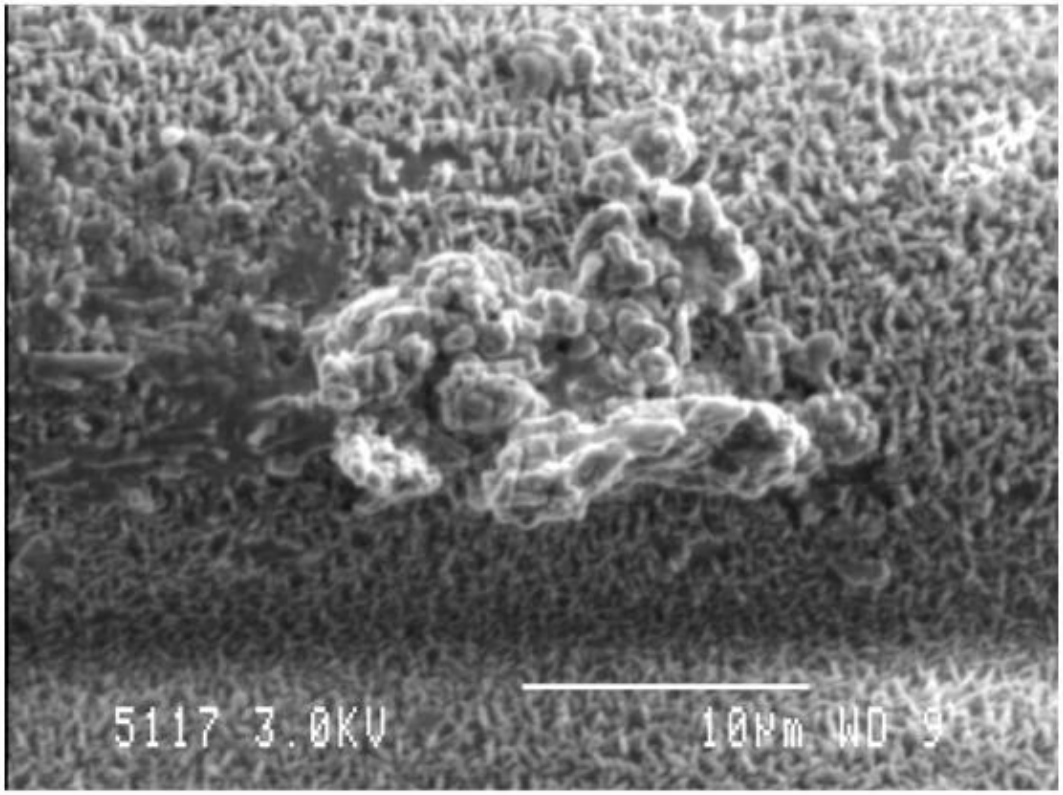
Lytic phages were isolated from the bacterial genera *Serratia* and *Streptococcus* in rumen fluid as far back as 1966 (Adams *et al*., 1966), but little emphasis has been placed on understanding rumen viruses since then. While some research on isolating phages was done in the 1970s and 1980s, only those with potential biotechnological applications were further characterized and kept in a culture collection (Gilbert and Klieve, 2015). More recently, Gilbert *et al*. (2017) isolated and obtained complete genome sequences for lytic phages capable of infecting *Bacteroides*, *Ruminococcus* and *Streptococcus* and all belonging to the order Caudovirales. Subsequently, five novel bacteriophages infecting *B. fibrisolvens* have been isolated and their genomes characterized (Friedersdorf *et al*., 2020). Within other ecosystems it is known that phages alter the ecology and evolution of microbial communities (Koskella and Brokhurst, 2014) by killing some bacteria and allowing exchange of genes via a process known as transduction. However, the effects of phages on the rumen microbiome remain to be determined.

3.6 The biofilm architecture

Like most other microbiomes in nature, the rumen microbiome is dominated by microbes existing within biofilms, which are defined as a consortia of microbes attached to a surface, encased in self-produced extracellular polymeric substances (EPS) (Figure 5; Cheng, McCowan and Costerton, 1979; Cheng and Costerton, 1980; Mcallister *et al*., 1994; Huws *et al*., 2013, 2014, 2016; Zhao *et al*., 2018). The biofilm phenotype has many advantages, including the concentration of digestive enzymes within

the EPS in proximity to the substrate, an arrangement that enables effective hydrolysis of plant material within the rumen (Minato *et al*., 1966; Wolin *et al*., 1997; Michalet-Doreau *et al*., 2001; Leng, 2014). The EPS is rich in DNA, protein and lipids, which possibly play a role in biofilm stability and also serve as a source of nutrients for the ruminant following their outflow from the rumen into the lower digestive tract (Shukla and Rao, 2017; Sugimoto *et al*., 2018). Although protein concentration in the EPS is greater than in the attached bacteria, very little consideration has been given to this structure in terms of its contribution to the nutrition of the host. It is therefore exceptionally important that the significance of the biofilm phenotype in the rumen is recognized.

Figure 5. Biofilm community on the surface of fresh perennial ryegrass following *in vitro* incubation in the presence of rumen fluid



Note: Scale bar: 10 µm.
Source: Huws *et al*., 2014. Reproduced with permission.

4. Trends in the diversity of microorganisms of relevance to ruminant digestion and the biotic factors driving these trends

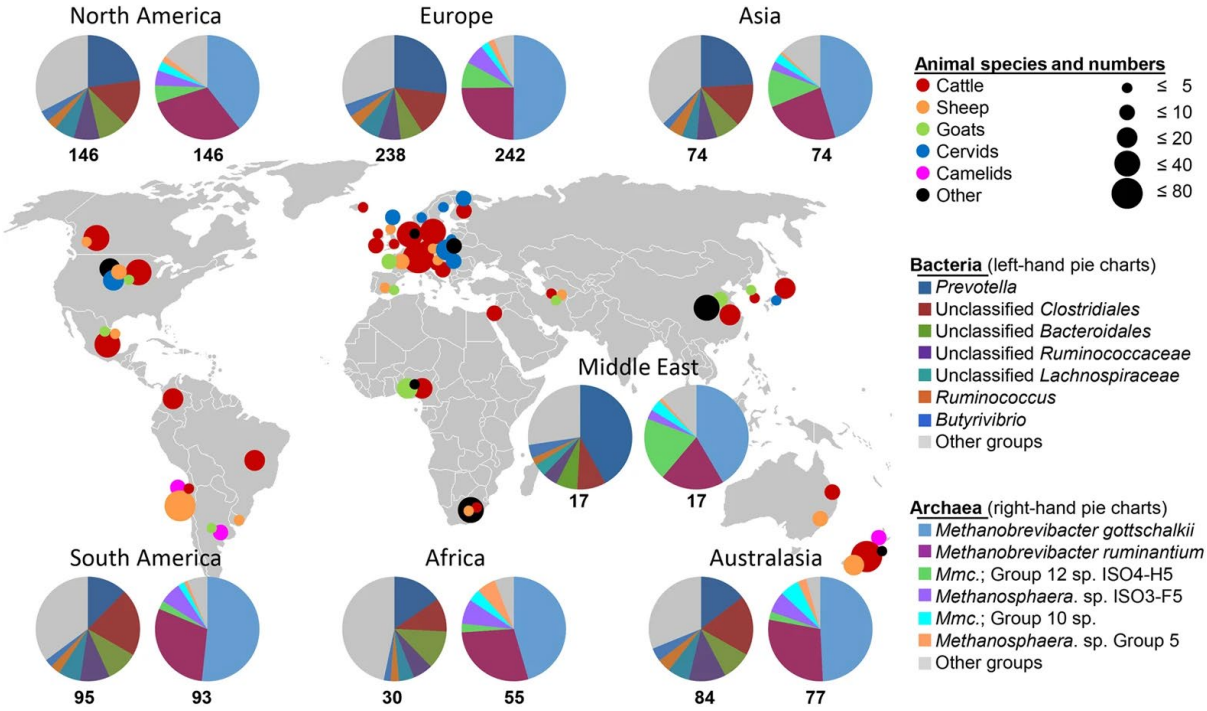
The previous section outlined our knowledge of which microorganisms are associated with ruminant digestion and the roles they play. In this section we describe our knowledge of their co-association and how that is related to geographical region, diet, animal species and genetics.

While individual rumen microbes have been isolated and characterized since the 1960s, it is only in recent decades that studies have investigated their global diversity, prompted by the advent of advanced DNA-sequencing technologies. Among these, the study undertaken by Henderson *et al.* (2015) currently represents the most geographically broad sampling to date, with data obtained from bacteria, archaea and protozoa populations in 742 rumen samples from 32 animal species and 35 countries (Figure 6). This study identified that there is a core microbial community that varies with both diet and host. Most strikingly, it found that similar bacteria and archaea dominated in nearly all samples but that only 14 percent of bacteria were identifiable to a named species and only 30 percent to a formally recognized genus. Five known methanogen groups comprised 89 percent of the archaeal

communities identified worldwide. The protozoal sequence data were all (more than 99.9 percent) assigned to just 12 genus-equivalent protozoal groups.

In general, despite different diets and feeding strategies, Henderson *et al*. (2015) found that similar bacteria were abundant in rumen communities worldwide, with 89 percent of all data arising from just 30 bacterial groups found in over 90 percent of the samples, with organisms identified as belonging to the *Prevotella*, *Butyrivibrio* and *Ruminococcus* genera identified in all samples. Among archaea, *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* were found in almost all samples. The protozoal genera *Entodinium* and *Epidinium* occurred in over 90 percent of samples worldwide.

Figure 6. Global Rumen Census samples, hosts and major bacterial and archaeal community compositions in different regions



Source: Henderson *et al*. 2015. Reproduced with permission.

Henderson *et al*. (2015) also identified clear links between the type of diet consumed by the ruminant and the composition of the microorganisms in the rumen, with the most notable differences in microbiome abundances observed between those in animals fed forage-based diets and those in animals fed concentrate-based[11] diets. Forage-based diets were found to be associated with increased numbers of unclassified Bacteroidales and Ruminococcaceae bacteria, while concentrate diets were found to be associated with increased numbers of bacteria belonging to the genus *Prevotella* and unclassified Succinivibrionaceae bacteria (Henderson *et al*., 2015). There was some evidence of specific microorganisms being more associated with specific hosts, for example unclassified Veillonellaceae were found to be more associated with sheep, deer and camelids, while members of the genus *Fibrobacter* were found to be more associated with bovines. This may reflect underlying differences in anatomy and feeding frequencies between these two groups (ibid.).

The rumen microbiome is not static across the lifetime of the animal. Newly born calves are usually described as “preruminants”. Unlike in the adult, the abomasum is the largest part of their digestive tract, reflecting the fact that the immature digestive system functions in a manner more similar to a young monogastric animal than to an adult ruminant (Huws et al., 2018). The transition from preruminant to ruminant occurs between four and eight weeks of age and is tightly linked to the

[5] Concentrates are feeds that are low in fibre and high in energy compared to forages, with varying levels of protein content.

colonization and establishment of an early-life microbiome. Indeed, microbial derived VFAs have been shown to stimulate the development of the rumen mucosa (Mentschel *et al*., 2001), and differences in metabolic utilization between the early and the adult rumen microbiome have been demonstrated, for example a higher number of lactate-utilizing bacteria in the early rumen (Jami *et al*., 2013*;* Koringa *et al*., 2019).

Evidence suggests that a core group of microbes establishes early in life and persists until adulthood, including well-known rumen microbes from the Ruminococcaceae, Bacteroidaceae and Prevotellaceae families (Furman *et al*., 2020), although their numbers change over time (Wang *et al*., 2019), and there are suggestions that there are distinct colonization phases over the period between birth and adulthood (Rey *et al*., 2014; Zhang *et al*., 2019). However, the microbial assemblages observed in early life have varied between studies (Jami *et al*., 2013; Zhang *et al*., 2019; Malmuthuge, Liang and Guan, 2019). These differences may be caused by differences in management, with evidence indicating that practices such birthing method (Furman *et al.,* 2020), preweaning feeding (Abecia *et al*., 2017), weaning age (Meale *et al*., 2017), early-life (post-weaning) diet (Dill-McFarland *et al*., 2019) and inoculation with rumen fluid (Palma-Hidalgo *et al*., 2021) affect the early-life microbiome as well as by the environment itself.

Evidence has also emerged that host genetics influences the rumen microbiome (Roehe *et al*., 2016; Difford *et al*., 2018; Li *et al*., 2019b; Wallace *et al*., 2019; Zhang *et al*., 2020). These studies have identified host genetic markers uniquely or more frequently associated with the abundance of specific commensal microbes. This suggests that the host's genotype partly determines the establishment of these (presumably beneficial) microbes. Estimates of the total proportion of rumen microbes selected for by the host vary from 6 percent for bacterial and 12 percent for archaeal taxa (Difford *et al.,* 2018) to 34 percent for all microbial taxa (Li *et al*., 2019b) and 0.5–1.2 percent for all microbial genes (Roehe *et al*., 2016). The organisms whose abundances are associated with host genetics include well-known rumen taxa from the bacterial orders *Bacteriodales* and *Clostridiales* as well as other typically minor groups and, interestingly, rumen fungal taxa from the genus *Neocallimastix* (Wallace *et al*., 2019). This is supported by evidence from other studies on the association between microbial abundances and host attributes and between microbial abundances and performance traits (Jami *et al*., 2014*;* Xue *et al*., 2018), feed efficiency (Jami, White and Mizrahi, 2014*;* Sasson *et al*., 2017; Li *et al*. 2019a) and methane emissions (Pinares-Patiño *et al*., 2011*;* Roehe *et al*., 2016; Difford *et al*., 2018; Li *et al*., 2019a; Wallace *et al*., 2019; Zhang *et al*., 2020; Smith *et al*, 2021; Martínez-Álvaro *et al*., 2022; Smith *et al*., 2022). However, evidence is based on descriptive results from DNA-based studies. Proof of causality will require integrative holistic studies, including cultivation and 'omic-based studies.

Worryingly, as animal management practices become more industrialized and homogenized globally, the risk of losing this localized diversity may increase. For instance, supplementing the base diet with easily digestible carbohydrates, as is common in more intensive farming settings (e.g those with high stocking densities and high energy inputs), has been observed to be associated with a simplification of the rumen microbial community (Fernando *et al*., 2010), resulting in lower bacterial diversity and lower concentrations of fibrolytic microbes (Belanche *et al*., 2012). Similarly, increased feed efficiency in ruminants (a key goal in ruminant agriculture given the need to feed a growing world population sustainably) has been linked to a reduction in the richness of the gene content of the rumen microbiome (Shabat *et al*., 2016), and feed-additive strategies aimed at reducing methane production from ruminants have been linked to the formation of alternative, stable microbial community states (Mizrahi *et al*., 2021), which may drive further homogeneity globally in ruminant microbiomes. These developments create a potential risk to the resilience of the microbial rumen community. A consequent narrowing of the function of the microbiome would in turn increase the likelihood that host animals would be less able to withstand environmental perturbations. Another risk associated with trends of this kind would be a reduction in the availability of enzymes that could potentially be exploited for industrial processes.

