

September 2012

	منظمة الأغذية والزراعة للأمم المتحدة	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
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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

MICRO-ORGANISMS AND RUMINANT DIGESTION: STATE OF KNOWLEDGE, TRENDS AND FUTURE PROSPECTS

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Abbreviations and Acronyms

ARISA	automated ribosomal intergenic spacer region
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
FAO	Food and Agriculture Organization of the United Nations
GHG	greenhouse gas
GMOs	genetically modified rumen microorganisms
IAEA	International Atomic Energy Agency
ILRI	International Livestock Research Institute
INRA	Institut National de la Recherche Agronomique
IPCC	Intergovernmental Panel on Climate Change
mRNA	messenger ribonucleic acid
PCR	polymerase chain reaction
qPCR	quantitative real-time polymerase chain reaction
RCC	Rumen cluster C
RNA	ribonucleic acid
rrn	R RNA gene
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SARA	sub-acute ruminal acidosis

Acknowledgement

The authors would like to thank Dr Evelyne Forano, INRA, Clermont-Ferrand-Theix, France; Dr Jan Kopečný, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic; Dr Yu Zhongtang, Department of Animal Sciences, the Ohio State University, Columbus, United States of America; and Mr Qu Liang and colleagues at the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (AGE) for reviewing the document and for their suggestions. The preparation of this document was coordinated, managed and supervised by Dr Harinder P.S. Makkar with the support of Dr Philippe Ankers from Livestock Production Systems Branch of Animal Production and Health Division and of other officers: Ms Linda Collette and Ms Kim-Anh Tempelman from the Secretariat of the Commission on Genetic Resources for Food and Agriculture, and Dr Irene Hoffmann and Ms Beate Scherf from the Animal Genetic Resources Branch. The support of these officers from FAO is also gratefully acknowledged.

MICRO-ORGANISMS AND RUMINANT DIGESTION: STATE OF KNOWLEDGE, TRENDS AND FUTURE PROSPECTS

I. EXECUTIVE SUMMARY

This report has been prepared at the request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture to provide policy makers, researchers and livestock nutritionists and producers with:

1. a historical account of the progress that has been made in rumen microbiology research;
2. our current understanding of the rumen microbial ecosystem; and
3. the opportunity these new DNA sequencing technologies provide for improving productivity of livestock and the impacts of the enterprises on the environment.

During the last decade, an increase in the human population, decrease in arable land due to soil degradation, urbanization, industrialization, and associated increase in the demand for livestock products has brought about dramatic changes in the global ruminant livestock sector. These changes include a shift in the size of regional livestock populations and in the types of management and feeding systems under which ruminant livestock are held. There will be increased demand of a wider range of quality attributes from animal agriculture, not just of the products themselves but also of the methods used in their production. The livestock sector will therefore need to respond to new challenges of increasing livestock productivity while protecting the environment and human health and conserving biodiversity and natural resources.

The importance of rumen microbial ecology and diversity of microorganisms in the ruminant forestomach has gained increasing attention in response to recent trends in global livestock production. The microorganisms in the digestive tracts of ruminant livestock have a profound influence on the conversion of feed into end-products which can impact on the animal and the environment. As the livestock sector grows in numbers and productivity, particularly in developing countries there will be an increasing need to understand these processes for better management and use of both the feed-base and other natural resources that underpin the development of sustainable feeding systems.

Until recently, knowledge of ruminant gut microbiology was primarily obtained using classical culture based techniques which probably only account for 10 to 20 percent of the rumen microbial population. The gut microbiota and its collective genomes (termed the microbiome) is estimated to contain 100 times more genes than the host animal and provides the ruminant with genetic and metabolic capabilities that the host has not had to evolve on its own, including capabilities to hydrolyze and ferment inaccessible nutrients and toxins. Advances in molecular microbial ecology based on 16 r RNA gene (rrn) phylogeny have enabled the identification and quantification of the normal microbiota in the rumen. This system of microbial classification coupled with deep sequencing of DNA from the rumen has revealed the presence of complex communities that have co-evolved with the ruminant host in response to the environmental conditions (feedbase, etc.) and gut physiology of the animal. While there are differences in gut microbial communities between animal species there is also new evidence that the bacterial microbiome and metabolic potentials in the rumen are different between animals of the same breed when fed the same diet and viewed in relation to nutrient utilisation.

In a recent study the microbial diversity of bacteria and archaea (methanogens) in the rumen of predominantly domesticated livestock was assessed by analysing all the curated rrn sequences deposited in the Ribosomal Database Project database in 2010 which included > 13 000 bacterial and > 3 500 archaeal rrn sequences at that time which formed the basis of the analysis. Rarefaction analysis of these sequences showed that the current coverage of the diversity at the species level was 71 percent for bacteria and 65 percent for archaea. These data indicate that about a 5 and 7 fold increase in bacterial and archaeal sequences respectively is required to achieve full coverage of the

diversity of these microorganisms. The structure of the rumen microbiome at the phylum level is in general similar but the differences in rumen microbial diversity between ruminant species is more apparent at the genus level and lower. Recent data from Asian breeds of cattle show a distinctive rumen bacterial community compared with Holstein cattle, supporting the notion of host genotype as an important factor shaping the composition.

Based on the analysis of global data sets available in public databases, the majority (> 90%) of rumen archaea (methanogens) are affiliated with genera; *Methanobrevibacter* (> 60%), *Methanomicrobium* (~ 15%), and a group of uncultured rumen archaea commonly referred to as rumen cluster C (RCC, ~16%) or Thermoplasmatales-Affiliated Lineage which are of unknown function. Metagenomic approaches enabled the Commonwealth Scientific and Industrial Research Organisation (CSIRO) to recently reconstruct the genome of RCC methanogens from a mixed culture without the need to isolate the organism. This revealed a novel metabolic activity by which the RCC methanogens produce greater amounts of methane relative to the autotrophic *Methanobrevibacter*.

Importantly the RCC methanogens were the predominant population in several ruminant species (yak, cattle and sheep) raised in a unique agro-economical region of central China. The total methanogen population in these animals was more similar to each other compared with the structure expected in ruminants on conventional production diets in industrialized regions. This provides further evidence of the interplay between the environmental conditions and physiology of the animal which governs the acquisition, colonization and ultimate structure of the microbiome in the mature animal. The composition of the methanogen population in the maturing rumen is of significance to the amount of methane (greenhouse gas, GHG) emitted since some methanogens produce more methane depending on the metabolic pathway that is encoded by their genome. Therefore environmental conditions may dictate the populations of methanogens that are acquired at birth which imprint the structure of the methanogen community in the mature animal and hence methane producing potential.

Furthermore it is also evident that there is genetic diversity within rumen bacterial species of practical and economic importance. For example, our studies are demonstrating that the rumen bacterium *Synergistes jonesii*, which detoxifies the economically important forage tree legume, is genetically diverse based on geographic region. This bacterium was introduced from overseas ruminants into Australian cattle because they lacked the organism and were susceptible to intoxication. The genetic diversity resident in this species provides further opportunity to introduce more potent detoxifying strains into ruminants in regions dependent upon leucaena production systems for sustainable production.

Major innovations in the field such as metagenomics have arisen in the last decade with the advent of affordable nucleic-acid based-technologies and rapid evolution of DNA sequencing platforms that are culture independent for studying diversity of complex microbial ecosystems. These technologies have the potential to capture and study the entire microbiome (the predominant genomes) from the complex microbial community in the rumen and to determine function (“What they are doing”) in addition to structure (“Who’s there”) of the community. Rapid advances in the development of publically available annotation tools and computing platforms for assigning function to genes has also made the genome-sequencing of individual microorganisms and interpretation both affordable and available to the broader research community through ‘in-house’ and commercial services. This has led to an increase in the number of rumen microorganisms (> 20) that have sequenced genomes which are publically available, but there is little information available on the genomic make-up of rumen anaerobic fungi and ciliate protozoa and no genomes from these organisms have been published. To address these issues, a Rumen Microbial Genomics Network³ has been formed comprising a consortium of advanced rumen microbiology laboratories, large publicly funded DNA sequencing institutions and curators of international public culture collections. The consortium will facilitate the sequencing and development of rumen microbial genomics approaches through access to methods, genome sequences and metagenome data relevant to the rumen microbial community. The reference genome information of more than 1 000 rumen microbial isolates will establish a publically accessible catalogue (database) of rumen microbial genes and assign function to these genes as a framework for characterising the rumen microbiome in different ruminant genotypes and under varying dietary and environmental conditions.

³ RMG.Network@agresearch.co.nz

This information will be used to support international efforts to initiate genome-enabled research aimed at understanding rumen function in order to find a balance between food production and GHG emissions. It is possible that this reference collection will be biased towards microorganisms from ruminants in industrialised production systems unless a co-ordinated effort is initiated to engage laboratories from countries and regions where the animals have evolved and adapted to natural environment particularly in tropical regions.

Recent research indicates that ruminants and their gut microbiota have co-evolved while adapting to their climatic and botanical environment. It is likely therefore that the gut microbiota of indigenous breeds may differ significantly from introduced ruminants, which may have resulted in the transmission of distinctive microbial populations between adapted and non-adapted breeds. If this is the case it is important to define and preserve the diversity of indigenous ruminants (domesticated and wild) which will provide a benchmark before intermingling of populations occurs through the introduction of imported genotypes. An opportunity exists for ruminant laboratories in emerging and developing countries to provide DNA from rumen samples and cultures of rumen isolates from local adapted breeds as contributions to this catalogue which will then represent a broader geographical census of microorganisms that are relevant to many agro-economic zones and environment conditions. This material should be collected from animals under the natural grazing systems in which they have evolved so that the data generated would provide a “fingerprint” of the adaptive state of these ecosystems. Samples from these animals in controlled nutritional studies where animals are being fed formulated diets will demonstrate how the indigenous rumen respond to the production systems being advocated from other agro-ecological regions. In addition, nutrition laboratories in Asia, South America and Africa (e.g. International Livestock Research Institute, ILRI) with an interest in rumen microbiology would benefit from future interactions with the advanced labs via intergovernmental exchange programmes and aid projects where local scientists are advised and trained in the latest techniques in molecular microbial ecology. Involvement of international organizations such as FAO, ILRI and the World Bank would be highly desirable to:

1. identify target countries and agro-economic regions for inclusion in such programmes and projects;
2. mobilize resources;
3. co-ordinate capacity-building activities; and
4. provide guidance on matters related to the conservation and sustainable use of micro-organisms and to the manipulation of rumen microbiota for enhancing digestion of feed in the rumen.

II. INTRODUCTION

The importance of rumen microbial ecology and diversity of microorganisms in the ruminant forestomach was highlighted by McSweeney and Makkar (2005) in response to recent trends in global livestock production. They recognized that during the last decade, an increase in the human population, decrease in arable land due to soil degradation, urbanization, industrialization, and associated increase in the demand for livestock products has brought about dramatic changes in the global ruminant livestock sector. These changes include a shift in the size of regional livestock populations and in the types of management and feeding systems under which ruminant livestock are held. There will be increased demand of a wider range of quality attributes from animal agriculture, not just of the products themselves but also of the methods used in their production. The livestock sector will therefore need to respond to new challenges of increasing livestock productivity while protecting the environment and human health and conserving biodiversity and natural resources.

The microorganisms in the digestive tracts of ruminant livestock have a profound influence on the conversion of feed into end-products which can impact on the animal and the environment. As the livestock sector grows in numbers and productivity particularly in developing countries there will be an increasing need to understand these processes for better management and use of both feed and other natural resources that underpin the development of sustainable feeding systems. Microbial ecology has

developed as a specialized research field in microbiology and focuses on studying the role of microbes on a variety of ecosystems, including the gastro-intestinal tract. It is worthwhile reiterating that the study of microbial ecology in gastro-intestinal tract ecosystems involves investigation of the organisms present (abundance and diversity), their activity (usually determined *in vitro*, but *in vivo* activity or expression of activity is required), and their relationship with each other and the host animal (synergistic and competitive interactions) (Hungate, 1960).

Until recently, knowledge of ruminant gut microbiology was primarily obtained using classical culture based techniques, such as isolation, enumeration and nutritional characterization, which probably only account for 10 to 20 percent of the rumen microbial population. Now, new nucleic acid-based technologies that are culture independent can be employed to examine microbial ecology and diversity to determine “Who’s there” and “What they are doing”. These technologies have the potential to revolutionize the understanding of rumen function and will overcome the limitations of classical based techniques, including isolation and taxonomic identification of strains important to efficient rumen function and better understanding of the roles of microorganisms in relation to achieving high productivity and decreasing environmental pollutants and contamination of the food chain.

Scope of the study

This report has been prepared at the request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture and the scope of the study is to provide policy makers, researchers and livestock nutritionists and producers with a historical account of the progress that has been made in rumen microbiology research and our current understanding of the rumen microbial ecosystem and its role in improving productivity of livestock and the environmental and health impacts of these enterprises. Major innovations in the field have arisen in the last decade with the advent of nucleic-acid based-technologies for studying complex microbial ecosystems. These molecular methods as well as new generation sequencing technologies developed in mapping the human genome are now being deployed to study microbial ecosystems, which provide the potential to capture and study the entire microbiome (the predominant genomes) from the complex microbial community in the rumen. An explosion of knowledge in the field of microbial ecology is underway. However, the opportunity is to ensure these technologies are used to improve ruminant production through a better understanding of microbial function and ecology.

III. RUMEN MICROBIOLOGY IN HISTORICAL PERSPECTIVE

The rumen microbial ecosystem is an anaerobic environment, which defines the microorganisms that have adapted to this lifestyle yet. It was Pasteur (1860) who described those microorganisms could survive and prosper in the absence of oxygen using the process of fermentation. A more complete history of the development of gut microbial ecology as a discipline was provided by Bryant (1996) who is regarded as one of the main leaders in the development of this field during the 20th century. Progress in the field of gut microbiology was slow and until about 1940 – only spore formers and non-spore formers of clinical importance had been isolated and described, probably due to the popularity of Petri dish and the ease of cultivating aerobic bacteria. Attempts to inoculate and incubate plates under anaerobic conditions were unsuccessful until the anaerobic glove box was developed (Aranki *et al.*, 1969). However, it is Robert E. Hungate who perfected the art of anaerobic culture and is recognized as the father of modern anaerobic microbial ecology. His understanding of the principle of redox potential and achieving low potentials in anaerobic media led to the development of procedures for media preparation enabling enumeration and isolation of anaerobic bacteria. The roll tube technique, with its numerous modifications and improvements since its original description (Hungate, 1950), was considerably superior to other anaerobic methods and contributed much to our understanding of anaerobes. Despite the advent of the anaerobic cabinet, with its many advantages, modifications of the roll tube technique are still widely used and are standard procedures for anaerobe laboratories. Importantly, these techniques have provided the tools that have enabled microbial ecologists,

particularly those working in gut ecology, to advance this field of research. Hungate (1960) also emphasized enumeration of species in their natural habitat, fermentation rates, and turnover times of various intermediates in the ecosystem.

The technological impetus for major advances in our knowledge of rumen microbial ecology during the latter half of the 20th century has been derived from three main sources:

1. the development of anaerobic culture techniques and their application to the study of the rumen microbial ecosystem by Hungate, Bryant and others;
2. the use of rodent experimental models to define relationships between intestinal bacteria and the host by Dubos, Savage and others; and
3. the development of gnotobiotic technology by which germ-free or defined-microbiota animal models could be derived and maintained (Hobson and Steward, 1997; Chaucheyras-Durand *et al.*, 2010).

However, it is already clear that the advent of use of molecular ecology and genomics technologies in the 21st century has and will continue to generate major advances in our knowledge and provide, for the first time, not simply a refinement or increased understanding, but an in depth description of the ruminant gastrointestinal ecosystem. A major step forward was the phylogenetic studies of bacteria based on nucleic acid sequence-analysis of the 16S rRNA gene which were initiated by Carl Woese at the University of Illinois in the 1970's. This approach led to the discovery of the third microbial kingdom, the Archaeobacteria, which includes the methanogens. Subsequently the taxonomy of all living organisms has been redefined into three main domains – Archaea, Bacteria and Eucarya (Woese *et al.*, 1990). All three domains are represented in the rumen i.e. Bacteria (eubacteria), Archaea (methanogens) and Eucarya (ciliate protozoa and anaerobic rumen fungi).

IV. CURRENT UNDERSTANDING OF THE ECOLOGY AND FUNCTIONS OF RUMEN MICRO-ORGANISMS

Status of knowledge on the roles of rumen micro organisms

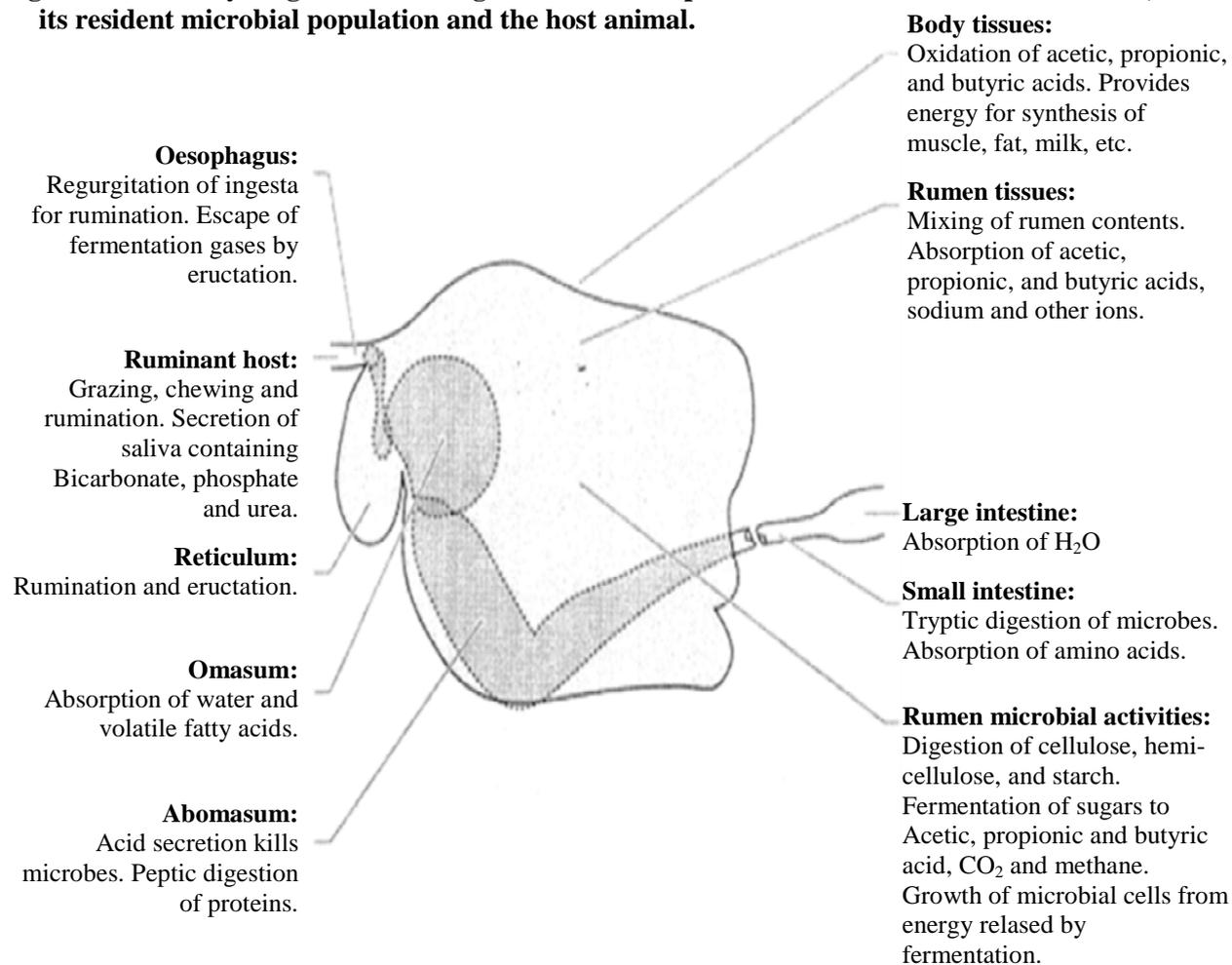
Feed digestion and physiology

Many animals across a wide range of orders have a portion of their digestive tract adapted to accommodate a microbial population which aids in digestion and provides a variety of nutritional and health benefits. Microbial populations have been described in the gut of herbivores, omnivores and carnivores and in all zoological classes. This complex, mixed, microbial culture (comprising bacteria, ciliate and flagellate protozoa, anaerobic phycomycete fungi and bacteriophage) forms a closely integrated ecological unit with each other and the host animal, as well as playing a vital role in the nutritional, physiological, immunological and protective functions of the host. Development of microbial populations in the digestive tract of higher animals commences soon after birth and involves a complex process of microbial succession and many microbial – host interactions which, eventually resulting in dense, stable microbial populations inhabiting characteristic regions of the gut. The rumen is one of the most extensively studied and well-documented gut ecosystem because of the importance of ruminants (cattle, sheep, goats, camels and yak) to human nutrition and the major role played by rumen microbes in nutrition of the ruminant animal.

