13 Rumen Microbiome and Plant Secondary Metabolites (PSM): Inhibition of Methanogenesis and Improving Nutrient Utilization

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Abstract

Plants contain a large number of secondary compounds which are not required for the primary activities of plants but act as a defense against pathogenic microbes and dust particles. These plant secondary metabolites (PSM) include saponins, tannins, essential oils, alkaloids, terpene compounds, etc. These PSM have strong anti-methanogenic activity, and a few of them have also fiber degradation stimulating activity, but many of these have no effect on feed degradation or have an adverse effect on nutrient release. A proper combination of these PSM might have a balanced activity against methane inhibition and improve fiber degradation, making the process of livestock production economic and eco-friendly.

Keywords

Rumen microbes · Diversity · Methanogenesis · Improving fiber degradation · Saponins · Tannins · Essential oils

13.1 Introduction

There are several thousands (about 200,000) of secondary metabolites are present in plants, which protect them from the invasion of foreign particles and pathogenic microbes. Primary metabolites of plants are directly involved in the growth and development of a plant, while secondary metabolites are compounds produced in other metabolic pathways and are not essential to the functioning of the plants.

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These compounds are synthesized during secondary metabolism. Therefore, these are classified as plant secondary metabolites. The major plant secondary metabolites are saponins, tannins, essential oils, and alkaloids.

13.2 Plant Secondary Metabolites (PSM)

13.2.1 Saponins

The naturally occurring chemicals are found in plants, which have soap-like qualities and produce lather with water just like detergent, and are present in more than 100 families of plants. In the digestive tract, saponins produce an emulsification of fat-soluble molecules. Specifically, saponins bind to bile acids and help eliminate them from the body, preventing cholesterol from being reabsorbed. In addition to this, saponins can help boost the immune system, exhibit an antioxidant effect, and may even support bone strength. Saponins have several qualities that act against cancer cells. In particular, some saponins have an antioxidant effect and may be directly toxic to cancer cells. Other beneficial characteristics of saponins include encouraging normal detoxification. Saponins found in oats and spinach support digestion by accelerating the body's ability to absorb calcium and silicon. They modify ruminal fermentation by suppressing ruminal protozoa and selectively inhibiting some bacteria. The symbiosis of protozoa with methanogenic bacteria in the rumen is well established, and the selective suppression of protozoa has been suggested to be a promising approach to reduce methane production.

Using *Yucca* as a source of saponins, Sliwiński et al. (2002) did not record methane reduction, while a decrease was observed in other studies. On the other hand, Holtshausen et al. (2009) at 1% of *Yucca* extract in the diet (saponin content 0.06% in diet) did not observe a reduction in methane production. The saponin content in the *Yucca* extract used in the study is not given. Although *Yucca* extracts used in the studies of Santoso et al. (2004) and Holtshausen et al. (2009) were obtained from the same commercial company, the products used could be different. However, it may be noted that Holtshausen et al. (2009) used 100-fold higher amount of the extract than that used by Santoso et al. (2004), and also in the former study, saponins used were fivefold higher than the amount of the extract, but no methane reduction was observed. Although these results are difficult to explain, the difference in effects could be due to different diets used; effects might be higher for silage-based diet used by Santoso et al. (2004). Hess et al. (2004) used dried fruits of *Sapindus saponaria* and recorded a decrease in methane production. The level of saponins in the diet in this study was 0.75%, which is much higher than the levels of *Yucca* extracts/saponins that elicited methane reduction.

Saponins are reported in plants like soapnut/soapwort and a few others. The saponins are soluble in water and form foam. These contain glycosides, steroid, and terpenoid glycosides as one of the major constituents. It can act as a detergent and a fire extinguisher. These consist of a polycyclic aglycones, which are either choline steroids or triterpenoids which are attached with C3 and ether bonds to sugar side

chains (Güçlü-Ustündağ and Mazza 2007). Some of the saponins reduce feed intake and growth rate of nonruminant animals, while others are not very harmful.

Saponins are not harmful in tropical forage legumes, but they are common in several temperate forage legumes. Alfalfa contains several saponins (medicagenic acid, soyasapogenol A, soyasapogenol B, lucernic acid), and the seeds and foliage of chickpeas (*Cicer arietinum*), soybeans, and common beans contain saponins. *Yucca* contains sarsaponins and are occasionally grazed by cattle. In monogastric animals like poultry and swine, poisoning is reported due to saponins: irritated mucous membranes of the mouth and digestive tract, reduced feed intake, decreased performance, anorexia, weight loss, rough hair coat, gastroenteritis, diarrhea, and possibly abortion. On consuming fresh alfalfa, saponins cause bloat in ruminants. Bloat only occurs in animals grazing temperate legumes that contain saponins but not in livestock grazing tropical legumes or temperate legumes like bird's-foot trefoil that does not contain saponins. However, low-saponin cultivars of alfalfa can cause bloating.

13.2.2 Tannins

Tannins are defined as phenolic compounds of high molecular weight ranging from 500 Da to more than 3000 Da which are found in plant leaves, bark, fruit, wood, and roots. These are used in making leather, and gallic acid is employed in the production of inks. Tannins in tea give the beverage its astringency. Teas with high levels of tannins have a bitter taste especially in green and black tea. Tannins also remove harmful microbes from the body and fight against harmful bacteria, viruses, and fungi. On regular use, tannic acid can cause side effects such as stomach irritation, nausea, vomiting, and liver damage.

Tannins are active for plant defense mechanisms against mammalian herbivores, birds, and insects (Hassanpour et al., 2011). Tannins are soluble in water (20–35°C), leaving aside high molecular weight tannins. These are water-soluble polyphenolic substances and have the ability to bind with proteins that form insoluble or soluble tannin-protein complexes. Tannins are able to make complexes with polysaccharides (cellulose, hemicelluloses, and pectin) and nucleic acids, steroids, alkaloids, and saponins (Chaichi Semsari et al., 2011). Now it has also been observed that tannins have beneficial effects on animals in having antimicrobial and anthelmintic effects in ruminants (Hassanpour et al. 2011).

As per chemical structure and properties, tannins are divided into two main groups: hydrolysable (HT) and condensed tannins (CT) (Chaichi Semsari et al. 2011). The chemical structures of HTs (gallotannins and ellagitannins) are molecules which contain carbohydrate, generally D-glucose, as a central core (Min and Hart 2003). The hydrolysable groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Haslem 1989).

In the dry season of tropics, the quality of lignocellulosic/tanniniferous feeds is poorer, and livestock production is limited due to poor availability of nutrients,

i.e., 2.5–7.0% crude protein (dry matter basis) and low dry matter digestibility of 40–50%. The ruminants lose weight and milk production drops (Patra and Saxena 2010). Improved animal performance has been frequently reported in response to the use of high-quality tanniniferous forages as supplements for ruminants fed with low-quality roughage diets. In addition to that, moderate amounts of CTs have been reported to exert beneficial effects on protein metabolism in ruminants, decreasing rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine (Hervás et al. 2003). The CT may enable dietary protein bypass from the rumen for digestion in the lower digestive tract (Hassanpour et al. 2011). An increase in flow of metabolizable protein or essential amino acids to the small intestine has been observed in animals grazing forages of high-CT content compared to those grazing a low-CT diet (Waghorn 2008). The plant secondary metabolites have beneficial effects on protein metabolism in ruminants, decreasing rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine.