The risk of losing rumen microbial diversity and of being unable to rectify detrimental effects by reintroducing the lost microbes, highlights the need for action to conserve this diversity. The issue has prompted calls for initiatives to capture a better representation of rumen microbiomes in culture, both to preserve their uniqueness and to better understand their adaptations and potential biotechnological

potential (Creevey *et al*., 2014; Seshadri *et al*., 2018; Huws *et al*., 2018). These points were highlighted by the respondents to the expert survey conducted for this study (see Section 1) when asked to comment on the status and trends of the diversity of microorganisms of relevance to ruminant digestion, related challenges and enablers needed to overcome the challenges (Box. 1).

Box 1: Expert views on status and trends of the diversity of microorganisms of relevance to ruminant digestion

Survey respondents commented that although our general knowledge of microorganism diversity has increased, we are still unable to explain how this diversity influences digestion, feed efficiency, methane emissions, health, etc. They stated that an increasing diversity of microbes have been found to be essential and relevant to feed digestion and function. Important ruminal microorganisms are being identified for optimum utilization of roughage feed, particularly in the tropics, where feed is more fibrous. The number of publications addressing the diversity of rumen microorganisms is growing exponentially.

However, this diversity is mostly revealed by sequencing-based analyses and only very few of the rumen microbes identified have been recovered and preserved as cultures. There are still technical limitations at both fundamental and applied levels, and many researchers and curators of microbial collections are unable to cultivate fastidious gastrointestinal-tract microorganisms. Information for non-bacterial taxa contributing to the diversity of microorganisms of relevance to ruminant digestion is still limited. As we do not yet have a full picture of the diversity rumen microbes and their functions, the use of these microbes in the development of direct-fed microbials remains limited.

The experts stated that a wide range of rumen microbial diversity is being maintained in the world's production systems, where a variety of different ruminant species are fed on a variety of different diets, and that there is high likelihood that the status of this diversity will remain healthy over the next decade. Strong interest and collaborative efforts among researchers, growing emphasis on cultivation/isolation and exponential research output in this area are contributing to the maintenance of this diversity.

Some respondents highlighted that future trends in animal production are likely to shift away from traditional breeds and diets towards standardized production systems that have less variety of animal species, breeds and diets, with a consequent reduction in the diversity of rumen microbes on a global scale. They also stated that the healthy status of the diversity of microorganisms of relevance to ruminant digestion worldwide is threatened by the fact that only a small fraction of rumen microbes are preserved as cultures, with very few dedicated culture collections available.

Source: Responses to the expert survey conducted for the present study.

5. Management and conservation of rumen microorganisms

One of the early cases in which an understanding of the rumen microbiome led to an ability to effectively manipulate was the "leucaena story". *Leucaena leucocephala* is a leguminous plant, high in protein, that is used as a ruminant feed in tropical countries. However, the plant also has toxic properties that cause symptoms such as salivation, live-weight losses and generally poor animal performance. The toxic effect of *L. leucocephala* is largely caused by to a compound called mimosine, which is converted in the rumen to 4-hydroxy-4(H)-pyridone (DHP) (Wallace, 2008). The rumen microbiome of goats in Hawaii was shown to be able to tolerate the toxic effects of *L. leucocephala* (Jones and Megarrity, 1986). Further investigations revealed that the goats' rumens contained a bacterium, *Synergistes jonesii,* that was capable of degrading DHP. Administering *S. jonesii* to ruminants allows them to feed on *L. leucocephala* without suffering toxic effects. This is a major success story whereby an understanding the role of the rumen bacteria transformed livestock nutrition, with *S. jonesii* now being used as an inoculum in many tropical countries (Wallace, 2008).

Since the time of the success with leucaena, there have been a few further cases in which the rumen microbiome has been effectively manipulated, particularly with respect to the mitigation of methane emissions. Four main ways of reducing methane emissions from ruminants are widely acknowledged: enhancing ruminant management; breeding ruminants that emit less methane; adapting feeding

strategies; and reducing flock/herd sizes (an option that could have far-reaching consequences for those whose food security depends on ruminant production). Breeding and dietary strategies have shown potential to reduce methane emissions via beneficial changes in the rumen microbiome. However, as noted above, developing this line of innovation and understanding any trade-offs it may bring will require more time and data.

5.1 Ruminant breeding for climate change mitigation through manipulation of the rumen microbiome

Ruminant breeding has changed over time, with welfare and health traits becoming as important as classical production traits (Miglior *et al*., 2017). Recent global data show the potential to breed ruminants with decreased methane emissions (Pickering *et al*., 2015; de Haas *et al*., 2017; Beauchemin *et al*., 2020; Smith *et al*., 2021; Smith *et al*., 2022). This approach can potentially reduce methane emissions by up to 30 percent based on daily methane emissions (g/day), methane yield (g/kg of dry matter intake) and methane intensity 0.70 (g/kg or litre of product produced), because the host genome influences the rumen microbiome (Roehe *et al*., 2016; Difford *et al*., 2018; Li *et al*., 2019; Wallace *et al*., 2019; Zhang *et al*., 2020; Smith *et al*, 2021; Martínez-Álvaro *et al*., 2022; Smith *et al*., 2022).

Roehe *et al*. (2016) and Martínez-Álvaro *et al.* (2022) showed that some rumen microbes are heritable and that a number of rumen microbial genes are linked to low methane emissions (e.g. *cofG*, *bcd*, *pccb*, *ABC.P.E.P*, *TSTA3* and *RP-L35*). They suggested that breeding ruminants with a microbiome containing these microbial genes was possible and offered a major step change for breeding programmes. Li *et al*. (2019) also showed that the abundance of some microbes is heritable, with a modest heritability of ≥ 0.15, and 12 bovine chromosomes to be linked to this heritability, again indicating the possibility of breeding animals with a "utopian" microbiome. Rumen size has also been shown to change in these breeding programmes, with sheep that emit high levels of methane often having larger rumen volumes than low emitters (Goopy *et al*., 2014). Breeding programmes aiming to breed sheep that emit low levels of methane have also unveiled differences in feeding behaviour between sheep with different methane emission levels. Sheep emitting less methane have been shown to eat more frequently and in smaller amounts, resulting in a smaller rumen, whereas high emitters have been shown to eat larger amounts more often, resulting in a larger rumen (Goopy *et al*., 2014). This difference in eating behaviour has been found to be consistent in generations of sheep bred with differential methane emissions, suggesting that feeding behaviour underlies the breeding programmes (Johnson *et al*., 2022). Breeding for low-emitting ruminants seems to be a way of obtaining a desirable rumen microbiome. However, the mechanisms underlying this need further clarification and the data supporting it as a strategy need to be expanded, for example to confirm that reductions are obtained under different diets and geographies, and to definitively investigate any potential trade-offs.

5.2 Ruminant dietary interventions for climate change mitigation through manipulation of the rumen microbiome

It has been shown for some time that the effect of diet on the rumen microbiome is likely to be the main influencer of methane emission levels in ruminants (Henderson *et al*., 2015). Diet is the most amenable way of instigating immediate change in the rumen microbiome to enhance the ruminant phenotype. Dietary interventions to manage the rumen microbiome in order to achieve reductions in methane emissions can be broadly grouped into the following categories: plant-based strategies (e.g. forage management and feeding plants that are high in secondary compounds, such as tannins), targeted methane inhibitors (such as 3-NOP, which is commercially known as Bovaer®), oils and oilseeds, and hydrogen sinks (e.g. either chemicals or microbes that utilize hydrogen so that there is less available for methanogeneis) (Figure 7). It should also be noted that basal diets can change the outcomes of such strategies and also that utilizing more than one approach may enhance outcomes.

Enhanced forage quality and digestibility is known to increase methane (g/day) emissions and feeding less-mature grass can decrease methane emissions by up to 13 percent based on methane emission intensity for a given amount of milk produced (g CH_4/kg milk) (de Souza Congio *et al*., 2021; Arndt *et al*., 2022; Beauchemin *et al*., 2022 Figure 2). However, while it is known that plant maturity influences methane emissions, the mechanisms of action in terms of changes in the rumen microbiome are unknown.

It has been shown that including legumes in ruminant feeding systems can reduce methane emissions by approximately 18 percent based on methane emission intensity for a given amount of milk produced (g CH_4/kg milk) because of the plant secondary compounds, particularly the tannins, found in legumes (Enriquez-Hidalgo *et al*., 2014; Eugène, Klumpp and Sauvant, 2021; Arndt *et al*., 2022; Beauchemin *et al*., 2022). Plant tannins are polyphenolic compounds, normally identified as condensed or hydrolysable in terms of their chemical structure, with an affinity to bind to proteins and lipids. They are antimicrobial in nature, and a recent study showed that feeding the tannin-rich tropical plants *Calliandra calothyrsus*, *Gliricidia sepium* and *Leucaena leucocephala* reduced the densities of plant-attached *Fibrobacter succinogenes* and methanogenic archaea in the rumen, the latter resulting in reduced methane production (Rira *et al*., 2022).

A recent review article (Ku-Vera *et al*., 2020) brought together data on the effects of condensed tannins from various plant sources on the rumen microbiome; some findings were similar to those obtained by Rira *et al*. (2022), but the effects were not consistent, with some tannins found to cause increases in methanogens, for example. Although feeding legumes rich in tannins is promising, it should be noted that most data are from *in vitro* experiments and that more *in vivo* research is required in order to evaluate the benefits of including legumes in the ruminant diet. Data on the feeding of multispecies swards containing legumes and herbs to beef cattle show that it increases average daily weight gain, which would be expected to decrease methane intensity, as the animals reach slaughter weight more quickly (Boland *et al*., 2013).

Methane inhibitors have been developed as more microbiome-targeted tools for reducing methane emissions from ruminants. The most well studied is 3-NOP (Beauchemin *et al*., 2020; Arndt *et al*., 2022; Beauchemin *et al*., 2022). 3-NOP inhibits the last step in rumen methanogenesis, resulting in an approximately 35 percent reduction in methane emissions, based on yield and intensity, without negatively affecting animal health and welfare (Hristov *et al*., 2015). 3-NOP is now approved for use in Australia, Brazil, Chile and the European Union (where is has generally been classified as a feed additive) and is under consideration by regulating bodies in many other countries. It faces delays in some countries, such as Canada and the United States of America, because of its classification as a veterinary drug. Encouragingly, 3-NOP seems to have minimal effects on the diversity of rumen protozoa and bacteria (Romero-Perez *et al*., 2014; Haisan *et al*., 2016).

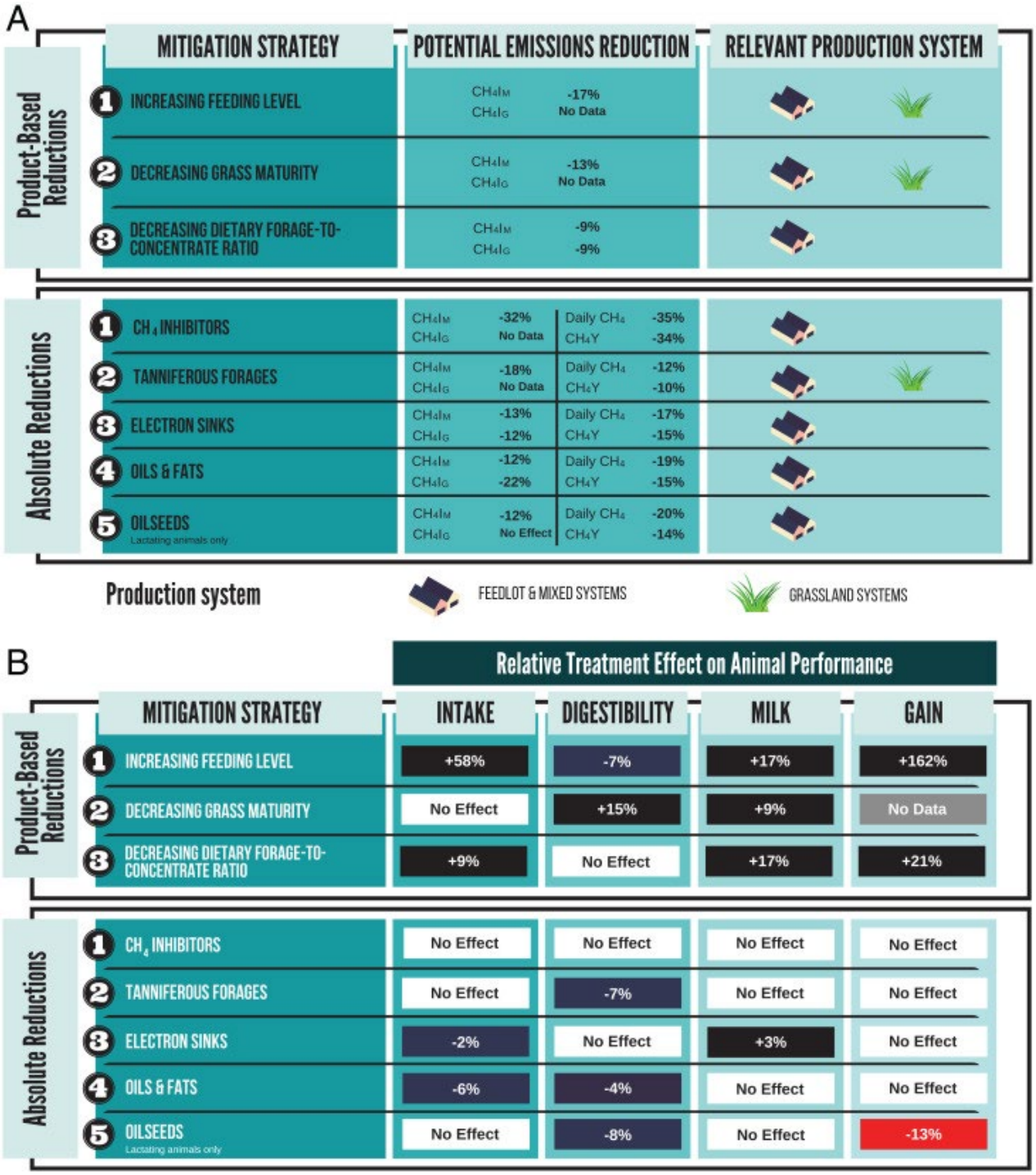
More recently, it has been found that feeding the seaweeds *Asparagopsis taxiformis*, *Alaria esculenta*, *Ascophyllum nodosum* and *Chondrus crispus* as part of the diet of beef and dairy cattle has the potential to reduce methane emissions from the rumen by 10 to 80 percent (based on yield), with dietary inclusion of *A. taxiformis* providing the largest reductions (Wang, Bach and McAllister, 2008; Machado *et al*., 2015; Kinley *et al*., 2016; Ramin *et al*., 2018; Abbott *et al*., 2020; Lean *et al*., 2021; Roque *et al*., 2021; Beauchemin *et al*., 2022). This reduction is largely attributed to the bioactive compound bromoform, which is found in several seaweed species, especially red seaweeds such as *Asparagopsis* spp., with the mechanism of action shown to be inhibition of methanogenesis (Abbott *et al*., 2020). Studies have shown that the supplementation of ruminant feed with macroalgae affects the diversity of the rumen community in the short-term but that the effect lessens over time, possibly because of adaptation (Roque *et al*., 2019). Likewise, as bromoform is carcinogenic, further studies assessing its safety in terms of animal and human health are required.

