

Rumen microbial ecosystem

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The inhabitants of the rumen microbial eco-system, a complex consortium of different microbial groups living in symbiotic relationship with the host, act synergistically for the bioconversion of lignocellulosic feeds into volatile fatty acids which serve as a source of energy for the animals. The constraints, imposed by the host and the feed consumed by the animal, under which these microbes have to function, have been discussed. The eco-system is specialized and buffered in a narrow range of pH, which helps the animal to maintain a very well stabilized eco-system which is not disturbed by the incoming microbial contaminants into the fermentation sac (rumen) through feed and water intake. The microbial ecosystem is well studied for the rumen of domesticated animals like cattle, sheep and goat, but it is poorly studied in buffalo and wild ruminants. The necessity to use molecular biology techniques for identification and characterization of rumen microbes has been emphasized in this review.

In tropical countries of the world, the ruminants are fed on lignocellulosic agricultural by-products like cereal straws, stovers, sugarcane bagasse, tree foliages and cakes of oil seeds like groundnut, cotton, mohua, neem and mustard. The efficiency of ruminants to utilize such a wide variety of feeds is due to highly diversified rumen microbial eco-system consisting of bacteria (10^{10} – 10^{11} cells/ml, representing more than 50 genera), ciliate protozoa (10^4 – 10^6 /ml, from 25 genera), anaerobic fungi (10^3 – 10^5 zoospores/ml, representing five genera) and bacteriophages (10^8 – 10^9 /ml)¹. These numbers might even be larger as majority of them are non-culturable. The synergism and antagonism among the different groups of microbes and even among different genera of the same group is so diverse and complicated that it is difficult to quantify the role played by any particular group of microbes present in the rumen. The net result of these reactions going on in the rumen is responsible for the bioconversion of feed into such form that is utilizable by the animal as a source of energy (short chain volatile fatty acids). These microbes survive in the rumen under different constraints which may be either natural or feed associated as some of the feeds contain a significant amount of anti-nutritional factors, which sometimes limit the growth of some of these natural microbial inhabitants.

Constraints in the rumen

Natural environmental constraints

The microbial eco-system of the rumen is stable and at the same time dynamic. The eco-system is stable as it is well established and has been performing the function of bioconversion of feed into volatile fatty acids. In a healthy ruminant the contamination of the eco-system does not occur in spite of the fact that millions of microbes invade the rumen everyday through feed, drinking water and air. The eco-system is dynamic as the microbial population changes considerably on change of diet so as to adapt it to the new feed ingredients. This happens because the rumen microbes are adapted to survive in a set of constraints prevalent in the rumen and any contaminant which cannot survive these constraints is eliminated. The major environmental constraints are anaerobiosis, high buffering capacity and osmotic pressure and saprophytic competition among the microbes for their survival.

The anaerobiosis inside the rumen is one of the major constraints in the rumen eco-system, which helps in conserving the energy ultimately to be used by the host animal. The amount of energy released by electron transfer reaction depends upon the terminal electron acceptor. In aerobic microbes, the terminal electron acceptor is oxygen and the release of energy in the form of ATP is much higher compared to energy released when the terminal electron acceptor is an organic compound as in the case of anaerobic bacteria². The anaerobic conditions in the rumen are maintained by gases generated during fermentation, e.g. carbon dioxide, methane and traces of hydrogen. Some of the oxygen entrapped in the feed consumed by the animal is utilized by the facultative anaerobes present in the rumen and thus a perfect anaerobic condition is generated and maintained. Therefore, only the microbes which are able to tolerate such a low redox potential (–350 mV) are able to survive in the rumen and the rest are eliminated from the system. High buffering capacity and osmotic pressure too limit the growth of invading microbes. Some of the rumen microbes produce antimicrobial compounds which limit the growth of other microbes present in the eco-system^{3,4}.

Feed-associated constraints

The livestock are fed mainly on agro-industrial by-products, which contain a lot of anti-nutritional factors which may

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act as inhibitors for some of the rumen microbes. The anti-nutritional compounds like tannins, lignins, saponins, mimosine, etc. are synthesized in the plants to protect them against the invading microbes. Therefore these compounds have anti-microbial activity. Therefore when consumed by the animals, these compounds limit the growth of different types of microbes (useful and undesirable) in the rumen.

Tannins. Tannins are phenolic compounds with molecular weight ranging from 500 to 3000 Da, which are water soluble, able to precipitate proteins, alkaloids and gelatin. Tannins are usually secondary metabolites of plants which are not involved in primary metabolic pathways of plants. Based on the differences in their chemical structure and relative ratios of different phenolic monomers, tannins are classified into two groups, e.g. hydrolysable and condensed tannins. The hydrolysable tannins are mostly the esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar core (usually glucose), which are readily hydrolysed by acids or enzymes into monomeric products, on the other hand the condensed tannins are polymeric proanthocyanidins.