The ruminant foregut or stomach has evolved into three pregastric fermentation chambers (rumen, reticulum and omasum) of which the rumen is by far the largest. Ingested plant material is hydrolysed and fermented in the rumen, and microbial cells and undigested plant particles pass into the abomasum where gastric digestion begins (Figure 1). The most distinctive feature of ruminants, rumination, where foregut digesta is regurgitated, rechewed and reswallowed in a frequent regular pattern repeated up to 500 times per day and enables reduction in particle size (comminution) and exposure of maximal surface area to microbial attack. The mutualistic microbial fermentation is based on digestion of the

plant cell wall by cellulases and hemicellulases, synthesis of microbial proteins from poor quality dietary (forage) protein and nonprotein nitrogen mainly via ammonia as precursor, synthesis of vitamins B and K, as well as detoxification of phytotoxins and mycotoxins. In turn, the host animal provides a mechanism for the selection and harvesting of feed, maintaining a high level of nutrient supply (10–18 percent dry matter), temperature regulation (38–41 °C), pH control (6–7) by buffer in saliva, osmotic control (250–350 milliosmole) and removal of soluble inhibitory end-products of digestion as well as undigested particulate matter (residence time 1–2 days) and microbial cells, and provision of some nutrients (urea, phosphate and bicarbonate through saliva and the rumen wall) (Table 1).

Figure 1. Summary diagram describing interrelationships between the ruminant forestomach, its resident microbial population and the host animal.



Source: after Hungate R.E., 1985.

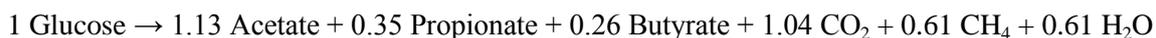
Table 1. Summary of physical, chemical, and microbiological characteristics of the rumen ecosystem

Physical criteria	Range characteristics
pH	5.5–6.9 (mean 6.4)
Redox potential	-350 to – 400 mV
Temperature	38-41 °C
Osmolality	250-350 milliosmole/kg ⁻¹
Dry matter	10-18%
Chemical criteria	Range characteristics
Gas phase (%)	CO ₂ , 65; CH ₄ 27; N ₂ 7; O ₂ 0.6; H ₂ 0.2
Volatile fatty acids (mmol L ⁻¹)	Acetate 60-90 Propionate 15-30 Butyrate 10-25 Branched-chain and higher 2-5
Nonvolatile acids (mmol L ⁻¹)	Lactate < 10
Amino acids and oligopeptides	< 1 mmol L ⁻¹ present 2-3 h post feeding
Ammonia	2-12 mmol L ⁻¹
Soluble carbohydrates	< 1 mmol L ⁻¹ present 2-3 h post feeding
Insoluble polysaccharides	
Dietary (cellulose, hemicelluloses, pectin)	Always present
Endogenous (mucopolysaccharides)	Always present
Lignin	Always present
Minerals	High Na; generally good supply
Trace elements/vitamins	Always present; good supply of B vitamins
Growth factors	Good supply; branched-chain fatty acids, long-chain fatty acids, purines, pyrimidines, other unknown
Microbiological* criteria	Range characteristics
Bacteria	10 ¹⁰ -10 ¹¹ g ⁻¹ (> 200 species)
Ciliate protozoa	10 ⁴ -10 ⁶ g ⁻¹ (25 general)
Anaerobic fungi	10 ³ -10 ⁵ g ⁻¹ (5 general)
Bacteriophage	10 ⁷ -10 ⁹ g particles ml ⁻¹

*Diversity is based on culturable microbes. *Source:* adapted from Mackie, R.I. *et al.* (1999). *Note:* mmol L = millimole per liter; mV = millivolts.

Fermentative microbes, mainly bacteria, hydrolyse plant polymers (starch, cellulose, hemicellulose, pectins and protein) to short oligomers and monomers. These soluble substrates are transported into the microorganism by specific transport mechanisms and fermented, resulting in synthesis of microbial cells and production of fermentation end-products (acetate, propionate, butyrate, carbon dioxide and hydrogen). Hydrogen and formate are produced by many microbes in the rumen where the hydrogen is quantitatively converted to methane by methanogenic archaea, resulting in undetectable or low levels of free hydrogen in the gas phase. Although acetogenic and syntrophic bacteria that also consume hydrogen have been isolated, they are of minor quantitative importance in the rumen.

The predominant microorganisms in the rumen are obligate anaerobes. Fermentation of feedstuffs in the rumen yields short-chain volatile fatty acids, primarily acetic, propionic and butyric acids, carbon dioxide, methane, ammonia and occasionally lactic acid. Some of the change in free energy is used to drive microbial growth, but heat is also evolved. The overall fermentation equation for an animal consuming a high roughage diet is:



The molar proportions in which the principal volatile fatty acids are formed in the rumen are 65 acetic, 20 propionic, 12 butyric and 3 higher and branched-chain volatile fatty acids. The volatile fatty acids provide 60–80 percent of the daily metabolizable energy intake in ruminant animals and provide the energetic foundation for the mutually beneficial association between the rumen microbes and the host animal. It follows therefore that ruminants are characterized by low blood glucose levels and rely heavily on gluconeogenesis for provision of glucogenic precursors. The quality and quantity of rumen fermentation products is dependent on the digestive interactions between amount and quality of feed ingested and the types and activities of the rumen microbes, which in turn, has an enormous impact on nutrient output and performance of ruminant animals.

The microbial environment in the rumen has been examined in some detail and allowing for variation in the nature and amount of feed ingested, serves as a good model for other gastrointestinal ecosystems in both herbivores and nonherbivores. A summary of some of the approximate physical, chemical, and microbiological characteristics of grazing cattle and sheep is presented in Table 1. Although the physical and chemical parameters of the rumen environment illustrate the complexities that must be considered in media selection and design, it is significant that some rumen bacteria remain to be isolated and characterized. The current state of knowledge on the microbial digestion of macro-nutrients in the rumen is provided below.

Fibre digestion: an excellent overview of fibre digestion in the rumen and the future opportunities was reviewed by Krause *et al.*, (2003) and remains a primary reference source in terms of microbiology, ecology and genomics. The degradation of plant cell walls by ruminants is of major economic importance in the developed as well as developing world. Rumen fermentation is unique in that efficient plant cell wall degradation relies on the cooperation between microorganisms that produce fibrolytic enzymes and the host animal that provides an anaerobic fermentation chamber. Increasing the efficiency with which the rumen microbiota degrades fibre has been the subject of extensive research for at least the last 100 years. Fibre digestion in the rumen is not optimal, and is demonstrated is supported by the fact that fibre recovered from faeces is fermentable. This view is confirmed by the knowledge that mechanical and chemical pretreatments improve fibre degradation, as well as more recent research, which has demonstrated increased fibre digestion when plant lignin composition is modified by genetic manipulation.

Plant cell wall hydrolysis is carried out by specialist bacteria (mainly the genera *Ruminococcus* and *Fibrobacter*), ciliate protozoa and anaerobic fungi. Bacteria are the most important group although indirect estimates suggest that protozoa may be responsible for 30–40 percent of overall fibre digestion under certain conditions, while the role of fungi is unclear. Cellulase enzyme systems are complex and comprise a number of endo- and exocellulases, cellodextrinases and β -glucosidase activities. The first step in the degradation of an insoluble substrate, such as the plant cell wall or cellulose, is attachment, and factors that regulate this are under investigation. Also, molecular mechanisms involved in adherence of fibre-degrading bacteria and their enzymes to insoluble substrates are being determined (see Morgavi *et al.*, 2012). Xylan is a more heterogeneous polymer than cellulose and is broken down by a variety of enzymes having endo- and exoxylanase, β -glucosidase and a range of debranching activities. This hemicellulose-degrading ability is more widely distributed among rumen microbes than cellulolytic activity.

Starch and pectin degradation: starch is rapidly and extensively degraded in the rumen. Starch granules are rapidly engulfed by the Entodiniomorphid protozoa and converted to an iodophilic storage polymer, as are soluble sugars by the Holotrich protozoa. Degradation of dietary starch by bacteria, protozoa and fungi occurs by combined activity of debranching, -linked endo- and exo-amylase and glucosidase enzymes. Maltodextrins and glucose are the products of enzymatic starch hydrolysis. Pectin (polygalacturonic acid) is hydrolysed by pectin esterase and polygalacturonase enzymes of bacteria and protozoa. Anaerobic fungi are weakly pectinolytic.

Lipid degradation: dietary lipids (triglycerides, galactolipids and phospholipids) are rapidly hydrolysed in the rumen to glycerol, free fatty acids and galactose. *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica* are actively lipolytic while the long-chain free fatty acids are isomerized and hydrogenated by a range of bacteria (*Butyrivibrio fibrisolvens*, *Treponema bryantii*, *Eubacterium sp.* and *Ruminococcus albus*). Protozoa are also active in lipid hydrolysis and may be responsible for 30–40 percent of the total ruminal activity although this may be confounded by activities of attached and engulfed bacteria. Hydrogenation (saturation) of unsaturated free fatty acids serves as an electron sink but importantly results in detoxification of inhibitory (uncoupling) effects of unsaturated free fatty acids. On roughage diets hydrogenation capacity results in a high proportion of saturated free fatty acids in body (depot) fat but this capacity may be exceeded with high intakes of unsaturated fatty acids resulting in an increase in quantity of unsaturated fatty acids deposited. Further anaerobic degradation of long-chain fatty acids to acetate requires longer residence times (slower turnover) than occurs in the rumen, resulting in their outflow and absorption in the small intestine. The health

implications of rumen lipid metabolism on food products from ruminant animals and microbial metabolism involved is discussed below.

Protein degradation: metabolism of dietary protein to ammonia by ruminal proteolytic bacteria is an efficient process, which provides cellulolytic bacteria with a nitrogenous source for protein synthesis. Many rumen bacteria utilize ammonia, urea or other nonprotein nitrogenous compounds as sole nitrogen source and 60–80 percent of bacterial protein is synthesized from ammonia as precursor. Oligopeptides (di- and tripeptides), rather than amino acids, account for the remaining 20 to 30 percent of bacterial protein synthesized. However excessive conversion of dietary protein to ammonia can deprive the host animal of the nutritional value of the consumed amino acids (Leng and Nolan, 1984). Thus, there is considerable interest in regulating microbial metabolism of proteins so that rumen supply of nutrients to the host animal is optimised.

Proteolytic activity is widely distributed among the predominant ruminal bacteria including *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *Streptococcus bovis* and *Ruminobacter amylophilus* (Hazlewood *et al.*, 1983). Rumen bacterial proteases are primarily of the cysteine (65–80%) and serine (30–40%) types based on inhibitor studies. Protein breakdown results in production of peptides and amino acids. Peptides may be further metabolised by peptidolytic bacteria such as *Prevotella ruminicola* and several other species. Peptides are usually broken down to amino acids before assimilation by microorganisms. The rate of peptide metabolism differs according to the amino acids at the N-terminus of the peptide sequence (Wallace and McKain, 1991; Walker *et al.*, 2003; Wang *et al.*, 2004). However, some bacteria possess carboxypeptidases which cleave amino acid at the C-terminus of a peptide (Prins *et al.*, 1983). The predominant microbial aminopeptidase activity in rumen cleaves dipeptides rather than single amino acids from peptide chains. These types of enzymes are classified as dipeptidyl peptidases as the first step of peptide hydrolysis (Wallace *et al.*, 1993, 1997; Wang and Hsu, 2005). However, the whole peptide utilisation profile might change in different rumen fluid when the bacterial community consists of a large population of *Streptococcus bovis* (Russell and Robinson, 1984).

Although the form of amino acids presented in the rumen will be peptides rather than free amino acids, there is a small population of deaminating bacteria in the rumen fluid. Amino acids produced in excess of the amount incorporated into microbial protein are rapidly deaminated to produce ammonia, carbon dioxide and corresponding fatty acids (Krause and Russell, 1996). Ammonia and branched-chain volatile fatty acids are essential nutrients, especially for cellulolytic bacteria. Most of the studies on the uptake of amino acids and peptides have indicated the preference for peptides rather than free amino acids (Wallace and McKain, 1991; Bach *et al.*, 2005). The isolation of aminolytic bacteria from the genera *Megasphaera*, *Eubacterium* and *Streptococcus* showed neutral and acidic amino acids being assimilated more readily by the isolated strains (Scheifinger *et al.*, 1976). McSweeney *et al.* (1993) studied a ruminal bacteria, *Synergistes jonesii*, which is capable of degrading the pyridinediol toxin and solely utilises amino acids as its carbon source.

Protozoa engulf bacteria, fungi and other smaller protozoa. This activity plays a significant role in intraruminal nitrogen recycling and the efficiency of protein synthesis in the rumen. Protozoa play a major role in the ingestion of particulate protein, including plant (supplementary) protein and a lesser role in uptake of soluble protein, peptides and amino acids. Protozoa have mixed protease activity similar to the bacteria and rapidly deaminate amino acids. Isolation and characterization of ammonia hyperproducing bacteria and investigation of their role in rumen fermentation of peptides and amino acids are current research topics. Fungi also have proteolytic activity, mostly trypsin-like metalloprotease. Recent evidence suggests a role for plant proteases in initial proteolysis of plant proteins (Kingston-Smith *et al.*, 2005).

Detoxification of phytotoxins

In many countries, grazing herbivores are exposed to toxic forages. Animals that are foregut fermenters can often detoxify or reduce the toxicity of these plants by microbial metabolism although microbial biotransformation of certain compounds in the gut can also enhance the toxicity. An important reason for the evolution of foregut fermentation is detoxification of phytotoxins and mycotoxins. Phytotoxins occur in many common feeds, including grains, protein supplements and

forages. They range from tannins, alkaloids, goitrogens, gossypol, saponins, glucosinolates, mimosine and cyanogens to nitrate and oxalate (Table 2). However, in most cases the microorganisms involved have not been isolated and identified. In many instances, the rumen microbiota provide a protective function and effectively modify or degrade a wide variety of toxic compounds. In some cases the opposite can occur, with production of toxic metabolites from innocuous compounds (McSweeney *et al.*, 2003). However, prior exposure of rumen bacteria to many of the plant toxicants increases the rate of subsequent detoxification and thus adaptation is an important factor to consider. Utilisation of the toxin as a source of energy is usually the most important factor driving adaptation in the rumen. However, the toxin degrading population can also be selected for indirectly and enriched by manipulating the diet to provide other energy yielding substrates, preferred sources of nitrogen, growth factors and substrates that can act as electron donors or acceptors in the metabolism of the toxin. This ability can be modified and deliberately managed as a system to detoxify feedstuffs both naturally by adaptation or inoculation, and through modern genetic engineering technology.

The ability of the ruminal ecosystem to adapt and increase its capacity to detoxify a plant toxin in response to the amount of toxin consumed is a major factor determining the pathogenesis of plant toxicity in these forestomach fermenters (Krause *et al.*, 2005). In many cases, the degradative pathway for a toxin involves a consortium of microorganisms since the enzymes involved may not be present in one organism. Even when a single species of ruminal bacteria is capable of degrading a toxin, there are probably several distinct strains of the species present in the rumen which all contribute to the detoxification (see Allison *et al.*, 1992). The initial rate of metabolism of a particular toxin in the rumen is usually a function of the level of expression of enzymes that degrade or transform the toxin and the number of organisms producing these enzymes. The size of the population of toxin degrading microorganisms in the naive rumen is determined by its ability to derive energy for growth from the normal feed constituents and other less obvious traits, which enable it to compete with other organisms (McSweeney *et al.*, 2005 a, b). The population is likely to increase in size when a toxic substrate is available and can be exploited as an additional source of energy, which the remainder of the rumen microbial ecosystem cannot use. The adaptive response of rumen microorganisms to the presence of a plant toxin may also involve the induction of an enzyme(s) involved in the detoxification process.

Rumen microbial ecology of animals fed phenolic-rich forage: the most complete description of a ruminal microbial response to a plant secondary compound involves animals consuming tannin-rich diets. The predominant class of tannins in forages is the condensed tannins, which do not appear to be degraded by anaerobic microorganisms (Makkar *et al.*, 1995a). Recent research has therefore focused on the inhibitory effects of condensed tannins on microbial populations, their mode of action, and the adaptive responses of the ruminal community to these compounds. Tannins are generally regarded as inhibitory to the growth of microorganisms by reducing the availability and digestibility of macronutrients and minerals (Chung *et al.*, 1998; McSweeney *et al.*, 1999; Molan *et al.*, 2001), impeding cell wall function (O'Donovan and Brooker, 2001), and interfering with the catalytic activity of extracellular enzymes (Bae *et al.*, 1993; Bell *et al.*, 1965) and cell bound enzymes (Makkar *et al.*, 1988). However, plants that are rich in tannins often contain simpler phenolics that are overlooked in their potential antimicrobial activity. Indeed some simple phenolics appear to be more toxic to bacteria than higher molecular weight hydrolysable tannins and condensed tannins, although other studies have demonstrated that oligomeric proanthocyanidins are better inhibitors of bacteria than the simpler phenolics, catechin and epicatechin (Sivaswamy and Mahadevan, 1986; Waage *et al.*, 1984).

Molecular ecology studies have confirmed that Gram-negative bacterial groups (Enterobacteriaceae and *Bacteroides* species) predominate in the presence of dietary tannins and that there is a corresponding decrease in the Gram-positive *Clostridium leptum* group and other Gram-positive bacteria (Smith and Mackie, 2004). Metabolic fingerprint patterns revealed that functional activities of culturable fecal bacteria were affected by the presence of tannins. Calliandra and Lotus condensed tannins reduce the population of cellulolytic and proteolytic bacteria in the rumen although the proteolytic bacteria did not appear to be as greatly affected (McSweeney *et al.*, 1999, 2001; Min *et al.*, 2002).