The possibility of using hydrogen sinks alone or in combination with dietary additives is substantial. It has already been shown that supplementation with phloroglucinol together with 3-NOP promotes the capture of excess hydrogen from methanogenesis and generates valuable metabolites for the host (Martinez-Fernandez *et al*., 2017; Figure 7). DFMs are feed products containing a source of viable and naturally occurring microbes (Brashears, Amezquita and Jaroni, 2005). Their use as a dietary intervention, alone or in combination with methane inhibitors, offers a mechanism for redirecting hydrogen into energy (Box 2). For example, the potential use of lactic acid bacteria (LAB) in methane mitigation strategies has been recognized (Hristov *et al*., 2013; Takahashi, 2013; Jeyanathan, Martin and Morgavi, 2014; Knapp *et al*., 2014; Varnava, Ronimus and Sarojini, 2017; Doyle *et al*., 2019; Ban and Guan, 2021). However, research on the topic has been limited, with data showing that some hydrogen sinks are less effective.

The addition of acetogenic rumen bacteria to remove excess hydrogen has also been widely suggested as an intervention that may work if combined with an additive that inhibits methanogenesis (Wright and Klieve, 2011). Nollet *et al*. (1998) examined the addition of the cell-free supernatant of *Lactobacillus plantarum* 80 to ruminal samples *in vitro* and noted a 30.6 percent reduction in methane production when the supernatant was combined with an acetogenic bacterium, *Peptostreptococcus productus* ATCC 35244. A subsequent *in vivo* study showed that the LP80 supernatant in combination with *P. productus* reduced methane emissions by 80 percent (mmol/6h) during the initial three days, but unfortunately the reduction was not persistent (Nollet et al. (1998). Cao *et al*. (2010a) investigated the effect of *Lactobacillus plantarum* Chikuso-1 on an ensiled total mixed ration and showed that methane production decreased by 8.6 percent compared with untreated silage. Further testing *in vivo* showed that silage inoculated with *L. plantarum* Chikuso-1 increased digestibility and decreased ruminal methane yield by 24.7 percent in sheep compared with a non-inoculated control silage (Cao *et al*., 2010b). The bacterium *Propionibacterium* utilizes lactate to produce propionate, but when tested *in vivo* as a DFM supplementation it was found not to affect total VFA production or enteric methane production in beef heifers fed high-forage diets (Vyas *et al*., 2014) or finishing cattle fed high-concentrate diets (Narvaez *et al*., 2014). These studies did not report a change in rumen propionate concentrations and hypothesized that this lack of change was caused by the moderate persistency of the strains and/or the pre-existing high level of propionate production from starch fermentation (Narvaez *et al*., 2015; Vyas *et al*., 2014; Vyas *et al*., 2015). Jeyanathan *et al*. (2016) also screened 45 bacteria, including strains of LAB, Bifidobacteria and Propionibacteria, *in vitro* for their ability to reduce methanogenesis and then selected 3 strains for *in vivo* trials in sheep, with one strain, *L. pentosus* D31, resulting in a 13 percent reduction in methane yield over four weeks following dosing with 6×10^{10} cfu/animal/day. Astuti *et al*. (2018) also evaluated 14 strains of *L. plantarum in vitro* and identified strain U32 as the most inhibitory of methane emissions. However, other studies, such as those by Ellis *et al.* (2016) which fed lactating dairy cows with silages inoculated with *LAB strains*, did not see an effect on methane emissions when expressed as g/d, g/DMI and g/kg milk. Nonetheless, the results suggest that some specific DFMs fed directly to ruminants have the potential to alter ruminal fermentation in a way that leads to improved production parameters and reduced methane production, with the unexplored possibility that use in addition to a methane-inhibiting additives may have an enhanced effect on methane and animal production. Therefore, isolation and characterization of potential DFMs from the rumen gastrointestinal tract is a valid pursuit and should be a focus of future research.

The overall feed additive market is a multimillion-dollar business. However, the market share of DFMs and other rumen manipulation additives is probably very small given that only a few such products have been commercialized. Natural products such as red seaweed are on the market, as are oils such as linseed and nitrate-based products, but it is hard to know how much is used for methane reduction.

Figure 7. Mitigation strategies and their effect on methane (CH_4) emissions (A) and animal performance metrics (B)



Notes: CH_4I_M = CH_4 emission intensity for milk (g CH_4 kg of milk^{-1}); CH_4I_G = CH_4 emission intensity for weight gain (g CH_4 kg of weight gain for growing animals^{-1}); daily CH_4 = daily CH_4 emissions (g animal^{-1} d^{-1}); digestibility = apparent digestibility of neutral detergent fibre (%); gain = average daily gain (kg d^{-1}); intake = dry matter intake (kg d^{-1}); milk = milk yield (kg d^{-1}); when numeric values are shown a significant effect was observed (adjusted $P < 0.05$) and no effect when adjusted $P \geq 0.05$.
Source: Arndt *et al*. 2021. Reproduced with permission.

5.3. Conservation and culture collections

To date, most studies investigating the effective management of the rumen microbiome have been correlative, and those identifying the underlying mechanistic basis of an effective management regime have been scarce. Moving towards an understanding of causation, at the whole rumen level, is essential if the management of the rumen microbiome is to contribute effectively to efforts to meet the global challenges of food security and climate change. Respondents to the survey of expert opinion also made the point that we must enhance our knowledge substantially and underlined the need to obtain pure cultures of rumen microbes as a transformative step towards understanding the rumen microbiome and ultimately developing further innovations for its management (Box 1 and Box 2). Specifically, obtaining pure isolates of rumen microbes allows us to test hypotheses arising from correlative studies that indicate that a particular microbe may be important for a particular phenotype, i.e. to move from correlation to causation. The Hungate Collection has provided a major step change in this respect. For example, the CowPI tool was designed, based on the initial Hungate Collection data (Wilkinson *et al*., 2018), to allow functional inferences to be made from less costly rDNA-based diversity data, providing a paradigm shift that enabled stakeholders with less infrastructure to enhance their understanding of rumen function. Enhanced culture collections will also improve our ability to understand how dietary additives work at a molecular level, a necessary requirement for regulatory approval. This is in addition to the fact that pure cultures of isolates offer a major source of bioactive compounds for potential exploitation by the biotechnology industry. Nonetheless, the authors are not aware of any within-country policies or frameworks enabling the conservation of microbes important in the digestive processes within the rumen, and none were reported by the expert survey respondents (Box 2).

Box 2: Expert views on the current status of the implementation of activities aimed at promoting sustainable use and conservation of microorganisms of relevance to ruminant digestion

Survey respondents noted that there is insufficient knowledge of the activities of rumen microorganisms *in vivo*. This is partly because of the time-consuming and expensive nature of maintaining a collection of isolates. They also noted that there are not enough isolates to allow effective study of microbial function, especially as conservation of rumen microorganisms is based on anaerobic microbiology and most rumen species are uncultured, and that there is as yet no publicly available culture collection for rumen anaerobic fungi. They also noted that they were not aware of any within-country policies underpinning the conservation of microbes important for rumen digestion.

Source: Responses to the expert survey conducted for the present study.

Because of the need for more comprehensive culture collections of rumen microbes, a new GRA flagship project (RUMEN Gateway) to culture them has been endorsed (Box. 3). This project aims to fill the gaps in culture collections and ensure that the microbes and their phenotypes and genotypes are available to all stakeholders, allowing a transformative step change in our ability to understand, manage and exploit the rumen microbiome (Box 3). Nonetheless, this flagship requires enhanced infrastructure to enable academics from the global North, and especially the global South, to be involved.

Box 3. Flagship project "Expansion, analysis and exploitation of the Hungate rumen microbial culture collection"

Members of the Global Research Alliance on Agricultural Greenhouse Gases' (GRA's) Rumen Microbial Genetics network have recently proposed a GRA flagship project on culturomics, and GRA member states supported the establishment of a project called "Expansion, analysis and exploitation of the Hungate rumen microbial culture collection"[12] led by Queen's University Belfast and involving many of the institutions listed in Table 2.

[12] https://globalresearchalliance.org/flagship-projects/mining-rumen-data/

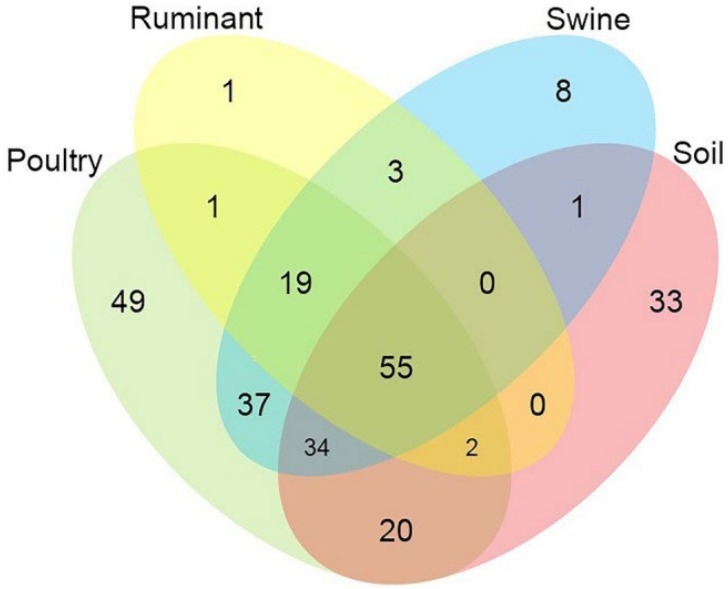
The project builds on the step change in rumen microbiome knowledge provided by the Hungate Collection (Seshadri *et al*., 2018), which allowed the expansion of the rumen microbial genome catalogue from 14 to 501 but also identified major gaps in the diversity available in culture collections globally. It brings the global scientific community together to share, culture and analyse further rumen samples from around the world and to drive future innovations that will reduce greenhouse gas emissions from livestock. It will result in:
- the world's most comprehensive rumen culture collection, alongside phenotype and genotypic biological and bioinformatic resources built by the world's most knowledgeable anaerobic microbiologists;
- free access to the collection so that it can be scientifically mined for novel bioactives and direct-fed microbials to reduce methane emissions, enhance nitrogen-use efficiency and increase productivity in a sustainable manner; and
- a network of "hubs" that are able to isolate, phenotype and genotype novel rumen bacteria (or the latter two only if novel cultures are already available) from cultures or samples sent to them.

6. Livestock gastrointestinal microbiomes and their implications for One Health

The One Health High Level Expert Panel defined One Health as an integrated, unif*ying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.*[4] *One Health recognizes that the health of humans, domestic and wild animals, plants and the wider environment (including ecosystems) is closely linked and interdependent.* The interconnectedness of microbes across the human-animal-environmental axis has been demonstrated by many researchers, for example by Pal *et al*. (2016), who showed that many bacterial genera (29–84 percent of total detected genera) were shared across the microbiomes of human and animal gastrointestinal tracts and of wastewater/sludge. This highlights the need to consider these microbiomes within the wider connotations on One Health challenges, such as spread of AMR.

AMR is major One Health challenge, as AMR bacteria are commonly found across environments and therefore pose a threat to the health of humans, animals and soils. According to the World Health Organization, the economic impact of uncontrolled AMR will result in a dramatic rise in health expenditures and damage to food systems and livelihoods, leading to increasing levels of poverty and inequality (WHO, 2019). Consequently, the number of studies characterizing AMR gene abundance and diversity in microbiomes, including human, livestock and environment microbiomes, is on the rise. For example, Sabino *et al*. (2019) analysed 435 ruminal bacterial genomes and found a high abundance of genes encoding tetracycline resistance and evidence that the tet(W) gene (encoding tetracycline resistance) is located in a novel integrative and conjugative element (ICE) in several ruminal bacterial genomes; ICEs are commonly transmitted to other microbes with relative ease. Another study on resistome analysis of global livestock and soil microbiomes observed that 55 resistance genes were shared between pig, poultry, ruminant and farmed soil microbiomes from 37 countries (Lawther *et al*., 2022; Figure 8). It is therefore important to consider the significance of rumen bacteria in the spread of AMR in addition to their roles in nutrition and GHG emissions.

Figure 8: Venn diagram showing the number of shared and unique antimicrobial resistance genes across soil and pig, poultry and ruminant gastrointestinal tract microbiomes



Source: Lawther *et al*., 2022. Reproduced with permission.

Conversely, the diversity and abundance of species within livestock gastrointestinal tract microbiomes present a rich source for bioprospecting and the discovery of novel compounds important for addressing some of these One Health problems. These compounds include, but are not limited to, enzymes of industrial importance, such as ulvan lyases, glycosyl hydrolasese (GHs) and esterases, probiotics, metabolites useful as postbiotics, and novel antimicrobials for improving animal health and production efficiency and combatting the AMR problem. Indeed, antibiotic-producing bacteria are abundant in nature, especially in the rumen (Azevedo *et al*., 2015; Oyama *et al*., 2017; Oyama *et al*., 2022; Anderson and Fernando, 2021; de Oliveira *et al*., 2022) and are ripe for therapeutic development to treat many diseases.

7. Policies and regulation

A small number of policies facilitate our efforts to understand, manage and conserve rumen microbes, with the main ones being those related to GHG emissions and climate change. However, there are some that potentially inhibit our ability to exchange and conserve rumen microbes globally, for example the Nagoya Protocol.

7.1 Global climate policies

The intergovernmental Panel on Climate Change (IPCC) has played an important role in facilitating debates and processes around the development of climate change policies. IPCC's first report in 1990 fed into the drafting of the United Nations Framework Convention on Climate Change (UNFCCC) in 1991. This was signed by 166 nations at the Earth Summit in Rio de Janeiro in 1992 and entered into force in 1994. The UNFCCC did not contain any specific GHG targets, but it contained key foundations for subsequent climate change debates and processes. The UNFCCC's Kyoto Protocol, adopted in 1997, provided a first step towards more substantial GHG reductions. The Kyoto Protocol set binding targets for developed countries to reduce GHG emissions by 8 percent below 1990 levels by 2012.

A specific focus on the role of the agriculture sectors in climate change mitigation came only with the adoption of the Koronivia Joint Work on Agriculture[13] (Decision 4/CP.236) in 2015. The Koroniva Joint Work on Agriculture includes workshops on the following themes: improved soil carbon, soil

[8] http://www.fao.org/koronivia/en/

health and soil fertility under grassland and cropland as well as integrated systems, including water management; improved nutrient use and manure management towards sustainable and resilient agricultural systems; and improved livestock management systems.[14] The Koronivia workshop on improved livestock management systems, including agropastoral production systems and others, was held in 2020. A summary of the conclusions of the workshop is presented in Box 4.

Box 4: Outcomes of Koronovia workshop on "Improved livestock management systems, including agropastoral production systems and others" (UNFCCC TOPIC 2(e))

"Having considered the report on the workshop on topic 2(e) of the Koronivia road map, the SBSTA and the SBI also recognized that livestock management systems are very vulnerable to the impacts of climate change, and that sustainably managed livestock systems have high adaptive capacity and resilience to climate change while playing broad roles in safeguarding food and nutrition security, livelihoods, sustainability, nutrient cycling and carbon management. They noted that improving sustainable production and animal health, aiming to reduce greenhouse gas emissions in the livestock sector while enhancing sinks on pasture and grazing lands, can contribute to achieving long-term climate objectives, taking into account different systems and national circumstances."

Notes: SBSTA = Subsidiary Body for Scientific and Technological Advice; SBI = Subsidiary Body for Implementation.
Source: UNFCCC, 2021 (paragraph 6).