Tannins are most effective against the fibre-degrading bacteria. McSweeney *et al.*⁵ observed that in animals fed tannin rich *Calliandra calothyrsus*, the population of *Ruminococcus* spp. and *Fibrobacter* spp. was reduced considerably, but fungi, protozoa and proteolytic bacteria were less affected by this diet. Sotohy *et al.*⁶ reported that the number of total bacteria in the rumen of goats decreased significantly when the animals were fed tannin-rich plant (*Acacia nilotica*) and the decrease in the numbers was directly proportional to the level of this feed in the diet. Condensed tannins from the leaves of sainfoin (*Onobrychis viciifolia*) inhibited growth and protease activity in *Butyrivibrio fibrisolvens* A38 and *Streptococcus bovis* 45S1, the growth of *Prevotella ruminicola* B14 and *Ruminobacter amylophilus* WP225 was not much affected⁷.

It was perhaps for the first time that Brooker *et al.*⁸ isolated a Gram-positive facultative anaerobe which could grow in a medium containing 2.5% tannic acid or condensed tannins from the rumen liquor of feral goats browsing on *Acacia* spp leaves. The bacterium was named as *Streptococcus caprinus*. It was not a major inhabitant of the rumen and was present at a population density of 2×10^6 per ml of rumen fluid.

Odenyo and Osuji⁹ isolated three strains of tannin-tolerant bacteria from sheep, goat and an antelope and observed that the isolates could tolerate tannins up to 8 g/litre in the medium. Their growth increased when soluble carbohydrates are included in the growth medium. Nelson *et al.*¹⁰ isolated six strains from the rumen contents of Sardinian sheep (*Ovis aries*), Honduran and Colombian goats (*Capra hircus*), white tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*).

Four of the isolates belonged to the genus *Streptococcus*, most closely related to *S. bovis* and *S. gallolyticus*.

The ruminants which continuously feed upon diets rich in tannins usually develop a microflora which is tolerant to high tannins. The feral goats and camel fed on *Acacia* and *Calliandra calothyrsus*, which contain high level of tannins, are capable of tolerating tannins in diet due to the presence of high numbers of tannin-resistant bacteria like *Streptococcus caprinus* and *Selenomonas ruminantium* K2 (ref. 11). The other isolated bacteria belong to the genera *Butyrivibrio* sp. and *Lactobacillus* sp. As confirmed by 16S rDNA restriction fragment length polymorphism (RFLP) analysis, *S. caprinus* and *S. gallolyticus* have been shown to be synonyms based on their 16S rRNA sequence similarity (98.3%) and DNA-DNA homology (>70%) and this bacterium has been preferred to be named as *Streptococcus gallolyticus*¹².

Feral goats fed mulga (*Acacia aneura*) were more resistant to tannins than sheep fed grass, but the capability to digest or tolerate tannins in diet increased when rumen flora was trans-inoculated into the rumen of sheep, indicating that goats contain microbial populations which could degrade tannins efficiently¹³. In another study in Indonesia, Wiryawan *et al.*¹⁴ isolated five types of tannin-degrading bacteria, which could tolerate 3% tannic acid or 1% condensed tannins in their growth medium and reduce the tannin content by 52% in 72 h of growth. Inoculation of these bacteria at the rate of 3×10^{11} cfu into the rumen of unadapted goats could help in improving feed digestibility and live weight gain in the animals.

As discussed above tannins do have harmful effect on rumen microbes, but on prolonged feeding some of the tannin-degrading bacteria proliferate and increase in numbers and make the animals more tolerant to higher levels of tannins in their diet.

Saponins. Saponins are steroidal sapogenin covalently linked to oligosaccharide moiety. The saponins cause haemolysis of red blood cells perhaps by increasing the permeability of the plasma membrane and inhibit smooth muscle activity¹⁵. The saponins of lucerne origin had a detrimental effect on rumen fermentation and caused a reduction in total volatile fatty acid production and acetate to propionate ratio from 1.93 to 1.37 in the presence of 1% saponin in the medium¹⁶. The rumen microbes are able to deglycosylate the saponins to release the steroid moiety, which affects rumen fermentation. *Yucca schidigera* extract has been found to alter rumen fermentation favourably, increase animal growth and milk production^{17,18}. The yucca extract is also inhibitory for some of the rumen ciliate protozoa and bacteria¹⁹. Wang *et al.*²⁰ also confirmed the anti-protozoal activity of yucca extract in RUSITEC experiments and reported an increase in the protease activity of the rumen microbes. Thalib *et al.*²¹ studied the effect of saponins of *Sapindus rarak* fruit on rumen microbes of sheep and reported that the methanol extract of

seeds caused a 57% reduction in the number of protozoa and 69% increase in bacterial population which resulted in improved feed conversion efficiency and better gain in body weight of the animals.