13.2.3 Essential Oils

A concentrated hydrophobic liquid extracted from plants containing aromatic compounds is known as essential oil, which is also known as volatile oils, ethereal oils, aetherolea, or simply as the oils of the plants. These oils are extracted by steam distillation and are used in perfumes, soaps, cosmetics, household cleaning products, and flavoring of foods and drinks. The essential oils are used in aroma therapy for skin treatment. Improper use of essential oils may cause harms including allergic reactions and skin irritation, and children may be particularly susceptible to the toxic effects of improper use. The essential oils are also named after the plants from which these are extracted like garlic oil, mentha oil, sweet orange oil, lemon oil, cedarwood oil, clove oil, eucalyptus oil, jasmine oil, rose oil, etc., which are used for various reasons.

Patra and Yu (2012) studied five EOs with different chemical structures, e.g., clove oil (CLO) containing eugenol (phenylpropanoid), eucalyptus oil (EUO) cineole (bicyclic monoterpenoid), garlic oil (GAO) alliin and allicin (organosulfur compounds), origanum oil (ORO) thymol (monoterpenoid monocyclic phenol), and peppermint oil (PEO) menthol (monoterpenoid monocyclic nonphenol) on different fermentation characteristics and rumen microbiome in the rumen. This study demonstrated that different EOs vary in their potencies in modulating rumen microbial populations and fermentation.

All the EOs exhibited an adverse effect on the three rumen cellulolytic bacteria, i.e., *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus*, which were significantly reduced by all EOs. The *F. succinogenes* population suffered from more inhibition than the populations of *R. flavefaciens* and *R. albus* for all EOs. The results showed that EOs could significantly decrease methane production, ammonia production, and the abundance and diversity of archaea with increasing doses. Calsamiglia et al. (2007) compiled a review on the effect of essential oils to

Table 13.1 Essential oils with antimicrobial activity, their active components, and susceptible microorganisms

Essential oil	Name	Active components	Susceptible microorganisms	References
Allium sativum	Garlic	Allicin, diallyl sulfite	Enteropathogenic bacteria	Ross et al. (2001)
Cinnamomum cassia	Cassia	Cinnamaldehyde	*Escherichia coli, Staphylococcus aureus*	–
Origanum vulgare	Oregano	Carvacrol, thymol	Gram-positive and gram-negative bacteria	Sivropoulou et al. (1996) and Dorman and Deans (2000)
Syzygium aromaticum	Clove	Eugenol	*E. coli, Staph. Aureus, L. monocytogenes, S. enteritidis, C. jejuni*	Smith-Palmer et al. (1998)
Zingiber officinale, ginger zingiberene	Ginger	Zingerone	Gram-positive and gram-negative bacteria	–

[a]*Based on* Calsamiglia et al. (2007)

study their effect on methane production and microbial ecosystem, and results are summarized in Table 13.1.

13.2.4 Rumen Microbiome

Herbivorous animals retain within their gastrointestinal tract microbiome that specialize in hydrolysis and fermentation of lignocellulosic plant biomass. The gut microflora is exceedingly diverse and contains representatives of all three domains – Eukarya, Archaea, and Bacteria.

Next-generation sequencing (NGS) and DNA microarrays have revealed that diversity and population density and range of microbial communities present in any ecosystem are much diverse and complex than explained based on customary culture-based methods and conventional molecular biological methods.

13.2.5 Conventional Techniques

The rumen microbial ecosystem consists of a vast majority of different microbes like bacteria, protozoa, fungi, bacteriophages, archaea, and mycoplasma, but a large number of them (more than 90%) are not culturable. Therefore, until now we have been playing only with less than 10% of the total microbes present in the ecosystem, while all these microbes had been active biologically in the system. Some of the genera present in the ecosystem are listed below (based on Kamra 2005).

13.2.6 Rumen Microbes

Bacteria (adapted from Kamra 2005)

Cellulose degraders: *Fibrobacter succinogenes* (*Bacteroides succinogenes*), *Ruminococcus flavefaciens, Ruminococcus albus, Clostridium longisporum, Clostridium lochheadii, Eubacterium cellulosolvens* (*Cillobacterium cellulosolvens*)

Hemicellulose degraders: *Butyrivibrio fibrisolvens, Prevotella ruminicola (Bacteroides ruminicola), Eubacterium xylanophilum, E. uniformis*

Starch degraders: *Streptococcus bovis, Ruminobacter amylophilus (Bacteroides amylophilus*), (*Bacteroides ruminicola*)

Sugar/dextrin fermenters: *Succinivibrio dextrinosolvens, Succinivibrio amylolytica, Selenomonas ruminantium, Lactobacillus acidophilus, L. casei, L. fermentum, L. plantarum, L. brevis, L. helveticus, Bifidobacterium globosum, B. longum, B. thermophilum, B. ruminantium*

Pectin degraders: *Treponema saccharophilum, Lachnospira multiparus*

Protein degraders: *Prevotella ruminicola, Ruminobacter amylophilus, Clostridium bifermentans*

Urea hydrolysers: *Megasphaera elsdenii, Micrococcus*

Acid utilizers: *Megasphaera elsdenii* (*Peptostreptococcus elsdenii*), *Wolinella succinogenes* (*Vibrio succinogenes*), *Veillonella gazogenes* (*Veillonella alcalescens, Micrococcus lactolytica*)

Oxalic acid degraders: *Oxalobacter formigenes*

Sulfate-reducing bacteria: *Desulfovibrio desulfuricans, Desulfotomaculum ruminis*

Succinic acid utilizers: *Succiniclasticum ruminis*

Lipolytic bacteria: *Anaerovibrio lipolytica*

Acetogenic bacteria: *Eubacterium limosum, Acetitomaculum ruminis*

Tannin degraders: *Streptococcus caprinus, Eubacterium oxidoreducens*

Mimosine degraders: *Synergistes jonesii*

Archaea

Archaea: *Methanobrevibacter ruminantium, Methanobacterium formicicum, Methanosarcina barkeri, Methanomicrobium mobile*

Mycoplasma

Anaeroplasma bactoclasticum, Anaeroplasma abactoclasticum

Protozoa

Rumen anaerobic protozoa (ciliates): *Isotricha prostoma, I. intestinalis, Dasytricha ruminantium, Oligoisotricha bubali*

Entodiniomorphid protozoa: *Entodinium bovis, E. bubalum, E. caudatum, E. longinucleatum, Diplodinium dendatum, D. indicum, Eremoplastron asiaticus, E. bubalus, Eudiplodinium maggii, Ostracodinium trivesiculatum, Polyplastron multivesiculatum, Metadinium medium, Epidinium caudatum, Ophryoscolex caudatus, Caloscolex camelicus*

Rumen Fungi

Rumen anaerobic fungi: *Neocallimastix frontalis, N. patriciarum, N. hurleyensis, Sphaeromonas communis* (*Caecomyces communis*), *Caecomyces equi, Orpinomyces bovis, Anaeromyces mucronatus* (*Ruminomyces mucronatus*), *Ruminomyces elegans, Piromyces communis*

The ruminants consume lignocellulosic feeds like cereal straws and stovers, sugarcane-based agricultural by-products, and mature green fodders in India and other tropical countries. The ruminants are not able to digest these feeds by themselves, but microbes (bacteria, ciliate protozoa, fungi, and archaea present in the fermentation sacs of the gastrointestinal tract like rumen, pseudo rumen, and caecum) help in digesting these feeds and convert them into volatile fatty acids (the major source of energy for the ruminants) and microbial protein which serve as nitrogen source in the ruminant animals (Kamra 2005).