The 2015 Paris Agreement (UNFCCC, 2016) set a maximum 1.5 °C global warming target (above pre-industrialization levels), with methane from ruminants having a reduction target of 11–30 percent by 2030 and 24–47 percent by 2050 compared with 2010 levels (Arndt *et al.,* 2022). These targets have shaped country-specific policies. Discussing national policies in detail is beyond the scope of this paper, but most note a target of reaching net zero by 2050 at the latest. These policies have shaped research funding calls and subsequently guided rumen microbiome innovations, such as targeted additives.

According to FAO, most (79 percent) of national mitigation contributions cover forestry and many cover the crop (51 percent) and livestock (36 percent) subsectors (Crumpler *et al*., 2021). Livestock-related measures include mitigation actions related to livestock and grasslands, ranging from improved feeding practices to pasture restoration (ibid.). Although some governments, for example those of New Zealand and Ireland, have prioritized research into the rumen microbiome in terms of managing methane emissions and feed efficiency, this is not the case for many countries. The rumen microbiome is thus not always at the forefront of policies targeting the reduction of GHG emissions.

7.2 The Nagoya Protocol

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity (Nagoya Protocol) is a 2010 supplementary agreement to the 1992 Convention on Biological Diversity (CBD). It was instigated to ensure the fair and equitable sharing of benefits arising out of the utilization of genetic resources, thereby contributing to the conservation and sustainable use of biodiversity. It obliges its contracting parties to take measures in relation to access to genetic resources, benefit-sharing and compliance. As of April 2022, it had been ratified by 137 parties, which include 136 UN member states and the European Union.

The Nagoya Protocol has increased the level of bureaucracy with respect to the exchange of samples/microbes and is therefore of major concern to those involved in the monitoring, collection and understanding of microbial biodiversity and potentially inhibits advances in research and innovation. The experts that responded to the survey conducted as part of this study noted that implementation of GHG reduction policies may be stalled by the requirements of the Nagoya Protocol.

[9] https://unfccc.int/topics/land-use/workstreams/agriculture

7.3 Access to information

Most funding agents and publishing journals have an open-access policy. Specifically, when manuscripts involving "omic" data are submitted to journals, all data must be publicly available when the articles are submitted for review. This is not the case for novel microbial isolates, and this results in poor open-access sharing of isolates for continued research and societal benefit. One of the key recommendations of this paper is that open-access policy needs to be expanded to microbial isolates, at prepublication or publication stage. Most openly accessible microbial isolates, including ruminant gastrointestinal tract isolates are deposited in openly accessible culture collections, mainly housed in the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures in Germany or the American Type Culture Collection (ATCC) in the United States of America. These culture collections are pivotal to maintaining global microbial genetic diversity and ensuring open access to all stakeholders. However, many isolates are not deposited in culture collections, as there is no legal obligation to do so. Also, if any microbe has potential commercial use, concerns about intellectual property infringements will mean that it will not be deposited in these culture collections, and many will also remain in individual storage even after patenting and publication. A key example of this issue as it relates to ruminant gut isolates is the fact that of the 410 microbes whose genomes were sequenced and made openly accessible by the Hungate Collection, only about 40 are attributed to cultures available in DSMZ or ATTCC, with 370 remaining in individual freezers (Seshadri *et al*., 2018), although there are plans to make these open-access at some point in the future. This is one example of many and highlights the risk of losing rumen microbial diversity, as ensuring culturability and the sharing of isolates depends on research infrastructure and on willingness to share. Likewise, rumen microbe-specific databases and pipelines need to integrate all genomic and phenotypic data from these isolates, thus ensuring that such activities have maximum impact.

7.4 Dietary interventions

Dietary interventions such as the feeding of plants containing tannins do not require regulatory approval. However, when dietary interventions involve the use of products classed as additives or probiotics, for example, these require approval by regulatory bodies. Such requirements ensure both that the products can be fed safely to ruminants and that claims made about their impacts on methane emissions or on sustainability can be relied on. Approval procedures take a long time and require a lot of data. While they are needed in order to ensure product safety, they can be a major barrier to the timely uptake of innovations and are out of sync in terms of global challenges and their timeframes. Feed additives are at different stages of regulatory approval in different countries (Table 1). Labelling of food products arising from feeding ruminants feed additives as having been produced with reduced methane emissions is a "new area" that is often tightly regulated, and in many countries approval requires a lot of time. The first meat products from *A. taxiformis*-fed cattle were due to be launched in Sweden in mid 2022.[15] It should also be noted that approaches based on feed additives will be challenging to implement in developing countries because of the cost implications and a lack of funding or policies to support their implementation. In these countries, improving ruminant efficiency and/or feeding tannin-rich plants is a less costly and more easily implementable solution. However, the latter will not decrease methane emissions to the same extent. However, improvements in production in developing countries will reduce their impacts on the climate by reducing methane intensity substantially.

[11] https://www.esmmagazine.com/retail/coop-sweden-to-launch-methane-reduced-beef-177522

Table 1. Status of regulatory approval for dietary interventions in ruminants

Dietary intervention	Regulatory approval to feed	Countries with approval
Plants containing secondary compounds e.g tannins	Yes for all ruminants	Not required
CH_4 inhibitor: 3-NOP (Bovaer®)	Yes in dairy	Europe, Brazil, Chile and Australia
CH_4 inhibitor: *Asparagopsis taxiformis*)	Yes for all ruminants	Most as feed ingredient
Oils and oilseeds	Yes for all ruminants	All

Box 5: Expert views on the current status of development and implementation of policies, legislation and institutional arrangements for the management of microorganisms of relevance to ruminant digestion

More than half of the expert survey respondents indicated that they believed that there was currently no activity in relation to the development of policies legislation and institutional arrangements for the management of microorganisms of relevance to ruminant digestion in their respective jurisdictions. They cited differences in policy on the transfer of materials and intellectual property issues among institutions as factors that hinder the development and implementation of agreements between countries. It was generally agreed that although much paperwork would be required, it is feasible to develop policies and legislation in this area.

Source: Responses to the expert survey conducted for the present study.

8. Key institutions involved in the management of microorganisms of relevance to ruminant digestion

The main academic organizations with major capacity and capability to isolate and maintain gastrointestinal tract microbes are listed in Table 2. These partners all hold personal culture collections, with a small number of microbial isolates being deposited in culture collections.

Table 2. Main academic institutions with capacity and capability to expand research in microbial function and maintain culture collections for gastrointestinal tract microbial genetic diversity

Institution	Academic/s
Agriculture and Agri-Food Canada (AAFC), Canada	Prof Tim McAllister
Agrosavia, Colombia	Dr Hugo Jiminez
AgResearch, New Zealand	Dr William Kelly
	Dr Graeme Attwood
	Dr Peter Janssen
	Dr Sinead Leahy
Ben-Gurion University, Israel	Prof Itzik Mizrahi
Chinese Agricultural University, China	Prof Shengguo Mao
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia	Dr Chris McSweeney
	Dr Stuart Denman
National Research Institute for Agriculture, Food and Environment (INRAE), France	Dr Diego Morgavi
	Dr Cecile Martin
	Dr Evelyn Forano
Nanjing Agricultural University, China	Prof Weiyun Zhu
	Prof Yanfen Chen
Queen's University Belfast, United Kingdom	Prof Sharon Huws
	Prof Chris Creevey
Spanish National Research Council (CSIC), Spain	Dr David Yanez-Ruiz
Teagasc, Ireland	Prof Sinead Waters
UC Davis, California, United States of America	Dr Matthias Hess
Madison State University, United States of America	Dr Hilario Mantovani
	Dr Garret Suen
University of Alberta, Canada	Prof Leluo Guan
University Illinois Urbana-Champaign, United States of America	Prof Rod Mackie
Wageningen University & Research, Netherlands	Prof Hauke Smidt

Other universities, research organizations and governmental or international networks that contribute to the sustainable use and conservation of microorganisms of relevance to ruminant digestion worldwide include the following: the International Institute of Tropical Agriculture (IITA)-Bioscience, Nigeria; the Environmental Sustainability Research Centre (ESRC), United Kingdom; the Rowett Research Institute, University of Aberdeen, United Kingdom; the University of Ljubljana, Slovenia; the Centre of Biosciences, Institute of Animal Physiology, Slovak Academy of Sciences, Slovakia; and the O'Malley Lab, University of California Santa Barbara, United States of America. The GRA

(see above) is an important global network, as it brings countries together to find ways to grow more food without increasing GHG emissions.[16] It has a Livestock Research Group (LRG), [17] whose work is underpinned by, *inter alia*, the RMG. Industry stakeholders may also hold pure culture isolates from the rumen (e.g. DSM novozymes), but for intellectual property reasons it is difficult to know what they hold, and this information is unlikely to become publicly available.

Where stakeholder collaboration is concerned, several major projects (e.g. the EU Horizon 2020 projects MASTER[11] and Holoruminant[12]) play an important role. However, the expert survey respondents noted that it was more challenging for those from low- and middle-income countries to collaborate, largely because of inadequate funding (Box 6).

Box 6: Expert views on the current status of collaboration between organizations that contribute to the sustainable use and conservation of microorganisms of relevance to ruminant digestion

The survey respondents stated that research collaboration exists and offer opportunities for further development of research innovation. Several large organizations, international projects and networks support collaboration among laboratories to increase research on the sustainable use and conservation of microorganisms of relevance to ruminant digestion, especially in Europe. However, there is much less collaboration across continents, especially with low- and middle-income countries. The reason for this may be that this area of research is not considered a high priority topic for many scientific foundations or that it is of low relevance to some countries, and that thus very limited funding is available in many countries. Availability of funding to actively participate in these initiatives is still a challenge for many, both in developed countries and in low- and middle-income countries.

Respondents generally agreed that, as well as from funding challenges, difficultly with paperwork for material and technology transfer agreements, intellectual property issues and administrative barriers also hinder collaboration among organizations. However, they noted that most colleagues were happy to collaborate, generally making it easy to share or obtain strains from other groups.

The respondents also stated that there was a need to review and amend the Nagoya Protocol with a view to enabling easier transfer of genetic material between countries. They also noted the need to develop international funding programmes that allow for money to be made available to international partners outside Europe, where most initiatives are currently based.

Source: Responses to the expert survey conducted for the present study.

9. Gaps and weaknesses

9.1 Research gaps

Research and technology have advanced in the past 20 years, allowing better definition of what an "optimal" rumen microbiome that would contribute to goals such as methane mitigation would look like and of how such a microbiome can be obtained. However, there are still major gaps in our scientific understanding and in our ability to deliver societal impact. For example, for the past 20 years or so we have focused on nucleic acid-based sequencing of the rumen microbiome to evaluate diversity and function with different hypotheses in mind. However, while such studies have been useful in terms of correlating the rumen microbiome to the host phenotype, they often cannot confirm whether a link to a particular microbe/gene is real, i.e. they do not allow us to move from correlation to causation. This step can be made if the isolate or a close relative is available in pure culture, as this enables hypotheses based on "omic" studies to be tested. The field of culturing microbes has largely been neglected in the "omic" era. However, the need for advanced culturing and subsequent genome sequencing (culturomics) has never been greater. Indeed, culturomics within other ecosystems, such as the human gastrointestinal tract, is rapidly advancing, as most scientists now recognize that

[17] https://globalresearchalliance.org/research/livestock/ [171] https://www.master-h2020.eu/
[12]https://holoruminant.eu/

sequencing alone cannot provide the detailed information required to test hypothesis (Lagier *et al*., 2018; Forster *et al*., 2019).

The potential to use isolates as DFMs, particularly in combination with methane-reducing additives, could further reduce methane output and enhance production by redirecting hydrogen to key VFAs. The microbial genomes will also substantially enhance our ability to infer function from diversity-based sequencing studies through enhancing the CowPI database.[18] Likewise, isolates are important for assessing AMR risks, which are key to future One-Health challenges, and for enabling the discovery and exploitation of bioactive compounds.

Of course, culturing should be undertaken for all rumen microbes, thus enabling a whole system approach. However, the rumen protozoa in particular are more challenging to culture outside the rumen. Overall, understanding the current diversity of microorganisms of relevance to rumen digestion so that any future losses in diversity can be tracked is a key research gap.

9.2 Conservation and culture collections

Open-access policies, particularly those relating to the publication of work involving pure cultures, do not currently require deposition of isolates in culture collections. This means that isolates remain in academic freezers. We recommend that journals should insist that isolates are deposited before publication, allowing other stakeholders to access them. As noted above, without such policies, such microbes sit in freezers across the world, and this poses a major risk that they will lose viability and be lost to the scientific community. Clearly, such a policy would have to ensure that culture collections had the infrastructure and capability required to deal with an increase in the number of microbes being deposited.

9.3 Policies and regulation

Fair and equitable access to rumen microbial genetic resources is a major area requiring change. The Nagoya Protocol is a major barrier to the exchange of genetic material, owing to the level of bureaucracy and paperwork required to obtain samples/microbes from parties to the protocol. Given the significance of such exchanges to efforts to meet global challenges, such as improving food security and mitigating climate change, reducing such barriers is crucial. Consequently, we recommend that a standard, simple procedure for the development of policies, legislation and institutional arrangements related to the exchange of rumen microbial genetic resources is developed. We also suggest a review of intellectual property laws related to microorganisms, which currently hinder act as barriers to collaboration, slowing research progress.

Clearly, the livestock sector is under extreme pressure to provide innovative solutions to reduce methane emissions from ruminants within a very short timeframe. Providing innovative feed additives that can help meet this challenge requires a change in the regulatory framework so that they can be approved more quickly – but still with the necessary evaluation of their efficacy and safety. Dietary interventions to reduce methane emissions can be costly for the farmer to use, and therefore all stakeholders need urgently to consider how costs could be returned to the farmer, either via the consumer's (or the supermarkets') pocket or via approaches such as governmental carbon frameworks whereby farmers are paid based on their net carbon status. Science will soon have developed a range of innovations that can reduce ruminant methane emissions. However, they will not be adopted unless farmers can recoup costs.

10. Potential ways in which the Commission and its members could contribute to addressing gaps and weaknesses in the sustainable use and conservation of microorganisms of relevance to ruminant digestion

Based on a review of the available scientific data, current policies and regulations, and the opinions expressed by experts, we recommend the following potential ways in which the Commission and its

[18] https://www.cowpi.org/

members could contribute to addressing gaps and weaknesses in the sustainable use and conservation of microorganisms of relevance to ruminant digestion:

- establishing a global expert group to work on the prioritization of activities related to the management of micro-organisms of relevance to ruminant digestion and on the identification of threats to the sustainable use and conservation of these organisms;
- ensuring adequate resourcing global research initiatives for the culture, cataloguing and management of rumen microbes;
- promoting open-access policies ensuring that all pure culture microbial isolates must be deposited in culture collections before publication of any data related the respective organism(s);
- enhancing the capacity of global culture collections to deal with the increased demand that having an open policy requiring isolate deposition in a culture collection would bring;
- promoting the funding of research on innovations in the management of the rumen microbiome, particularly with respect to ruminant breeding and dietary innovations;
- improving funding opportunities for database development for isolate genomes and phenotypes while also enhancing the computational expertise available to translate these underpinning data into enhanced metagenomic annotations and ultimately enable inference of fermentative capacity and nutrient availability to the host;
- instigating a change to the Nagoya Protocol to enable ease of sample/microbial exchange globally; and
  providing stimulus to encourage global collaboration, especially collaboration involving low- and middle-income countries; and
- providing stimulus to encourage global collaboration, especially collaboration involving low- and middle-income countries.