Mimosine. A tropical browse leguminous plant, *Leucaena leucocephala* contains a toxic compound, mimosine, which restricts its nutritional use in domestic ruminants and monogastric animals. Some of the rumen microbes are able to degrade mimosine and its pyridine diol derivatives, i.e. 3,4-dihydroxypyridine and 2,3-dihydroxypyridine (3,4-DHP and 2,3-DHP). 3,4-DHP is a potent goitrogen of the thiouracil type, which is excreted in the urine. The Hawaiian goats fed on *Leucaena* fodder were found to contain a microflora which was able to degrade 3,4-DHP. The trans-inoculation of mixed inoculum from these goats to Australian ruminants sensitive to mimosine toxicity resulted in the transfer of the capability of degrading DHP completely and efficiently²². Allison *et al.*²³ isolated a bacterium capable of degrading DHP from the rumen of goats resistant to mimosine toxicity and named it as *Synergistes jonesii*. Four strains of *S. jonesii* have been isolated and all of them are Gram-negative, rod-shaped and obligate anaerobic. The inoculation of this culture in the rumen of mimosine intolerant animals extends protection against mimosine²⁴, indicating that some of the microbes are able to fill a specific niche in the rumen depending upon the availability of a specific component for its survival in the rumen. The bacterium isolated is an obligate anaerobe, Gram-negative, non-motile oval rod and non-spore forming. It can ferment 2,3- and 3,4-DHP, arginine and histidine, but carbohydrates are not fermented. An oligonucleotide probe which targets specific region of 16S rRNA, has been developed. This will help in studying the population of this microbe, its establishment in the new environment when inoculated and its colonization²⁵.

Nitrate–nitrite. Certain crops like oat, wheat, barley, etc. accumulate nitrates up to the concentration which may be fatal to the animal. The nitrate entering the rumen gets metabolized into nitrite which is absorbed into the blood rapidly and oxidizes oxyhaemoglobin to methemoglobin, thus reducing the oxygen-carrying capacity of red blood cells. Though some nitrite is metabolized further into ammonia, depending upon its level toxicity may occur. The rate of metabolism of nitrate and nitrite depends on a number of factors like particular microbial population, enzyme activity (nitrate reductase), pH, H donor, etc. Takahashi *et al.*²⁶ reported that the rate of nitrate and nitrite reduction was faster when the animals were fed on the diet rich in readily available carbohydrates, because the anaerobic microbes use fermentation products of carbohydrates like formate, pyruvate, lactate, glycine, etc. as a substrate for the reduction reaction. Five groups of bacteria have been isolated from a sheep adapted to nitrate²⁷. Two groups belonged to selenomonads, third group could not be classi-

fied, fourth group identified as *Anaerovibrio* spp and the fifth was lactate-fermenting short rods.

Microbial diversity of rumen

Bacteria

Ruminants in India are fed mainly on lignocellulosic agricultural by-products which are rich in cellulose, hemicellulose, lignin, starch, protein and a very small quantity of oils. The rumen harbours various types of bacteria which are active in degradation of these components of the feed (Table 1). The interaction among themselves and with other microbial groups in the rumen are also responsible for synergistic effect on the production of volatile fatty acids and microbial proteins in the rumen.

Some of the common features of bacteria found in the rumen of animals fed on high roughage diet are as follows:

- Majority of the bacteria are Gram-negative. The number of Gram-positive bacteria tends to increase on increasing high energy diets in the ration.
- Most of the bacteria are obligate anaerobes. Some of them are so sensitive to oxygen that these are killed on exposure to oxygen. A few rumen bacteria require a very low redox potential (indicating a high degree of anaerobiosis) and grow at a redox potential lower than –350 mV.
- The optimum pH for the growth of rumen bacteria lies between 6.0 and 6.9.
- The optimum temperature is 39°C.
- The bacteria can tolerate a considerably higher level of organic acids without affecting adversely their metabolism.

The bacteria isolated from the rumen of Indian cattle and buffalo were represented by Ruminococci to the extent of 50–94% on feeding of 15% sugarcane molasses and 2–3% urea to the animals²⁸. Some of the cellulose-degrading bacteria isolated from the rumen of buffalo include *Fibrobacter succinogenes*, *Ruminococcus albus* and *R. flavefaciens* as reviewed by Jalaludin *et al.*²⁹.