13.3 Fiber Degradation and Microbial Diversity

The rumen microbiome can be studied in more details by rRNA sequencing and consists of several thousand microbes belonging to three different domains like Bacteria, Archaea, and Eukarya (fungi and protozoa). Bacteria are the most diverse and represent about 95% of total microbes (Flint et al. 2008). *Prevotella* was the predominant bacterium representing about 30% of the total rumen bacteria for cellulose degradation. The already known and the most important key fibrolytic bacteria, viz., *R. flavefaciens*, *R. albus*, *F. succinogenes*, *Butyrivibrio*, *Clostridium*, *and Eubacterium,* represented only ~2% of ruminal bacterial 16S rRNA.

Like any other microenvironment, the rumen microbial ecosystem too has more than 90% of microbes which are unculturable; therefore, till date it is not possible to understand the mechanism of its functioning, complexity, and interaction among the microbes. By advancement in molecular biology tools, like the construction of 16S rDNA or 16S rRNA libraries, metagenomics, metatranscriptomics, etc., the researchers have tried to explore the unculturable microbes of rumen. Looking at the richness of rumen microbiome in terms of diversity and microbial enzymes, the metagenomic studies on rumen microbiome are an emerging research area. The metagenomic analysis of rumen microbiome of Surti buffalo fed on four different feeding schedules using pyrosequencing has been reported by Singh et al. (2011). The sequences were analyzed using Metagenome Rapid Annotation using Subsystems Technology (MG-RAST). The distribution of phylotypes and environmental gene tags (EGTs) detected within each rumen sample was dominated by Bacteroidetes/Chlorobi, Firmicutes, and Proteobacteria in all the samples irrespective of the type of diet, and most of the genes belonged to Bacteroidetes/Chlorobi group. A report from the author's laboratory from the rumen of Murrah buffalo also showed dominance of these three phylotypes followed by Actinobacteria representing bacteria-specific EGTs (~about 80% of total EGTs) with the abundance of supporting EGTs represented by *Bacteroides* and *Ruminococcus* with very few *Fibrobacter* which is considered to be the major fiber-degrading bacteria in the

rumen (unpublished data). The Surti buffalo rumen microbiome was dominated by carbohydrate metabolism (20%) in high-fiber diet (100% roughage) and lowest (13%) in high-concentrate diet (75% concentrate) (Singh et al. 2011).

It has been observed in the literature that F/B (Firmicutes/Bacteroidetes) ratio was higher in high-fiber diet (low TDN diet). This higher F/B ratio indicated higher fiber utilization which was supposed to be due to an increase in the population density of *Ruminococcus flavefaciens*. Similarly, in our study, a numerically higher F/B ratio and higher *Ruminococcus* count (by real-time PCR) were observed with a high-fiber diet (Kala et al. 2017). The majority of bacterial genera reported were able to degrade lignocellulosic feeds. *Prevotella* was the predominant bacterium representing about 30% of the total rumen bacteria. *Ruminococcus* and *Fibrobacter* were only 2–3% of rumen bacteria community of buffaloes irrespective of the diet. Predominance of *Prevotella* and very little representation of *Ruminococcus* and *Fibrobacter* were also found in the goat rumen microbiome as reported in the literature. The already known three most important key fibrolytic bacteria, viz., *R. flavefaciens*, *R. albus*, and *F. succinogenes*, represented only ~2% of the ruminal bacterial 16S rRNA. Very little representation of these fibrolytic bacteria might be the reason for no impact of diet variation on these microbes in most of the studies.

13.3.1 Fungi

Among the rumen microbes, anaerobic fungi are the most efficient fiber degraders, in spite of the fact that fungi contribute only a small fraction of total biomass. It might be because all the fungi reported are fibrolytic, and the enzymes required for fiber degradation have high specific activities. In addition to that, the fungi make the substrate ready for degradation of feed by bacteria and protozoa. The fiber-degrading enzymes secreted by the rumen fungi are more active as compared to rumen bacteria. Only six genera of rumen anaerobic fungi have been identified so far, namely, *Neocallimastix*, *Piromyces* (previously known as *Piromonas*), *Caecomyces* (previously known as *Sphaeromonas*), *Orpinomyces*, *Anaeromyces* (previously known as *Ruminomyces*), and *Cyllamyces* (Akin and Rigsby 1987).

13.3.2 Methanogens

The archaea appear to be the most important microorganisms in the rumen as these have the capacity of converting carbon dioxide and hydrogen into methane. Only eight species of ruminal methanogens have been isolated in pure cultures: *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, *Methanoculleus olentangyi*, and *Methanosarcina barkeri* (Janssen and Kirs 2008).

The herbivore gut, especially the rumen, is essentially obligatory anaerobic fermentation chamber hosting a highly dynamic state of microorganisms (archaea,

bacteria, fungi, and protozoa) that helps in the digestion of the ingested plant biomass. The rumen microbial population is very dense, comprising of around 10^{10} bacteria/ml, 10^6 protozoa/ml, and 10^3 fungi/ml. Henderson et al. (2015) looked into the microbial diversity of the rumen from 35 countries to study the effect of diet, host species, and geography in which the animals are reared. The archaeal diversity of these animals is highly conserved which make it possible to mitigate methane emission by controlling only the dominant species of methanogens. The microbial community composition is more affected by diet (Kala et al. 2017), but the hosts are less influential in the diversity of microbes in the rumen. But by the latest technique of meta-transgenomics, 23 genera of methanogens/hydrogen utilizers have been reported, i.e., *Methanobrevibacter*, *Methanothermobacter*, *Methanoplanus*, *Sulfolobus*, *Methanosarcina*, *Methanospirillum*, *Pyrococcus*, *Methanoculleus*, *Aciduliprofundum*, *Methanoregula*, *Methanosphaera*, *Methanosphaerula*, *Methanococcoides*, *Methanocaldococcus*, *Methanocorpusculum*, *Thermoplasma*, *Methanococcus*, *Methanobacterium*, etc. In archaea, Euryarchaeota was the most predominant phylum comprising major methane-producing archaea; *Methanobrevibacter* was the most abundant genus in the rumen of buffaloes. Also, the community structure of methanogenic archaea was not influenced by TDN level in the diet (Kala et al. 2017).

There appears no correlation of the number of methanogens with methanogenesis in the rumen; it was hypothesized that more H_2 production contributes to the higher methane emissions in cattle as compared to yak (Mi et al. 2017).

13.4 Global Warming and Methanogenesis

Methane, a greenhouse gas (GHG), is emitted from different sources including natural sources, viz., wetlands, oceans and freshwater ecosystem, wild fires, and the digestive system of wild herbivores and herbivorous insects. Methane-generating agriculture activities include wild or captive animals (St-Pierre and Wright 2012), domesticated ruminants (Kelly et al. 2016; Yatoo et al. 2018), animal manure (Ozbayram et al. 2018), burning of crop residues, and paddy fields. Methanogenesis is an anaerobic respiration process that produces methane as an end product. In general aerobic respiration, the glucose is converted to CO_2, O_2, and H_2O. In contrast, during hydrogenotrophic methanogenesis, a multistep metabolic cycle, H_2 is oxidized to H^+, and CO_2 is reduced to CH_4. Notably, the methanogenic archaea live in endosymbiosis with certain ciliates in the gut ecosystem. This is because many anaerobic ciliate protozoa possess hydrogenosomes that generate molecular hydrogen that is consumed by methanogenic archaea (Lewis et al. 2018). Methanogens also thrive in the cytoplasm of anaerobic unicellular eukaryotes and in the gastrointestinal tracts of animals and humans. The protozoa-associated methanogen community is a less explored area that should be viewed as a strategy to mitigate methane emission. The methanogenesis is a low energy-yielding process catalyzed by obligate methane producers or methanogens. The energy yield is ≤ 1 ATP per methane generated (Lyu et al. 2018). Methane production through enteric fermentation is of

concern due to its contribution to the accumulation of greenhouse gases (GHG) in the atmosphere. Methane emissions from various major sources is as wetland (217 Tg), fossil fuel (96 Tg), geological and fresh water (94 Tg), gut fermentation of domestic ruminants (89 Tg), livestock manure and sewage (75 Tg), rice cultivation and plant biomass burning (71 Tg) per year.