Annex 1: Responses received to the expert survey

This annex outlines the results of a survey sent to members of the Rumen Microbial Genomics network of the Global Research Alliance to solicit their opinions on the sustainable use and conservation of microorganisms of relevance to ruminant digestion. The Rumen Microbial Genomics Network (RMG) is a global forum for researchers and stakeholders using genomics approaches to understand microorganisms of relevance to rumen digestion as they relate to enteric methane emissions, animal health and productivity (Figure A1).

Figure A1. Countries of Residence of RMG network members contacted for the survey.

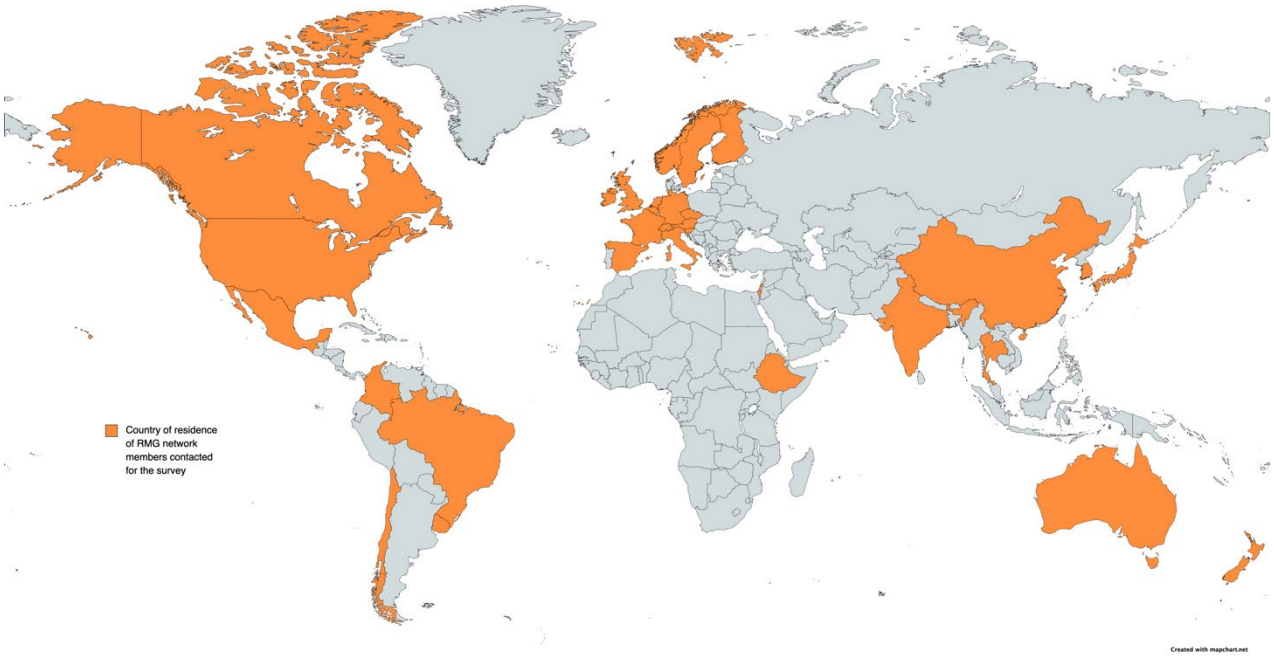
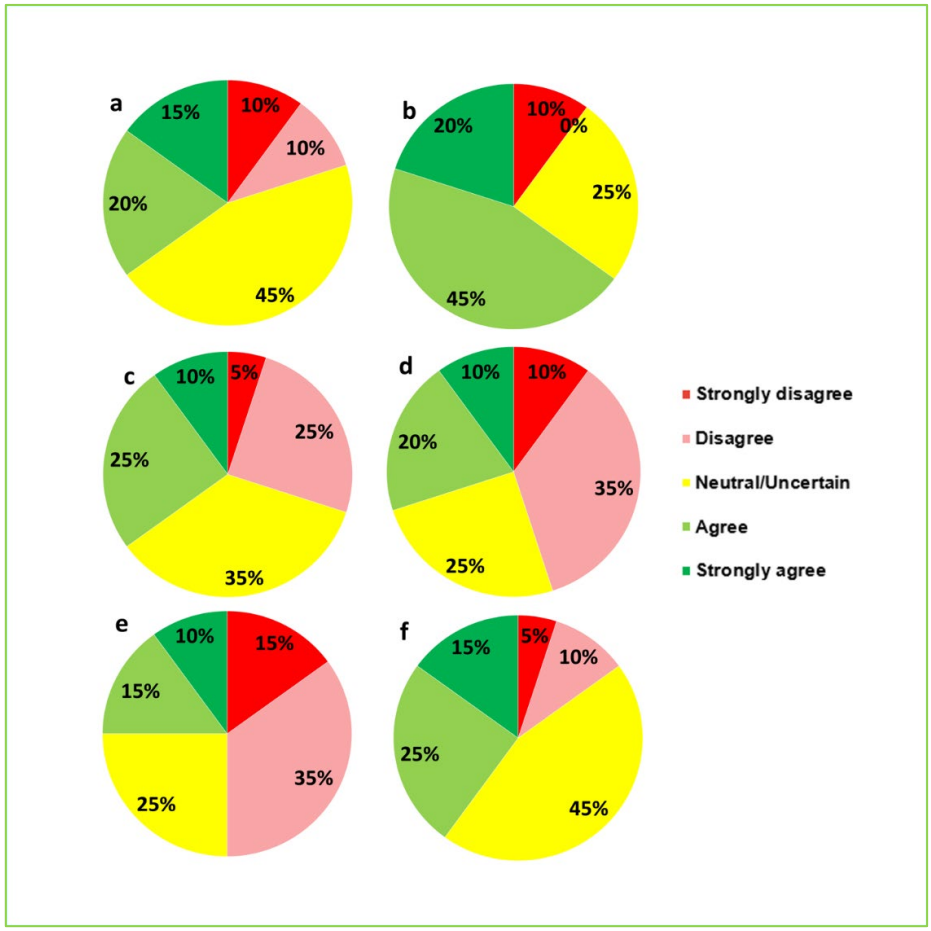


Table A1. Geographical regions and stakeholder groups of the survey respondents

By region	Number of responses	By stakeholder group	Number of responses
Africa	2	Academia	8
Europe	4	Industry	2
North America	2	Government	2
Latin America and the Caribbean	2	Not known	8
Not known	8		

Figure A2. Distribution of responses to the survey questions



Notes: The pie charts refer, respectively, to the following statements: a) *"The current status of the diversity of microorganisms of relevance to ruminant digestion worldwide is healthy"*; b) "*The current trends in diversity of microorganisms of relevance to ruminant digestion worldwide suggests that their status will remain healthy over the next decade*"; c) "*The current status of the implementation of activities aimed at promoting sustainable use and conservation of microorganisms of relevance to ruminant digestion is good"* d) "*The current status of development of policies, legislation and institutional arrangements in your jurisdiction for the management of microorganisms of relevance to ruminant digestion is healthy"*; e) "*The current status of *implementation* of policies, legislation and institutional arrangements in your jurisdiction for the management of microorganisms of relevance to ruminant digestion is healthy"* ; f) "*The current status of collaboration between organisations that contribute to the sustainable use and conservation of microorganisms of relevance to ruminant digestion worldwide is healthy".*

References

Abbott, D.W., Aasen, I.M., Beauchemin, K.A., Grondahl, F., Gruninger, R., Hayes, M., Huws, S., Kenny, D.A., Krizsan, S.J., Kirwan, S.F., Lind, V., Meyer, U., Ramin, M., Theodoridou, K, von Soosten, D., Walsh, P.J., Waters, S. & Xing, X. 2020. Seaweed and seaweed bioactives for mitigation of enteric methane: challenges and opportunities. *Animals (Basel)*, 10(12): 2432. https://doi.org/10.3390/ani10122432

Abecia, L., Jiménez, E., Martinez-Fernandez, G., Martín-García, A.I., Ramos-Morales, E., Pinloche, E., Denman, S.E., Newbold, C.J. & Yáñez-Ruiz, D.R. 2017. Natural and artificial feeding management before weaning promote different rumen microbial colonization but not differences in gene expression levels at the rumen epithelium of newborn goats. *PLoS ONE*, 12(8): e0182235. https://doi.org/10.1371/journal.pone.0182235

Adams, J.C., Gazaway, J.A., Brailsford, M.D., Hartman, P.A. and Jacobson, N.L. 1966. Isolation of bacteriophages from the bovine rumen. *Experientia*, 22(11): 717–718. https://doi.org/10.1007/BF01901335

Anderson, C.L. & Fernando, S.C. 2021. Insights into rumen microbial biosynthetic gene cluster diversity through genome-resolved metagenomics. *Communications Biology,* 4: 818. https://doi.org/10.1038/s42003-021-02331-7

Arndt, C., Hristov, A.N., Price, W.J., McClelland, S.C., Pelaez, A.M., Cueva, S.F., Oh J. *et al.* 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences of the United States of America*, 119(20): e2111294119. https://doi.org/10.1073/pnas.2111294119

Astuti, W.D., Wiryawan, K.G., Wina, E., Widyastuti, Y., Suharti, S. and Ridwan, R. 2018. Effects of selected Lactobacillus plantarum as probiotic on in vitro ruminal fermentation and microbial population. *Pak J Nutr*, 17(3), pp.131-139. https://dx.doi.org/10.3923/pjn.2018.131.139

Attwood, G.T. & Leahy, S.C. 2020. The rumen archaea. In: C.S. McSweeney & D.I. Mackie, eds. *Improving rumen function*. pp. 133–189. Cambridge, UK, Burleigh Dodds Publishing.

Auffret, M.D., Dewhurst, R.J., Duthie, C.A., Rooke, J.A., Wallace, R., Freeman, T.C., Stewart, R., Watson, M. & Roehe, R. 2017. The rumen microbiome as a reservoir of antimicrobial resistance and pathogenicity genes is directly affected by diet in beef cattle. *Microbiome*, 5: 159. https://doi.org/10.1186/s40168-017-0378-z

Auffret, M.D, Stewart, R., Dewhurst, R.J., Duthie, C.A., Rooke J.A., Wallace, R.J., Freeman, T.C., Snelling, T.J., Watson, M. & Roehe, R. 2018. Identification, comparison, and validation of robust rumen microbial biomarkers for methane emissions using diverse *Bos Taurus* breeds and basal diets. *Frontiers in Microbiology*, 8: 2642. https://doi.org/10.3389/fmicb.2017.02642.

Azevedo, A.C., Bento, C.B., Ruiz, J.C., Queiroz, M.V. & Mantovani, H.C. 2015. Distribution and genetic diversity of bacteriocin gene clusters in rumen microbial genomes. *Applied and Environmental Microbiology*, 81(20): 7290–7304. https://doi.org/10.1128/AEM.01223

Ban, Y. & Guan, L.L. 2021. Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. *Journal of Animal Science and Biotechnology*, 12(1): 109. https://doi.org/10.1186/s40104-021-00630-x

Barr, D.J.S. 1980. An outline for the reclassification of the *Chytridiales*, and for a new order, the *Spizellomycetales*. *Canadian Journal of Botany*, 58: 2380–2394. https://doi.org/10.1139/b80-276

Barr, D.J.S. 1988. How modern systematics relates to the rumen fungi. *Biosystems*, 21(3-4): 351–356. https://doi.org/10.1016/0303-2647(88)90032-9

Bauchop, T. & Mountfort, D.O. 1981. Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogens. *Applied and Environmental Microbiology*. 42(6): 1103–1110. https://doi.org/10.1128/aem.42.6.1103-1110.1981

Beauchemin, K.A., Ungerfeld, E.M., Abdalla, A.L., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C. *et al.* 2022. Invited review: Current enteric methane mitigation options. *Journal of Dairy Science*, 105(12): 9297–9326. doi: 10.3168/jds.2022-22091.

Beauchemin, K.A., Ungerfeld, E.M., Eckard, R.J. & Wang, M. 2020. Review: fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. *Animal*, 14(S1): S2–S16. https://doi.org/10.1017/S1751731119003100

Belanche, A, de la Fuente, G. & Newbold, C.J. 2014. Study of methanogen communities associated with different rumen protozoal populations. *FEMS Microbiology Ecology*, 90(3): 663–677. https://doi.org/10.1111/1574-6941.12423

Belanche, A., Doreau, M., Edwards, J.E., Moorby, J.M., Pinloche, E. & Newbold, C.J. 2012. Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. *The Journal of Nutrition*, 142(9): 1684–1692. https://doi.org/10.3945/jn.112.159574

Berry, D. & Widder, S. 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Frontiers in Microbiology*, 5: 219. https://doi.org/10.3389/fmicb.2014.00219

Boland, T.M., Quinlan, C., Pierce, K.M., Lynch, M.B., Kenny, D.A., Kelly, A.K. & Purcell, P.J. 2013. The effect of pasture pregrazing herbage mass on methane emissions, ruminal fermentation, and average daily gain of grazing beef heifers. *Journal of Animal Science*. 91: 3867–3874. https://doi.org/10.2527/jas.2013-5900

Brashears, M.M., Amezquita, A. & Jaroni, D. 2005. Lactic acid bacteria and their uses in animal feeding to improve food safety. *Advances in Food and Nutrition Research*, 50: 1–31. https://doi.org/10.1016/S1043-4526(05)50001-9

Cain, M., Lynch, J., Allen, M.R., Fuglestvedt, J.S., Frame, D.J. & Macey, A.H. 2019. Improved calculation of warming-equivalent emissions for short-lived climate pollutants. *npj Climate and Atmospheric Science*, 2: 29. https://doi.org/10.1038/s41612-019-0086-4.

Cao, Y, Takahashi, T, Horiguchi, K. & Yoshida, N. 2010a. Effect of adding lactic acid bacteria and molasses on fermentation quality and *in vitro* ruminal digestion of total mixed ration silage prepared with whole crop rice. *Grassland Science,* 56(1), 19–25. https://doi.org/10.1111/j.1744-697X.2009.00168.x

Cao, Y, Takahashi, T, Horiguchi, K, Yoshida, N. & Cai, Y. 2010b. Methane emissons from sheep fed fermented or non-fermented total mixed ration containing whole-crop rice and rice bran. *Animal Feed Science and Technology,* 157(1-2): 72–78. https://doi.org/10.1016/j.anifeedsci.2010.02.004

CIEL (Centre for Innovation Excellence in Livestock). 2022. *Net zero and livestock: how farmers can reduce emissions*. York, UK. https://www.cielivestock.co.uk/expertise/net-zero-carbon-uk-livestock/report-april-2022/

Cheng, K.J., McCowan, R.P. & Costerton, J.W. 1979. Adherent epithelial bacteria in ruminants and their roles in digestive tract function. *The American Journal of Clinical Nutrition*, 32(1): 139–148. https://doi.org/10.1093/ajcn/32.1.139

Cheng, K-J & Costerton, J.W. 1980. Adherent rumen bacteria—their role in the digestion of plant material, urea and epithelial cells. In: Y. Ruckebusch & P. Thivend, eds. *Digestive physiology and metabolism in ruminants*. Proceedings of the 5th International Symposium on Ruminant Physiology, held at Clermont—Ferrand, on 3rd−7th September, 1979. pp 227–250. Dordrecht, Netherlands, Springer.