Table 2. Rumen microbial detoxification/modification/tolerance reactions to plant associated compounds

Plant associated compound/toxic ruminal metabolite of plant compound	Modification/activity	Microorganisms involved in degradation of toxin (geographical location)	References
Non-protein amino acids			
Mimosine/3,4dihydroxypyridine Lathrogens - diaminobutyric acid - oxalyldiaminopropionic acid	Benzenoid ring cleavage of 3,4 DHP Modification not determined	<i>Synergistes jonesii</i> (Hawaii, Indonesia) Unidentified isolates (North America, Africa, Australia)	Allison <i>et al.</i> , 1992 Rasmussen <i>et al.</i> , 1993; McSweeney <i>et al.</i> , 2005 a, b
Aliphatic nitro compounds			
3-nitro-1-propionic acid 3-nitro-1-propanol	Reduction of the nitro group and deamination to β -alanine Reduction to 3-amino-1-propanol	<i>Megasphaera elsdenii</i> , <i>Coprococcus sp.</i> , <i>Selenomonas sp.</i> (North America)	Anderson <i>et al.</i> , 1993; Majak and Cheng, 1981
Nitrate/Nitrite	Reduction of nitrate to nitrite Reduction of nitrite to ammonia	<i>Selenomonas sp.</i> No isolates identified (North America)	Lewis, 1951 a, b; Allison and Reddy, 1984
Pyrrolizidine alkaloids			
Heliotrine	Ester hydrolysis of carbon side chain, Reduction of 1,2 double bond of the heterocyclic ring	<i>Peptococcus heliotrinireducens</i> (Australia)	Russell and Smith, 1968; Lanigan, 1976
Cyanide glycoside	Glycoside hydrolysis Cyanide metabolised to thiocyanate	Unknown organisms (North America)	Majak and Cheng, 1984, 1987; Onwuka <i>et al.</i> , 1992
Tansy ragwort (<i>Senecio jacobaea</i>) Pyrrolizidine alkaloids		Unknown organisms (North America)	Lodge-Ivey <i>et al.</i> , 2005; Rattray and Craig, 2007
Phenolics			
Hydrolysable tannin	Ester hydrolysis	<i>Selenomonas ruminantium</i> <i>Streptococcus gallolyticus</i> <i>Streptococcus spp.</i> (North America)	Osawa and Walsh, 1993; Skene and Brooker, 1995; Nelson <i>et al.</i> , 1995, 1998
Trihydroxybenzenoids (e.g. gallate)	Dehydroxylation Ring cleavage	<i>Eubacterium oxidoreducens</i> <i>Streptococcus bovis</i> <i>Syntrophococcus sucromutans</i> <i>Coprococcus sp.</i> (North America)	Tsai <i>et al.</i> , 1976; Krumholz and Bryant, 1986 a, b
Flavonoid glycosides	Glycoside hydrolysis Heterocyclic ring cleavage	<i>Selenomonas sp.</i> <i>Butyrivibrio sp.</i> <i>Peptococcus sp.</i> <i>Eubacterium oxidoreducens</i> <i>Butyrivibrio sp.</i> (North America)	Simpson <i>et al.</i> , 1969; Cheng <i>et al.</i> , 1969; Krumholz and Bryant, 1986 a, b
Condensed and hydrolysable tannins	Tannin tolerance (exopolysaccharide)	<i>Streptococcus gallolyticus</i> , <i>Streptococcus</i>	Nelson <i>et al.</i> , 1998;

Plant associated compound/toxic ruminal metabolite of plant compound	Modification/activity	Microorganisms involved in degradation of toxin (geographical location)	References
	production)	<i>bovis</i> , <i>Clostridium sp.</i> <i>Prevotella ruminicola</i> <i>Selenomonas ruminantium</i> (North America, Africa, Australia, New Zealand)	Brooker <i>et al.</i> , 1994; Jones <i>et al.</i> , 1994; Skene and Brooker, 1995; Molan <i>et al.</i> , 2001; Odenyo <i>et al.</i> , 2001
Saponins			
Sapogenins	Glycoside hydrolysis Hydrogenation Unknown organisms (Africa, New Zealand)	Teferedegne <i>et al.</i> , 1999; Makkar and Becker, 1997; Newbold <i>et al.</i> , 1997	Wang <i>et al.</i> , 1998; Meagher <i>et al.</i> , 2001; Mathison <i>et al.</i> , 1999
Trans-aconitic acid	Oxidation	<i>Acidaminococcus fermentans</i> (North America)	Cook <i>et al.</i> , 1994
Cardiac glycosides (cardenolides)	Unknown	Unknown organisms	Westermarck, 1959
Phytoestrogens			
Isoflavones -formononetin, daidzein, genistein, biochanin, coumestrol	Demethylation Heterocyclic ring cleavage	No isolates identified	Adler and Weitzkin-Neiman, 1970; Nilsson, 1961, 1962; Nilsson <i>et al.</i> , 1967; Batterham <i>et al.</i> , 1965; Braden <i>et al.</i> , 1967; Schoefer <i>et al.</i> , 2002
Oxalate	Metabolised to formate	<i>Oxalobacter formigenes</i> (North America)	Dawson <i>et al.</i> , 1980; Allison <i>et al.</i> , 1985; Baetz and Allison, 1992
Mycotoxins			
Trichothecenes T-2 toxin, HT-2 toxin, Deoxynivalenol, diacetoxyscirpenol, ochratoxin	Deepoxidation Deesterification Isovaleryl deesterification	<i>Butyrivibrio fibrisolvens</i> <i>Selenomonas ruminantium</i>	Mackie and White, 1990; Westlake <i>et al.</i> , 1987, 1989; Mobashar <i>et al.</i> , 2010
Thiol compounds Glucosinolates Thiocyanate, Oxazolidine-2-thiones	Glycoside hydrolysis to aglycone Aglycone modification not determined	Unknown	Duncan and Milne, 1992; Geertman <i>et al.</i> , 1994; Lanzani <i>et al.</i> 1974; Majak <i>et al.</i> , 1991; Nugon-Baudon <i>et al.</i> , 1990
Halogenated compounds			
Fluoroacetate	Defluorination by dehalorespiration	<i>Synergistetes sp.</i> (Australia)	Camboim <i>et al.</i> , 2012; Davis <i>et al.</i> , 2011, 2012

Source: adapted from McSweeney *et al* 2003. Note: HT = hydrolysable tannins.

It appears that both condensed tannins and hydrolysable tannins induce the secretion of extracellular polysaccharide that separates the microbial cell wall from reactive tannin, and formation of a thick glycocalyx or glycoprotein which has high binding affinity for tannin in tannin-resistant bacteria (Krause *et al.*, 2003). Bacteria with the tannin-tolerant phenotype appear to be represented by several genera although closely related bacterial strains of the same species can differ significantly in their tolerance (Molan *et al.*, 2001). Although the population of tannin-tolerant microorganisms may increase in ruminants fed tannin-rich diets, these changes do not appear to compensate for tannin-induced reduction in digestion of nutrients. It is not surprising therefore that the strategy of inoculating tannin-tolerant bacteria into ruminants fed tannin-rich diets has not improved the productivity of these ruminants.

Although tannins may reduce the abundance of predominant microbial populations in the gut, several studies have shown that the effect on ruminal metabolism was insufficient to alter the efficiency of microbial protein synthesis (see McSweeney *et al.*, 2001). While it is well established that forage tannins reduce the digestibility of the macro- and micro- nutrients (Barry *et al.*, 1986; Waghorn *et al.*, 1987; McSweeney *et al.*, 1988; Rubanza *et al.*, 2005) additional studies are required to determine the impact of tannins on efficiency of microbial protein synthesis (amount of microbial protein synthesized per unit of organic matter digested) in the rumen under varying feeding conditions. Although some evidence exist from in vitro studies that tannins enhance efficiency of microbial protein synthesis (Makkar *et al.*, 1995b; Getachew *et al.*, 2001).

There have been several claims that herbivore gut microorganisms adapted to tannin-rich diets may have the ability to degrade protein that is complexed with tannin and thus reduce the primary anti-nutritional effect of these diets (Foley *et al.*, 1999). Current research indicates that access to the bound protein is mainly dependent upon the type and activity of tannins involved in the complex and tannin type and activity determine the extent of binding of proteins and tannins. Anaerobic bacteria have been isolated from many herbivorous animals, which can dissociate hydrolysable tannins-protein complexes but not protein complexed with condensed tannins (Osawa, 1990; Osawa, 1992; Osawa *et al.*, 1993; Nemoto *et al.*, 1995; McSweeney *et al.*, 1999). It has been suggested that degradation of hydrolysable tannins-protein complexes is because depolymerising enzymes (tannin acylhydrolases; esterases) cleave the ester linkages in the hydrolysable tannins rather than directly affecting the hydrogen bonds between the phenolic sub-units of the polymer and the carbonyl groups of peptides of the protein (Skene and Brooker, 1995). The mechanism by which tannin acylhydrolases degrade hydrolysable tannins without being bound and inactivated by the tannin remains unclear. Microorganisms which can degrade condensed tannins-protein complexes under anaerobic conditions may not exist although rumen inoculum from a variety of wild and domesticated ruminants in Africa has shown differences in protein fermenting ability of tannin-rich browse plants (Odenyo *et al.*, 1999). This may indicate that there are ruminal populations which are more efficient at digesting protein in tannin containing forages that are not complexed and thus requires further investigation.

Ruminal disorders

Ruminal acidosis: ruminal acidosis is the most common digestive disturbance in ruminant livestock when their diet is suddenly changed from forage to concentrate or when excessive amounts of highly fermented diet are eaten. In the acute form, lactic acid accumulates in the rumen, due to an imbalance in microbial populations and an associated decrease in pH, causing metabolic acidosis (Dawson *et al.*, 1997). *Streptococcus bovis* has been identified as the main bacterial species involved in the production of lactic acid from rapid growth on the highly fermentable forms of carbohydrate (Mackie and Gilchrist, 1979; Russell and Hino, 1985; Dawson *et al.*, 1997). If adaptation to the grain diet is gradual, populations of lactic acid-consuming bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminantium* convert the lactic acid to propionic acid and prevent a rapid decline in pH (Nocek, 1997). The broader microbiological changes associated with acute lactic acidosis are based primarily on culture based investigations (Goat *et al.*, 1998; Nagaraja and Titgemeyer, 2007) and probably do not provide a complete understanding of the organisms that are responsible. Acute acidosis is mainly a problem in developed countries in ruminants fed large amounts of concentrates to achieve high levels of productivity.

In sub-acute ruminal acidosis (SARA), lactic acid does not accumulate during low-pH conditions and other factors such as microbial population change, increased gut permeability, bacterial lipopolysaccharides, and inflammatory and immune responses may have a role in the etiology of SARA (Plaizier *et al.*, 2008). In contrast to lactic acidosis, which is characterized by low pH and increased lactate, SARA seems to be an intermediate state where microbial fermentations are instable and unpredictably oriented to butyrate, propionate, or both at the expense of acetate (Lettat *et al.*, 2010). A recent study explored the microbial changes in the rumen of cows with experimentally induced SARA using either grain or alfalfa pellets (Khafipour *et al.*, 2009). The analysis indicated that the main change in the rumen microbial populations was a decrease in Bacteroidetes bacteria (primarily *Prevotella albensis*, *Prevotella brevis*, and *Prevotella ruminicola*), but the proportion of Bacteroidetes was greater in alfalfa pellet-induced SARA than in grain-related SARA. Also, *Streptococcus bovis* and *Escherichia coli* were markedly increased in severe grain-induced SARA, whereas mild grain-induced SARA was associated with an increase in *Megasphaera elsdenii*, and alfalfa pellet-induced SARA was dominated by *Prevotella albensis*. The authors of this work have concluded that *Escherichia coli* (*E. coli*) may be a significant contributing population in the onset of SARA. A recent study has shown that probiotic bacteria (Propionibacteria and Lactobacilli) were ineffective in ameliorating lactic acidosis but some of the probiotics may be effective in reducing occurrence of butyric and propionic SARA in sheep (Lettat *et al.*, 2012).

Environmental pollutants and their effects on climate change

Greenhouse gases: in 2007 the Intergovernmental Panel on Climate Change (IPCC) reported that the global average surface temperature had increased by around $0.74 \pm 0.18^\circ\text{C}$ over the twentieth century. The root cause of this climate change is now recognised to be a number of GHGs including carbon dioxide, methane and nitrous oxide and their warming potential is shown in Table 3 (Thorpe, 2009). The IPCC report (2007a) indicates that agriculture contributes about 47 percent and 58 percent of total anthropogenic emissions of CH_4 and N_2O , respectively, but there is a range of uncertainty in the estimates. CH_4 from enteric fermentation (38%) and N_2O emissions from soils (32%) are the main sources of non- CO_2 emissions from agriculture (US-EPA, 2006).

Table 3. Trends and warming potential of greenhouse gases relevant to ruminant livestock

Greenhouse gases	Abundance		Trend	Annual emission (late 1990s)	Lifetime (years)	Global warming potential
	1750	1998				
Carbon dioxide	280	367	2.0	7 GtC	5–200	1
Methane	700	1 745	7.0	600 TgCH ₄	8.4	23
Nitrous oxide	270	314	0.8	16.4 TgN	120	296

Source: adapted from Thorpe (2009).

Note: GtC = gigatonne carbon; TgCH₄ = teragrams of methane; TgN = teragrams of nitrogen.

Methane is a potent GHG that has been implicated in global warming and the warming potential of CO_2 has recently been revised from 23 to 27 (IPCC, 2007b; Moss *et al.*, 2000). Approximately 600 Tg of methane is released into the atmosphere each year and 55 to 70 percent of this is anthropogenic (Thorpe, 2009). Enteric fermentation of ruminant livestock is the largest source of anthropogenic methane emissions contributing between 20 percent and 25 percent (Thorpe, 2009). During enteric fermentation archaea in the rumen (known as methanogens) produce methane through the stepwise reduction of CO_2 ($4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$) (Thauer *et al.*, 1993). As well as contributing significantly to GHG emissions, the process of methanogenesis is energetically wasteful representing a loss of between 2 and 12 percent ingested feed energy for ruminant livestock (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995). Cattle are the major contributors to enteric methane emissions globally and the main countries involved in cattle production and related methane emissions are summarized in Tables 4 and 5. The increase in nitrous oxide emissions is primarily due to agricultural enterprises. Nitrous oxide is generated by the microbial transformation of nitrogen in soils and manures, and is often enhanced where available nitrogen exceeds plant requirements, especially under wet conditions (IPCC, 2007b). The main driver for increasing emissions is management of manure from cattle, poultry and swine production, and manure application to soils.

There is increasing concern regarding the contributions of ruminant animals to emissions of environmental pollutants (FAO, 2006), including emissions of nitrogen generally and ammonia and nitrous oxide specifically. Agricultural N₂O emissions are projected to increase by 35 to 60 percent up to 2030 due to increased nitrogen fertilizer use and increased animal manure production (FAO, 2003). Apart from the contribution of nitrous oxide from manures to global GHG concentrations, concerns also include the contribution of manure to the eutrophication of aquatic environments and effects of ammonia on air quality. Data from lactating cows have concluded that the major factor determining total nitrogen excretion as manure (feces plus urine) in high producing animals is total dietary nitrogen intake (Castillo *et al.*, 2000; Yan *et al.*, 2006). Nutritional management of ruminants for improved utilization of absorbed nitrogenous compounds will reduce not only the amount of manure nitrogen excreted but also the portion excreted as more volatile urinary urea nitrogen. As restrictions on nitrogen losses from animal production facilities increase, there will be a need to more precisely formulate diets that meet the requirements for specific amino acids and minimize nitrogen excretion. Rates and amounts of rumen ammonia production reflect the solubility and fermentability of the dietary and endogenous sources of nitrogen.

While dietary manipulation and improved feeding systems can reduce methane and nitrogenous emissions, a better understanding of the microorganisms involved in these processes in the rumen will provide a basis for interventions which improve efficiency of digestion and reduce the amount of pollutants from ruminant livestock.

Table 4. Top ten enteric methane emitting countries—and emissions by source in tara gram per annum (status 2004)

	Cattle	Buffalo	Sheep	Goats	Other animals	Total*
India	8.6	4.89	0.31	0.6	0.08	14.48
Brazil	9.6	0.06	0.07	0.16	10.33	7.23
China	4.7	1.14	0.79	0.92	0.77	8.32
United States of America	5.1	0	0.05	0.01	0.2	5.36
Argentina	2.5	0	0.06	0.02	0.07	2.65
Pakistan	1.1	1.28	0.12	0.27	0.10	2.87
Australia	1.2	0	0.76	0	0	1.96
Sudan	1.4	0	0.24	0.21	0.19	2.04
Russian Federation	1.6	0	0.12	0	0.05	1.77
Ethiopia	1.2	0	0.06	0.05	0.07	1.38
Total developing	43.76	8.60	3.46	3.74	2.99	62.55
Total developed	17.55	0.04	2.49	0.16	0.74	20.98
Total	61.31	8.64	5.95	3.9	3.73	83.53

* based on data from Intergovernmental Panel on Climate Change for 2004. *Source:* adapted from Thorpe (2009).

Table 5. Enteric emissions of methane from cattle in countries with national herds exceeding 20 million head of cattle

	Population (millions)		Change (%)	Global population (%)	Annual methane emissions (teragram)		
	1984	2004			1984	2004	IPCC2004*
Brazil	127.7	192	50.3	14	6.90	10.37	9.6
India	195.2	185.5	-5.0	14	6.83	6.49	8.6
China	59.0	106.5	80.5	8	2.07	3.73	4.7
United States of America	113.4	94.9	-16.3	7	6.58	5.50	5.1
Argentina	54.6	50.8	-7	4	2.95	2.74	2.5
Sudan	21.0	38.3	82.4	3	0.74	1.34	1.2
Ethiopia	N/A	35.5	N/A	3	N/A	1.24	1.2
Mexico	30.5	30.8	1.0	2	1.07	1.08	1.6
Australia	22.1	26.4	19.5	2	1.19	1.43	1.4
Colombia	23.4	25.3	8.1	2	0.82	0.89	1.3
Russian Federation	N/A	24.8	N/A	2	N/A	1.36	1.6
Bangladesh	21.9	24.5	11.9	2	0.77	0.86	1.1
Pakistan	16.4	23.8	45.1	2	0.57	0.83	1.1
Developing	827.7	1,018.4	23.0	76.3	32.43	39.82	43.76
Developed	426.1	316.1	-25.8	23.7	23.77	17.67	17.55
Total	1,253.8	1,334.5	6.4		56.2	57.49	61.31

* based on data from Intergovernmental Panel on Climate Change (IPCC) for 2004. *Source:* adapted from Thorpe (2009).

Safety of animal products

The transmission of gut microorganisms from ruminant animals and their impacts on human health has been reviewed by Vanselow and McSweeney (2007). Both human health and disease are often linked to ruminant animals: health through the nutritive value of meat and dairy products, and disease predominantly through the direct spread of zoonotic organisms or the contamination of food and the environment with manure. Worldwide the meat industry has found itself in the midst of controversy over a number of large-scale food-borne contamination incidents. These include bovine spongiform encephalopathy (BSE), *E. coli* O157:H7 infection, *Salmonella typhimurium* DT 104 with multiple antibiotic resistance, and chemical residues, which have led to the perception that meat, is not always a “safe” product.

The animal industry not only produces meat but is also the source of manure and waste effluent which are used as fertiliser. Manure and effluent are potential sources of contamination by enteric pathogens to crops (both for animal and human consumption) and waterways (Wallace, 1999; McQuigge *et al.*, 2000). The upsurge in organic farming with increased use of manure could be a source of increased contamination if manure is not properly stored or composted (Himathongkham *et al.*, 1999; Guan and Holley, 2003; Duffy, 2003). It is paradoxical that incidences of food-borne disease are increasing in industrialised countries. The factors involved in this increasing incidence are complex: production and distribution systems for food have changed as well as eating and cooking habits, and there is increased movement of people globally (Altekruse and Swerdlow, 1996; Lederberg, 1997). Current intensive animal husbandry practises promote pathogens in the animal populations through contaminated feed (often by rodents or birds), and environmental (soil and water) contamination (Johnston, 1990; McEwan and Fedorka-Cray, 2002). Potential pathways for the spread of these organisms from animals to humans are shown in Figure 2. There is also concern that intensive animal production systems may be contributing to the evolution of antibiotic resistance in human infections through the transmission of resistant gut bacteria and associated genetic elements from animals to humans (Khachatourians, 1998; McEwan and Fedorka-Cray, 2002).

