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Review Article

Sheep and goat production: basic differences, impact on climate and molecular tools for rumen microbiome study

A.R.Agrawal[1], S. A. Karim[2], Rajiv Kumar[2], A.Sahoo[2] and P. J. John[1]

[1]Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India
[2]Central Sheep and Wool Research Institute, Avikanagar, Tonk, Rajasthan, India
Corresponding author e-mail:

K e y w o r d s

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A B S T R A C T

Small ruminants are able to utilize the lingo-cellulosic materials and convert them to animal products of high nutritional value viz. meat, milk, wool/fur, hide and manure. However ruminants are also contributing towards green house gas emission. Like large ruminants, these fore gut fermenters also harbor a dense and diverse microbial population belonging to different group of flora and fauna. The mammalian system is devoid of enzymes cellulase to degrade structural carbohydrate while ruminants are able to degrade them by the enzymes elaborated by symbiotic microbes inhabiting in the rumen. Importance of small ruminants in Indian economy lies in their smaller size, which is easy to graze and manage. Sheep and goat production is an integral component of rural economy of India and serves as major source of economic sustenance for weaker segments of the society in the hot semiarid and arid region. Sheep and goats are closely related since both belong to subfamily *Caprinae* whereas they are separate species. However, minor differences also exist in their anatomy, physiology, grazing behavior, etc. Sheep as individuals and breeds are more sensitive to environmental changes than other domestic animals but as a species they thrive everywhere. The inherent characteristics of goats such as resistance to dehydration, wider choice of vegetation, and wide-ranging feeding habits with preference for browse species, enable them to perform better than sheep in regions of scanty rainfall. Furthermore, goats appear to digest fiber more efficiently than sheep. Studies are required to delineate differences in microbial population between sheep and goat which might be helpful in enhancing productivity of the species reared under changing climatic conditions. Modern biotechnology based tools are widely applied to unravel complexities of microbial communities harboring rumen and their functions and interaction among different microbes.

Introduction

Herbivores, particularly ruminants, constitute large share of the domestic animals involved in production of food for human consumption. They are able to

utilize the lingo-cellulosic materials and convert them to animal products of high nutritional value viz. meat, milk, wool/fur, hide and manure. Moreover ruminants do not compete with human and monogastric livestock species for feed resources. They are fore gut fermenters harboring a dense and diverse microbial population which break down the lingo-cellulosic fibrous feeds. The mammalian system is devoid of enzymes cellulase to degrade structural carbohydrate while ruminants are able to degrade them by the enzymes elaborated by symbiotic microbes inhabiting in the rumen medium. However rumen, also called false stomach/fore-stomach, itself is devoid of glandular tissues and as such do not secret any enzyme for digestion of ingested food. In rumen fermentation hydrogen and CO_2 are contributed by ciliate protozoa, bacteria and fungi while methanogenic bacteria utilize the hydrogen to reduce CO_2 to produce methane.

Among the herbivores, the ruminants, particularly sheep, goat, cattle and buffaloes are dominant livestock species for human consumption. Animal husbandry has made sizeable contribution to human being in the past century. Animal products provide one-sixth of human food energy and more than one-third of the protein on global basis (Bradford, 1999). Livestock production in India is an integral part of traditional mixed farming (crop-livestock) and play important role in the agrarian economy. The importance of livestock increases with aridity of the environment since traditional crop production is a gamble in the region due to unfavorable agro-climatic condition. The livestock contribute milk, meat, fiber, manure, fuel, farm power and rural transport and thus have major role in rural economy by providing income and employment to small holder farmers and other weaker sections of society including women and landless laborers. Livestock is the major source of cash income for subsistence of the livestock owners in critical zones of the country. In today's competitive economy driven environment, the survival of small scale sustainable animal production has some inherent constrains. Moreover with economic empowerment simultaneous greater demand is being placed on limited and finite natural resources resulting in chaotic situation. It is now essential to ensure that the farmers not only produce for their own needs but also to meet the expanding demand of the market. This is possible only by application of appropriate technological interventions besides ensuring better access to credit and market. Unfortunately, there exist a wide gap in demand and supply of nutrients for the Indian livestock which is the major cause of concern in realizing optimal and sustained production. Therefore, in the present scenario, judicious utilization of feed resources from agriculture, Common Property Resources (CPR) and forest resource is of vital concern to meet the ever-increasing demand of animal derived protein.