Berseem supported maximum number of cellulose-degrading bacteria and were represented by *Ruminococcus albus* and *R. flavefaciens* (59.8%). *Bacteroides succinogenes* (*Fibrobacter succinogenes*) (19.2%), *Butyrivibrio fibrisolvens* (11.1%), *Clostridium lochheadii* (3.8%) and *C. longisporum* (1.3%)³⁰. The cellulose-degrading bacteria get stimulated (57.4% higher numbers than that in control) when buffaloes are fed *Saccharomyces cerevisiae* in their diets³¹ which might be due to release of some un-identified micro nutrients by the yeast which are essentially required by the cellulose-degrading bacteria. Recently Sahu *et al.*³² isolated a cellulose degrading bacteria from the faeces of chinkara (*Gazella gazella*) and identified it as *Ruminococcus flavefaciens*.

Table 1. Bacterial diversity of the rumen microbial ecosystem of domestic and wild animals

Substrate	Bacteria	Ref.
Bacteria active in carbohydrate utilization		
Cellulose	<i>Fibrobacter succinogenes</i>	100–101
	(<i>Bacteroides succinogenes</i>)	
	<i>Ruminococcus flavefaciens</i>	41, 102–104
	<i>Ruminococcus albus</i>	
	<i>Clostridium cellobioparum</i>	105
	<i>Clostridium longisporum</i>	106
	<i>Clostridium lochheadii</i>	
Hemicellulose	<i>Eubacterium cellulosolvens</i>	107, 108
	(<i>Cillobacterium cellulosolvens</i>)	
	<i>Butyrivibrio fibrisolvens</i>	109, 110
	<i>Prevotella ruminicola</i>	111
	(<i>Bacteroides ruminicola</i>)	
Starch	<i>Eubacterium xylanophilum</i> , <i>E. uniformis</i>	112
	<i>Streptococcus bovis</i>	113
	<i>Ruminobacter amylophilus</i> (<i>Bacteroides amylophilus</i>)	114, 115
	<i>Prevotella ruminicola</i> (<i>Bacteroides ruminicola</i>)	111
	<i>Succinivibrio dextrinosolvens</i>	110
Sugars/dextrins	<i>Succinivibrio amylolytica</i>	107
	<i>Selenomonas ruminantium</i>	116
	<i>Lactobacillus acidophilus</i> , <i>L. casei</i> , <i>L. fermentum</i> , <i>L. plantarum</i> , <i>L. brevis</i> , <i>L. helveticus</i>	37, 117
	<i>Bifidobacterium globosum</i> , <i>B. longum</i> , <i>B. thermophilum</i>	118
	<i>B. ruminale</i>	119
	<i>B. ruminantium</i>	120
	<i>Treponema saccharophilum</i>	121
Pectin	<i>Lachnospira multiparus</i>	110, 122
Bacteria active in nitrogen utilization		
Protein degraders	<i>Prevotella ruminicola</i>	
	<i>Ruminobacter amylophilus</i>	
	<i>Clostridium bifermentans</i>	123
Urea hydrolyzers	<i>Megasphaera elsdenii</i>	124, 125
Other bacteria		
Acid utilizers	<i>Megasphaera elsdenii</i>	126, 127
	(<i>Peptostreptococcus elsdenii</i>)	
	<i>Wollinella succinogenes</i> (<i>Vibrio succinogenes</i>)	128
	<i>Veillonella gazogenes</i> (<i>Veillonella alcalescens</i> , <i>Micrococcus lactolytica</i>)	129
	<i>Oxalobacter formigenes</i>	130
	<i>Desulphovibrio desulphuricans</i>	131, 132
	<i>Desulphatamaculum ruminis</i>	133
Lipolytic bacteria	<i>Succinilasticum ruminis</i>	134
	<i>Anaerovibrio lipolytica</i>	135
Acetogenic bacteria	<i>Eubacterium limosum</i>	136
	<i>Acetitomaculum ruminis</i>	137
Tannin degraders	<i>Streptococcus caprinus</i>	8
	<i>Eubacterium oxidoreducens</i>	138
Mimosine degraders	<i>Synergistes jonesii</i>	23
Methanogenic archaea	<i>Methanobrevibacter ruminantium</i>	139, 140
	<i>Methanobacterium formicicum</i>	
	<i>Methanosarcina barkeri</i>	141, 142
	<i>Methanomicrobium mobile</i>	143
Mycoplasma	<i>Anaeroplasmata bactoclasticum</i>	144
	<i>Anaeroplasmata abactoclasticum</i>	145

Archaea

Seven different species representing five genera of methanogens have been reported from the rumen of different animals, i.e. *Methanobacterium formicicum*, *Methanobacterium bryanti*, *Methanobrevibacter ruminantium*, *Methanobrevibacter smithii*, *Methanomicrobium mobile*, *Methanosarcina barkeri* and *Methanoculleus olentangyi*^{33,34}. Methanogens are present in the rumen in large numbers varying from 10⁷ to 10⁹ cells/ml of rumen liquor depending upon the type of diet given to the animals, especially the fibre content in the ration. The methanogens play a vital role in the rumen of scavenging molecular hydrogen generated during rumen fermentation, thereby making rumen fermentation a continuous process, but this leads to a significant loss of gross energy consumed by the animals.