Apart from the human health risks associated with contaminated animal products, there can be detrimental impacts on the industries and businesses associated with the incident. In recent years, legal proceedings have occurred against manufacturers, butchers and fast food chain operators because of large-scale food-poisoning incidents. Product recalls often have a profound effect on consumer confidence and preference for products.

Although it has been estimated that about 97 percent of human food-borne disease cases are caused by improper preparation and handling of food immediately prior to consumption (Biddle *et al.*, 1997), significant benefits should arise from reducing contamination of the product prior to cooking. In the case of *Campylobacter* on chicken carcasses, it has been estimated that a 100-fold reduction in the levels of microbial contamination would reduce the incidence of campylobacteriosis in humans by 30-fold (Rosenquist *et al.*, 2003). Therefore, the responsibility for food safety does not rest solely with food handlers. Farmers and animal scientists are also an integral part of the food industry and as such must realise that decisions they make may influence the safety of food.

Bacterial pathogens associated with the consumption of meat and dairy products include *Salmonella spp.*, enteropathogenic *E.coli*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Campylobacter coli*, *Yersinia enterocolitica*, *Clostridium perfringens* and *Clostridium botulinum*, *Staphylococcus aureus*, *Bacillus spp.*, *Brucella abortus*, *Mycobacterium bovis* and *Mycobacterium paratuberculosis*. Many of these organisms are present in the ruminant animal and can be a potential source of contamination of animal products. The chain of events from killing, processing, storage and food preparation can provide the conditions which enable multiplication of these contaminating organisms. Other bacteria such as *Clostridium botulinum*, *Staphylococcus aureus*, and *Bacillus spp.* are generally regarded as secondary contaminants that may contaminate meat during processing, while some organisms (e.g. *Staphylococcus aureus*) produce toxins that cannot be destroyed by cooking. Many of the pathogens associated with human food poisoning are not host specific and thus humans can share pathogens with other mammalian species as well as with birds, reptiles and amphibians. Control of these microorganisms in the food chain requires a basic understanding of their ecology and physiology.

The recently emerged agents of food-borne disease that have been associated with ruminants include: the prion of bovine spongiform encephalopathy, antibiotic resistant bacteria, *Salmonella Typhimurium* DT104 with multiple antibiotic resistance, and the enterohaemorrhagic *Escherichia coli* such as *E.coli* O157:H7, and *E.coli* O26.

Microbial resistance to antibiotics is a major concern for human health. The occurrence of resistance has arisen from the improper use of antibiotics in human medicine and in part due to their use in the agricultural sector. The use of antibiotics in ruminant feed as growth promotants and to prevent infection leads to the selection of antibiotic resistant bacterial populations, particularly in the gut of these animals. Organisms that develop resistance to a family of antibiotics can transmit that resistance through mobile genetic elements to other non-related bacteria or may simply transfer from animals to humans. Therefore in both animals and humans the use of antibiotics needs to be rationalised to extend the life/usefulness of these chemicals. The enteric gut bacteria of food-producing animals that are considered most likely to be transmitted from animals to humans are nontyphoid *Salmonella spp.*, *E. coli*, and *Campylobacter spp.* The predominant route of transfer for these bacteria is via the food chain, although spread to humans could occur directly on farm and at abattoirs from faecal contamination and poor hygiene (Figure 2).

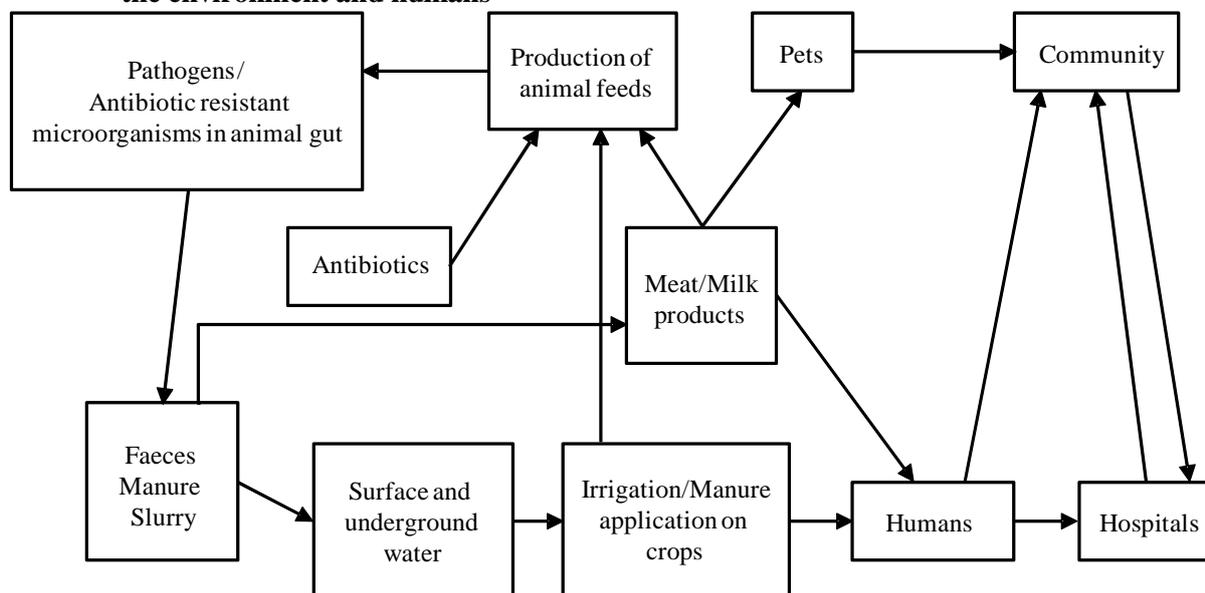
Definitive evidence of spread of antibiotic resistant bacterial strains from animals to humans is limited but based on molecular techniques, which demonstrate that both the bacterial strain in the animal and human are identical, and that the antibiotic gene sequence is also identical (van den Bogaard and Stobberingh, 2000). The available evidence indicates that antibiotic resistant *Salmonella* and *Campylobacter* have passed from animals to humans as well as horizontal transfer of resistance genes from animal enterococci and *E. coli* to human strains (McEwan and Fedorka-Cray, 2002).

Several examples are given of the emergence of antibiotic resistant-bacteria in livestock systems and their mode of transmission in the environment and humans. Contamination of the environment may also indirectly lead to the transfer of antibiotic resistance from animals to humans. Animal manures containing antibiotic resistant bacteria have contaminated waterways, and resistance has spread to aquatic organisms (Morinigo *et al.*, 1990; Young, 1993; Marshall *et al.*, 1996). It also appears that

antibiotic resistance from gut bacteria of animals has spread to bacteria in fish and crustacea (Hatha and Lakshmanaperumalsamy, 1995). Humans and animals share a range of bacterial species in their gastrointestinal tracts, which could be interpreted to indicate that certain strains of bacteria are quite promiscuous in their host range. However the few data available demonstrate that the host range of bacterial strains between species may be restrictive (Aarestrup *et al.*, 1997). The transfer of resistance strains from animals to humans is therefore dependent upon the host range of the animal strain.

Several countries have implemented strategies to arrest the rapid rise in antibiotic resistance and contain the problem. The main elements of these strategies involve a more rational use of antibiotics in human medicine and animal infections, the eventual removal of antibiotics from animal feeds, and the containment of spread of antibiotic-resistant organisms through improved hygiene practices in hospitals and industrial settings which impact on public health. Many countries have also implemented surveillance programmes for monitoring the use of antibiotics in agriculture and the occurrence of antibiotic-resistant strains in food and the environment (Williams and Heyman, 1998). The use of probiotic strains of bacteria as a strategy to control undesirable organisms in the gut of intensively reared livestock is seen as a more sustainable approach.

Figure 2. Simplified pathways for the flow of pathogens and antibiotic genes from livestock to the environment and humans



Source: adapted from Khachatourians, 1998.

Quality of animal products

Lipids and fats: Excessive intake of saturated fats in the human diet has adverse health impacts including cardiovascular disease and susceptibility to insulin resistance, obesity and cancer (World Health Organization, 2003; Mensink *et al.*, 2003; Nugent, 2004). Dietary lipids are rapidly hydrolysed and biohydrogenated in the rumen resulting in meat and milk products characterised by a high content of saturated fatty acids and low polyunsaturated fatty acids (Harfoot and Hazlewood, 1997). The majority of the 12:0 and 14:0 and a large amount of 16:0 fatty acids in the human diet are derived from ruminant meat and dairy products. Therefore altering the composition of lipids in milk fat is seen as a way of reducing chronic diseases in society that arise from poor nutrition and eating habits particularly in westernized societies. There is considerable interest in altering fatty acid composition of milk to decrease the content of medium-chain saturated fatty acids and increase the concentrations of several long chain bioactive lipids, including cis-9-18:1, trans-11-18:1 and cis-9,trans-11-18:2 is desirable (Pariza, 2004; Bauman *et al.*, 2005; Shingfield *et al.*, 2009).

Lipids in the diet of ruminants undergo hydrolysis and biohydrogenation in the rumen before being absorbed onto digesta that passes to the intestines or is incorporated into bacterial cells. The rumen is

therefore a major control point in determining the fatty acid composition of ruminant lipids. An understanding of the rumen microorganisms involved in biohydrogenation of lipids in different animal feeds and the metabolic pathways they employ in this process are essential to any strategy that seeks to improve the nutritional attributes of lipids in ruminant milk products.

The microorganisms with a central role in fatty acid metabolism in the rumen are the ciliate protozoa, which store unsaturated fatty acids (Devillard *et al.*, 2006) and several bacterial species (eg. *Butyrivibrio fibrisolvens*, *Butyrivibrio proteoclasticum*, *Propionibacterium acnes*, *Selenomonas ruminantium*, *Enterococcus faecium*, *Streptococcus bovis*, *Staphylococcus sp.*, and *Flavobacterium sp.*), which are primarily involved in biohydrogenation (see McKain *et al.*, 2010). Recent studies by McKain *et al.* (2010) indicate there are probably three systems for the metabolism of unsaturated fatty acids in ruminal biohydrogenating bacteria – one that reduces geometric isomers of 9,11 conjugated linoleic acid to *trans*-11-18:1, another that reduces 10,12 geometric isomers of conjugated linoleic acid to a mixture of 10- and 12-18:1 fatty acids, and a third, only found in *Butyrivibrio proteoclasticus*, that reduces a range of monoenoic fatty acids, including *trans*-10-18:1, to 18:0. Their experiments also indicated that *Propionibacterium acnes* hydrates 18:1 substrates to yield 10-O-18:0 as an end product. These studies of the rumen also provide insights into the likely bacteria and pathways for fatty acid metabolism that occur in the human colon which could influence human health (Devillard *et al.*, 2007).

Status of rumen microbial diversity research

An excellent review of the importance of rumen microbial ecology and the molecular methods for studying the ecosystem has been written by Mackie *et al.* (2007). They describe how the microbial community inhabiting the ruminant gastrointestinal tract is characterized by its high population density, wide diversity, and complexity of interactions. This complex, mixed, microbial culture can be considered the most metabolically adaptable and rapidly renewable component of the body, which plays a vital role in the normal nutritional, physiological, immunological and protective functions of the host animal. The gut microbiota and its collective genomes (termed the microbiome) is estimated to contain 100 times more genes than the host animal and provides the ruminant animal with genetic and metabolic capabilities that the host has not had to evolve on its own, including capabilities to hydrolyze and ferment inaccessible nutrients. Recent advances in molecular microbial ecology that allow identification and quantification of the normal microbiota suggest the presence of complex communities of co-evolved bacteria with the ruminant host. It is worthwhile emphasizing that the study of rumen microbial ecology involves investigation of the organisms present, their activity and their relationship with each other and the host animal.

Rumen microbial diversity

Diversity of protozoa, bacteria, archae, fungi and bacteriophage: in a recent study by Kim *et al.*, (2011a), the microbial diversity of bacteria and archaea in the rumen of predominantly domesticated livestock was assessed by analysing all the curated 16S rRNA gene (*rrn*) sequences deposited in the Ribosomal Database Project database in 2010. There were 13 478 bacterial and 3 516 archaeal *rrn* sequences in the database by late 2010, which formed the basis of the analysis (Table 6). The diversity of bacterial and archaeal species in the rumen is estimated to be approximately 7000 and 1500 species respectively of which about 30 percent are still not represented in databases.

The bacterial sequences were assigned to 5 271 operation taxonomic units (OTUs) at species level (0.03 phylogenetic distance) and genus levels (0.05 distance) which represented 19 existing phyla, and 180 genera, of which the *Firmicutes* (~56%), *Bacteroidetes* (~31%) and *Proteobacteria* (~4%) were the most predominant phyla.

More than 90 percent of the *Firmicutes* sequences were assigned to genera within the class *Clostridia* while streptococci were prominent within the class *Bacilli*. Within the *Clostridia*, *Lachnospiraceae* and *Ruminococcaceae* and *Veillonellaceae* were the largest families. The predominant genera included *Butyrivibrio*, *Acetivibrio*, *Ruminococcus*, *Succiniclasicum*, *Pseudobutyrvibrio* and *Mogibacterium*.

Table 6. Estimated bacterial and archaeal species in the rumen and current known coverage of sequences in public databases

Microbial group	Number of species observed (% coverage)	Estimated maximum number of species
Total Bacteria	5 271 (71)	7 426
<i>Firmicutes</i>	2 958 (74)	3 993
<i>Bacteroidetes</i>	1 610 (69)	2 344
<i>Proteobacteria</i>	226 (71)	320
Unclassified <i>Clostridiales</i>	606 (76)	798
Unclassified <i>Lachnospiraceae</i>	588 (66)	897
Unclassified <i>Ruminococcaceae</i>	524 (70)	750
Total Archaea	949 (65)	1 469

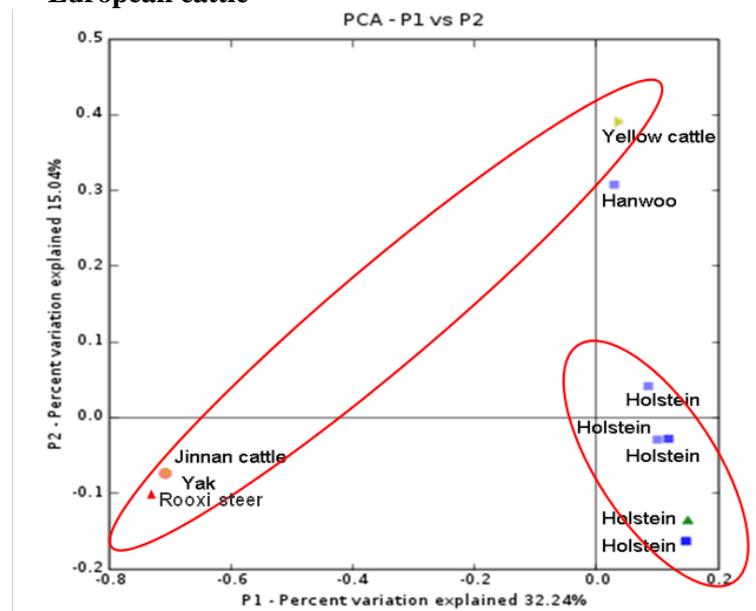
Source: Kim *et al.*, 2011b.

In the *Bacteroidetes* phylum the majority of sequences were assigned to class *Bacteroidia* and *Prevotella* was the most predominant genus. *Prevotella* is generally in high abundance in the rumen generally and represented by the species *Prevotella ruminicola*, *Prevotella brevis*, *Prevotella bryantii* and *Prevotella albensis*. All five classes of *Proteobacteria* were represented in the database of rumen sequences dataset with the *Gamma proteobacteria* being the dominant Class.

Nearly all the archaeal sequences were assigned to the phylum *Euryarchaeota* which was represented by about 670 genus-level OTUs and 1 000 species-level OTUs for which there are only 12 existing phyla. Rarefaction analysis of the sequences in the database indicated that the current coverage of the diversity at the species level was 71 percent for bacteria and 65 percent for archaea. These data indicate that about a 5 and 7 fold increase in bacterial and archaeal sequences respectively is required to achieve full coverage of the diversity of these microorganisms.

The diversity within the rumen is also affected by diet and whether the microorganisms are adhered to plant particles or planktonic (Kim *et al.*, 2011b). A ground-breaking paper by Ley *et al.*, (2008) on the evolution of mammals and their gut microbes showed that bacterial communities co-diversified with their hosts and that diet and host species both influence bacterial diversity, which increases from carnivory to omnivory to herbivory. While there are differences in gut microbial communities between animal species there is also new evidence that the bacterial microbiome and metabolic potentials in the rumen are markedly different between animals fed the same diet when viewed in relation to nutrient utilisation (Brulc *et al.*, 2009).

Figure 3. Principal component analysis of rumen bacterial microbiomes from Asian and European cattle

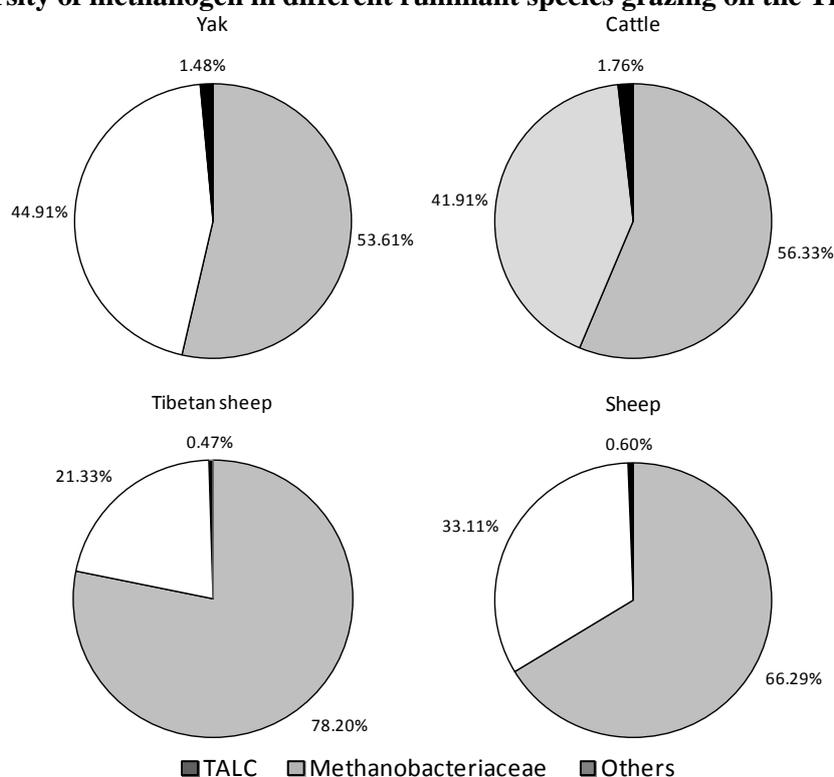


Source: Kailang Liu, unpublished data.

Recent data from China indicates that Asian species of cattle [Yunnan yellow cattle (*Bos taurus*), Jinnan cattle (*Bos taurus*), Yak (*Bos grunniens*) and Hanwoo (*Bos taurus coreanae*)] have a distinctive rumen bacterial community compared with Holstein cattle, supporting host genotype as an important factor shaping the composition (Kailang Liu, unpublished data; Figure 3). A superficial analysis of the bacterial diversity in the rumen of buffaloes has been reported (Pandya *et al.*, 2010) and the structure at the phylum level is similar to other domesticated ruminants but the differences in rumen microbial diversity between ruminant species is more apparent at the genus level and lower.