Importance of small ruminants in Indian economy lies in their smaller size, which is easy to graze and manage. Sheep and goat production is an integral component of rural economy of India and serves as major source of economic sustenance for weaker segments of the society in the hot semiarid and arid region. The species is traditionally reared by small and marginal farmers and land less laborers under extensive range management on CPR with top feed supplementation during lean season. As per last Census (2007), India was host to 71.5 and 140.5 million (m)

sheep and goats which increased to 74.0 and 154.0 m , respectively by 2010 (FAO, 2010). As per literature (Karim, 2008; Karim and Sankhyan, 2009) the slaughter rate of sheep and goats is 32% and 36 % hence a total of 23.68 and 55.44 m heads are slaughtered annually. Considering average carcass weight of 10 kg the total meat production by sheep and goats comes to 237 and 554 m kg respectively (Gadekar *et al,* 2011).

Hoofed mammals like sheep and goats belong to the highly successful order Artiodactyls, family Bovidae with wide geographical distribution. The main difference between ruminants and monogastric animal is that ruminant have four parts in stomach i.e. rumen, reticulum, abomasum and omassum. Food mixed with saliva in rumen and reticulum and separated in two layers solid and liquid. Then solid part of food regurgitated and mixes with saliva to break down in small particles. Fiber particles of food break in hexose monomers and further degraded by rumen microbes into volatile fatty acids like acetic acid, propionic acid, butyric acid etc. Polymers of amino acids and non structural carbohydrates are fermented to propionates. Then digested part of food moves towards stomach and from stomach it moves towards small intestine where the digestion is effected by the enzymes elaborate by the system followed by absorption of nutrients.

Sheep rearing is one of the oldest professions closely associated with human civilization wherein the domestication of animals was carried out during neolithic times along with the cultivation of cereals. First sheep and goat, followed by cattle and pig, and finally draft animals such as horse and asses were domesticated. Sheep ancestry status over the past 11,000 years has revealed that sheep are most important domestic animals, harboring huge genetic diversity (40 descript breeds in India) and substantial prospects for continued breeding (Acharya, 1982) to further boost meat and wool production for rising population. Human have molded sheep to suit diverse environments and to enhance the specialized production of meat, wool and milk. Earlier studies have identified particular regions of the sheep genome that appear to have changed rapidly in response to selection for genes controlling traits such as coat color, coat cover, body size, reproduction and especially, the lack of horns: one of the earliest goals of selective breeding (Kijas *et al*, 2012). Sheep (*Ovis aries*) are range fed ruminant and like all ruminants are even-toed ungulate. Domestic sheep are relatively smaller sized ruminants and depending on breed, sheep exhibit a wide range of heights and weights usually with a crimped hair called wool or hairy. Their rate of growth, mature weight and fecundity are heritable traits that are often used in selection for breeding. Ewes worldwide weigh between 45 and 100 kg and rams between 45 and 160 kg whereas in India the average weight of sheep is around 30-35 kg with coat cover ranging from hairy to wooly with fiber diameter of 20- 55 µ (Melinda *et al*, 2004).

Sheep and goats are closely related since both belong to subfamily *Caprinae* whereas they are separate species. Visual differences between sheep and goats include the beard and divided upper lip of goats. Sheep tails also hang down, even when short or docked, while the short tails of goats are held upwards. Sheep breeds are also often naturally polled (either in both sexes or just in the female), while naturally polled goats are rare (though many are polled artificially). Sheep are

primarily herbivorous mammals: most breeds are close grazers on surface vegetation and short height herbage, avoiding the taller woody parts of plant that goats readily consume. Sheep are predominantly grazers while the goats are browsers and in the process of grazing/browsing both the species resort to intensive selection on plant parts rich in nutrients and pick up a wide range of vegetation from the range land. Both lambs and kids are mono-gastric animals at birth while coming in contact with surrounding environment microbes inhabit their rumino-reticulum within two months transformation, lambs and kids became small ruminants.