There is a close association between entodiniomorphid protozoa and some of the bacteria, which attach to the pellicle of protozoa. Eleven species of entodiniomorphid protozoa have been found to have adhered methanogens like *Entodinium longinucleatum*, *Eudiplodinium maggii*, *Entodinium bursa* and *Eremoplastron bovis*. The methanogens attach themselves with the ciliate protozoa to get a constant supply of hydrogen. On pumping hydrogen in the rumen, the methanogens get detached from the protozoa³⁵.

Protozoa

The rumen protozoa were detected in domestic animals as early as nineteenth century by Gruby and Delafond³⁶. Not much work was done for several decades after their first report in the rumen. It was only after 1920 that the researchers paid any significant attention towards the identification, morphology and biochemical functions of protozoa in the rumen. At present a lot of information has been generated and compiled in the form of research papers, reviews, bulletins, books, symposium proceedings, etc.^{1,37–40}.

The ciliate protozoa of the rumen have been classified into two groups depending upon their morphological characteristics, i.e. holotrich and entodiniomorphid protozoa³⁷. Alternatively these can also be classified as soluble sugar utilizers, starch degraders and lignocellulose hydrolyzers. The holotrich protozoa are represented by 15 different genera in the rumen of different animals. Among these genera, *Isotricha*, *Dasytricha*, *Buetschlia* and *Charonina* are some which are widely distributed in the rumen of domestic and wild ruminants and hind gut fermentors^{41,42} (Table 2). Some of the non-ruminant pre-gastric fermentors like camel and hippopotamus also have a large number of holotrich protozoa^{43,44}.

The enzymatic profile of holotrich protozoa indicates that they have amylase, invertase, pectin esterase and polygalacturonase in sufficiently large quantities for using starch, pectin and soluble sugars as energy source^{45–48}. The en-

Table 2. Protozoal diversity of the rumen microbial ecosystem of domestic and wild animals

Protozoa	Host	Ref.
Holotrich protozoa		
<i>Isotricha prostoma</i>	Sheep	146
	Zebu cattle	147
	Blackbuck	148
	Water buffalo	54
	Red deer	149
	Goat	150
<i>I. intestinalis</i>	Cattle	151
	Bison	146
	Mouse deer	147
	Red deer	149
<i>Dasytricha ruminantium</i>	Sheep	152
	Blackbuck	148
	Water buffalo	54
<i>Oligoisotricha bubali</i>	Water buffalo	59
	Cattle	61
Entodiniomorphid protozoa		
<i>Entodinium bovis</i>	Yugoslavian cattle	153
	Zebu cattle and water buffalo	154
<i>E. bubalum</i>	Water buffalo	59
<i>E. bursa</i>	Cattle sheep and goat	92
<i>E. caudatum</i>	Cattle sheep and goat	155
<i>E. chatterjeei</i>	Indian goat	150
	Water buffalo	154
<i>E. longinucleatum</i>	Cattle, water buffalo	154
<i>Diplodinium dendatum</i>	Widely distributed	151
<i>D. indicum</i>	Indian cattle	156
<i>Eremoplastron asiaticus</i>	Indian cattle	156
<i>E. bubalus</i>	Brazilian cattle and water buffalo	54
<i>Eudiplodinium maggii</i>	Widely distributed	157
<i>Ostracodinium trivesiculatum</i>	Cattle and water buffalo	54
<i>Polyplastron multivesiculatum</i>	Cattle, sheep, goat, blackbuck	148, 158–160
<i>Metadinium medium</i>	Cattle	161
	Water buffalo	54
	African reedbuck	162
<i>Epidinium caudatum</i>	Cattle, sheep, goat	161
<i>Ophryoscolex caudatus</i>	Widely distributed	150, 158, 161
<i>Caloscolex camelicus</i>	Dromedary camel	163

zymes responsible for cellulose and hemicellulose degradation have also been reported in the holotrich protozoa but the levels are very low compared to those present in the entodiniomorphid protozoa⁴⁹.

The microbiology of rumen of cattle and sheep has been extensively studied and reviewed by many workers from time to time^{37,50}, but studies on rumen microbes of buffalo are limited^{29,51,52} and the experiments on relative microbiology of cattle and buffalo on similar diets and same environmental conditions are rare. The total number of ciliate protozoa is lower in buffalo than that in cattle, but the ciliate protozoa represented in both the species of animals included *Entodinium*, *Diplodinium*, *Eremoplastron*, *Eudiplodinium*, *Elytroplastron*, *Metadinium*, *Ostracodinium*, *Epidinium*, *Dasytricha* and *Isotricha*⁵³.