Archaeal community composition: Based on the analysis of global data sets available in public databases, the majority (>90%) of rumen archaea are affiliated with genera; *Methanobrevibacter* (> 60%), *Methanomicrobium* (~ 15%), and a group of uncultured rumen archaea commonly referred to as rumen cluster C (RCC, ~16%) or Thermoplasmatales-Affiliated Lineage (Janssen and Kirs, 2008; St-Pierre and Wright, 2012). Within the genus *Methanobrevibacter*, two major clades *M. gottschalkii* (contains *M. gottschalkii*, *M. thaueri*, and *M. millerae*) and *M. ruminantium* (*M. ruminantium* and *M. olleyae*) dominant in the rumen. Members of other groups of methanogens, including *Methanimicrococcus spp.*, *Methanosphaera spp.*, and *Methanobacterium spp.* appear in fewer animals and/or are in low abundance. The metabolic activities of the RCC clade are unknown but CSIRO has recently isolated a representative of this group and have shown they produce methane by reducing organic compounds containing methyl groups but lack the ability to autotrophically form methane from CO₂ and H₂. This means they produce more methane than autotrophic methanogens per mole of H₂ consumed and thus a high abundance of these archaea in the rumen may result in a greater production of methane. Indeed in collaborative studies between CSIRO and the University of Langzhou we have observed that the methanogen population in ruminants foraging on the Tibetan plateau are uncharacteristically dominated by members of RCC (Figure 4). The dominance of these methanogens has not been observed previously and may have arisen through a combination of factors including climatic conditions, altitude, diet and relatively pristine condition of the natural environment.

Figure 4. Diversity of methanogen in different ruminant species grazing on the Tibetan Plateau



Source: Huang *et al.*, unpublished data.

A recent review of the literature by St-Pierre and Wright (2012) examined differences in methanogen community between different ruminant species, breeds, diets or geographical locations. The community structure of archaea in ruminant animals varies significantly in abundance and diversity of the genera and

species that occur commonly in the rumen (described above). This variation can be due to differences in diet, environment, health, animal genotype, and animal age although the methods of detection (e.g. primer sets, extraction methods, DNA vs. RNA etc) are also probably contributing to some of the differences (McSweeney *et al.*, 2009; see Janssen and Kirs, 2008; Zhou *et al.*, 2009, 2010, 2011). It is generally accepted that the majority of rumen archaea probably use hydrogen and carbon dioxide as the main substrates to drive methane formation in the rumen but there are few studies to confirm this observation and whether organic forms of carbon in some diets also contribute significantly as precursors for methane. The broad diversity of methanogens in the rumen would suggest that factors other than availability of hydrogen and carbon dioxide are determining the structure of the community. It is likely that diet and host physiological characteristics are playing a significant role in determining the relative abundance of sub populations. The influence of diet on rumen pH is likely to affect methanogen metabolism directly (Lana *et al.*, 1998), or indirectly through interactions with other microbial groups that have responded to the diet, and through the passage rate and physical characteristics of the digesta. The host physiology in terms of rumen capacity and bioactive compounds in saliva may also influence the species structure but there is little data to confirm these observations. Rumen protozoa harbour both ecto- and endosymbiotic methanogens which belong to the main rumen genera described previously. However there have not been enough studies to determine whether protozoan- associated methanogens are different from planktonic lineages.

Protozoal community structure: recent insights into the protozoal community structure in the rumen were discussed by McSweeney *et al.* (2006) with relatively few studies since to provide a greater understanding of the impacts of the diet and host species on protozoal ecology in the ruminant animal. Microscopy has been the method of choice in identifying and enumerating protozoal populations in rumen samples and is still a reliable method (Dehority, 2003). However, molecular techniques are becoming more prevalent in ecological studies of ruminal protozoa (Karnati *et al.*, 2003; Regensbogenova *et al.*, 2004a, b; Shin *et al.*, 2004; Sylvester *et al.*, 2004, 2005; Skillman *et al.*, 2006). *Entodinium* is the dominant genus in the rumen, but considerable protozoal diversity representing many different genera and species is found. Because of the numerical dominance of *Entodinium* species, which can account for as much as 95–99 percent of rumen protozoa in animals fed high grain diets, genus-specific primers, such as those published by Skillman *et al.* (2006), are required to effectively examine the complete protozoal diversity in the rumen. Moreover, given the high sequence similarity of 18S rDNA among different rumen protozoal species, other phylogenetic markers of higher sequence divergence, such as 28S rDNA or internal transcribed sequences (ITS), need to be developed for molecular ecological studies of rumen protozoa. Sylvester *et al.* (2004 and 2005) demonstrated the utility of polymerase chain reaction (PCR) - denaturing gradient gel electrophoresis (DGGE) in profiling protozoal communities in the rumen and duodenum by employing different protozoa-specific primers. They were able to show the impact of diets on protozoal diversity, and identified the major protozoal species (*Epidinium caudatum*, *Entodinium caudatum*, and *Isotricha prostoma*) by sequencing rDNA fragments recovered from predominant DGGE bands. More recently, comparison of diversity and structure of protozoa communities from hay-fed versus silage/grain-fed cattle also showed that the dominant species *Entodinium spp.* were more abundant in the silage/grain-fed cattle and diversity was higher for the hay-fed cattle (Tymensen *et al.*, 2012). Other genera common to both diets included *Isotricha*, *Dasytricha*, *Ostracodinium*, *Diplodinium*, and *Diploplastron* but there was a higher prevalence of *Dasytricha*, *Ostracodinium*, and *Diploplastron* in the hay-fed cattle. Genera that were unique in the hay-fed animals were *Eudiplodinium* and *Epidinium*, whereas *Ophryoscolex* and *Polyplastron* were unique to the silage/grain diet. A similar pattern was observed in Yunnan Yellow cattle which harboured the genera *Entodinium* (~67%; predominantly *E. caudatum*), *Dasytricha* (~6%), *Isotricha* (~9%), and *Diplodinium* (~18%) (Leng *et al.*, 2011).

With respect to ruminant nutrition, it is important to accurately quantify protozoan biomass in the rumen and its passage to the duodenum as a component of microbial protein flow to the intestines (Firkins and Yu, 2006). Real-time PCR assays specific for protozoal rDNA have been developed that can effectively quantify protozoan biomass in the rumen and duodenum both in terms of rDNA copy numbers and in nitrogen mass (Sylvester *et al.*, 2004, 2005; Skillman *et al.*, 2006).

Anaerobic Fungi Community Structure: anaerobic rumen fungi have been isolated from digesta and faeces of numerous herbivores including ruminant and monogastric animals and were recently

assigned to a new and separate phylum *Neocallimastigomycota* (Griffith *et al.*, 2010; Liggenstoffer *et al.*, 2010). Previously, six genera (*Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces*) were recognized but greater depth of sequence analysis with high throughput sequencing technology has provided new insights into this group of organisms and it is apparent that several new uncultured taxa are yet to be isolated. Currently, 18 species of anaerobic rumen fungi have been described based on morphology of thallus and zoospore ultra-structure but this should increase dramatically once pure cultures of the new taxa are obtained. The ability to accurately distinguish and classify these fungi in an *in vivo* environment is difficult due to their pleomorphic tendencies, and this is compounded for environmental samples where the fungi are intimately entwined with plant material. Visual identification also relies on the presence of both the mature and zoospore stages of the fungi. DNA-based molecular methods do not depend on the culturability of micro-organisms, and therefore offer an attractive alternative for the study of complex fungal community structures.

A rapid and specific qPCR technique has been developed to quantify the abundance of anaerobic rumen fungi but recently this method has been improved by the design of new primers which show greater coverage of the rumen fungal population (Denman *et al.*, 2008; Edwards *et al.*, 2008). However this method does not discriminate between the different fungal genera which is required for diversity analysis. To address this issue of community analysis, an automated ribosomal intergenic spacer region (ARISA) method for the detection and monitoring of anaerobic rumen fungi has been developed which is capable of clearly discriminating to the genus level between pure cultures (Denman and McSweeney, 2006; Edwards *et al.*, 2008). Using this method, significant changes in the composition of the anaerobic rumen fungi population were observed between animals fed a high fibre diet compared to those animals being fed a diet high in grain. The technique has also been used to study the dynamics of initial colonization of forage by anaerobic fungi (Edwards *et al.*, 2008). Through the use of ARISA monitoring, it is possible therefore to obtain a better understanding of the temporal interrelationships between diets and the different members of anaerobic rumen fungi community. However next-generation sequencing technology is likely to supersede ARISA analysis for studies of the ecology and diversity of anaerobic rumen fungi. For example, the phylogenetic diversity and community structure of members of the gut anaerobic fungi (phylum *Neocallimastigomycota*) were investigated in 30 different herbivore species using the internal transcribed spacer region 1 (ITS-1) ribosomal RNA (rRNA) region as the phylogenetic marker. More than one quarter of a million sequences representing all known anaerobic fungal genera were obtained in this study. Sequences affiliated with the genus *Piromyces* were the most abundant, while sequences affiliated with the genera *Cyllamyces* and *Orpinomyces* were the least abundant. However nearly 40 percent of the sequences obtained did not cluster with known genera and formed eight distinct novel anaerobic fungal lineages, some of which were widely distributed in the herbivores sampled. This study also examined the effect of physiological and environmental factors on the diversity and community structure of AF in the gut and concluded that the phylogenetic placement of the animal host has the greatest impact on shaping anaerobic fungal diversity and community composition. The use of next-generation deep-sequencing technology has greatly extended our understanding of the diversity of these unusual microorganisms in gut ecosystems and provided insights into the interactions with host genetics which may shape their role and impact on fibre digestion between animals.

Rumen Bacteriophage diversity: Bacteriophages are abundant (10^7 – 10^9 particles per ml) in the rumen ecosystem but the diversity of these viruses is poorly understood as well as their interactions with the other microorganisms in this ecosystem. They appear to influence other microbial population structure and density through bacterial lysis in the rumen as well as being intimately involved in the exchange of genetic information with other microbial populations (Klieve *et al.*, 1991; Klieve and Swain, 1993; Swain *et al.*, 1996; Klieve and Hegarty, 1999). The first comprehensive metagenomic analysis of the bovine rumen virome was reported recently in which 28 000 different viral genotypes were identified (Berg Miller *et al.*, 2012). The genotypes belonged to the following Families in descending order of prevalence; *Siphoviridae*, *Myoviridae*, *Podoviridae*, *Unclassified*, *Herpesviridae*, *Phycodnaviridae*, *Mimiviridae*, *Poxviridae*, *Baculoviridae*, *Iridoviridae*, *Polydnaviridae*, *Adenoviridae*, *Bicaudaviridae*. Prophages dominated lytic phages by 2:1. The sequence analysis indicated that the phages were

associated with the main bacterial phyla including *Firmicutes*, *Proteobacteria* and *Bacteroidetes* thus suggesting a role in shaping these bacterial communities. Rumen phage also influence the efficiency of digestion in the rumen through the spontaneous lysis of bacterial populations by lytic phage which will also influence protein supply to the animal from microbial protein synthesized in the rumen (Swain *et al.*, 1996).

Changes in rumen ecology as a function of age and host genetics

The developing rumen: Our current knowledge of the changes in rumen ecology of as the juvenile ruminant animal matures to adulthood is based primarily on cultivation studies and thus provides only a general understanding of the transition in microbial population with age. For example, over the past 20 years the most informative studies have been performed at the INRA laboratories in France where newly born lambs are raised in isolation immediately after birth to reveal the sequence of rumen colonization (Table 7; Bera-Maillet *et al.*, 2009; Fonty *et al.* 1983, 1987, 1989, 2007; Hobson and Fonty, 1997).

Table 7. Sequence in colonisation of the new-born rumen with different microbial groups

Age	Establishment of microbial group
First 2 days	Facultative / strict anaerobic bacteria
2 to 7 days	Fibrolitic bacteria and methanogens
7 to 10 days	Fungi
3 to 7 weeks	Protozoa

The first reported molecular diversity analysis of rumen microorganisms in lambs raised in isolation as described above revealed aspects of this animal model and rumen colonisation that had not previously been revealed using cultivation techniques alone (Gagen *et al.*, 2012). Firstly, an unexpected finding of this study was the presence of a low-abundant population of methanogens in the developing rumen isolated at 17 h which was diverse and similar to those found in the mature rumen of conventionally raised sheep. The earliest reported age at which methanogens have been found in the rumen is 30 h (Morvan *et al.*, 1994), though it was previously accepted that methanogens colonize the developing rumen (both bovine and ovine) 3 to 4 days after birth and establish at numbers similar to those in adults, from the first week onwards (Fonty *et al.*, 1987, 1997; Anderson *et al.*, 1987). Family level analysis of the bacterial populations in the study by Gagen *et al.*, (2012) showed that *Bacteroidaceae*, *Ruminococcaceae*, *Butyrivibrio* and *Johnsonella* bacteria that were in high abundance in the new born lamb were much lower or not detectable in the adult animals while the reverse occurred with several families including *Prevotellaceae*. These studies may indicate that methanogens, which colonise the rumen from a very young age, are maintained throughout rumen development and life, unlike the bacterial populations. If this is the case then variation in methane production between ruminant animals may be influenced by the methanogen populations that colonise the rumen soon after birth. Therefore the diversity of microbial populations that are dominant in some ruminants in different geographical regions may be transferred at birth to offspring which could then explain phenotypic variation between animals such as methane production. While acquisition and colonization of bacterial families may be more complex and dependent upon age and physiological state of the animal, host genetics and feed composition.

Host genetics and links to rumen microbiome structure and function: until recently, selection of animals for superior production traits, attributed the differences in productivity to host metabolism, physiology and behaviour of the animal in the environment without considering the role that the rumen microbiome might contribute to the variation between animals. However the highly acclaimed publication by Ley *et al.*, (2008) demonstrated there has been co-evolution of mammals (including ruminants) and their gut microbiota and that the dietary preference of the host and the genetic make-up of the animal influence bacterial diversity in the gut. In their study the patterns of microbial community structure matched the genotype of the animal group more than would be expected if co-evolution had not occurred. While definitive studies are still to be undertaken in ruminants there is

clear evidence in monogastric laboratory models that discrete genes of the host animal are involved in determining the colonization of the gut by key groups of bacteria (Benson *et al.*, 2010). Currently there is significant interest in breeding ruminants for low methane production as this trait appears to be heritable (Cottle *et al.*, 2011). The underlying mechanism governing methane production in these animals is still to be elucidated but experiments on the rumen microbial ecology in animals with varying feed conversion efficiencies have shown characteristic microbial patterns including methanogen community structure associated with both high and low feed conversion efficiencies (Guan *et al.*, 2008; Zhou *et al.*, 2010). Current research in several laboratories is aimed at identifying rumen microbial patterns that correlate with methane production and/or feed conversion efficiency which could be used as markers in genetic selection and breeding programs for improved productivity and reduced environmental impacts. If ruminants and their gut microbiota have co-evolved while adapting to their climatic and botanical environment then the gut microbiota of indigenous breeds may differ significantly from introduced ruminants which may have resulted in the transmission of distinctive microbial populations between adapted and non-adapted breeds. If this is the case it is important to define and preserve the diversity of indigenous ruminants (domesticated and wild) which will provide a benchmark before intermingling of populations occurs through the introduction of imported genotypes.

V. CURRENT TRENDS AND INNOVATIONS IN RUMEN MICROBIOLOGY

Conventional culture-based rumen ecology and molecular microbial ecology and the importance to ruminant livestock agriculture

Conventional culture-based rumen ecology vs. molecular microbial ecology: Recent developments in nucleic acid based techniques for use in rumen manipulation has been reviewed by Mackie *et al.* (2007) and McSweeney *et al.* (2009). During the 20th century, the study of rumen microbiology was primarily based on classical culture-based techniques, such as isolation, enumeration and nutritional characterization, which probably only account for 10 to 20 percent of the rumen microbial population. These traditional methods are time consuming and cumbersome, but have identified more than 200 species of bacteria and at least 100 species of protozoa and fungi inhabiting the rumen (Orpin and Joblin, 1997; Stewart *et al.*, 1998; Williams and Coleman, 1998; White *et al.*, 1999). Culturing microorganisms from the rumen will remain an important research activity into the future since isolation of novel microorganisms, and their physiological characterization and genomic sequence analysis will underpin the use and interpretation of the next-generation nucleic acid technologies. A recent paper on the isolation of rumen bacteria by a dilution approach using a new liquid culture medium has demonstrated that a large proportion of previously uncultured bacteria can be isolated when new culturing methods are applied and will remain a cornerstone of the strategy for describing the diversity of the rumen microbiota (Kenters *et al.*, 2011). New nucleic acid-based approaches can however be employed to examine microbial community structure using small subunit rRNA gene analysis (eg 16S and 18S rRNA) and to understand the function of complex microbial ecosystems through the combined analysis of multiple genomes (metagenomics) and the message from the expressed genes (mRNA) in the rumen (metatranscriptomics).

Several recent reviews and technical manuals have been written on the subject of molecular microbial ecology of animals which provide a broad perspective of the variety of techniques available and their potential application in the field of animal science which is beyond the scope of this report (see Zoetendal *et al.*, 2003, 2004, 2008; Makkar and McSweeney, 2005; McSweeney *et al.*, 2006; Mackie *et al.*, 2007; Morgavi *et al.*, 2012). The classical culture-based methods for studying rumen microbes are described in these reviews since a laboratory that intends using molecular techniques for studying the rumen must have a capacity to grow and isolate microorganisms under anaerobic conditions. There is information on the basic techniques and protocols in molecular ecology such as DNA extraction from environmental samples, the quantitative real-time polymerase chain reaction (qPCR), oligonucleotide probe and primer design, and DNA fingerprinting amongst others, as well as the

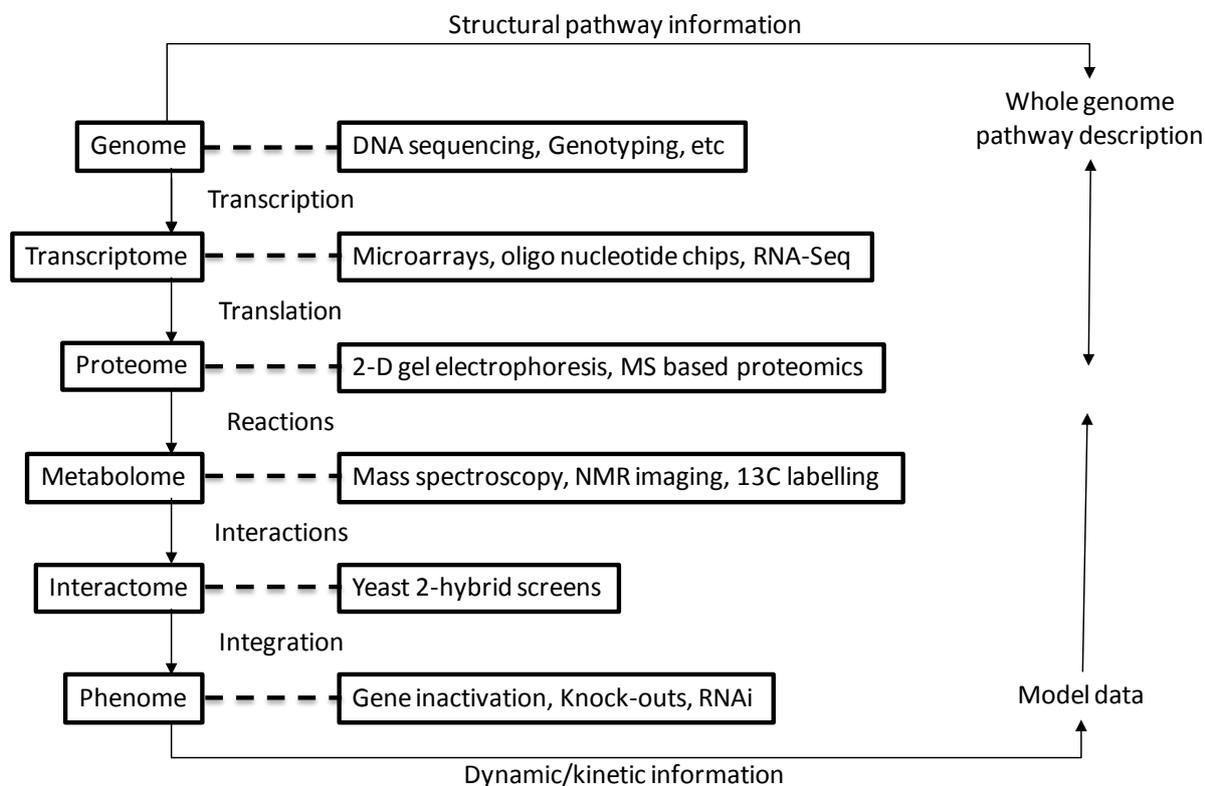
application of these techniques to microbial detection and identification. Specialised techniques such as denaturing gradient gel electrophoresis and 16S/18S ribosomal DNA libraries for studying complex communities that contain unculturable organisms are also compared. Many of these techniques are used to identify and enumerate the populations of organisms that are present in a sample. Techniques such as fluorescent *in-situ* hybridization which use 16S rRNA-targeted oligonucleotide probes combined with epifluorescent light microscopy or confocal laser microscopy provide knowledge about location and spatial relationships of microorganisms in their natural environment that are often essential to understanding the function of these organisms.