Cheng, Y.F., Edwards, J.E., Allison G.G., Zhu, W.Y. & Theodorou, M.K. 2009. Diversity and activity of enriched ruminal cultures of anaerobic fungi and methanogens grown together on lignocellulose in consecutive batch culture. *Bioresource Technology*, 100(20): 4821–48218. https://doi.org/10.1016/j.biortech.2009.04.031

Creevey, C.J., Kelly, W.J., Henderson, G. & Leahy, S.C. 2014. Determining the culturability of the rumen bacterial microbiome. *Microbial Biotechnology*, 7(5): 467–479. https://doi.org/10.1016/j.biortech.2009.04.031

Crumpler, K., Abi Khalil, R., Tanganelli, E., Rai, N., Roffredi, L., Meybeck, A., Umulisa, V., Wolf, J. & Bernoux, M. 2021. *2021 (Interim) Global update report – agriculture, forestry and fisheries in the Nationally Determined Contributions*. Environment and Natural Resources Management Working Paper No. 91. Rome, FAO. https://doi.org/10.4060/cb7442en

de Haas, Y., Pszczola, M., Soyeurt, H., Wall, E. & Lassen, J. 2017. Invited review: Phenotypes to genetically reduce greenhouse gas emissions in dairying. *Journal of Dairy Science*, 100(2): 855–870. https://doi.org/10.3168/jds.2016-11246

de Oliveira, I.M.F., Godoy-Santos, F., Oyama, L.B., Moreira, S.M., Dias, R.G., Huws S.A., Creevey, C.J. & Mantovani, H.C. 2022. Whole-genome sequencing and comparative genomic analysis of antimicrobial producing *Streptococcus lutetiensis* from the rumen. *Microorganisms*. 10(3): 551. https://doi.org/10.3390/microorganisms10030551

de Souza Congio, S.F., Bannik, A. & Mogollon, O.L.M. 2021. Enteric methane mitigation strategies for ruminal livestock systems in the Latin America and Caribbean region. *Journal of Cleaner Production*, 312: 127693. https://doi.org/10.1016/j.jclepro.2021.127693

Dey, A., Sehgal, J.P., Puniya, A.K. & Singh, K. 2004. Influence of an anaerobic fungal culture (*Orpinomyces* sp.) administration on growth rate, ruminal fermentation and nutrient digestion in calves. *Asian-Australasian Journal of Animal Science*, 17(6): 820–824. https://doi.org/10.5713/ajas.2004.820

Difford, G.F., Plichta, D.R., Løvendahl, P., Lassen, J., Noel, S.J., Højberg, O., Wright, A.G. *et al.* 2018. Host genetics and the rumen microbiome jointly associate with methane emissions in dairy cows. PLoS Genetics, 14(10): e1007580. https://doi.org/10.1371/journal.pgen.1007580

Dill-McFarland, K.A., Weimer, P.J., Breaker, J.D. & Suen, G. 2019. Diet influences early microbiota development in dairy calves without long-term impacts on milk production. *Applied and Environmental Microbiology*, 85(2): e02141-18. https://doi.org/10.1128/AEM.02141

Doyle, N., Mbandlwa, P., Kelly, W.J., Attwood, G., Li, Y., Ross, R.P., Stanton, C. & Leahy, S. 2019. Use of lactic acid bacteria to reduce methane production in ruminants, a critical review. *Frontiers in Microbiology*, 10: 2207. https://doi.org/10.3389/fmicb.2019.02207

Edwards, J.E, Forster, R.J., Callaghan, T.M., Dollhofer, V., Dagar, S.S., Cheng, Y., Chang, J. *et al.* 2017. PCR and omics based techniques to study the diversity, ecology and biology of anaerobic fungi: Insights, challenges and opportunities. *Frontiers in Microbiology*, 8: 1657. https://doi.org/10.3389/fmicb.2017.01657.

Elekwachi, C.O., Wang, Z., Wu, X, Rabee, A. & Forster, R.J. 2017. Total rRNA-seq analysis gives insight into bacterial, fungal, protozoal and archaeal communities in the rumen using an optimized RNA isolation method. *Frontiers in Microbiology*, 8: 1814. https://doi.org/10.3389/fmicb.2017.01814

Enriquez-Hidalgo, D., Gilliland, T., Deighton, M.H., O'Donovan, M.O. & Hennessy, D. 2014. Milk production and enteric methane emissions by dairy cows grazing fertilized perennial ryegrass pasture with or without inclusion of white clover. *Journal of Dairy Science*, 97: 1400–1412. http://dx.doi.org/10.3168/jds.2013-7034.

Eugène, M., Klumpp, K. & Sauvant, D. 2021. Methane mitigating options with forages fed to ruminants. *Grass Forage Science*, 76(2): 196–201. https://doi.org/10.1111/gfs.12540.

FAO, IFAD, UNICEF, WFP & WHO. 2022. *The State of Food Security and Nutrition in the World 2022. Repurposing food and agricultural policies to make healthy diets more affordable*. Rome, FAO. https://doi.org/10.4060/cc0639en

Fernando, S.C., Purvis, H.T., Najar, F.Z., Sukharnikov, L. O., Krehbiel, C. R., Nagaraja, T. G., Roe, B.A. & da Silva, U. 2010. Rumen microbial population dynamics during adaptation to a high-

grain diet. *Applied and Environmental Microbiology,* 76: 7482–7490. https://doi.org/10.1128/AEM.00388-10

Forster, S., Kumar, N., Anonye, B. O., Almeida, A., Viciani, E., Stares, M.D., Dunn, M. *et al*. 2019. A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nature Biotechnology,* 37: 186–192. https://doi.org/10.1038/s41587-018-0009-7

Friedersdorff, J.C.A., Kingston-Smith, A.H., Pachebat, J.A., Cookson, A.R., Rooke, D. & Creevey, C.J. 2020. The isolation and genome sequencing of five novel bacteriophages from the rumen active against *Butyrivibrio fibrisolvens*. *Frontiers in Microbiology*, 11: 1588. https://doi.org/10.3389/fmicb.2020.01588

Furman, O., Shenhav, L., Sasson, G., Kokou, F., Honig, H., Jacoby, S., Hertz, T. *et al*. 2020. Stochasticity constrained by deterministic effects of diet and age drive rumen microbiome assembly dynamics. *Nature Communications*, 11: 1904. https://doi.org/10.1038/s41467-020-15652-8

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G. 2013. *Tackling climate change through livestock – a global assessment of emissions and mitigation opportunities*. Rome. FAO. https://www.fao.org/3/i3437e/i3437e.pdf

Gilbert, R.A. and Klieve, A.V. 2015. Ruminal viruses (bacteriophages, archaeaphages). In Rumen microbiology: *From evolution to revolution* (pp. 121-141). Springer, New Delhi. https://doi.org/10.1007/978-81-322-2401-3_9

Gilbert, R.A., Kelly, W.J., Altermann, E., Leahy, S.C., Minchin, C., Ouwerkerk, D. & Klieve, A.V. 2017. Toward understanding phage: host interactions in the rumen; complete genome sequences of lytic phages infecting rumen bacteria. *Frontiers in Microbiology*, 8: 2340. https://doi.org/10.3389/fmicb.2017.02340

Goopy, J.P., Donaldson, A., Hegarty, R., Vercoe, P.E., Haynes, F., Barnett, M. & Oddy, V.H. 2014. Low-methane yield sheep have smaller rumens and shorter rumen retention time. *British Journal of Nutrition*, 111(4): 578–585. https://doi.org/10.1017/S0007114513002936

Gordon, G.L. & Phillips, M.W. 1998. The role of anaerobic gut fungi in ruminants. *Nutrition Research Reviews*, 11(1): 133–168. https://doi.org/10.1079/NRR19980009

Griswold, K.E., White, B.A. & Mackie R.I. 1999. Diversity of extracellular proteolytic activities among *Prevotella* species from the rumen. *Current Microbiology*, 39(4): 187–94. https://doi.org/10.1007/s002849900443

Gruby, D. & Delafond, H.M.O. 1843. Recherches sur des animalcules se developpant en grand nombre dans l'estomac et dans les intestins, pendant la digestion des animaux herbivores et carnivores. *Comptes Rendus*, 17: 1304–1308.

Haisan, J., Sun, Y., Guan, L., Beauchemin, K.A., Iwaasa, A., Duval, S., Kindermann, M., Barreda, D.R. & Oba, M. 2016. The effects of feeding 3-nitrooxypropanol at two doses on milk production, rumen fermentation, plasma metabolites, nutrient digestibility, and methane emissions in lactating Holstein cows. *Animal Production Science*, 57: 282–289.

Hassan, A., Gado, H., Anele, U.Y., Berasain, M.A.M. & Salem, A.Z.M. 2019. Influence of dietary probiotic inclusion on growth performance, nutrient utilization, ruminal fermentation activities and methane production in growing lambs. *Animal Biotechnology*, 31(4): 365–372. https://doi.org/10.1080/10495398.2019.1604380

Hegarty, R.S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. *Australian Journal of Agricultural Research*, 50: 1321–1328. https://doi.org/10.1071/AR99008

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Global Rumen Census Collaborators & Janssen, P.H. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, 5: 14567. https://doi.org/10.1038/srep14567.

Hess, M., Sczyrba, A., Egan, R., Kim, T.W., Chokhawala, H., Schroth, G., Luo, S. *et al.* 2011. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. *Science*, 331: 463–467. doi: 10.1126/science.1200387

Hess, M., Paul, S.S., Puniya, A.K., van der Giezen, M., Shaw, C., Edwards, J.E. & Fliegerová, K. 2020. Anaerobic fungi: past, present, and future. *Frontiers in Microbiology*, 11: 584893. https://doi.org/10.3389/fmicb.2020.584893

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S. *et al*. 2007. A higher-level phylogenetic classification of the Fungi. *Mycological Research*, 111(5): 509–547. https://doi.org/10.1016/j.mycres.2007.03.004

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P. *et al.* 2013. Special topics--Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of Animal Science*, 91(11): 5045–5069. https://doi.org/10.2527/jas.2013-6583

Hristov, A.N., Oh, J., Giallongo, F., Frederick, T.W., Harper, M.T., Weeks, H.L., Branco, A.F., *et al*. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. *Proceedings of the National Academy of Sciences of the United States of America*, 112(34): 10663–10668. https://doi.org/10.1073/pnas.1504124112

Hungate, R.E. 1966. *The rumen and its microbes*. New York, USA, Academic Press.

Huws, S.A., Creevey, C.J., Oyama, L.B., Mizrahi, I., Denman, S.E., Popova, M., Muñoz-Tamayo, R. *et al.* 2018. Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. *Frontiers in Microbiology*, 9: 2161. https://doi.org/10.3389/fmicb.2018.02161

Huws, S.A., Kim, E.J., Kingston-Smith, A.H., Lee, M.R., Muetzel, S.M., Cookson, A.R., Newbold, C.J., Wallace, R.J. & Scollan, N.D. 2009. Rumen protozoa are rich in polyunsaturated fatty acids due to the ingestion of chloroplasts. *FEMS Microbiology Ecology*, 69(3): 461–471. https://doi.org/10.1111/j.1574-6941.2009.00717.x

Huws, S.A., Mayorga, O.L., Theodorou, M.K., Onime, L.A., Kim, E.J., Cookson, A.H., Newbold C.J. & Kingston-Smith A.H. 2013. Successional colonization of perennial ryegrass by rumen bacteria. *Letters in Applied Microbiology*, 56(3): 186–196. https://doi.org/10.1111/lam.12033

Huws, S.A., Mayorga, O.L., Theodorou, M.K., Kim, E.J., Cookson A., Newbold, J.C. & Kingston-Smith, A.H. 2014. Differential colonization of plant parts by the rumen microbiota is likely to be due to different forage chemistries. *Journal of Microbial & Biochemical Technology*, 6(2): 80–86. doi: 10.4172/1948-5948.1000126.

Huws, S.A., Edwards, J.E., Creevey, C.J., Rees Stevens, P., Lin, W., Girdwood, S.E., Pachebat, J.A. & Kingston-Smith, A.H. 2016. Temporal dynamics of metabolically active rumen bacteria colonizing fresh perennial ryegrass. *FEMS Microbiology Ecology*, 92(1): fiv137. https://doi.org/10.1093/femsec/fiv137

Huws, S.A., Lee, M.R., Kingston-Smith, A.H., Kim, E.J., Scott, M.B., Tweed, J.K. & Scollan, N.D. 2012. Ruminal protozoal contribution to the duodenal flow of fatty acids following feeding of steers on forages differing in chloroplast content. *British Journal of Nutrition*, 108: 2207–2214. https://doi.org/10.1017/S0007114512000335

Huws, S.A., Edwards, J.E., Lin, W., Rubino, F., Alston, M., Swarbreck, D., Caim, S. *et al.* 2021. Microbiomes attached to fresh perennial ryegrass are temporally resilient and adapt to changing ecological niches. *Microbiome*, 9: 143. https://doi.org/10.1186/s40168-021-01087-w.