The first chamber in alimentary canal is rumino-reticulum of small ruminants and it is also biggest part of rumen. Microbial fermentation of food occurs in it. Reticulum is the smaller part and continuous with rumen. In rumino-reticulum the digestion is a very complex process and occurs through fermentation by microbes and digestion of cellulose and other carbohydrates proceeds here.

There are different prokaryotes (Eubacteria, archea), eukaryotes (Protozoa and fungi) and viruses harbors the rumen. Protozoa engulf other microbes through phagocytosis for their nutrient requirement. Hydrolization of structural carbohydrate and non carbohydrates occurs by microbial enzymes and these are fermented to volatile fatty acids and polymer of amino acids breaks into peptides and amino acids through microbial enzyme and stores as cell biomass. Nitrogen sources present in feed used directly by microbes with in small quantity. If nitrogen is required in excess amount, then proteins are also fermented to produce energy and yield ammonia. There is not dominant role of lignin, lipid, vitamins and minerals to produce energy. Rumen microbial ecosystem is a complex medium of different microbial groups living in a symbiotic relationship with the host and capable of degrading structural carbohydrates (Wolin and Miller, 1988). Rumen microbial ecosystem is specialized and buffered in a narrow range of pH thus stabilizing the system (Kamra, 2005).

Rumen protozoa play important role in feed fermentation particularly in methanogenesis. The rumen protozoa are categorized into two broad heads viz. holotrichs and entodiniomorphhids and they can also be classified as soluble sugar/starch degrader and ligno-cellulose hydrolyser. The ability of sheep and goats to utilize wide range of conventional feed, top feed and otherwise toxic herbage is possible because of some microbial adaptation in rumino-reticulum. In process of rumen microbial degradation, 12-15 % digestible energy is wasted as rumen gases particularly CH_4. In rumen fermentation hydrogen and CO_2 are contributed by ciliate protozoa, bacteria and fungi while methanogenic bacteria utilize the hydrogen to reduce CO_2 to produce methane. Hence elimination of organism contributing to synthesis of hydrogen in the rumen or methane synthesizing microbes will save digestible energy loss in waste full fermentation and increase digestible energy availability to the host animals for better production. However rumen bacteria cannot be effectively eliminated by chemical treatments because of associated adverse effects to the host animals hence alternatively the elimination of protozoa and fungi by suitable treatments without deleterious effects is the method of choice.

Climate change is no longer a distant threat as inter-governmental panel on climate change (IPCC) has amply demonstrated that we are already experiencing climate change induced by human activities. Industrial revolution based on combustion of hydrocarbons (initially coal followed by petroleum and natural gases) has increased emission of Green House Gases (GHG) leading to accumulation of carbon dioxide in the atmosphere precipitating global warming and climate change. Methane is a green house gas whose atmospheric concentration has increased dramatically over the last century. CH_4 is emitted from variety of both human related (anthropogenic) activities and natural sources. Human related activities include fossil fuel production, animal husbandry (enteric fermentation from livestock and manure management), rice cultivation, biomass burning and atmosphere. It is estimated that 60 % global CH_4 emission is related to human activities. Natural sources of CH_4 include wasteland, gas hydrates, permafrost, termites, ocean, fresh water bodies, wasteland, soils and sources such as wild fires. The domestic animal population has increased by 0.5-2.0 % annually during last century and one of the results of this population increase is that emission from livestock has become a significant source of atmospheric methane. In fact, domestic animals currently account for about 15 % of annual anthropogenic methane emission.

Worldwide distribution of sheep indicates their ability to adapt to a variety of environments. However, the preferred environment is on the lighter sandy soils in hot semiarid and arid, drier tropics, rather than in the wet humid tropics: in India they perform best and thrive well in large numbers in the dry tropics. The inherent characteristics of goats such as resistance to dehydration, wider choice of vegetation, and wide-ranging feeding habits with preference for browse species, enable them to perform better than sheep in regions that receive less than 750 mm of rainfall (Khan *et al*, 2003). However proliferation of sheep has provided breeds or types adapted to almost every climate, from snow-covered hills to hot semiarid/arid environment, but sheep are essentially grazers and prefer to graze on short plants/ground coverage as a result they thrive best on rangelands with a low-growing plant population that usually occurs in the drier areas.