‘Omics’ approaches to understanding rumen microbial function

The introduction of powerful new technologies over the past two decades has led to many advances in microbiology. The first, Genomics (the mapping and sequencing of genomes and analysis of gene and gene function) has revolutionized the biological sciences. Genomics offers the capacity of rapidly investigating all biological features of a selected organism without prior development of a specific genetic system for the chosen organism. Currently, more than 2 900 genomes are published and more than 11 000 genome projects are listed in the Genomes Online Database⁴. These numbers are increasing rapidly based on new sequencing technologies and dramatic reductions in the cost of sequencing.

Fundamental biological processes can now be studied by applying the full range of *omics* technologies (genomics, transcriptomics, proteomics, metabolomics, and beyond). A schematic summary of these *omics* technologies used to gather information on numerous levels is presented in Figure 5.

Figure 5. “Omics” technologies involving the genome, transcriptome, proteome, metabolome, interactome, and phenome



Omics” technologies gather information on numerous levels, including the genome, transcriptome (entirety of all genes that are converted into transcripts [i.e., mRNA molecules]), proteome (entirety of all proteins found in a given cell), metabolome (entirety of all metabolism products and intermediates

⁴ www.genomesonline.org

in a cell), interactome (set of molecules, such as biologically active metabolism products, that interact with a given protein), and phenome (entirety of all observable characteristics of an organism) levels.

Rapid development in nucleic acid sequencing technologies and annotation platforms has made the genome-sequencing of individual microorganisms and interpretation both affordable and available to the broader research community through ‘in-house’ and commercial services. This has led to a marked increase in the number of rumen microorganisms that have sequenced genomes (Table 8; Morgavi *et al.*, 2012). There is little information available on the genomic make-up of rumen anaerobic fungi and ciliate protozoa and no genomes from these organisms have been published. However, a Rumen Microbial Genomics Network⁵ has been formed that will coordinate and accelerate the sequencing and development of rumen microbial genomics approaches for access to methods, genome sequences and metagenome data relevant to the rumen microbial community. The Rumen Microbiology group at AgResearch, New Zealand obtained in 2011 support from the US Department of Energy Joint Genome Institute through their Community Sequencing Programme for a major sequencing project titled “The Hungate 1 000: a catalogue of reference genomes from the rumen microbiome”. The Hungate 1 000 project aims to produce a reference set of 1 000 rumen microbial genome sequences from cultivated rumen bacteria and methanogenic archaea, together with representative cultures of rumen anaerobic fungi and ciliate protozoa that represent the broad diversity of organisms in the rumen and of functional significance. The reference genome information will be used to support international efforts to develop methane mitigation and rumen adaptation technologies, as well as to initiate genome-enabled research aimed at understanding rumen function in order to find a balance between food production and GHG emissions.

Table 8. Publically available genome sequences of rumen bacteria and archaea

Organism	Family	Reference
Fibrolitic bacteria		
<i>Butyrivibrio proteoclasticus</i> B316	<i>Lachnospiraceae</i>	Kelly <i>et al.</i> (2010)
<i>Eubacterium cellulosolvens</i> 6	<i>Lachnospiraceae</i>	
	<i>Prevotellaceae</i>	
<i>Prevotella bryantii</i> B ₁₄	<i>Prevotellaceae</i>	Purushe <i>et al.</i> (2010)
<i>Prevotella ruminicola</i> 23	<i>Fibrobacteraceae</i>	Purushe <i>et al.</i> (2010)
<i>Fibrobacter succinogenes</i> S85	<i>Ruminococcaceae</i>	Purushe <i>et al.</i> (2010)
<i>Ruminococcus albus</i> 7	<i>Ruminococcaceae</i>	Suen <i>et al.</i> (2011)
<i>Ruminococcus albus</i> 8	<i>Ruminococcaceae</i>	
<i>Ruminococcus flavefaciens</i> FD-1	<i>Ruminococcaceae</i>	Berg Miller <i>et al.</i> (2009)
<i>Ruminococcus flavefaciens</i> 007C	<i>Methanobacteriaceae</i>	
Other bacteria		
<i>Actinobacillus succinogenes</i> 130Z	<i>Pasteurellaceae</i>	
<i>Desulfotomaculum ruminis</i> DSM 2154	<i>Peptococcaceae</i>	
<i>Desulfovibrio desulfuricans</i> subsp. <i>desulfuricans</i> str. ATCC 27774	<i>Desulfovibrionaceae</i>	
<i>Basfia succiniciproducens</i> MBEL55E	<i>Pasteurellaceae</i>	
<i>Mannheimia succiniciproducens</i>	<i>Pasteurellaceae</i>	Hong <i>et al.</i> (2004)
<i>Slackia heliotrinireducens</i> DSM 20476	<i>Coriobacteriaceae</i>	Hong <i>et al.</i> (2004)
<i>Treponema saccharophilum</i> DSM 2985	<i>Spirochaetacea</i>	Pukall <i>et al.</i> (2009)
<i>Wolinella succinogenes</i> strain DSM 1740	<i>Helicobacteriaceae</i>	
<i>Megasphaera elsdenii</i> DSM 20460	<i>Veillonellaceae</i>	Baar <i>et al.</i> (2003)
<i>Lactobacillus ruminis</i> RF3	<i>Lactobacillaceae</i>	Marx <i>et al.</i> (2011)
Methanogens		
<i>Methanobrevibacter ruminantium</i> M1	<i>Methanobacteriaceae</i>	

Source: adapted from Morgavi *et al.*, 2012.

This sequence information, which can be linked to defined species of known function, is essential and a prerequisite for interpretation of metagenomic (metatranscriptomic) data sets. Metagenomic technologies, which provide the potential to capture and study the entire microbiome (the predominant

⁵ RMG.Network@agresearch.co.nz

genomes) from a complex microbial community in the rumen is being widely adopted globally by many advanced laboratories to study important microbial ecosystems for environmental, health and productivity benefits. Also rapid high-throughput technologies such as microarray analysis as well as new generation sequencing technologies developed in mapping the human genome are now being deployed to study microbial ecosystems. An explosion of knowledge in the field of rumen microbial ecology is therefore predicted. However the field is rapidly moving to a functional analysis of the microbes in the ecosystem and some of the next-generation sequencing methods are being employed to measure genes expression of the entire rumen microbiome.

Over the last decade, molecular ecology studies of the rumen microbiota have focused almost entirely on identifying and enumerating populations. While these analyses are very informative for determining the composition of the microbial community and monitoring changes in population size, they can only infer function based on these observations. The next major step in understanding the 'microbial activity' of the rumen is to link structural analysis of the microbial community with measurements of functional gene abundance and activity. A DNA-based approach which has gained rapid adoption as a way of studying community function is metagenomic analysis. Metagenomics is defined as the study of collected genomes from an ecosystem that can be used to examine the phylogenetic, physical and functional properties of microbial communities (Handelsman, 2004). The principle approaches in methagenomics involves either functional or sequence based analyses of the extracted DNA from the microbial community. In functional studies DNA fragments are cloned into a suitable host (e.g. *E. coli*) using a fosmid or bacterial artificial chromosome vector which results in a library of many thousands of clones. The clone library can then be screened using specific DNA sequences in a PCR or hybridization based approach for genes encoding discrete steps in known metabolic pathways or enzymatic activities. Also activity-driven assays can be performed to screen the library for novel enzymes or specific activities and function. The advantage of this approach is that the activity assay may identify clones with novel gene sequences encoding for functions which would have been missed by DNA screening, based on current sequence information in the database. Clones identified in either screening strategy are then subjected to a variety of sequencing technologies and computational analyses which enables the assembly and annotation of large inserts (> 40kb) of genomic DNA from the clones. The principle steps in sequence-drive analysis is the extraction of DNA from the microbial community followed by analysis on high throughput sequencing platforms (e.g. Roche FLX pyrosequencer, Illumina Hi Seq 2000 sequencer) and barcode "pyrotagging" procedures for pooling of samples. The DNA sequences generated from this analysis are assembled and annotated by computational analyses with references to known genomes deposited in public databases. Ultimately metagenomic analysis creates a catalogue of genomic information which can be interrogated to gain insights into the predominant genes, how they are regulated and their contribution to ecosystem function. Functional gene analysis and annotation is undertaken by using a bioinformatic pipeline to transform the DNA-sequence data via translation to amino acid sequence, similarity searches and annotation using public databases and functional data. For example, the DNA sequence data from a rumen sample can be binned (grouped on similarity and frequency of occurrence) and annotated using various gene identification and database tools (e.g. ContigExpress, Phylopythia, MEGAN and MG-Rast), to classify the genes within known enzyme families or clusters of orthologous genes (COGs) and metabolic pathways. By using this process, a catalogue of the genetic capacity of the rumen ecosystem can be determined as well as providing an avenue to identify novel genes or genes of unknown function from the environment (Attwood *et al.*, 2008; Brulc *et al.*, 2009; Hess *et al.*, 2011, Bayer and White, 2012).

Another way of describing the activity of functionally discrete microbial groups in vivo is to measure the expression of genes that define the function. Currently there are few published studies of gene expression in rumen microorganisms under either in-vitro or in-vivo conditions (Bera-Maillet *et al.*, 2009; Krause *et al.*, 2005; Guo *et al.*, 2008). Gene expression can be sensitively analyzed by amplification of messenger RNA (mRNA) using reverse transcription-PCR (RT-PCR) to produce cDNA as template for further analysis. Metatranscriptomics is an emerging technology which is used to assess what genes are globally expressed in a microbial ecosystem by analysis of all the mRNAs in the sample and has been applied recently in the rumen of muskoxen and the human gut (Gosalbes *et al.*, 2011; Qi *et al.*, 2011). To date, only a few studies using functional metagenomic approaches have been reported for rumen

microorganisms and primarily for investigating the genes involved in polysaccharide degradation (Ferrer *et al.*, 2005, 2007), lipases (Liu *et al.*, 2009; Bayer *et al.*, 2010), polyphenol oxidase (Beloqui *et al.*, 2006), an enzyme capable of degrading an halogenated organophosphorus insecticide (Math *et al.*, 2010), phytases (Huang *et al.*, 2010) and novel enzyme activities (Zhu *et al.*, 2007). We are currently applying a metatranscriptomic approach to evaluate the genes that are upregulated in response to inhibition of methanogenesis in the rumen (see Mitsumori *et al.*, 2011).

Fuelled by the rapidly growing genomic DNA sequence database, proteomics (the large scale analysis of proteins) has become one of the most important disciplines for characterizing gene function, for building functional linkages between protein molecules, and to provide insight into the mechanisms of biological and regulatory processes in a high throughput mode. It is now possible to examine the simultaneous expression of more than 1 000 proteins using mass spectrometry technology coupled with various separation methods. Genomics decodes sequence information of an organism and provides a “parts catalogue” while proteomics attempts to elucidate the functions and relationships of the individual components and predict outcomes of the modules they form on a higher level (Kolmeder *et al.*, 2012).

Rumen microbial manipulation

Technologies that are currently used to modify rumen function or are the focus of recent research include, antimicrobial compounds, probiotics, plant extracts and inoculants consisting of natural or genetically modified rumen microorganisms (GMOs) and vaccines.

Anti-microbial agents and microbial feed additives: the application of antibiotic or antimicrobial feed additives to enhance animal growth rate and feed efficiency was established some 50 years ago and today is widely practiced in the livestock industry. Several possible mechanisms have been proposed to explain the growth-promoting effects of antimicrobial feed additives, all of which involve the gut microbiota and their interaction with the host animal. The use of antibiotics to manipulate the intestinal microbiota and their interaction with the host animal has a number of disadvantages. Firstly, broad-spectrum antibiotics can inhibit beneficial indigenous bacteria. Secondly, consumers are concerned about antibiotic residues in animal products and development of antibiotic resistance in foodborne pathogens. This could potentially result in untreatable human disease from antibiotic resistant pathogens. Thus there is much interest in developing alternative, non-antibiotic strategies using probiotic bacteria, bacteriocin-like compounds and plant extracts to enhance health and performance of livestock as well as improving food safety.

Several excellent reviews on anti-microbial agents and microbial feed additives for ruminants have already been undertaken (Nagaraja, 1995; Newman and Jacques, 1995; Newbold, 1995; Calsamiglia *et al.*, 2007). These agents are used mainly in cattle production systems. Ionophore antibiotics are the most common anti-microbial agent used in ruminant production, and improvements in feed conversion efficiency and growth are attributed mainly to changes in the structure of the microbial community to Gram negative bacteria and shift in fermentation to greater amounts of propionate production. Monensin has gained wide acceptance but other ionophores in use include lasalocid, laidlomycin, lysocellin, narasin, salinomycin and tetronasin. In general terms, ionophore antibiotics disturb the flow of cations across the cell membrane of gram positive bacteria thus producing a bacteriostatic effect which alters rumen microbial populations and fermentation patterns. The primary changes in rumen function due to ionophore feeding are:

1. increased propionate and decreased methane production;
2. decreased proteolysis and deamination of amino acids; and
3. decreased lactic acid production and froth development.

These ruminal effects improve productivity through increased efficiency of energy and nitrogen metabolism in ruminal disorders associated with grain feeding. However the effect of monensin on rumen microbial populations is still unresolved and needs further investigation (Weimer *et al.*, 2008). Monensin has resulted in improvements in microbial protein synthesis and nitrogen digestion in sheep fed concentrate diets and these responses are associated with decreased rumen protozoa numbers (Rogers *et al.*, 1997; Garcia *et al.*, 2000). While there is growing resistance to the use of ionophores due to the concerns about the development of antibiotic resistance, there is a strong view that ionophores

do not contribute to resistance associated with important antibiotics used in human medicine (Callaway *et al.*, 2003). However ionophores (like the other antibiotics used as growth promoters) have been banned in Europe since 2006.

Plant extracts: essential oils from plants are receiving considerable attention as alternatives to growth-promoting rumen modifiers and antibiotics since the introduction of the ban on antibiotics as feed additives by the European Union (Calsamiglia *et al.*, 2007). A range of plant essential oils including garlic oil, cinnamon oil, eugenol, capsaicin, anise oil have all demonstrated beneficial effects on rumen fermentation that may improve productivity or reduce methane production but these results need to be confirmed in vivo under commercial production conditions (Castillejos *et al.*, 2007).

Bacteriocins: the production of antagonistic substances is a common component of intermicrobial competitive interactions and many of these substances function in the regulation and control of microbial populations. One class, the bacteriocins, is a heterogeneous group of small proteins that often display a high degree of target specificity, although some have a wide spectrum of activity (Kalmakoff *et al.*, 1997). Antagonistic bacteriocin-like activity between *Ruminococcus albus* and *Ruminococcus flavefaciens* was demonstrated in mixed fibrolytic cultures growing on cellobiose and cellulose by Odenyo *et al.* (1994). A series of surveys showed an unusually high incidence of bacteriocin production among rumen bacteria in general and by *Butyrivibrio fibrisolvens* in particular (Kalmokoff and Teather, 1997). Three unique bacteriocins have been purified and characterized from strains of *Butyrivibrio fibrisolvens* and *Streptococcus bovis* (Kalmokoff and Teather, 1997; Kalmakoff *et al.*, 1999; Whitford *et al.*, 2001), demonstrating that rumen bacteria produce all three known types of bacteriocins. This research demonstrated that bacteriocins have potential for the manipulation of rumen fermentation but there have been few in-vivo trials to demonstrate their efficacy.

Probiotic bacteria: bacterial pathogens associated with the consumption of contaminated meat products have become a serious concern in animal agriculture. Unique serotypes of *E. coli* such as O157:H7 have resulted in haemorrhagic colitis and haemolytic uremic syndrome in humans (Griffin, 1995). Non-pathogenic *E. coli* isolated from cattle and used as probiotics reduced the level of carriage of *E. coli* O157:H7 in experimentally infected animals (Zhao *et al.*, 1998; Zhao *et al.*, 2000). Similarly, dosing cattle with *Clostridium*, *Lactobacillus*, *Enterococcus* and other lactic acid bacteria reduced shedding of *E. coli* O157:H7 in ruminants (Takahashi *et al.*, 2000; Lema *et al.*, 2001).

Inclusion of strains of *Saccharomyces cerevisiae* and *Aspergillus oryzae* in the diet has stimulated the total number of rumen bacteria and numbers of cellulolytic rumen bacteria. This has resulted in increased rates of digestion but has not consistently resulted in production responses with all diets (Newbold, 1995; Jouany *et al.*, 1998; Corona *et al.*, 1999).

Inoculants of natural ruminant microorganisms: the introduction of naturally occurring ruminal organisms into the ruminant gut has been investigated in the context of protection from plant toxicity, decreased susceptibility to rumen acidosis, improved fibre digestion and control of the shedding of pathogenic gut bacteria.