IPCC (Intergovernmental Panel on Climate Change). 2021. Climate *change 2021: the physical science basis*. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change V. Masson-Delmotte, P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou, eds. Cambridge,

United Kingdom and New York, USA, Cambridge University Press. https://www.ipcc.ch/report/ar6/wg1

Jami, E., Israel, A., Kotser, A. & Mizrahi, I., 2013. Exploring the bovine rumen bacterial community from birth to adulthood. *The ISME Journal*, *7*(6): 1069–1079. https://doi.org/10.1038/ismej.2013.2

Jami, E., White, B.A. & Mizrahi, I. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PloS ONE*, *9*(1): e85423. https://doi.org/10.1371/journal.pone.0085423

Jeyanathan, J., Martin, C. & Morgavi, D.P. 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. *Animal* 8(2): 250–261. https://doi.org/10.1017/S1751731113002085

Jeyanathan, J., Martin, C. and Morgavi, D.P. 2016. Screening of bacterial direct-fed microbials for their antimethanogenic potential *in vitro* and assessment of their effect on ruminal fermentation and microbial profiles in sheep. *Journal of Animal Science*, *94*(2):.739–750. https://doi.org/10.2527/jas.2015-9682

Johnson K.A. & Johnson D.E. 1995. Methane emissions from cattle. *Journal of Animal Science*, 73(8): 2483–2492. https://doi.org/10.2527/1995.7382483x

Johnson, P.L., Hickey, S., Knowler, K., Wing, J., Bryson, B., Hall, M., Jonker, A., Janssen, P.H., Dodds, K.G., McEwan, J.C. and Rowe, S.J. 2022. Genetic parameters for residual feed intake, methane emissions, and body composition in New Zealand maternal sheep. Frontiers in Genetics, 13. https://doi.org/10.3389%2Ffgene.2022.911639

Jones, R.J. & Megarrity, R.G. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome toxicity of Leucaena. *Australian Veterinary Journal*, 63(8): 259–262. https://doi.org/10.1111/j.1751-0813.1986.tb02990.x

Kenters, N., Henderson, G., Jeyanathan, J., Kittelmann, S. & Janssen, P.H. 2011. Isolation of previously uncultured rumen bacteria by dilution to extinction using a new liquid culture medium. *Journal of Microbiological Methods*, 84(1): 52–60. https://doi.org/10.1016/j.mimet.2010.10.011

Kingston-Smith, A.H., Edwards, J.E., Huws, S.A., Kim, E.J. & Abberton, M. 2010. Plant-based strategies towards minimising 'livestock's long shadow'. *Proceedings of the Nutrition Society*, 69(4): 613–20. https://doi.org/10.1017/S0029665110001953

Kinley, R.D., de Nys, R., Vucko, M.J., Machado, L. & Tomkins, N.W. 2016. The red macroalgae *Asparagopsis taxiformis* is a potent natural anti-methanogenic that reduces methane production during in vitro fermentation with rumen fluid. *Animal Production Science*, 56(3): 282–289. http://dx.doi.org/10.1071/AN15576

Kittelmann, S. & Janssen, P.H. 2011. Characterization of rumen ciliate community composition in domestic sheep, deer, and cattle, feeding on varying diets, by means of PCR-DGGE and clone libraries. *FEMS Microbiology Ecology*, 75(3): 468–481. https://doi.org/10.1111/j.1574-6941.2010.01022.x

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P. & Tricarico, J.M. 2014. Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science,* 97(6): 3231–3261. https://doi.org/10.3168/jds.2013-7234

Koringa, P.G., Thakkar, J.R., Pandit, R.J., Hinsu, A.T., Parekh, M.J., Shah, R.K., Jakhesara, S.J. & Joshi, C.G. 2019. Metagenomic characterisation of ruminal bacterial diversity in buffaloes from birth to adulthood using 16S rRNA gene amplicon sequencing. *Functional & Integrative Genomics*, 19(2): 237–247. https://doi.org/10.1007/s10142-018-0640-x

Koskella, B. & Brockhurst, M.A. 2014. Bacteria–phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiology Reviews*, *38*(5): 916–931. https://doi.org/10.1111/1574-6976.12072

Ku-Vera, J.C., Jiménez-Ocampo, R., Valencia-Salazar, S.S., Montoya-Flores, M.D., Molina-Botero, I.C., Arango J., Gómez-Bravo C.A., Aguilar-Pérez C.F. & Solorio-Sánchez, FJ. 2020. Role of secondary plant metabolites on enteric methane mitigation in ruminants. *Frontiers in Veterinary Science*, 7: 584. https://doi.org/10.3389/fvets.2020.00584

Lagier, J.C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., Fenollar, F., Levasseur, A., Rollain, J.-M., Fournier, P.-E. & Raoult, D. 2018. Culturing the human microbiota and culturomics. *Nature Reviews Microbiology,* 16: 540–550. https://doi.org/10.1038/s41579-018-0041-0

Lawther, K., Santos, F.G., Oyama, L.B., Rubino, F., Morrison, S., Creevey, C.J., McGrath, J.W. & Huws, S.A. 2022. Resistome analysis of global livestock and soil microbiomes. *Frontiers in Microbiology*, 13: 897905. https://doi.org/10.3389/fmicb.2022.897905

Leahy, S.C., Janssen, P.H., Attwood, G.T., Mackie, R.I., McAllister, T.A. & Kelly, W.J. 2022. Electron flow: key to mitigating ruminant methanogenesis. *Trends in Microbiology*, 30(3): 209–212. https://doi.org/10.1016/j.tim.2021.12.005

Lean, I.J., Golder, H.M., Grant, T.M.D. & Moate, P.J. 2021. A meta-analysis of effects of dietary seaweed on beef and dairy cattle performance and methane yield. *PLoS ONE*, 16(7): e0249053. https://doi.org/10.1371/journal.pone.0249053

Lee, S, Ha, J.K. & Cheng, K.J. 2000. Influence of an anaerobic fungal culture administration on in vivo ruminal fermentation and nutrient digestion. *Animal Feed Science and Technology*, 88(3-4): 201–217. https://doi.org/10.1016/S0377-8401(00)00216-9

Leng, R.A. 2014. Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. *Animal Production Science*, 54(5): 519–543. https://doi.org/10.1071/AN13381

Li, F., Hitch, T.C., Chen, Y., Creevey, C.J. & Guan, L.L. 2019a. Comparative metagenomic and metatranscriptomic analyses reveal the breed effect on the rumen microbiome and its associations with feed efficiency in beef cattle. *Microbiome*, *7*: 6. https://doi.org/10.1186/s40168-019-0618-5

Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., Fitzsimmons, C., Plastow, G. & Guan, L.L. 2019b. Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle. *Microbiome*, 7: 92. https://doi.org/10.1186/s40168-019-0699-1

Li, Z., Wang, X., Zhang, Y., Yu, Z., Zhang, T., Dai, X., Pan, X. *et al*. 2022. Genomic insights into the phylogeny and biomass-degrading enzymes of rumen ciliates. *ISME Journal*, 16(12): 2775–2787. https://doi.org/10.1038/s41396-022-01306-8

Lynch, J., Cain, M., Pierrehumbert, R. & Allen, M. 2020. Demonstrating GWP*: a means of reporting warming-equivalent emissions that captures the contrasting impacts of short- and long-lived climate pollutants. *Environmental Research Letters*, 15(4): 044023. https://doi.org/10.1088/1748-9326/ab6d7e

Machado, L., Kinley, R.D., Magnusson, M., de Nys R. & Tomkins, N.W. 2015. The potential of macroalgae for beef production systems in Northern Australia. *Journal of Applied Phycology*, 27: 2001–2005. https://doi.org/10.1007/s10811-014-0439-7

Malmuthuge, N., Liang, G. & Guan, L.L. Regulation of rumen development in neonatal ruminants through microbial metagenomes and host transcriptomes. *Genome Biology,* 20: 172. https://doi.org/10.1186/s13059-019-1786-0

Martínez-Álvaro, M., Auffret, M.D., Duthie, C.A., Dewhurst, R.J., Cleveland, M.A., Watson, M. & Roehe, R. 2022. Bovine host genome acts on rumen microbiome function linked to methane emissions. *Communications Biology*, 5: 350. https://doi.org/10.1038/s42003-022-03293-0.

Martinez-Fernandez, G., Denman, S.E., Cheung, J. & McSweeney, C.S. 2017. Phloroglucinol degradation in the rumen promotes the capture of excess hydrogen generated from methanogenesis inhibition. *Frontiers in Microbiology*, 8: 1871. https://doi.org/10.3389/fmicb.2017.01871

Mayorga, O.L., Kingston-Smith, A.H., Kim, E.J., Allison, G.G., Wilkinson, T.J., Hegarty, M.J., Theodorou, M.K., Newbold, C.J. & Huws, S.A. 2016. Temporal metagenomic and metabolomic characterization of fresh perennial ryegrass degradation by rumen bacteria. *Frontiers in Microbiology*, 7: 1854. https://doi.org/10.3389/fmicb.2016.01854

McAllister, T.A., Bae, H.D., Jones, G.A. & Cheng, K.J. 1994. Microbial attachment and feed digestion in the rumen. *Journal of Animal Science*, 72(11): 3004–3018. https://doi.org/10.2527/1994.72113004x

Meale, S.J., Li, S.C., Azevedo, P., Derakhshani, H., DeVries, T.J., Plaizier, J.C., Steele, M.A. & Khafipour, E. 2017. Weaning age influences the severity of gastrointestinal microbiome shifts in dairy calves. *Scientific Reports*, 7(1): 198. https://doi.org/10.1038/s41598-017-00223-7

Mentschel, J., Leiser, R., Mülling, C., Pfarrer, C. & Claus, R. 2001. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. *Archiv für Tierernaehrung*, 55(2): 85–102. https://doi.org/10.1080/17450390109386185

Michalet-Doreau, B., Fernandez, I., Peyron, C., Millet, L. & Fonty, G. 2001. Fibrolytic activities and cellulolytic bacterial community structure in the solid and liquid phases of rumen contents. *Reproduction Nutrition Development*, 41(2): 187–194. https://doi.org/10.1051/rnd:2001122

Miglior, F., Fleming, A., Malchiodi, F., Brito, L.F., Martin, P. & Baes, C.F. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. *Journal of Dairy Science*, 100(12):10251–10271. https://doi.org/10.3168/jds.2017-12968

Minato, H., Endo, A., Ootomo, Y. & Uemura, T. 1966. Ecological treatise on the rumen fermentation II. The amylolytic and cellulolytic activities of the fractionated bacterial portions attached to the rumen solids. *The Journal of General and Applied Microbiology*, 12(1): 53–69. https://doi.org/10.2323/jgam.12.53

Mizrahi, I., Wallace, R.J. & Moraïs, S. 2021.The rumen microbiome: balancing food security and environmental impacts. *Nature Reviews Microbiology,* 19(9): 553–566. https://doi.org/10.1038/s41579-021-00543-6

Morgavi, D.P., Forano, E., Martin, C. & Newbold, C.J. 2010. Microbial ecosystem and methanogenesis in ruminants. *Animal*, 4(7): 1024–1036. https://doi.org/10.1017/S1751731110000546

Narvaez, N., Alazzeh, A.Y., Wang, Y. & McAllister, T.A. 2014. Effect of *Propionibacterium acidipropionici* P169 on growth performance and rumen metabolism of beef cattle fed a corn- and corn dried distillers' grains with solubles-based finishing diet. *Canadian Journal of Animal Science*, 94(2): 363–369. https://doi.org/10.4141/cjas2013-130.

Newbold, C.J., De La Fuente, G., Belanche, A., Ramos-Morales, E. & McEwan, N.R. 2015. The role of ciliate protozoa in the rumen. *Frontiers in Microbiology*, 6: 1313. https://doi.org/10.3389/fmicb.2015.01313.

Nollet, L., Mbanzamihigo, L., Demeyer, D. &Verstraete, W. 1998. Effect of the addition of *Peptostreptococcus productus* ATCC 35244 on reductive acetogenesis in the ruminal ecosystem after inhibition of methanogenesis by cell-free supernatant of *Lactobacillus plantarum* 80. *Animal Feed Science and Technology*, 71(1-2): 49–66. https://doi.org/10.1016/S0377-8401(97)00135-1

Orpin, C.G. 1974. The rumen flagellate *Callimastix frontalis*: does sequestration occur? *Journal of General Microbiology*, 84(2): 395–398. https://doi.org/10.1099/00221287-84-2-395

Orpin, C.G. 1977a. The rumen flagellate *Piromonas communis*: its life-history and invasion of plant material in the rumen. *Journal of General Microbiology*, 99(1): 107–117. https://doi.org/10.1099/00221287-99-1-107

Orpin, C.G. 1977b. Invasion of plant tissue in the rumen by the flagellate *Neocallimastix frontalis*. *Journal of General Microbiology*, 98(2): 423–430. https://doi.org/10.1099/00221287-98-2-423

Oyama, L.B., Girdwood, S.E., Cookson, A.R., Fernandez-Fuentes, N., Privé F., Vallin, H.E. & Wilkinson, T.J., 2017. The rumen microbiome: an underexplored resource for novel antimicrobial discovery. *NPJ Biofilms Microbiomes*, 3: 33. https://doi.org/10.1038/s41522-017-0042-1

Oyama, L.B., Olleik, H., Teixeira, A.C.N., Guidini, M.M., Pickup, J.A., Hui, B.Y.P., Vidal, N., Cookson, A.R., Vallin, H., Wilkinson, T. and Bazzolli, D. 2022. In silico identification of two peptides with antibacterial activity against multidrug-resistant Staphylococcus aureus. *npj Biofilms and Microbiomes*, 8(1):1–14. https://doi.org/10.1038/s41522-022-00320-0

Pal, C., Bengtsson-Palme, J., Kristiansson, E. & Larsson, D.G.J. 2016. The structure and diversity of human, animal and environmental resistomes. *Microbiome*, 4: 54. https://doi.org/10.1186/s40168-016-0199-5

Palevich, N., Kelly, W.J., Leahy, S.C., Denman, S., Altermann, E., Rakonjac, J. & Attwood, G.T. 2019. Comparative genomics of rumen *Butyrivibrio* spp. uncovers a continuum of polysaccharide-degrading capabilities. *Applied and Environmental Microbiology*, 86(1): e01993-19. https://doi.org/10.1128/AEM.01993-19

Palma-Hidalgo, J.M., Jiménez, E., Popova, M., Morgavi, D.P., Martín-García, A.I., Yáñez-Ruiz, D.R. & Belanche, A. 2021. Inoculation with rumen fluid in early life accelerates the rumen microbial development and favours the weaning process in goats. *Animal Microbiome*, 3: 11. https://doi.org/10.1186/s42523-021-00073-9

Park, T., Wijeratne, S., Meulia, T., Firkins, J.L. and Yu, Z. 2021. The macronuclear genome of anaerobic ciliate *Entodinium caudatum* reveals its biological features adapted to the distinct rumen environment. *Genomics*, 113(3):.1416–1427. https://doi.org/10.1016/j.ygeno.2021.03.014

Parks, D.H., Rinke, C., Chuvochina, M., Chaumeil, P.A., Woodcroft, B.J., Evans, P.N., Hugenholtz, P. & Tyson, G.W. 2017. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nature Microbiology*, 2: 1533–1542. https://doi.org/10.1038/s41564-017-0012-7.

Paul, S.S., Kamra, D.N., Sastry, V.R.B., Sahu, N.P. & Agarwal, N. 2004. Effect of administration of an anaerobic gut fungus isolated from wild blue bull (*Boselaphus tragocamelus*) to buffaloes (*Bubalus bubalis*) on in vivo ruminal fermentation and digestion of nutrients. *Animal Feed Science and Technology*, 115: 143–157. https://doi.org/10.1016/j.anifeedsci.2004.01.010

Pidcock, S.E., Skvortsov, T., Santos, F.G., Courtney, S.J., Sui-Ting, K., Creevey, C.J. & Huws, S.A. 2021. Phylogenetic systematics of *Butyrivibrio* and *Pseudobutyrivibrio* genomes illustrate vast taxonomic diversity, open genomes and an abundance of carbohydrate-active enzyme family isoforms. *Microbial Genomics*, 7(10): 000638. https://doi.org/10.1099/mgen.0.000638

Pinares-Patiño, C.S., Ebrahimi, S.H., McEwan, J.C., Dodds, K.G., Clark, H. & Luo, D. 2011. Is rumen retention time implicated in sheep differences in methane emission? *Proceedings of the New Zealand Society of Animal Production*, 71: 219–222.