Rumen methanogen population in mid-lactating cows maintained on a total mixed ration (forage 35%, corn silage 30%, and concentrate 35%) consisted of unassigned *Methanobrevibacter* sp. (36.6%), *Methanosphaera* sp. (26.8%), *Methanobrevibacter ruminantium* (21.9%), and *Methanosarcinales* (14.6%) (Whittford et al. 2001). The numbers of methanogens per gram of rumen contents estimated by using denaturing gradient gel electrophoresis (DGGE) and 16sRNA clone gene library analysis were found to be higher than that estimated by conventional culturing methods (10^6cells per ml) and the reports of Orpin (1988) who reported methanogen population ranging from 10^6 to 10^7 cells/ml of rumen contents. 16S rRNA gene libraries constructed from pooled PCR products obtained from rumen contents of Holstein and Jersey rumen, fed analogous diets, showed that sequences representing *Methanobrevibacter millerae* were more prominent in Jersey cattle, while *Methanosphaera* sp. and novel uncultured methanogens dominated in Holstein species. *Methanobrevibacter ruminantium* was detected in both the species (King et al. 2011). Using NGS, a core methanogenic archaea community comprising of *Methanobrevibacter* (*Mbr.*) *smithii*, *Mbr. thaueri*, *Mbr. ruminantium*, and *Mbr. Millerae* was identified in primiparous Holstein, Jersey, and Holstein-Jersey crossbred cattle (Cersosimo et al. 2016). The study shows that a core methanogenic community is present among dairy cattle breeds, which is influenced by factors such as the breed of animal and lactation stage (Cersosimo et al. 2016).

Non-dairy or beef cattle might have a difference in their methanogenic archaea. Methanogen populations were studies in Hereford-cross beef cattle fed with different sources of starch. *Methanobrevibacter*-related methanogens were found to be the predominant genera, of which *M. ruminantium* was the most abundant being 51.2%, Thermoplasmatales-related methanogens were around 37.8%, while other unassigned genera such as Methanosarcinales were around 9.4% of total methanogens (Wright et al. 2007). Two novel species of formate-utilizing archaea, viz., *Methanobrevibacter millerae* sp. nov., and *Methanobrevibacter olleyae* sp. nov., have been isolated from sheep and cow rumen (Rea et al. 2007). *Methanobrevibacter* spp. and *Methanosphaera stadtmanae* phylotypes were reported in crossbred Karan Fries cattle (Sirohi et al. 2013). Archaeal sequences corresponding to *M. Ruminantium* and *M. millerae* have been identified from rumen of yak (*Bos grunniens*) (An et al. 2005).

Water and swamp buffaloes are two important species used for milk and meat (Singh et al. 2009) production. *Methanomicrobium mobile* was found as dominating methanogen in Murrah (Chaudhary and Sirohi 2009) and Surti (Singh et al. 2012) buffaloes. *Methanobrevibacter*-related phylotypes were more common in Mediterranean buffaloes (Franzolin et al. 2012). Like other ruminants, the methanogens are reported from sheep, whose diversity varies depending on the breed and feeding habits in sheep. 16S rRNA gene sequence analysis of mixed rumen

microbiome of Marino sheep from Australia harbored *Methanobrevibacter*-related archaea, especially the *M. millerae* as predominant species (Wright et al. 2004). In another study, *Methanobrevibacter* sp., especially *Methanobrevibacter gottschalkii*, and *Methanobacterium* sp. were found in sheep (Wright et al. 2008).

13.5 Camelids and Other Non-ruminants/Pseudo-Ruminants

Camelids perform better on the vegetation of poor quality and arid tropical or arid temperate zones. In view of their characteristic dietary habits and substantial production performance, interest is in unravelling their gut microbiota. 16S rRNA analysis of fecal samples of Bactrian camels (*Camelus bactrianus*) kept in zoos revealed the presence of *Methanobrevibacter* sp. as dominating methanogens (Turnbull et al. 2012). Fecal samples of Sumatran orangutans, given frugivorous diets at the Perth Zoo, revealed the presence of 37 different methanogen-specific 16S rRNA sequences. Major methanogenic genera detected included *Methanosphaera stadtmanae*, *Methanobrevibacter smithii*, and *Methanobacterium beijingense* (Facey et al. 2012). Real-time PCR analysis showed the foregut of hoatzin (*Opisthocomus hoazin*) was found to have methanogenic *Methanobrevibacter ruminantium* (Wright et al. 2009) (Table 13.2).

Table 13.2 Diversity of methanogenic archaea in the rumen of different animals

Methanogens	Characteristics	References
Total methanogens	*Methanosphaera stadtmanae* from human feces, methanogens, *Methanobrevibacter gottschalkii*, *Methanobrevibacter thaueri*, *Methanobrevibacter woesei*, and *Methanobrevibacter wolinii* have been cultured from the feces of horse, cow, goose, and sheep, respectively	Dridi et al. (2009)
General	Domain – Archaea, phylum – Euryarchaeota, pseudomurein present in *Brevibacterium*, and *Methanobacterium* and protein in *Methanomicrobium*, heteropolysaccharide in *Methanosarcina,* and protein in *Methanomicrobium*	Balch et al. (1979)
Methanobacterium, *Methanobrevibacter ruminantium*	Rod shaped, forms methane from hydrogen and carbon dioxide, and formate as substrates	Balch et al. (1979)
Methanobacterium formicicum	Rod shaped, non-motile	Balch et al. (1979)
Methanomicrobium mobile	Rod shaped, forms methane from hydrogen and carbon dioxide, and formate as substrates	Balch et al. (1979)
Methanosarcina barkeri	*Methanosarcina barkeri* and *M. mazei* produce methane from hydrogen and carbon dioxide, acetate, methylamines, and methanol	Rouviere et al. (1983)
Methanosarcina mazei	Same as above except for hydrogen and carbon dioxide	Balch et al. (1979)

The complex microbiome of the gut ecosystem converts indigestible plant biomass to microbial proteins, short-chain organic acids, and gases such as CO_2, H_2, and CH_4. Methanogenic archaea are of particular interest as they eliminate surplus metabolic hydrogen generated during plant fiber digestion. The control of methanogenesis is because of the current need to minimize methane emission from ruminant livestock.

Methanogens are prokaryotic microorganisms of domain Archaea, which fall within kingdom Euryarchaeota (Woese et al. 1990), that produce methane as the major end product through complex biochemical pathways. The methanogens are strictly anaerobic archaea, which occupy a wide range of anoxic habitats and harsh conditions and play an important role in biogeochemical cycles with potential biotechnological applications (Sharma et al. 2018). Another concern is that methane is produced through the normal process of fiber digestion; hence, it is the wastage of energy generated from dietary forage. Methanogens reduce hydrogen levels via the production of methane, thereby stimulating food fermentation by saccharolytic bacteria. On the other hand, colonization by archaea is suggested to promote gastrointestinal and metabolic diseases such as colorectal cancer, inflammatory bowel disease, and obesity. Compared to other forms of respiration, the methanogens operate at a very low reducing potential.