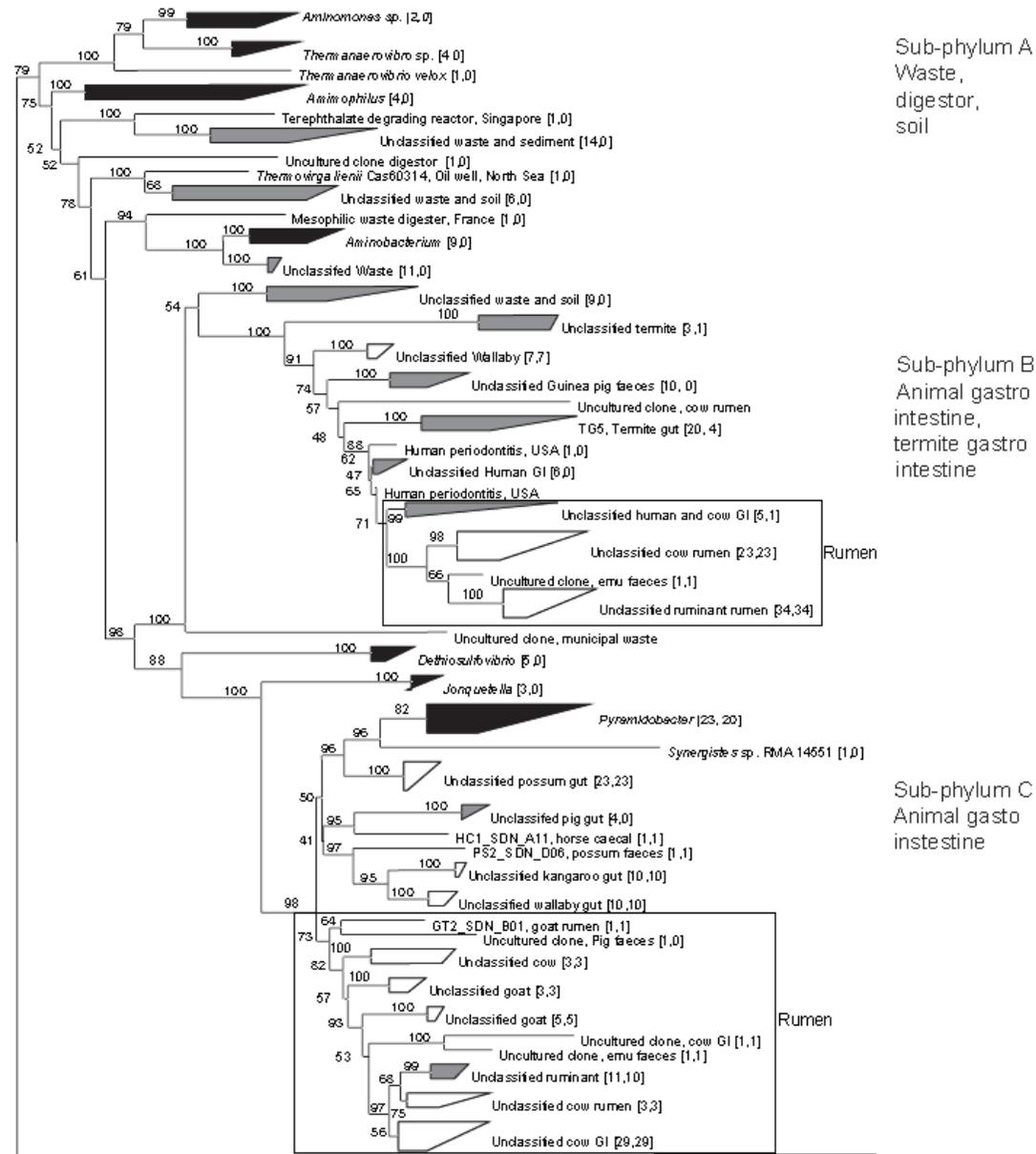
Plant toxicity: the most notable example of ruminal modification with a natural bacterium is the use of the ruminal bacterium *Synergistes jonesii* to detoxify the tropical browse legume *Leucaena leucocephala* (Jones and Megarrity, 1986). It was demonstrated that ruminants in several populations on different continents harboured bacterium (*S. jonesii*) which is capable of degrading the leucaena toxin, mimosine (Jones, 1981; Jones and Lowry, 1984). However, this organism was not present in ruminant populations from other geographic regions, but could be introduced to these animals (Jones and Megarrity, 1986; Quirk *et al.*, 1988). Recent research in the CSIRO laboratories has demonstrated that the species *S. jonesii* is comprised of different strains that are genetically distinct based on the geographic location and ruminant animals which harbour these organisms. Variations in the 16S rRNA gene sequence for this species of bacterium are shown in Table 9. It is planned to isolate strains from these geographic regions and evaluate the variation in ability to degrade the leucaena toxin which will provide further evidence that the genetic diversity can be identified and used to improve productivity. The ecology of *S. jonesii* is remarkable in that the organism appears to be transferable between cattle, sheep and goats and can establish in the rumen after being cultured in the laboratory (Jones and Megarrity, 1983; Allison *et al.*, 1990). Many other attempts to colonise the rumen with different genera of laboratory strains of bacteria have failed and thus the microbial ecology of mimosine

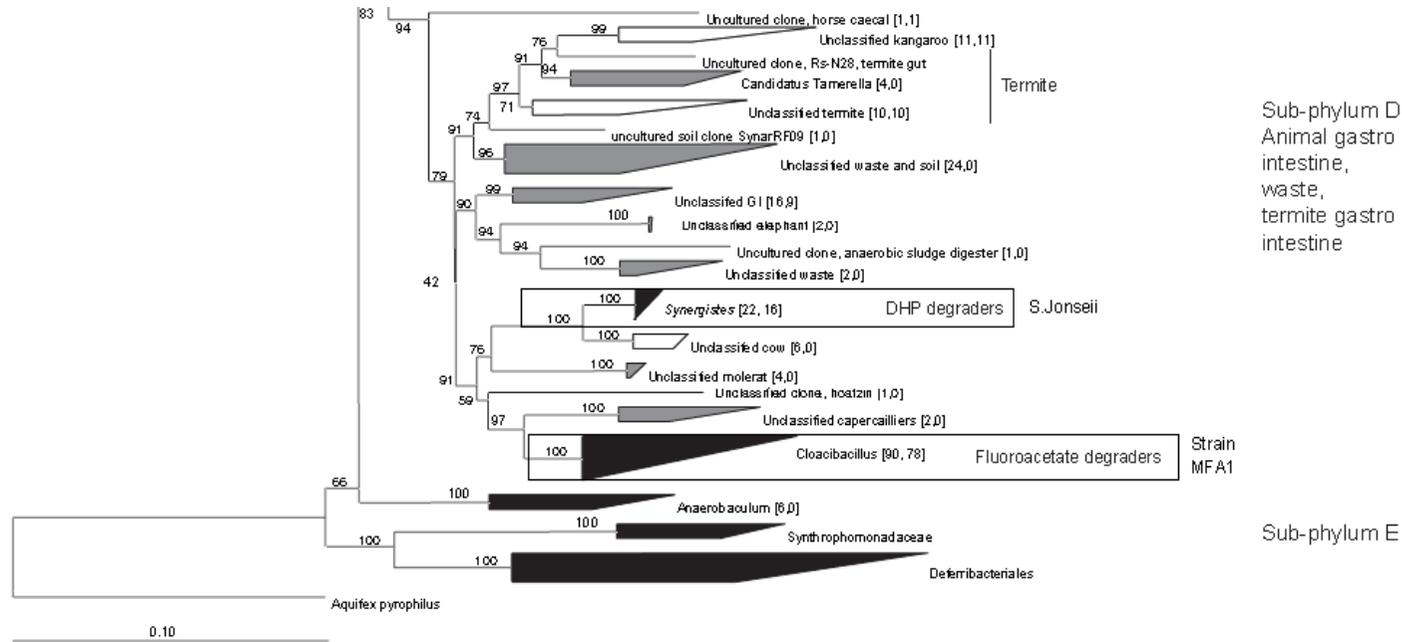
detoxification may be exceptional. The remarkable success of the use of inoculants of *S. jonesii* to protect ruminants from leucaena toxicity has encouraged researchers to use this approach for other plant related toxicities. Recently a *Synergistes* bacterium that is closely related to *S. jonesii* has been identified in ruminants and other herbivores and is capable of degrading the plant toxin fluoroacetate (Figure 6; Davis *et al.*, 2012).

Table 9. 16S rDNA Sequence variations to *S. jonesii* in ruminants in different geographical regions

Sequence position	ATCC (strain 78-1)	Thangool (Australia)	other Australian cattle	Thai buffalo
192	A	C	-	-
196	C	T	-	-
198	T	A	-	-
234	A	G	-	G
256	G	T	-	-
272	A	G	-	-
335	G	A	-	-
348	T	C	-	-
363	C	T	-	-
369	T	G	-	-
382,383	CG	GA	-	-
399	A	C	-	-
403	A	G	-	-
497	A	C	-	G
506	G	T	-	-
512,513	AA	CC	-	-
518,519,520	CAG	TGA	-	-
533	T	C	-	-
543,544	TT	CG	-	-
553,554,555	TAA	CGG	-	-
775	A	-	-	C
852	A	-	-	G
905	T	-	-	G
907	C	-	A	-
910	T	-	G	-
913	C	-	T	-
915	T	-	G	-
916	G	-	A	-
918	A	-	G	-
919	A	-	T	-
920	A	-	G	-
922	A	-	G	-
924	T	-	G	-
927	A	-	G	-
928	A	-	G	-
929,930	AG	-	TA	-
936	T	-	C	-

Figure 6. Phylogenetic tree of the *Synergistetes* phylum and placement of *S. jonesii* and *Synergistes*





This bacterium is being investigated for use as an inoculant to protect grazing ruminants from toxicity in northern Australia. The discovery of fluoroacetate degrading bacteria in the gastro-intestinal tract of herbivores including ruminants is an exciting development because previously it was suggested that anaerobic bacteria of this phenotype did not exist in nature. This observation led to a large research program on developing genetically modified fluoroacetate degrading rumen bacteria that could be used as inoculants to protect animals at risk from poisoning from this compound.

Ruminal disorders: in acute lactic acidosis, inoculation of ruminants with lactate-utilising bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminantium* and starch degrading bacteria that may compete with *Streptococcus bovis* has been suggested and evaluated successfully by reducing lactate concentration and pH with *Megasphaera elsdenii* NCIMB 41125 (Kung and Hession, 1995; Owens *et al.*, 1998, Meissner *et al.*, 2010). The use of *Prevotella* strains has also been considered as a preventative therapy for SARA since this Family of bacteria decreased in the sub acute form of the disease (Khafipour *et al.*, 2009). Other probiotic bacteria have also shown some efficacy in limiting acidosis (Chaucheyras-Durand and Durand, 2010; Lettat *et al.*, 2012). Control of the growth of lactate producing *Streptococcus bovis* with bacteriophages is also proposed (Klieve *et al.*, 1999). This technology has not been adopted on a wide scale due to cost and efficacy but also because monensin which is often included in feedlot diets is effective in controlling lactate producing microbial populations (Newbold and Wallace, 1988). Restrictions on the use of rumen modifiers in Europe have increased the attractiveness of probiotics as a therapy in ruminant feeding systems.

Fibre digestion: highly fibrolytic *Ruminococcus* strains have been evaluated for the ability to colonize the rumen and enhance fibre digestion when used as inoculants (Krause *et al.*, 2001a). Tracking systems based on strain-specific 16S rDNA sequences indicate that inoculated *Ruminococcus* strains did not persist for longer than 3 weeks before reaching undetectable levels. However these trials demonstrated that some improvement in digestibility of cellulose may occur when highly fibrolytic bacteria are dosed into the rumen.

Inoculants of recombinant ruminal microorganisms; a most successful project involving recombinant ruminal bacteria involved reducing toxicity from forage plants that contained fluoroacetate. A gene encoding a dehalogenase for fluoroacetate from the soil bacterium *Moraxella* species has been introduced into *Butyrivibrio fibrisolvens* OB156 and AR14 (Gregg *et al.*, 1994). The modified organisms detoxified fluoroacetate (Gregg *et al.*, 1994) and survived in the rumen of sheep for five months without loss of the gene (Gregg, 1995; Gregg *et al.*, 1996). Similar results have been confirmed in experiments with cattle (Padmanabha *et al.*, 2004). However, these experiments demonstrated that the population of the GMO differed between animals and fluctuated substantially within an animal from day to day.

A major research effort was directed at constructing recombinant ruminal bacteria with enhanced fibre degrading capacity more than 10 years ago. *Butyrivibrio fibrisolvens* H17c was transformed with a plasmid containing a xylanase gene from *Neocallimastix patriciarum* (Gilbert *et al.*, 1992; Xue *et al.*, 1995 and 1997). Although this GMO had enhanced xylan degrading capacity, it failed to establish in the rumen (Krause *et al.*, 2001b). Attempts to introduce cellulases (CelA and CelD) from *Neocallimastix patriciarum* (Xue *et al.*, 1992a, b; Denman *et al.*, 1996) and *Ruminococcus albus* (Vercoe and Gregg, 1995) and an acetylxylan esterase from *Neocallimastix patriciarum* (Dalrymple *et al.*, 1997) into *Butyrivibrio fibrisolvens* using the same strategy were not successful at the time.

One approach to supplementing diets of dairy cattle with limiting amino acids is to use recombinant DNA technology to engineer stable helical peptides enriched in essential limiting amino acids in bacteria. Using rDNA technology to create a gene encoding for a protein enriched in leucine (Leu), lysine (Lys), methionine (Met) and threonine (Thr), Beauregard *et al.* (1995) expressed a fusion protein in *E. coli* that was helical in structure, had the predicted molecular weight of 11kDa and the expected content (57%) of the essential amino acids (Met, Thr, Leu and Lys). This technology could potentially be used as a means of delivering peptides enriched in limiting amino acids for dairy cows as intracellular storage peptides in engineered rumen bacteria.

Although there has been a substantial research effort during the last decade, there are still major technical difficulties restricting the utility of recombinant technology for the rumen (McSweeney *et al.*

1999). Rapid progress is being impeded by a lack of transformation systems for different genera and species of bacteria (Flint, 1994), insufficient control of gene expression and efficiency of enzyme production and secretion, genetic instability; and poor competitive fitness of GMOs.

The fluoroacetate degrading GMO demonstrates that the approach is technically feasible, but environmental and regulatory concerns must be addressed if this technology is to be adopted. The spread of recombinant organisms from the target host animal to other herbivores and their impact on dietary preference and grazing behaviour grazing is of primary concern.

Most of the recombinant DNA approaches to modifying the rumen are speculative in nature and will be technically and ecologically challenging as well as meeting considerable consumer resistance.

Methane mitigation strategies for ruminant production systems: Compounds that inhibit methanogenesis in the rumen have been extensively reviewed (see Nagaraja *et al.*, 1997; Cottle *et al.*, 2011). These agents have three different modes of action:

1. selective inhibition of specific microorganisms;
2. alternative electron acceptors which reduce the extent of CO₂ reduction to methane; and
3. inhibition of the enzymes involved in the methyl transfer reaction.

It has been demonstrated that the DNA polymerases in Archaea can also be specifically inhibited by chemicals (Ishino *et al.*, 1998). Ionophores and defaunating agents are specific inhibitors of Gram positive bacteria and protozoa respectively and one of the indirect responses is a reduction in methane production. These agents also have effects on nitrogen metabolism and the pathways of volatile fatty acid production in the rumen. It is therefore difficult to apportion animal production responses to changes in methanogenesis *per se* in animal trials which involve these agents. Similarly, agents such as unsaturated long chain fatty acids, nitrate and sulfite which act as alternative electron acceptors to CO₂ also have broader effects on rumen metabolism that are not specific to methanogenesis. Therefore animal trials involving agents which specifically inhibit enzymes involved in methane production (e.g. bromochloromethane) are probably the most reliable for interpretation of the effects of inhibition of methanogenesis on digestive and animal performance parameters (McCrabb, 2000; McCrabb *et al.*, 1997, Tomkins and Hunter, 2004; Tomkins *et al.*, 2009; Mitsumori *et al.*, 2011). These studies indicate that improvements in animal productivity are variable. If increased growth rates and further improvements in feed efficiency are to be achieved, the mechanisms that causes reduced feed intake in animals treated with methane inhibitors needs to be determined. One possible mechanism is that inhibition of methanogenesis could result in the accumulation of H₂ in the rumen with detrimental effects on microbial metabolism.

Other functional groups of microbes in the rumen can also have an influence on methane production either because they are involved in hydrogen metabolism or because they affect the numbers of methanogens or other members of the microbiota which produce large amounts of H₂. Research approaches including vaccination, enzyme inhibitors, phage, homoacetogens, defaunation, feed supplements, use of plant bioactive compounds such as tannins and saponins, and animal selection are currently being investigated (Cottle *et al.*, 2011). It is likely that more than one strategy will be required to enable ruminant production systems to lower methane emissions significantly, and different mitigation strategies may be suitable for different farming practices and systems. It should also be noted that any strategy aiming at improving the animal productivity will lead to a decrease in methane production per kg of animal product.

Recent studies using denaturing gradient gel electrophoresis and qRT-PCR analysis indicate that supplementation of diets with dry corn distillers grain with solubles, condensed tannin, extruded linseed alters the diversity of rumen methanogens without affecting total methanogen numbers (Mohammed *et al.*, 2011; Popova *et al.*, 2011). By contrast supplementation of cattle with soya oil resulted in decrease in abundance of methanogens but diversity did not change (Lillis *et al.*, 2011). Treatments with cinnamaldehyde, garlic oil or juniper berry oil did not alter abundance of methanogens but diversity of *Methanosphaera stadtmanae*, *Methanobrevibacter smithii* and some uncultured groups of archaea changed (Ohene-Adjei *et al.*, 2008). The authors noted that shifts in the diversity of methanogenic archaea produced with the essential oil supplementation might have been

associated with changes in associated protozoal species. Changes in diversity of the rumen methanogen population are likely to also influence the amount of methane produced.

Research carried out on different mitigation strategies for reducing methane emissions from dairy cattle with an expected timeline for development is summarized in Table 10.

Table 10. Methods of reducing methane emissions from dairy cows and expected timeline

Timeline for development	Mitigation practice for the dairy industry	Expected reduction in methane (%)
Immediate	Feeding oils and oilseeds	5–20
	Higher grain diets	5–10
	Using legumes rather than grasses	-15
	High sugar grasses; high digestible grasses	5–10
	Using corn silage or small grain silage rather than grass silage or grass hay	5–10
	Ionophores	5–10
	Herd management to reduce animal numbers	5–20
	Best management practices that increase milk production per cow	5–20
5 years	Rumen modifiers (yeast, enzymes, directly fed microbials)	5–15
	Plant extracts (tannins, saponins)	5–20
	Animal selection for increased feed conversion efficiency	10–20
10 years	Vaccines	10–20
	Strategies that alter rumen microbial populations	30–60

Source: reproduced from a 2011 presentation by Beauchemin and co-workers (Agriculture and Agri-Food Canada).

Lignocellulose and biomass degradation : Rumen microbes are a rich source of plant polysaccharide degrading enzymes, and the Hungate 1 000 project described above offers the possibility of identifying novel enzymes for the degradation of lignocellulose, and improving our understanding of biomass degradation processes in nature. Many of the specialized plant cell wall degrading bacteria from the rumen (e.g. *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*) have evolved genomes containing large sets of genes that code for a wide diversity of polysaccharide-hydrolyzing enzymes. These enzymes termed glycosyl hydrolases include endoglucanases, exoglucanases, and cellobiohydrolases, which acting in concert can hydrolyze cellulose to glucose, and also xylosidases, and xylanases, which together can hydrolyze xylan to xylose. Interestingly, these putative glycoside hydrolases occur in large numbers in the genomes (>100 genes per genome) and are often in clusters or operons. Bioinformatic analysis of this complement of glycosyl hydrolases from sequenced genomes using the Carbohydrate-Active Enzyme (CAZy) database, followed by overexpression of the protein and subsequent biochemical characterization has been a productive approach to analyzing their diversity and function (Dodd *et al.*, 2010; Moon *et al.*, 2011; Kabel *et al.*, 2011). Other approaches have involved functional screening of clone libraries (Findley *et al.*, 2011) as well as high throughput screening of metagenomic sequences retrieved by direct sequencing of DNA from fiber incubated in the rumen (Hess *et al.*, 2011).

Vaccination: vaccination against rumen microorganisms has been the focus of several research groups over the last decade. This approach is an appealing concept for manipulating the rumen microbiota because of the ease of integration with management and a relatively low cost of treatment and is therefore dealt with here in detail. There is also considerable benefit for extensive grazing systems, because of the potential for a single treatment or limited number of booster injections to provide longevity of action. Williams *et al.* (2008) examined changes in rumen protozoal numbers in sheep that were vaccinated with two protozoal formulations containing either whole fixed Entodinium or mixed rumen protozoa cells with the aim of decreasing the number and/or activity of protozoa in the rumen. However, rumen protozoa were not decreased by the vaccination and there was also no difference between treatments in rumen fermentation and production responses. Serum antibodies raised to ciliate protozoa had an immobilizing effect on a mixed rumen ciliate population *in vitro* but

the amount of antibody generated by vaccination was inadequate for a response *in vivo*. It is likely that the vaccine could be improved if specific protozoal antigens were identified and used.