Dehority⁵⁴ studied the ciliate protozoa in the rumen of Brazilian water buffalo and found that 49 species of ciliate protozoa were present, out of which eight species were described for the first time. Four new species belonged to the genus *Diplodinium* (*Ostracodinium*), i.e. *O. brazili*, *O. esalqum*, *O. nucleolobum* and *O. tiete*; three new species of *Entodinium*, i.e. *E. ciculum*, *E. spinonucleatum* and *E. triangulum* and one new species of *Diplodinium* (*Eudiplodinium*) *E. bubalus* have also been reported. In addition to the above, new species of *Entodinium*, *Diplodinium*, *Ostracodinium*, *Eudiplodinium*, *Elytroplastron*, *Epidinium*, *Isotricha*, *Dasytricha*, *Charonina* and *Buetschlia* have also been found in the rumen of buffalo which are commonly found in other domestic and wild ruminants. In another study 17 species of ciliate protozoa have been detected in Malaysian water buffalo and 15 in Kedah Kelantan cattle⁵⁵. There was no difference in the total number of ciliates in two species of animals. *Metadinium ypsilon* and *Ostracodinium trivesiculatum* have been found to be present in both the species, but other species reported in Malaysian ruminants are similar to those reported in other tropical and temperate ruminants⁵⁶.

Dumag⁵⁷ studied the ciliate protozoa in the rumen of Philippine carabaos (*Bubalus bubalis*) and found 59 different species of ciliates, out of which 21 belonged to *Entodinium*, ten to *Diplodinium*, eight to *Epidinium*, four to *Ostracodinium* and *Eremoplastron*, three to *Metadinium* and *Eodinium* and one each to *Enoploplastron*, *Diploplastron* and *Eudiplodinium*. Three species belonged to holotrich protozoa, e.g. *Isotricha intestinalis*, *I. prostoma* and *Dasytricha ruminantium*. Most of these species have also been reported in the rumen of cattle³⁷. *Metadinium medium* has been reported to be present only in the rumen of buffalo²⁹. Four new species of ciliate protozoa which have been reported to be present in the rumen of buffalo are *Entodinium ogimotoi*, *E. bubalum*, *E. fujitai* and *E. tsunodai*⁵⁸. *Oligoisotricha bubali* has been considered to be restricted only in buffalo rumen^{59,60}, but it has also been detected in the rumen of cattle in the areas of Tennessee, USA. This protozoan has been observed in low numbers (<1% of total protozoa) to very high numbers (>70% of total protozoa) in the rumen of cattle fed different rations⁶¹. Corn silage-concentrate diet has been found to stimulate the growth of this protozoan in the rumen of cattle.

Eight genera of ciliate protozoa have been observed in the rumen of cattle and buffalo fed on wheat straw and concentrate mixture, e.g. *Isotricha*, *Dasytricha*, *Metadinium*, *Diplodinium*, *Eudiplodinium*, *Ophryoscolex*, *Entodinium*, and *Epidinium*. *Oscillospira guillermundii* was always found in the rumen of buffalo⁶². The protozoa production rate, estimated by use of ³⁵S as indicator, showed that it was lower in buffalo (25.4–27.5 mg/min) than that in the rumen of cattle calf (30.5 mg/min)⁶³. There was no difference in the numbers of ciliate protozoa in the rumen liquor of cattle and buffalo^{62,64}.

Effect of defaunation on animal performance

- There is no pH stabilization in the rumen in the absence of ciliate protozoa and therefore, a low pH is always observed⁶⁵.
- There is an increase in the level of lactic acid and propionic acid in the rumen liquor^{66,67}.
- Ammonia nitrogen decreases significantly on defaunation⁶⁸.
- Methanogenesis is reduced considerably in the absence of ciliate protozoa^{69,70}.
- There is a significant increase in the numbers of bacteria and fungi in the rumen liquor when protozoa are eliminated, perhaps to take over the several general functions of protozoa in the rumen.
- There is an increased feed conversion efficiency on some diets, especially the high roughage diets^{71,72}. This might be due to the absence of any specific function of ciliate protozoa on high roughage based diet.

Fungi

The flagellates were observed in the rumen as early as 1910, but were believed to be flagellate protozoa^{73,74} and were placed in the genera *Callimastix*, *Sphaeromonas*, *Oikomonas*, etc. These flagellates were discovered as fungi for the first time in mid-seventies by Orpin⁷⁵, who identified it as *Neocallimastix frontalis*. The flagellate zoospore grew into a mycelium which in turn transformed into a reproductive stage of rhizoids bearing zoosporangium. This was confirmed to be a true fungus by the presence of chitin in its cell wall by Orpin⁷⁶. The isolated organism was similar in life cycle and morphology to a chytridiomycete fungus, but this was a first report that it was a strict anaerobic fungus. Several strains of anaerobic fungi have been reported in the rumen of different ruminant species (Table 3).