Draft genome sequences of methanogens, namely, *Methanobacterium bryantii*, *Methanosarcina spelaei*, *Methanosphaera cuniculi*, and *Methanocorpusculum parvum*, represent a diverse set of isolates capable of methylotrophic, acetoclastic, and hydrogenotrophic methanogenesis. The genome analysis of these methanogenic archaea displays a shift toward energy conservation. In addition, the analysis of their membrane proteins and transporters distinguished various energy conservation modes. The analysis of the predicted membrane proteins and transporters distinguished differing energy conservation methods utilized during methanogenesis, such as chemiosmotic coupling in *Msar. spelaei* and electron bifurcation linked to chemiosmotic coupling in *Mbac. bryantii* and *Msph. cuniculi* (Gilmore et al. 2017).

The interest in methane-producing microorganisms has resulted from the fact that methane has a connection with global warming and that around 6% of the ingested fiber is wasted in the form of methane (Johnson and Johnson 1995). Geographical locations, species, and diet of animals have an impact on methanogenic populations. In addition, methanogenesis is of great concern in evolving strategies to control and manipulate its production. Rumen methanogens differ depending on diet and geographical location of the host, as does the methanogenesis. Further, it is relatively difficult to measure methane emissions from the rumen; it can be estimated from the stoichiometry of the fermentation of volatile fatty acids. The methane production of the forage-fed cattle is 1.5-fold greater than the concentrate-fed cattle.

13.6 Effect of PSM on Rumen Fungi

13.6.1 Application of Metagenomics in the Rumen

There were many questions unanswered before the advent of metagenomics like the diversity of rumen/gut of animals, their role in fiber degradation, the exact enzyme profile needed for degradation of lignocellulosic feed, generation of methane in the rumen, etc. It does not mean that all these questions have been answered, but the majority of pathways are now easy to understand.

- It is now confirmed that the earlier known fiber-degrading bacteria, like *Ruminococcus*, *Fibrobacter*, *Butyrivibrio*, etc., constituting only 3–4% of the total microbial biomass, are not the only microbes responsible for the degradation of fibrous feeds. There are several other groups like *Prevotella* and Firmicutes, contributing a significant part of total biomass, which play a vital role in fiber degradation.
- Esterases are another group of enzymes which break the bond between lignin and carbohydrates and release the latter free for degradation by cellulolytic microbes and release of energy for the utilization by the animals.
- The archaeal diversity of the ruminants are highly conserved which makes it possible to mitigate methane emission by controlling only the dominant species of methanogens.
- In the majority of the cases, there is no correlation between methanogens and methane synthesis. By the use of metagenomic techniques, it has now been confirmed that hydrogen-producing microbes determine the quantity of methane synthesis. If the hydrogen emitters are inhibited (as in defaunation), methane synthesis is reduced considerably.

13.6.2 Methanogenesis in Rumen

As discussed above, the chemical composition of plants reveals that in addition to normal constituents like cellulose, hemicellulose, soluble sugars, proteins, fats, etc., there are also some unique molecules present like saponins, tannins, essential oils, alkaloids, etc. As these molecules are not synthesized as a result of primary metabolism of plants, such compounds are classified as plant secondary metabolites, which are usually meant for providing protection to the plants against predators, pathogens, invaders, etc. Several thousands of such metabolites have been identified. Majority of these compounds fall in the category of lignins, tannins, saponins, terpenoids/volatile essential oils, alkaloids, etc. These plant secondary metabolites have antimicrobial activity, but their mechanism of action and inhibition of microbial growth are very specific, and therefore these are active against a specific group of microbes. This specificity of these plant secondary metabolites against microbial

groups can be used for selective manipulation of rumen fermentation. The methanogens which are classified as archaea have a distinctly different chemical composition of the cell walls from that of the other true bacteria present in the rumen. Therefore, there is a possibility that any one of the plant secondary compounds might act as a selective inhibitor of methanogens and can be used as a feed additive for the manipulation of rumen fermentation. The role of tannins, saponins, and essential oils has been in the inhibition of methanogens or the process of methanogenesis in the rumen.

In the "RUMEN-UP" project in Europe, 450 plants (mainly foliage) have been screened in vitro for their potential to inhibit methanogenesis by the rumen microbes at a concentration of 50 mg/500 mg of substrate incubated in 120 ml serum bottles. The selection of plants was restricted to those which are either growing or could be grown in European countries, therefore excluding most of the plants growing in the tropical regions of the world. Out of 450 plants examined in this project, 35 plants inhibited methane more than 15% and only six (*Carduus pycnocephalus*, *Populus tremula*, *Prunus avium*, *Quercus robur*, *Rheum nobile* Hook. F. and Thoms., and *Salix caprea*) more than 25% in comparison to their controls.

Another major project has been funded by FAO/IAEA Joint Division of the United Nations, which ran for 6 years (2003–2009) at eight international locations on using rumen molecular biology techniques to search for methods to inhibit methane emission by the livestock. The Indian participant of the project (IVRI, Izatnagar) worked on plant secondary metabolites to determine their potential as a rumen modifier to reduce methane emission. Various combinations of plants containing secondary metabolites have been tested in in vivo using buffalo as an experimental animal, and it has been observed that in vivo methane emission by the animal can be inhibited to the extent of 20–30% without affecting feed conversion efficiency and health of animals (Table 13.3).

13.6.3 In Vivo Feeding Trials

Most of the studies on the effect of plant secondary metabolites have been conducted in in vitro conditions. There are only a few experiments conducted to report methane inhibition in vivo. The results indicate that there are many plants which contain secondary metabolites and are active against rumen methanogenesis. Many times methane inhibition by secondary metabolites in in vitro conditions might not be translated into similar effects in in vivo conditions. This might happen due to improper selection of the dose of these metabolites in the ration of animals.

An in vivo experiment in sheep with tea saponins revealed that saponins inhibited protozoa, methane emission, and improved rumen fermentation, where the reduction of methane emission was mediated through an inhibitory effect on protozoa (Zhou et al. 2010). Some other experiments indicate positive results of including plant/plant extracts on inhibition of methanogenesis. In one experiment, *Terminalia chebula*, *Allium sativum*, and the mixture of these two plants were fed to sheep at the rate of 1% of DMI resulted in decreased ($p = 0.09$) methane production

Table 13.3 Effect of plant extracts on inhibition of in vitro methanogenesis*

Plant	Common name	Plant part	In vitro inhibition of methanogenesis (%)		
			Ethanol extract	Methanol extract	Water extract
Acacia concinna	Shikakai	Seed pulp	5.32	17.60	19.61
Allium cepa	Onion	Bulb	8.76	16.38	32.64
Allium sativum	Garlic	Bulb	61.31	69.73	19.88
Azadirachta indica	Neem	Seed cake	34.59	21.89	−14.80
Cannabis indica	Bhang	Leaves	34.42	30.67	3.33
Citrus limonum	Lemon	Peel extract	12.90	8.67	11.84
Emblica officinalis	Amla	Seed pulp	19.51	27.68	−26.69
Eugenia jambolana	Jamun	Leaves	5.61	24.27	5.66
Foeniculum vulgare	Fennel	Seed	39.42	70.72	−14.54
Mangifera indica	Mango	Leaves	23.17	35.67	9.15
Populus deltoides	Poplar	Leaves	8.49	85.86	−7.72
Psidium guajava	Guava	Leaves	81.79	9.29	9.44
Quercus incana	Oak	Leaves	−1.37	29.53	4.18
Sapindus mukorossi	Soapnut	Seed pulp	95.80	20.18	39.40
Syzygium aromaticum	Clove	Flower bud	46.96	85.61	2.37
Terminalia belerica	Baheda	Seed pulp	5.54	28.11	13.18
Terminalia chebula	Harad	Seed pulp	58.54	99.79	6.43
Trachyspermum ammi	Ajwain	Seed	42.28	−2.68	−11.35