Vaccination with a multivalent polyclonal antibody preparation against *Streptococcus bovis* reduced the effects of lactic acidosis (Gill *et al.*, 2000; Shu *et al.*, 2000). Similarly, preparations of polyclonal antibodies against *Streptococcus bovis* or *Fusobacterium necrophorum* were successful in reducing rumen concentrations of target bacteria and increasing pH in steers fed high-grain diets (DiLorenzo *et al.*, 2006). Vaccination against the human pathogen *E. coli* O157 with virulence factor proteins from the bacterium resulted in significantly less shedding of *E. coli* O157 from calves challenged with the organism (Potter *et al.*, 2004). In relation to reducing greenhouse emissions there has been a novel immunization approach to decrease the numbers and/or activity of methanogenic archaea in the rumen. Williams *et al.* (2009) vaccinated sheep with an anti-methanogen vaccine that was based on three strains belonging to the genus *Methanobrevibacter* and produced a 7.7 percent decrease in methane production per kg of dry matter intake. Wright *et al.* (2008) also found that less than 20 percent of the different species of methanogens detected in those sheep were closely related to the methanogens in the vaccine. On the basis of these findings, it was suggested that greater methane abatement might be possible if a greater proportion of the methanogen species/strains were targeted by the vaccine. There is thus a need for a better knowledge on the rumen methanogens (and their physiology) to target the most efficient species. In a recent report to the cattle industry in Australia, Lean *et al.* (2011) stated:

“While proof of concept has been achieved, vaccinal approaches to controlling disorders such as acidosis have not progressed greatly. The challenge in managing acidosis or reducing methane production is that effective control of the challenge relies on disposal of hydrogen into safe, efficient sinks in the rumen. In the case of acidosis, the removal of lactic acid is critical due to the very low pKa of lactic acid. However, organisms other than Streptococcus bovis have the potential to form lactic acid and the condition may reflect the generation of vaso-active substances including histamine and lipo-polysaccharides derived from the death of coliforms rather than the increased growth of Streptococcus bovis populations. Therefore, vaccination to control one population of bacteria, may not change the risk of the disorder as the problem is much more related to an abundance of one or several substrates eg starch and sugars, rather than one particular bacterial population.”

Such observations reinforce the importance of understanding the ruminal ecosystem, to ensure that new intervention strategies are appropriately directed and are effective.

In ruminant animals, all of the plant cell wall hydrolysis is carried out by symbiotic rumen microbes predominantly bacteria. The primary components of the plant cell wall are cellulose, hemicellulose and lignin. Lignin presents a barrier to hydrolysis and is not really degraded to any extent within the dynamic constraints of rumen turnover and passage. However efficient degradation of cellulose and hemicellulose occurs in this system (Krause *et al.*, 2003). Cellulose degradation is catalysed by enzymes called cellulases, amongst the most diverse enzymes catalyzing a single reaction, that hydrolyse the β -1,4-linkage between glucose residues in then insoluble cellulose molecule. The diversity of the cellulases is likely a reflection of the extreme diversity of their natural substrate, the plant cell wall. Hydrolysis of hemicellulose is carried out by perhaps a more elaborate and complex set of enzymes that include endoxylanases, β -xylosidases and a suite of debranching enzymes including arabinofuranosidases, glucuronidases and multiple esterases (Dodd *et al.*, 2011). Almost all enzymes that degrade insoluble substrates contain a substrate binding domain termed a carbohydrate binding module (CBM) which is usually linked to the catalytic domain by a flexible linker peptide (Wilson, 2011). Many aerobic microorganisms, such as the filamentous fungi, use the free cellulase mechanism in which they secrete a set of individual cellulases that act synergistically to degrade crystalline cellulose. Many anaerobic bacteria especially those in the Firmicutes, such as Clostridia, use cellulosomes and have been described in some Ruminococcus species. These are large multi-enzyme complexes comprise cohesin-dockerin domains that are highly ordered and mediate cellulosome assembly and cell surface attachment via scaffoldins. Other bacteria, especially anaerobic thermophiles, secrete large multidomain - often multifunctional - proteins that contain CBM's that are bound to the external surface of the bacterial cell wall. Current research in our laboratories with *R. albus* and *Prevotella* species suggests that this cellulolytic mechanism is common in the rumen. In addition, *Fibrobacter succinogenes*, a major and highly efficient cellulose degrader found in the rumen, appears

to use a novel mechanism that utilizes different binding domains and multiple, processive endocellulases but no exocellulase (Wilson, 2011). Because of the potential for biomass as a source of renewable fuels and chemicals there has been renewed interest in research in the area and it seems likely that this increase in our knowledge and understanding of the diversity of organisms and the molecular mechanisms employed in degrading the plant cell wall can also be used to improve and promote more efficient and sustainable forage feeding systems for ruminant animals.

VI. LOOKING FORWARD: PREPARING FOR THE FUTURE

Possible future research and main gaps in scientific knowledge

The world's livestock sector is amidst a massive transformation, fuelled by high demand for meat and milk, which is likely to double over the next two decades especially in developing countries. The major driving force behind this soaring demand for livestock products is a combination of population growth, urbanization and income growth, especially in transition economies in Asia, South America and Africa. The challenge is how to enhance animal productivity without adversely affecting the environment. The global demand for animal protein especially from the emerging economies will underpin much of the future profitability and viability of livestock production systems. The form and plane of nutrition that animals must efficiently convert into food products are two major factors limiting productivity; and there are also concerns about the significant contribution of livestock to global GHG emissions.

A large proportion of the global ruminant population is located in tropical environments, where animals feed predominantly on low quality highly fibrous forages which contain secondary plant compounds which are toxic or anti-nutritional in activity. However, in most cases the microorganisms involved have not been isolated and identified. The viability and public acceptance of these ruminant production systems is critically dependent on measurable improvements in feed digestion and nutrient retention, with reduced GHG emissions and nitrogen excretion. We believe that research focussed on rumen microbial community structure and function can advance our ability to either select, or adapt, host and/or gut microbial contributions to the efficient conversion of feedstuffs; and will drive improvements in ruminant productivity, product quality and safety, and environmental sustainability. The challenge in the next 5-10 years is for ruminant breeders, nutritionists and rumen microbiologists to work collaboratively to delivery products and services that can be used to:

1. inform government policy on ruminant GHG emissions and mitigation;
2. provide microbial and/or feed based interventions that improve feed digestion and nutrient retention by ruminants and measurably reduce GHG emissions.

The benefits will be the accelerated adaptation of global livestock industries to the key environmental and economic drivers challenging their sustainability and profitability.

For example, the potential impact of nutrient supply on host tissue metabolism and production performance is an area of animal science, which is complex, but the experiments are now being conducted on the influence of nutrients on gene expression in animal tissue function such as muscle development. At the FAO-IAEA International Symposium on gene-based technologies, held from 6 to 10 October 2003 in Vienna, Austria, it was concluded that DNA marker assisted selection of animal genotypes used in combination with conventional breeding and selection programs could be used to make rapid progress in developing superior phenotypes of production importance (Makkar and Viljoen, 2005). In several countries research programs are being developed to examine for the first time the rumen microbial community structure and function in relation to host genetics as a component of these genetic selection programs for low methane emissions or superior feed conversion efficiency. A publication by Benson *et al.* (2010) has provided clear evidence in monogastric animals for the importance of host genetic control in shaping individual gut microbiome diversity in mammals which is a key step toward understanding the factors that govern the assemblages of a gut microbiota that may be associated with superior digestive potential or reduced GHG emissions.

A priority area identified at the FAO/IAEA International Symposium on “Applications of Gene-based Technologies for Improving Animal Production and Health in Developing Countries” was the characteristic of survival and superior productivity during seasonal nutrient deprivation, which is experienced in many developing countries in the tropics. The following process was outlined as an approach to developing ruminant animals, which show superior nutritional resilience during nutrient deprivation and which could be used as a model for selecting low methane emitting ruminants and high feed conversion efficiency. The selection programme for resilience was as follows:

1. characterise local adapted breeds for “nutritional resilience” using phenotypic attributes e.g. adaptive behaviour, metabolic rate, muscle and bone structure;
2. identify the genes (QTLs, SNPs) that account for significant variance in weight loss and compensatory growth during periods of nutrient deprivation followed by improved nutrition; and
3. consider possible positive and negative implications of selecting for “nutritional resilience” e.g. toughness, feed conversion efficiency, and female reproductive performance and lactation.

Based on the advances in DNA sequencing technology and data analysis pipelines it is now possible to characterize the rumen microbiome of large numbers of individual animals in a genetic selection programme in a cost effective manner as a fourth component of the process. A similar selection program could be envisaged for linking the rumen microbiome to host genetics in relation to nutrient use efficiency such as nitrogen fermentation, microbial protein synthesis and assimilation and conservation by the animal. Studies on gastrointestinal microbial ecology should therefore focus on diversity, community structure, function, and interactions with the host in order to fully understand the biology and functioning of the intestinal ecosystem in production, health and disease.

Rapid advances are being made and future plans are in place to sequence the genomes of more than 1 000 rumen microbial isolates in order to establish a catalogue of rumen microbial genes and assign function to these genes as a framework for characterising the rumen microbiome in different ruminant genotypes and under varying dietary and environmental conditions. This work is being initiated by a consortium of advanced rumen microbiology laboratories (Table 11), large publicly funded DNA sequencing institutions, curators of international public culture collections. An opportunity exists for ruminant laboratories in emerging and developing countries to provide cultures of rumen isolates from local adapted breeds as contributions to this catalogue which will then represent a broader geographical census of microorganisms that are relevant to many agro-economic zones and environment conditions. In addition nutrition laboratories in Asia, South America and Africa (e.g. International Livestock Research Institute, ILRI) with an interest in rumen microbiology would benefit from future interactions with the advanced labs via intergovernmental exchange programmes and aid projects. Several laboratories in these countries participated in a Joint FAO/IAEA Coordinated Research Project between 2004 and 2009 on the “Development and Use of Rumen Molecular Techniques for Predicting and Enhancing Productivity” which has resulted in the establishment of rumen microbiology capability in these countries. Some of these laboratories are now in a position to take the next step in technological development through the acquisition of skills in next generation sequencing or ‘omics’ analysis of the rumen ecosystem. It is possible that this reference collection will be biased towards microorganisms from ruminants in industrialised production systems unless a co-ordinated effort is initiated to engage laboratories from countries and regions where the animals have evolved and adapted to natural environment particularly in tropical regions.

Table 11. Major laboratories with advanced capability in rumen microbiology

Institution	Lead scientist/s
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia	Dr Chris McSweeney Prof Mark Morrison
AgResearch New Zealand	Dr Graeme Attwood Dr Peter Janssen
Department of Employment, Economic Development and Innovation (DEEDI), Queensland Government/ University of Queensland, Australia	Dr Athol Klieve
University of Illinois, United States of America	Prof Isaac Cann Prof Rod Mackie Prof Bryan White
Aberystwyth University, Wales, United Kingdom	Prof Jamie Newbold
University of Aberdeen, Scotland, United Kingdom	Prof John Wallace
Hokkaido University, Japan	Prof Yas Kobayashi Prof Satoshi Koike
National institute of Livestock and Grassland Science, Japan	Dr Makoto Mitsumori
Zhejiang University, China	Prof Jianxin Liu
Chinese Academy of Agricultural Research, Beijing, China	Prof Jiaqi Wang
Khon Kaen University, Thailand	Prof Metha Wanapat
Institut National de la Recherche Agronomique (INRA), Clermont Ferrand, France	Dr Evelyne Forano Dr Diego Morgavi
University of Vermont, United States of America	Dr Andre-Denis Wright
Ohio State University, United States of America	Dr Zhongtang Yu Dr Geoff Firkins
Indian Veterinary Research Institute, Izatnagar, India	Dr Devki Kamra
U.S. Department of Agriculture, Madison, United States of America	Dr Paul Weimer
Joint Genome Institute, University of California, United States of America	Dr Edward Rubin
Beijing Genomics Institute, China	Ms Liu Ying
Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada	Dr Tim McAllister

Fundamental gaps in knowledge which still exist but can be resolved with the new technologies that are available to study complex microbial ecosystems like the rumen. What are the keys microbial members and functions that are required for optimal rumen fermentation in different forage based production systems? What is the variance in these populations and functions in groups of animals? Does geographical location shape the rumen microbiome and is this a significant influence on rumen function? Do some animals carry rare members of the rumen community with key functions which protect the host from toxins and metabolic disorders? Does diversity in taxonomic groups in relation to a function such as methanogenesis result in significant variance in this function between animals which can be manipulated?

Bacteria, archaea, protozoa, fungi, and viruses are all present in the rumen. They interact with each other and such interactions affect the fluxes of dietary carbon and nitrogen and thus feed efficiency and outputs of pollutant (methane and nitrogen). In future studies two or more of the above microbial groups need to be investigated simultaneously to better understand the structure and dynamics of rumen microbiome that determine feed conversion to animal products. Technologies are available that permit concurrent analysis of multiple groups of rumen microbes. Studies on rumen microbiome are currently mainly focussed on genomics of isolates and metagenomics which are important and provide information on the genetic potential of rumen microbiome. However, metatranscriptomics, metaproteomics, and metabolomics will enable better understanding on how the potential of rumen function is materialized. In addition, a few studies have shown that changes in rumen function can be

compensated by opposite changes in the large intestine (e.g. reduced fiber digestion in the rumen could be compensated by increased fiber digestion in the colon of cattle). However, it remains to be determined to what extent changes in rumen function can be compensated under certain dietary conditions, especially in degradation of fiber and proteins. Thus, it is important to investigate how rumen interventions affect the intestinal microbiome (both diversity and function) of ruminant animals.

The challenge in the next 5-10 years is for animal breeders, nutritionists and rumen microbiologists to work collaboratively to delivery products and services that can be used to:

1. inform government policy on ruminant GHG emissions and mitigation;
2. provide microbial and/or feed based interventions that improve feed digestion and nutrient retention by ruminants and measurably reduce GHG emissions; and
3. develop strategies to enhance resilience of ruminants and their adaptation to ongoing global warming.

The benefits will be the accelerated adaptation of global livestock industries to the key environmental and economic drivers challenging their sustainability and profitability.

VII. PROPOSAL FOR AN OBSERVATORY OF RUMEN MICROBIAL BIODIVERSITY

The rumen microbial ecosystem represents a resource of microbial biodiversity which is largely untapped. Our current knowledge, culture collection resource and understanding of diversity have focused primarily on domesticated ruminants belonging to the *Bos taurus* species. The growing capability in rumen microbiology based in tropical regions and developing countries provides a unique opportunity to expand our horizons to wild ruminant species and animals such as the yak, buffalo, high altitude sheep and goats which are adapted to more hostile environments but are of economic and social significance to more remote communities that rely heavily on the productivity of ruminant livestock for their well being.

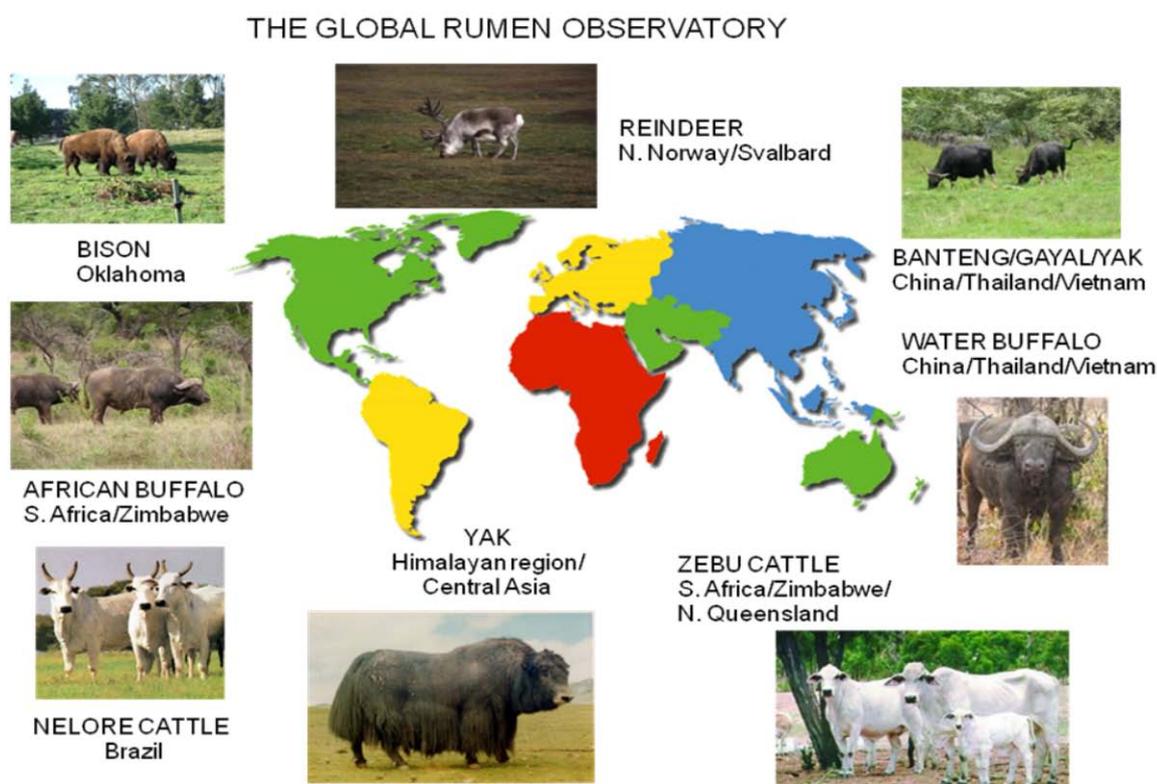
We propose that a Rumen Microbial Observatory Project involving ruminant researchers from emerging and developed economies be established to catalogue the diversity of rumen microbiota in domesticated and wild ruminant species of potential importance to farming systems. The involvement of developing country scientists focussed on diversity of indigenous ruminants and those adapted to their region would complement the existing initiatives of Rumen Microbial Diversity Network which has a stronger emphasis on domesticated ruminants in production systems within industrialized countries. Despite the wealth of biodiversity in ruminant animals, the study of rumen microbiology has been confined to studies of domesticated ruminants (mainly cattle, sheep and goats) in developed regions of the world with a tradition for the highly specialized techniques required for detailed analysis of this anaerobic ecosystem. In addition, virtually all published work is based on pen fed situations using total mixed diets containing various proportions of readily fermentable carbohydrate rather than grazing studies. In addition, many of these studies require the use of ruminally cannulated animals allowing frequent and reproducible access to the rumen in order to obtain timed samples. It can be stated unequivocally that most data and information obtained over the past 50 years is biased and has only sampled a very narrow proportion of the natural microbial diversity within these gut ecosystems. We hypothesize that the rumen microbial populations of these divergent ruminant species represents a special case for convergent evolution. However no comprehensive studies or analysis of the microbial populations at the cell and gene level have carried out to test this hypothesis. Thus we propose that a research programme based on a Global Rumen Microbial Observatory be established to systematically study this intriguing part of biology and biodiversity. This global study would require involvement and participation by an international group of scientists especially including those from less developed countries of the world that have been traditionally underrepresented in these endeavours so far. Furthermore co-ordination and involvement of scientists from developing countries would also provide an opportunity for a training program in both culture based anaerobic techniques and molecular analysis of complex microbial

ecosystems which employ the latest high through-put sequencing platforms. Involvement of international organizations such as FAO, ILRI, World Bank would be highly desirable to:

1. identify target countries and agro-economic regions for inclusion in the Rumen Microbial Observatory Project;
2. mobilize resources;
3. co-ordinate capacity-building activities; and
4. provide guidance on matters related to the conservation and sustainable use of micro-organisms and to the manipulation of rumen microbiota for enhancing digestion of feed in the rumen.

ILRI already has a preserved collection of rumen samples from a large numbers of ruminant animal species in Africa which may still be available for use in this project. An example of a simplified Global Rumen Microbial Observatory is presented in Figure 7 below. To make the best use of such an observatory, however, other important information should be included, such as breed or genetics of the host animals, feed and feeding, chemistry of the rumen, and geographic information.

Figure7. The Global Rumen Observatory



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