These obligate anaerobic fungi, found in the rumen and other parts of the gastro-intestinal tract of herbivorous animals, have an active and positive role to play in fibre degradation as evidenced by the presence of different en-

zymes involved in fibre degradation^{77,80}. The recent experiments conducted in the author's laboratory have shown that on removal of fungi from the rumen content, there is a significant reduction in *in vitro* gas production and degradation of fibrous feeds, indicating a positive role played by fungi in fibre degradation. The enzyme profile of various fungi studied indicates that a wide variety of enzymes required for lignocellulose degradation are excreted and it is confirmed by the scanning electron microscopic studies that these fungi prefer to get attached to the most lignified tissues of plant feed material⁸¹. The fibre-based diets stimulate the fungal growth in the rumen of buffalo⁸² in comparison to diets rich in easily fermentable carbohydrates. Pelleted diets usually have a shorter transit time through the gastro-intestinal tract, therefore do not support the growth of anaerobic fungi in the rumen. Large quantities of soluble sugars inhibit the germination of zoospores on plant tissues⁸³. This might be due to lowering of pH of the rumen liquor in the presence of high sugar concentration, which inhibits the production of zoospores in the rumen⁸⁴. Because of the presence of different enzymes like proteases and esterases in addition to cellulases and hemicellulases, the fungi have an additional advantages of better penetration of the lignocellulosic feeds over the cellulose-degrading bacteria⁸⁵.

Bacteriophages

Bacteriophages are the viruses of bacteria and are reported to be present in the rumen in large numbers. They are specific for different bacteria present in the rumen. They are also considered to be obligate pathogens for the bacteria as bacteriophages are capable of lysing bacteria. These phages help in bacterial mass turnover in the rumen, which may be considered not so useful for the animals on different feeding schedules⁸⁶, but by lysing the bacterial cells, the bacterial protein is easily made available to the animals as a source of amino acids.

The specificity of the bacteriophages for a particular rumen bacterium may be exploited for removal or killing by lysis of unwanted rumen bacteria from the ecosystem like *Streptococcus bovis* and methanogens^{87,88}.

The phage population in an animal at any time is specific for that animal as the animals kept on similar diet penned together in the same shed may have diverse population of these phages. The diurnal variation in the numbers of phage particles is also very diverse. There is a drop in numbers immediately on feeding followed by a gradual increase up to 8–10 h post feeding and then decline to reach the base level⁸⁹.

Inter-relation among different groups of microbes in the rumen

Some of the rumen microbes depend upon others for the supply of nutrients required by them, while others get an-

Table 3. Fungal diversity of the rumen microbial ecosystem of domestic and wild animals

Fungus	Source of isolation	Ref.
<i>Neocallimastix frontalis</i>	Cow	76
<i>N. patriciarum</i>	Sheep	164
<i>N. hurleyensis</i>	Cattle	165
<i>Sphaeromonas communis</i> (<i>Caecomycetes communis</i>)	Cattle	166, 167
<i>Caecomycetes equi</i>	Horse	168
<i>Orpinomyces bovis</i>	Cattle	169
<i>Anaeromyces mucronatus</i> (<i>Ruminomyces mucronatus</i>)	Cattle	170
<i>Ruminomyces elegans</i>	Cattle	171
<i>Piomyces communis</i> , <i>Piomyces mae</i> , <i>Piomyces dumbonica</i>	Horse, elephant	172

tagonized by the excretion of anti-microbial compounds. Thus a very sensitive equilibrium must exist among these microbes for optimum fermentation in the rumen. Some of the cellulose-degrading bacteria *Ruminococcus albus* and *R. flavefaciens* produce a soluble protein that inhibits cellulose degradation by rumen anaerobic fungi³ while some strains of chitinolytic bacteria inhibit the fungal growth⁹⁰. *R. albus* synthesizes a bacteriocin which inhibits the growth of *R. flavefaciens*⁴.

Polyplastron multivesulatum predates upon other ciliate protozoa present in the rumen like *Epidinium*, *Eudiplodinium*, *Diplodinium* and *Ostracodinium*⁹¹. *Entodinium bursa* has an obligate requirement for the spineless form of *E. caudatum*⁹². Protozoa in general are feeding upon rumen bacteria and play a critical role in bacterial protein turn over in the rumen. These interactions (growth promoting and antagonizing) among different microbes of the eco-system are important for maintaining a stable equilibrium which helps in optimal fermentative activity in the rumen and do not allow contaminated microbes to survive in the rumen.