Adapted from Patra et al. (2006)*, Agarwal et al. (2006), and *Kamra et al. 2008.

by 24, 11, and 23.5% in *T. chebula*, *A. sativum*, and the mixture of the two, respectively, when expressed as L/kg digestible DM intake (Patra and Saxena 2010). *T. chebula* is a rich source of tannin (4.89% of DM), whereas *A. sativum* is rich in essential oils. The data indicated that *T. chebula* was more effective as compared to garlic. The reason for low *A. sativum* activity might be due to the instability of allicin, the main secondary metabolite responsible for antimicrobial activity.

In another experiment, a mixture of three plants (Mix 3) fed to buffalo calves at the rate of 1, 2, and 3% of DMI resulted in a dose-dependent inhibition in methane emission (l/kg DDM) since percent inhibition increased with an increase in dose of the feed additive (Chaudhary and Sirohi 2009) without affecting dry matter digestibility at any of the levels of feed additives tested. The VFA and fiber-degrading enzyme activities were not affected, whereas there were a few changes in the rumen microbial profile as estimated by real-time PCR, but these were not responsible for any significant change in rumen fermentation. Many other experiments with growing animals have been conducted, out of which a few of these experiments have been listed in Table 13.4.

Table 13.4 Effect of plant extracts on inhibition of in vivo methane production (l/kg DDMI)

Plant	Methane inhibition (%)	Body weight gain (%)	Animal	References
Anti-methane	32	–	Buffalo	Kamra et al. (2010)[a]
Methane-suppressor	23	16	Buffalo	Kamra et al. (2012)[a]
BEO (blend of essential oils)	14.6	7.4	Buffalo	Yatoo et al. (2018)
Terminalia chebula	24.6	–	Sheep	Patra et al. (2011)
Ajwain oil and lemongrass oil in 1:1 ratio	16.7	No effect	Buffalo	Samal et al. (2016)
Garlic and soapnut in 2:1 ratio	12.9	No effect	Buffalo	
Garlic, soapnut, Harad and ajwain in 2:1:1:1 ratio	8.4	No effect		
Ficus benghalensis leaves	21.8		Sheep	Malik et al. (2017)
Artocarpus heterophyllus leaves	20.6		Sheep	
Azadirachta indica leaves	24.07		Sheep	

[a]Patents submitted

The results of experiments conducted so far with plant secondary metabolites indicate that there are some plants, which appear to have a good potential for use as a feed supplement to inhibit methane emission in the ruminants. There might be many more such plants which have not yet been tested. Therefore, screening of plants should be a continuous process to search for more useful plants, which can be used for rumen manipulation. In the secondary screening process, only selected plants should be tested in in vivo experiments to examine their potential for practical application.

13.7 Relation between Hydrogen Producers and Methanogens

Yak is a lower methane producer than cattle, in spite of the fact that both animals are fed similar diets and there are only small variations between the microbiomes of both animals. The methane and hydrogen yields in yak vs cattle are 0.26 vs 0.33 mmol methane/g dry matter intake and 0.28 vs 0.86 mmol/d hydrogen generation. Hydrogen recovery from cattle was significantly higher than that from yak (Mi et al. 2017). The relative abundance of methanogens was not different between the two animal species. It was hypothesized that more H_2 production is the reason for the higher methane emission in cattle as compared to yak. Kittlemann et al. (2013) were of the view that abundance of fibrolytic bacteria (major hydrogen producers) is related to the methanogen communities and consequently with methane production. Therefore, the abundance of methanogens does not have a direct correlation

with methane production, but the partial pressure of hydrogen is more important. Minimizing metabolic H_2 production in the rumen might reduce the availability of H_2 to methanogens. Suppression in ruminal H_2 producers is usually accompanied with a concurrent decrease in feed fermentation. This can be achieved by the intensification of propiogenesis and rumen biohydrogenation or promoting reductive acetogenesis in the rumen. Targeting H_2 utilizing protozoa or other microbes accountable for interspecies H_2 transfer to the methanogen can be a fruitful strategy to reduce methane emissions.

An Australian animal, the tammar wallaby (*Macropus eugenii*) harbors unique gut bacteria and produces 20% of the amount of methane produced by ruminants per unit of digestible energy intake. Pope et al. (2011) isolated a dominant bacterial species (WG-1) from wallaby which was affiliated to the family *Succinivibrionaceae* and implicated in lower methane emissions from starch-containing diets. Pure-culture studies confirm that the bacterium is capnophilic and produces succinate, further explaining a microbiological basis for lower methane emissions from macropodids. The abundance of WG-1 is variable in samples collected from animals in winter and spring; their results show that these bacteria will be numerically dominant when the plane of nutrition is rich in starch and soluble sugars.

As discussed above, hydrogen produced during fermentation of feed is responsible for methane production in the rumen, and the minimized metabolic H_2 production during enteric fermentation and diversion of H_2 away from the methanogenesis might result into useful energy-rich metabolites.

The most important process of methane reduction is killing of unproductive animals; this approach is neither ethical nor possible in India where the slaughter of cattle is not permissible in some of the states of country. Minimizing metabolic H_2 production in the rumen might reduce the availability of H_2 to methanogens. Suppression in ruminal H_2 producers is usually accompanied with concurrent decrease in feed fermentation and diversion of metabolic H_2 away from methanogenesis. This can be achieved by the intensification of propiogenesis and rumen biohydrogenation or promoting reductive acetogenesis in the rumen. Targeting H_2-utilizing protozoa or other microbes accounts for interspecies H_2 transfer to the methanogen which could be a good alternate for eradicating enteric methane emission. However, a significant reduction in rumen ciliates might lead to reduced fiber degradation. Another important way to tackle the emission of methane is to directly target rumen archaea through various approaches. By doing so, the enteric methane emission will decrease, and additional H_2 will also be adequate to stimulate alternate hydrogenotrophic pathways, i.e., reductive acetogenesis.

13.8 Future Perspectives

Although a large number of plants have been tested for their potential to reduce methanogenesis and/or increase degradability of feed under in vitro conditions, a few of them either alone or in combination have been evaluated in in vivo conditions and have been found that these plants containing secondary metabolites can cause

20–30% decrease in methane emission and result in improved growth rate of animals by 8–10% as compared to control animals. Therefore, further studies are required for screening more plants and feeding them in large number of animals for a longer duration for practical application to achieve eco-friendly and economic livestock production.