Puniya, A.K., Salem, A.Z.M., Kumar, S., Dagar, S.S., Griffith, G.W., Puniya, M., Ravella, S.R, Kumar, N., Dhewa, T. & Kumar, R. 2015. Role of live microbial feed supplements with reference to anaerobic fungi in ruminant productivity: a review. *Journal of Integrative Agriculture*, 14(3): 550–560. https://doi.org/10.1016/S2095-3119(14)60837-6

Ramin, M., Franco, M., Roleda, M., Aasen, I.M., Hetta, M. & Steinshamn, H. 2018. *In vitro* evaluation of utilisable crude protein and methane production for a diet in which grass silage was replaced by different levels and fractions of extracted seaweed proteins. *Animal Feed Science and Technology*, 255: 114225. https://doi.org/10.1016/j.anifeedsci.2019.114225

Rezaeian, M., Beakes, G.W. & Parker, D.S. 2004. Distribution and estimation of anaerobic zoosporic fungi along the digestive tracts of sheep. *Mycological Research*, 108(10): 1227–1233. https://doi.org/10.1017/S0953756204000929

Rey, M., Enjalbert, F., Combes, S., Cauquil, L., Bouchez, O. & Monteils, V. 2014. Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. *Journal of Applied Microbiology*. 116(2): 245–257. https://doi.org/10.1111/jam.12405

Rira, M., Morgavi, D.P., Popova, M., Maxin, G. & Doreau, M. 2022. Microbial colonisation of tannin-rich tropical plants: Interplay between degradability, methane production and tannin disappearance in the rumen. *Animal*, 16(8): 100589. https://doi.org/10.1016/j.animal.2022.100589

Roehe, R., Dewhurst, R.J., Duthie, C.A., Rooke, J.A., McKain, N., Ross, D.W., Hyslop, J.J. *et al.* 2016. Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance. *PloS Genetics*, 12(2): e1005846. https://doi.org/10.1371/journal.pgen.1005846

Romero-Perez, A., Okine, E.K., McGinn, S.M., Guan, L.L., Oba, M., Duval, S.M., Kindermann, M. & Beauchemin, K.A. 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. *Journal of Animal Science*, 92(10): 4682–4693. https://doi.org/10.2527/jas.2014-7573

Roque, B.M., Brooke, C.G., Ladau, J., Polley, T., Marsh, L.J., Najafi, N., Pandey, P. *et al.* 2019. Effect of the macroalgae *Asparagopsis taxiformis* on methane production and rumen microbiome assemblage. *Animal Microbiome*, 1: 3. https://doi.org/10.1186/s42523-019-0004-4

Roque, B.M., Venegas, M., Kinley, R.D., de Nys, R., Duarte, T.L., Yang, X. & Kebreab, E. 2021. Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS ONE*, 16(3): e0247820. https://doi.org/10.1371/journal.pone.0247820.

Roumpeka, D.D., Wallace, R.J., Escalettes, F., Fotheringham, I. & Watson, M. 2017. A Review of bioinformatics tools for bio-prospecting from metagenomic sequence data. *Frontiers in Genetics*, 8: 23. https://doi.org/10.3389/fgene.2017.00023.

Sabino, Y.N.V., Santana, M.F., Oyama, L.B., Santos, F.G., Moreira, A.J.S., Huws, S.A. & Mantovani, H.C. 2019. Characterization of antibiotic resistance genes in the species of the rumen microbiota. *Nature Communications*, 10: 5252. https://doi.org/10.1038/s41467-019-13118-0.

Sasson, G., Ben-Shabat, S. K. & Seroussi, E. Doron-Faigenboim, A., Shterzer, N., Yaacoby, S. & Berg Miller, M.E. 2017. Heritable bovine rumen bacteria are phylogenetically related and correlated with the cow's capacity to harvest energy from its feed. *MBio,* 8: 703–717. https://doi.org/10.1128/mBio.00703-17

Saxena, S., Sehgal, J.P., Puniya, A.K. & Singh, K. 2010. Effect of administration of rumen fungi on production performance of lactating buffaloes. *Beneficial Microbes*, 1(2): 183–188. https://doi.org/10.3920/BM2009.0018

Seshadri, R., Leahy, S.C., Attwood, G.T., Teh, K.H., Lambie, S.C., Cookson, A.L., Eloe-Fadrosh, E.A. *et al.* 2018. Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection. *Nature Biotechnology*, 36(4): 359–367. https://doi.org/10.1038/nbt.4110

Shabat, S.K.B., Sasson, G., Doron-Faigenboim, A., Durman, T., Yaacoby, S., Berg Miller, M.E., White, B.A., Shterzer, N. & Mizrahi, I. 2016. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. *The ISME journal*, 10(12): 2958–2972. https://doi.org/10.1038/ismej.2016.62

Shi, W., Moon, C.D., Leahy, S.C., Kang, D., Froula, J., Kittelmann, S., Fan, C. *et al.* 2014. Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. *Genome Research*, 24: 1517–1525. doi: 10.1101/gr.168245.113.

Shukla, S.K. & Rao, T.S. 2017. Staphylococcus aureus biofilm removal by targeting biofilm-associated extracellular proteins. *Indian Journal of Medical Research*, 146(Supplement): S1–S8. doi: 10.4103/ijmr.IJMR_410_15.

Smith, P.E, Waters, S.M, Kenny, D.A., Kirwan, S.F., Conroy, S. & Kelly, A.K. 2021. Effect of divergence in residual methane emissions on feed intake and efficiency, growth and carcass performance, and indices of rumen fermentation and methane emissions in finishing beef cattle. Journal of Animal Science, 99(11): skab275. https://doi.org/10.1093/jas/skab275

Smith P.E., Kelly A.K., Kenny D.A. & Waters S.M. 2022. Differences in the composition of the rumen microbiota of finishing beef cattle divergently ranked for residual methane emissions. *Frontiers in Microbiology*, 13: 855565. https://doi.org/10.3389/fmicb.2022.855565

Solomon, K.V., Haitjema, C.H., Henske, J.K., Gilmore, S.P., Borges-Rivera, D., Lipzen, A., Brewer, H.M. *et al.* 2016. Early-branching gut fungi possess a large, comprehensive array of biomass-degrading enzymes. *Science*, 351(6278): 1192–1195. https://doi.org/10.1126/science.aad1431

Stewart, R.D., Auffret, M.D., Warr, A., Wiser, A.H., Press, M.O., Langford, K.W., Liachko, I. *et al.* 2018. Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nature Communications*, 9: 870. https://doi.org/10.1038/s41467-018-03317-6

Sugimoto, S., Sato, F., Miyakawa, R., Chiba, A., Onodera, S., Hori, S. & Mizunoe, Y. 2018. Broad impact of extracellular DNA on biofilm formation by clinically isolated Methicillin-resistant and -sensitive strains of *Staphylococcus aureus*. *Scientific Reports*, 8: 2254. https://doi.org/10.1038/s41598-018-20485-z.

Svartström, O., Alneberg, J., Terrapon, N., Lombard, V., de Bruijn, I., Malmsten, J., Dalin, A.M. *et al.* 2017. Ninety-nine de novo assembled genomes from the moose (Alces alces) rumen microbiome provide new insights into microbial plant biomass degradation. *The ISME Journal*, 11: 2538–2551. https://doi.org/10.1038/ismej.2017.108

Takahashi, J. 2013. Lactic acid bacteria and mitigation of GHG emission from ruminant livestock. In: M. Kongo, ed. *Lactic acid bacteria - R & D for food, health and livestock purposes*, 455–46 pp. London, IntechOpen. http://dx.doi.org/10.5772/50358

Tapio, I., Snelling, T.J., Strozzi, F. & Wallace, R.J. 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. *Journal of Animal Science and Biotechnology*, 8: 7. https://doi.org/10.1186/s40104-017-0141-0

Thomas, M., Webb, M., Ghimire, S., Blair, A., Olson, K., Fenske, G.J., Fonder, A.T., Christopher-Hennings, J., Brake, D. & Scaria, J. 2017. Metagenomic characterization of the effect of feed additives on the gut microbiome and antibiotic resistome of feedlot cattle. *Scientific Reports*, 7: 12257. https://doi.org/10.1038/s41598-017-12481-6

Tripathi, V.K., Sehgal, J.P., Puniya, A.K. & Singh, K. 2007. Effect of administration of anaerobic fungi isolated from cattle and wild blue bull (*Boselaphus tragocamelus*) on growth rate and fibre utilization in buffalo calves. *Archives of Animal Nutrition*, 61(5): 416–423. https://doi.org/10.1080/17450390701556759.

Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S. & Banfield, J.F. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428: 37–43. https://doi.org/10.1038/nature02340

United Nations Department of Economic and Social Affairs, Population Division 2022. *World Population Prospects 2022: Summary of Results*. UN DESA/POP/2022/TR/NO. 3. New York, USA, United Nations. https:/a/www.un.org/development/desa/pd/content/World-Population-Prospects-2022

UNFCCC (United Nations Framework Agreement on Climate Change). 2016. The *Paris Agreement*. Bonn, Germany. https://unfccc.int/sites/default/files/resource/parisagreement_publication.pdf?download

UNFCCC. 2021. *Koronivia joint work on agriculture. Draft conclusions*. Subsidiary Body for Implementation/Subsidiary Body for Scientific and Technological Advice, Fifty-second to fifty-fifth session Glasgow, 31 October to 6 November 2021. FCCC/SB/2021/L.1. Bonn, Germany. https://unfccc.int/documents/309895

Ungerfeld, E.M. 2020. Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Frontiers in Microbiology*, 11: 589. https://doi.org/10.3389/fmicb.2020.00589.

Varnava, K.G., Ronimus, R.S. & Sarojini, V. 2017. A review on comparative mechanistic studies of antimicrobial peptides against archaea. *Biotechnology and Bioengineering,*114(11): 2457–2473. https://doi.org/10.1002/bit.26387

Vogels, G.D., Hoppe, W.F. & Stumm, C.K. 1980. Association of methanogenic bacteria with rumen ciliates. *Applied and Environmental Microbiology*, 40(3): 608–612.

Vyas, D., McGeough, E.J., Mohammed, R., McGinn, S.M., McAllister, T.A. & Beauchemin, K.A. 2014. Effects of *Propionibacterium* strains on ruminal fermentation, nutrient digestibility and methane emissions in beef cattle fed a corn grain finishing diet. *Animal*, 8(11): 1807–1815. https://doi.org/10.1017/S1751731114001657

Vyas, D., Alazzeh. A., McGinn, S.M., McAllister, T.A., Harstad, O.M., Holo, H. & Beauchemin, K.A. 2015. Enteric methane emissions in response to ruminal inoculation of *Propionibacterium* strains in beef cattle fed a mixed diet. *Animal Production Science*, 56(7): 1035–1040. https://doi.org/10.1071/AN14801

Wallace, J.R. 2008. Gut microbiology - broad genetic diversity, yet specific metabolic niches. *Animal*, 2(5): 661–668. https://doi.org/10.1017/S1751731108001687

Wallace, R.J., Rooke, J.A., McKain, N., Duthie, C.A., Hyslop, J.J., Ross, D.W., Waterhouse, A., Watson, M. & Roehe, R. 2015. The rumen microbial metagenome associated with high methane production in cattle. *BMC Genomics*, 16: 839. https://doi.org/10.1186/s12864-015-2032-0

Wallace, R.J., Sasson, G., Garnsworthy, P.C., Tapio, I., Gregson, E., Bani, P., Huhtanen, P. *et al.* 2019. A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. *Science Advances*, 5(7): eaav8391. doi: 10.1126/sciadv.aav8391.

Wang, L., Zhang, K., Zhang, C., Feng, Y., Zhang, X., Wang, X. & Wu, G. 2019. Dynamics and stabilization of the rumen microbiome in yearling Tibetan sheep. *Scientific Reports*, 9(1): 19620. https://doi.org/10.1038/s41598-019-56206-3

Wang, Y., Xu, Z., Bach, S.J. & McAllister, T.A. 2008. Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on *in vitro* ruminal digestion of mixed forage or barley grain. *Animal Feed Science and Technology*, 145(1-4): 375–395. https://doi.org/10.1016/j.anifeedsci.2007.03.013

WHO (World Health Organization). 2019. *No time to wait: Securing the future from drug-resistant infections.* Report to the Secretary-General of the United Nations. Geneva, Switzerland. https://www.who.int/publications/i/item/no-time-to-wait-securing-the-future-from-drug-resistant-infections

Wilkinson, T.J., Huws, S.A., Edwards, J.E., Kingston-Smith, A.H., Siu-Ting, K., Hughes, M., Rubino, F., Friedersdorff, M. & Creevey, C.J. 2018. CowPI: A rumen microbiome focussed version of the PICRUSt functional inference software. *Frontiers in Microbiology*, 9: 1095. https://doi.org/10.3389/fmicb.2018.01095

Williams, A.G. & Coleman, G.S. 1992. Role of protozoa in the rumen. In: P.N. Hobson & C.S. Stewart, eds. *The rumen protozoa*, pp. 317–347. New York, USA, Springer. https://doi.org/10.1007/978-94-009-1453-7_3

Williams A.G. & Coleman G.S. 1997. The rumen protozoa. In: P.N. Hobson & C.S. Stewart, eds, *The Rumen Microbial Ecosystem*, 73–139 pp. Dordrecht, Netherlands, Springer.

Williams, C.L., McEwan, N.R. & Huws, S.A. 2020. Rumen ciliated protozoa. In: R. Mackie & C. McSweeney, eds. *Improving rumen function*, London, Burleigh Dodds Scientific Publishing.

Williams, C.L., Thomas, B.J., McEwan, N.R., Rees Stevens, P., Creevey, C.J. & Huws, S.A. 2020. Rumen protozoa play a significant role in fungal predation and plant carbohydrate breakdown. *Frontiers in Microbiology*, 11: 720. https://doi.org/10.3389/fmicb.2020.00720

Wolin, M.J., Miller, T.L. & Stewart, C.S. 1997. Microbe-microbe interactions. In: P.N. Hobson & C.S. Stewart, eds, *The Rumen Microbial Ecosystem*, 467–491 pp. Dordrecht, Netherlands, Springer.

Wright A.-D.G. & Klieve A.V. 2011. Does the complexity of the rumen microbial ecology preclude methane mitigation. *Animal Feed Science and Technology,*166–167: 248–253. https://doi.org/10.1016/j.anifeedsci.2011.04.015

Xue, M., Sun, H., Wu, X., Guan, L.L. & Liu, J. 2018. Assessment of rumen microbiota from a large dairy cattle cohort reveals the pan and core bacteriomes contributing to varied phenotypes. *Applied and Environmental Microbiology*, 84(19): e00970-18. https://doi.org/10.1128/AEM.00970

Zhan, K., Gong, X., Chen, Y., Jiang, M., Yang, T. & Zhao, G. 2019. Short-chain fatty acids regulate the immune responses via G protein-coupled receptor 41 in bovine rumen epithelial cells. *Frontiers in Immunology*, 10: 2042. https://doi.org/10.3389/fimmu.2019.02042.

Zhang, K., Li, B., Guo, M., Liu, G., Yang, Y., Wang, X., Chen, Y. & Zhang, E. 2019. Maturation of the goat rumen microbiota involves three stages of microbial colonization. *Animals*, 9(12): 1028. https://doi.org/10.3390/ani9121028

Zhang, Q., Difford, G., Sahana, G., Løvendahl, P., Lassen, J., Lund, M.S, Guldbrandtsen, B. & Janss, L. 2020. Bayesian modeling reveals host genetics associated with rumen microbiota jointly influence methane emission in dairy cows. *ISME Journal*, 14(8): 2019–2033. https://doi.org/10.1038/s41396-020-0663-x

Zhao, S., Li G., Zheng, N., Wang, J. & Yu, Z. 2018. Steam explosion enhances digestibility and fermentation of corn stover by facilitating ruminal microbial colonization. *Bioresource Technology*, 253: 244–251. https://doi.org/10.1016/j.biortech.2018.01.024.

Zinder, S.H. 1993. Physiological ecology of methanogens. In: J.G. Ferry, ed. *Methanogenesis: ecology, physiology, biochemistry and genetics*, pp 128–206. New York, USA, Chapman & Hall.

Zinder, S.H. 1993. Physiological ecology of methanogens. In: J.G. Ferry, ed. *Methanogenesis: ecology,*