Molecular techniques for rumen microbiology

As discussed above, the rumen microbial eco-system is complex and is responsible for the bioconversion of lignocellulosic feeds into volatile fatty acids. Therefore the specific role of an individual rumen microbe is important as it has to compete with the other microbes of the eco-system for its survival. The enumeration of a specific species of bacterium in the ecosystem (to quantify its role in rumen fermentation) is difficult with the conventional techniques due to a large number of biochemical tests to be performed and imprecision of the technique even for the most predominant microbe present in the ecosystem. This is perhaps due to selection pressure of the medium used for enumeration as the relative numbers of these microbes will change when cultivated in a petri dish in comparison to that metabolically active in the ecosystem. In addition, very large proportion of rumen microbes (like in any other eco-system) is non-culturable, but is active in the rumen fermentation. Therefore, it is essential to search for some better technique of quantifying specific microbes in this ecosystem, which can take care of the above drawbacks of the conventional techniques of studying microbial ecology of the rumen.

A large number of bacteria present in the rumen are non-culturable as the number of total viable bacteria on a particular medium is much lower than the microscopic count of bacteria. There is no single culture medium available which can support growth of all the culturable bacteria of rumen. Therefore, whatever information is available on the culturable bacteria of the rumen is incomplete. The classification of rumen bacteria based on phenotypic characteristics and biochemical tests is not sufficient to study the diversity among the culturable organ-

isms. The studies on molecular biology of the known genera of rumen bacteria have shown that *Prevotella ruminicola*, *Butyrivibrio fibrisolvens* and *Ruminococcus* represent phylogenetically diverse groups of bacteria, in spite of the fact that the species of these genera appear to be phenotypically and biochemically similar⁹³. The variation in different groups of microbes due to change of diet is difficult to study by the conventional methods of isolation and characterization of culturable microbes. Therefore, such variations in the numbers of rumen bacteria can be studied easily by using different oligonucleotide DNA probes, homologous to some regions of bacterial 16S rRNA⁹⁴. A universal probe can be used to estimate total microbial mass and a species-specific probe can be used to determine relative proportion of total rRNA of a species of a particular bacterium. As the ratio of 16S rRNA : total cellular RNA does not change over the growth curve, 16S rRNA can be used as a measure of total bacterial population in the rumen⁹⁵. Specific DNA probes can also be used to enumerate a single bacterial strain in a mixed population if the concentration of this particular bacterium exceeds 10 million cells per ml of the rumen contents⁹⁶.

Tajima *et al.*⁹⁷ designed and revalidated PCR primers for the detection of 13 species of rumen bacteria and these primers have been used with real-time PCR for quantification of these bacteria in the rumen microbial eco-system. This group at the University of Illinois, USA has used real-time PCR for the first time in rumen microbial ecology and appears to be one of the most important techniques to study the variations in relative microbial numbers due to change in diet and to study the establishment of an improved strain of a specific bacterium in the rumen. In addition, these primer sets can also be used for preliminary identification of new bacterial isolates from the rumen of domestic and wild animals.

These new techniques may also help in understanding the mechanism of feed utilization in the rumen. Until recently *Streptococcus bovis* was considered to be the predominant starch utilizing bacteria present in the rumen of animals fed starch-rich diet⁹⁸, but the studies with real time PCR have shown that in majority of the animals *S. bovis* numbers were not affected when the diet was shifted from forage to grain based⁹⁹ and instead a lactate-utilizing bacterium, *Megasphaera elsdenii* got established, resulting in lower lactate and higher propionate contents in the rumen. For starch hydrolysis some other microbes might be active. Therefore, it appears that with use of newer molecular biology techniques, the metabolic roles assigned to different groups of bacteria may have to be re-established and the scenario of the rumen microbiology would be entirely different in the coming decades.

Status of rumen microbiology in India

The research in rumen microbiology in India started in the seventies, but it was limited to mixed culture rumen fer-

mentation or a very few scattered studies on pure cultures of rumen microbes were carried out^{30,62}. It was only in the nineties that pure culture studies were started in a few laboratories. Even now there are not more than four or five laboratories which have full facilities for the cultivation and maintenance of rumen microbes. A few bacterial and fungal cultures have been isolated from domestic (cattle and buffalo) and wild ruminants like blackbuck, chinkara, nilgai, hog deer and spotted deer^{32,80} in the author's laboratory.

Why is rumen microbiology neglected in India?

- This is a low-priority area of research for microbiologists, biotechnologists and molecular biologists.
- Although it is a high-priority area for animal nutritionists, there is a lack of coordination between nutritionists and microbiologists.
- No well-established culture collection in India to provide the required microbes for research.
- Relatively difficult to cultivate and maintain rumen microbes (especially ciliate protozoa and anaerobic rumen fungi).

Future research needs

- Studies on the microbial diversity of the rumen microbiota of domestic and wild ruminants.
- Isolation/selection of rumen microbes having high micro-crystalline cellulase activity.
- Identification of microbial isolates by using new molecular biology techniques.
- Development of genetically modified microbes and their establishment in the rumen of domestic ruminants.

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