References

Agarwal N, Kamra DN, Chaudhary LC, Patra AK (2006) Effect of *Sapindus mukorossi* extracts on *in vitro* methanogenesis and fermentation characteristics in buffalo rumen liquor. J Appl Anim Res 30:1–4

Akin DE, Rigsby LL (1987 Sep) Mixed fungal populations and lignocellulosic tissue degradation in the bovine rumen. Appl Environ Microbiol 53(9):1987–1995

An D, Dong X, Dong Z (2005) Prokaryote diversity in the rumen of yak (Bos grunniens) and Jinnan cattle (Bos taurus) estimated by 16S rDNA homology analyses. Anaerobe 11(4):207–215. Epub 2005 Mar 27

Balch WE, Fox GE, Magrum LJ (1979) Methanogens: reevaluation of a unique biological group, *Microbiol*. Rev 43(2):260–296

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A (2007) Invited review: essential oils as modifiers of rumen microbial fermentation. J Dairy Sci 90:2580–2595

Cersosimo LM, Bainbridge ML, Kraft J, Wright AD (2016) Influence of periparturient and postpartum diets on rumen methanogen communities in three breeds of primiparous dairy cows. BMC Microbiol 16:78. https://doi.org/10.1186/s12866-016-0694-7

Chaichi Semsari M, MaheriSis N, Sadaghian M, Eshratkhah B, Hassanpour S (2011) Effects of administration of industrial tannins on nutrient excretion parameters during naturally acquired mixed nematode infections in Moghani sheep. J Amer Sci 7(6):245–248

Chaudhary PP, Sirohi SK (2009). Dominance of *Methanomicrobium* phylotype in methanogen population present in Murrah buffaloes (Bubalus bubalis). Lett Appl Microbiol;49(2):274–277. doi: 10.1111/j.1472-765X.2009.02654.x.Epub 2009 May 27

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308–316. [PubMed].s

Dridi B, Henry M, El Kh'echine A, Raoult D, Drancourt M (2009) High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut

Facey HV, Northwood KS, Wright AD (2012) Molecular diversity of methanogens in fecal samples from captive Sumatran orangutans (*Pongo abelii*). Am J Primatol 74(5):408–413. https://doi.org/10.1002/ajp.21992

Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA (2008) (2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat Rev Microbiol 6(2):121–131. https://doi.org/10.1038/nrmicro1817

Franzolin R, St-Pierre B, Northwood K, Wright AD (2012) Analysis of rumen methanogen diversity in water buffaloes (*Bubalus bubalis*) under three different diets. Microb Ecol 64(1):131–139. https://doi.org/10.1007/s00248-012-0007-0

Gilmore SP, Henske JK, Sexton JA, Solomon KV, Seppälä S, Yoo JI, Huyett LM, Pressman A, Cogan JZ, Kivenson V, Peng X, Tan Y, Valentine DL, O'Malley MA (2017) Genomic analysis of methanogenic archaea reveals a shift towards energy conservation. BMC Genomics 18(1):639. https://doi.org/10.1186/s12864-017-4036-4

Güçlü-Ustündağ O, Mazza G (2007) Saponins: properties, applications and processing

Haslam E (1989) Plant polyphenols- vegetable tannins revisited. Cambridge University Press, Cambridge

Hassanpour S, Sadaghian M, MaheriSis N, Eshratkhah B, Chaichi SM (2011) Effect of condensed tannin on controlling faecal protein excretion in nematode-infected sheep: in vivo study. J Amer Sci 7(5):896–900

Henderson G, Cox F, Ganesh S, Jonker A, Young W (2015 Oct 9) Global rumen census collaborators, Janssen PH. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep 5:14567. https://doi.org/10.1038/srep14567. Erratum in: Sci Rep. 2016;6:19175. Yanez-Ruiz, David R [corrected to Yáñez-Ruiz, David R]; Pinares-Patino, Cesar [corrected to Pinares-Patiño, Cesar]; Munoz, Camila [corrected to Muñoz, Camila].

Hess HD, Beuret RA, Lötscher M, Hindrichsen IK, Machmüller A, Carulla JE, Lascano CE, Kreuzer M (2004) Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with *Sapindus saponaria* fruits and Cratylia argentea foliage. Anim Sci 79:177–189

Hervás G, Frutos P, Giráldez FJ, Mantecón ÁR, Del Pino MC (2003) Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. Anim Feed Sci Technol 109:65–78

Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA, Odongo NE, Cheeke PR, Benchaar C (2009 Jun) Feeding saponin-containing Yucca schidigera and *Quillaja saponaria* to decrease enteric methane production in dairy cows. J Dairy Sci 92(6):2809–2821. https://doi.org/10.3168/jds.2008-1843. Erratum in: J Dairy Sci. 2009 Jul;92(7):3543. Odongo, N E [added]

Janssen PH, Kirs M (2008) Structure of the archaeal community of the rumen. Appl Environ Microbiol 74(12):3619–3625. https://doi.org/10.1128/AEM.02812-07. Epub 2008 Apr 18

Johnson KA, Johnson DE (1995) Methane emissions from cattle. J Anim Sci 73(8):2483–2492

Kala A, Kamra DN, Kumar A, Agarwal N, Chaudhary LC, Joshi CG (2017) Impact of levels of total digestible nutrients on microbiome, enzyme profile and degradation of feeds in buffalo rumen. PLoS One. https://doi.org/10.1371/Journal.pone.0172051

Kamra DN, Patra AK, Chatterjee PN, Kumar R, Agarwal N, Chaudhary LC (2008) Effect of plant extracts on methanogenesis and microbial profile of the rumen of buffalo: a brief overview. Aust J Exp Agric Res Aust J Exp Agric 48:175–178

Kamra DN (2005) Rumen microbial ecosystem. Curr Sci 89:124–135

Kamra DN, Zadbuke S, Agarwal N, Choudhary LC, Bhar R (2010) Anti methane. Patent submitted

Kamra DN, PawarM, Agarwal N, Choudhary LC, Chaturvedi VB (2012) Methane Suppressor, Patent submitted

Kelly WJ, Pacheco DM, Li D, Attwood GT, Altermann E, Leahy SC (2016) The complete genome sequence of the rumen methanogen *Methanobrevibacter millerae* SM9. Stand Genomic Sci 11:49. https://doi.org/10.1186/s40793-016-0171-9. eCollection 2016

King EE, Smith RP, St-Pierre B, Wright AD (2011) Differences in the rumen methanogen populations of lactating Jersey and Holstein dairy cows under the same diet regimen. Appl Environ Microbiol 77(16):5682–5687. https://doi.org/10.1128/AEM.05130-11. Epub 2011 Jun 24

Kittelmann S, Seedorf H, Walters WA, Clemente JC, Knight R, Gordon JI, Janssen PH (2013) Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. PLoS One 8(2):e47879. https://doi.org/10.1371/journal.pone.0047879

Lewis WH, Sendra KM, Embley TM, Esteban GF (2018) Morphology and phylogeny of a new species of anaerobic ciliate, *Trimyemafinlayi* n. sp., with endosymbiotic methanogens. Front Microbiol 9:140. https://doi.org/10.3389/fmicb.2018.00140. eCollection 2018

Lyu Z, Shao N, Akinyemi T, Whitman WB (2018) Methanogenesis. Curr Biol 28(13):R727–R732. https://doi.org/10.1016/j.cub.2018.05.021

Malik PK, Kolte AP, Bakshi B, Baruah L, Bhatta R (2017). Enteric methane mitigation in sheep through selected tanniniferous tropical tree leaves. Livestock Science. 2016

Mi J, Zhou J, Huang X, Long R (2017) Lower Methane Emissions from Yak Compared with Cattle in Rusitec Fermenters. PLoS One 12(1):e0170044. https://doi.org/10.1371/journal.pone.0170044

Min BR, Hart SP (2003) Tannins for suppression of internal parasites. J Anim Sci 81:102–109

Orpin CG (1988) Nutrition and biochemistry of anaerobic Chytridiomycetes. Biosystems 21(3–4):365–370

Ozbayram EG, Ince O, Ince B, Harms H, Kleinsteuber S (2018). Comparison of Rumen and Manure microbiomes and implications for the inoculation of anaerobic digesters. Microorganisms 6(1). pii: E15. https://doi.org/10.3390/microorganisms6010015

Patra AK, Saxena J (2010) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J Sci Food Agric 91:24–37

Patra AK, Yu Z (2012) Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl Environ Microbiol 78(12):4271–4280. https://doi.org/10.1128/AEM.00309-12

Patra AK, Kamra DN, Agarwal N (2006) Effect of spices on rumen fermentation, methanogenesis and protozoa counts in *in vitro* gas production test. Int Congr Ser 1293:176–179

Patra AK, Kamra DN, Bhar R, Kumar R, Agarwal N (2011) Effect of *Terminalia chebula* and *Allium sativum* on *in vivo* methane emission by sheep. J Anim Physiol Anim Nutr 95:187–191

Pope PB, Smith W, Denman SE, Tringe SG, Barry K, Hugenholtz P, McSweeney CS, McHardy A, Morrison M (2011) Isolation of Succinivibrionaceae implicated in low methane emissions from tammar wallabies. Science 333:646–648

Rea S, Bowman JP, Popovski S, Pimm C, Wright AD (2007) *Methanobrevibacter millerae* sp. nov. and *Methanobrevibacter olleyae* sp. nov., methanogens from the ovine and bovine rumen that can utilize formate for growth. Int J Syst Evol Microbiol 57(Pt 3):450–456

Ross ZM, O'GaraEA HDJ, SleightholmeHV MDJ (2001) Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl Environ Microbiol 67:475–480

Rouviere W, Fiebig K, Hippe H (1983) Distribution of cytochromes in methanogenic bacteria. FEMS Microbiol Lett 20(3):407–410

Samal L, Chaudary LC, Agarwal N, Kamra DN (2016) Impact of phytogenic feed additives on growth performance, nutrient digestion and methanogenesis in growing buffaloes. Anim Prod Sci 55:1056–1063

Santoso B, Mwenya B, Sar C, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H, Takahashi J (2004) Effects of supplementing galacto-oligosaccharides, *Yucca schidigera* or nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. Livest Prod Sci 91:209–217

Sharma S, Ding Y, Jarrell KF, Brockhausen I (2018) Identification and characterization of the 4-epimerase AglW from the archaeon Methanococcus maripaludis. Glycoconj J 35(6):525–535. https://doi.org/10.1007/s10719-018-9845-4

Singh B, Chauhan MS, Singla SK, Gautam SK, Verma V, Manik RS, Singh AK, Sodhi M, Mukesh M (2009) Reproductive biotechniques in buffaloes (Bubalus bubalis): status, prospects and challenges. Reprod Fertil Dev 21(4):499–510. https://doi.org/10.1071/RD08172

Singh KM, Ahir VB, Tripathi AK, Ramani UV, Sajnani M, Koringa PG, Jakhesara S, Pandya, PR, Rank DN, Murty DS, Kothari RK, Joshi C.G. (2011). Metagenomic analysis of Surti buffalo (*Bubalus bubalis*) rumen: a preliminary study. Mol Biol Rep 39(4): 4841–4848 https://doi.org/10.1007/s11033-011-1278-0. Epub 2011 Sep 27

Singh KM, Ahir VB, Tripathi AK, Ramani UV, Sajnani M, Koringa PG, Jakhesara S, Pandya PR, Rank DN, Murty DS, Kothari RK, Joshi CG (2012 Apr) Metagenomic analysis of Surti buffalo (*Bubalus bubalis*) rumen: a preliminary study. Mol Biol Rep 39(4):4841–4848. https://doi.org/10.1007/s11033-011-1278-0. Epub 2011 Sep 27

Sirohi SK, Chaudhary PP, Singh N, Singh D, Puniya AK (2013) The 16S rRNA and mcrA gene based comparative diversity of methanogens in cattle fed on high fibre based diet. Gene 523(2):161–166. https://doi.org/10.1016/j.gene.2013.04.002. Epub 2013 Apr 18

Sliwiński BJ, Kreuzer M, Wettstein HR, Machmüller A (2002 Dec) Rumen fermentation and nitrogen balance of lambs fed diets containing plant extracts rich in tannins and saponins, and associated emissions of nitrogen and methane. Arch Tierernahr 56(6):379–392

Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M (1996) Antimicrobial and cytotoxic activities of *Origanum* essential oils. J Agric Food Chem 44:1202–1205

Smith-Palmer A, Stewart J, Fyfe L (1998 Feb) Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Appl Microbiol 26(2):118–122

St-Pierre B, Wright AD (2012 Jan 5) Molecular analysis of methanogenic archaea in the forestomach of the alpaca (*Vicugna pacos*). BMC Microbiol 12(1). https://doi.org/10.1186/1471-2180-12-1

Turnbull KL, Smith RP, St-Pierre B, Wright AD (2012) Molecular diversity of methanogens in fecal samples from Bactrian camels (*Camelus bactrianus*) at two zoos. Res Vet Sci 93(1):246–249. https://doi.org/10.1016/j.rvsc.2011.08.013. Epub 2011 Sep 13

Waghorn G (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production- Progress and challenges. Anim Feed Sci Technol 147:116–139

Whitford V, Ennos AR, Handley JF (2001). 'City form and natural process' – indicators for the ecological performance of urban areas and their application to Merseyside, UK. Landsc Urban Plan 57(2):91–103

Woese CR, Kandler O, Wheelis ML (1990 Jun) Towards a natural system of organisms: proposal for the domains archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 87(12):4576–4579

Wright AD, Williams AJ, Winder B, Christophersen CT, Rodgers SL, Smith KD (2004) Molecular diversity of rumen methanogens from sheep in Western Australia. Appl Environ Microbiol 70(3):1263–1270

Wright AD, Auckland CH, Lynn DH (2007) Molecular diversity of methanogens in feedlot cattle from Ontario and Prince Edward Island, Canada. Appl Environ Microbiol 73(13):4206–4210. Epub 2007 May 4

Wright AD, Ma X, Obispo NE (2008 Aug) Methanobrevibacter phylotypes are the dominant methanogens in sheep from Venezuela. Microb Ecol 56(2):390–394. https://doi.org/10.1007/s00248-007-9351-x. Epub 2007 Dec 29

Wright AD, Northwood KS, Obispo NE (2009) Rumen-like methanogens identified from the crop of the folivorous. South American bird, the hoatzin (Opisthocomus hoazin) 3(10):1120–1126. https://doi.org/10.1038/ismej.2009.41. Epub 2009 Apr 23

Yatoo MA, Chaudhary LC, Agarwal N, Chaturvedi VB, Kamra DN (2018) Effect of feeding of blend of essential oils on methane production, growth, and nutrient utilization in growing buffaloes. Asian-Australas J Anim Sci 31(5):672–676. https://doi.org/10.5713/ajas.16.0508. Epub 2017 Feb 23

Zhou Y-Y, Mao H-L, Jiang F et al (2010). Tea saponins inhibit ruminal methane emission through the inhibitory effect on protozoa in Hu sheep. In: Proceedings of Fourth Greenhouse Gases and Animal Agric. Conference