Various features of sheep and goats

Sheep may be distinguished from goats by the presence of a beard, strongly odoriferous tail-glands of the male, the absence of facial glands and lachrymal pits in the skull, the absence of foot glands in the hind feet. Domestic goats generally carry their tails up, while these are hanging in sheep. The body cover differs widely between the two species, hair in case of goats and hair/hairy wool or wool in sheep. Horn direction and spirals also exhibit variation in sheep and goat and skeletal differences also exist (Khan *et al*, 2003).

Sheep as individuals and breeds are more sensitive to environmental changes than other domestic animals but as a species they thrive everywhere. Worldwide distribution of sheep indicates their ability to adapt to a variety of environments. However, the preferred environment is lighter sandy soils in hot semiarid and arid, drier tropics, rather than in the wet humid tropics: in India they perform best and thrive well in large numbers in the dry tropics. The inherent characteristics of

sheep/goats such as resistance to dehydration, wider choice of vegetation, and wide-ranging feeding habits with preference for browse species, enable them to thrive in regions that receive less than 750 mm of rainfall. Proliferation of sheep has provided breeds or types adapted to almost every climate, from snow-covered hills to hot semiarid/arid environment, but sheep are essentially grazers and prefer to graze on short plants/ground coverage as a result they thrive best on rangeland with a low-growing plant population that usually occurs in the drier, but not the driest areas. As is the case with goats, sheep adapted to the humid environment appear to be smaller in size than those adapted to the drier climatic regions. The most telling difference, though not visible, is that sheep have 54 chromosomes and goats have 60. Male goats have a characteristic smell, which is quite different from the smell of a ram. The rams have a secretary gland on the hind feet which goats do not possess. Among the similarities the important features are: both are ruminants, ungulates, cloven-hooved, similar dentition, both have horned and hornless breeds and both species have some dairy breeds. In addition, both sheep and goats have been domesticated for thousands of years. Sheep are grazing animals while goats are essentially browsing animals. Goats have a competitive advantage over sheep in woodland and shrub land, are generally more active, selective, walk longer distances in search of feed and relish variety of feeds (Devendra, 1990). Sheep are less selective and utilize pasture more effectively. Another feature of the feeding behavior of goats is their discerning ability to taste. Goats can distinguish between bitter, sweet, salty and sour tastes, and show a higher tolerance for bitter taste than do sheep and cattle (Bell, 1959; Goatcher and Church, 1970). Sheep and goats behave differently on rangeland: goats are naturally curious and independent, while sheep tend to be more distant and aloof. Sheep have a stronger flocking instinct and become very agitated if they are separated from the rest of the flock. It is easier to keep sheep inside a fence than goats. Goats will seek shelter more readily than sheep.

Differences in digestion between grazers and browsers

First, differences between grazers and browsers exist in the structure of molars, which would be expected to influence chewing rates and longevity of teeth. Grazers like sheep tend to have wide muzzles, with lower incisors of similar size that project forward in a spatulate fashion (Janis and Ehrhardt, 1988). The greater incisor width of grazers should serve to maximize bite size (and thus harvest rate) when feeding on a continuous distribution of grasses (Illius and Gordon, 1987; Janis and Eharhardt, 1988). However, wider muzzles reduce the grazer's ability to select the smaller, more nutritious portion of grasses (Janis and Eharhardt, 1988). Australian workers (Wilson *et al*, 1975) used esophageal fistula to study the food preferences of captive feral goats compared with sheep at three grazing pressures (0.5, 0.25 and 0.17 animals per hectare). At low stocking rates, sheep ate 80 % herbs and 20 % browse, while goats preferred the reverse. At medium and high stocking rates, availability of herbs governed intake. Goats tended to select diets with appreciably higher nitrogen content than sheep, but *in vitro* digestibility of the nitrogen was not always as high. The significantly higher rumen volume in goats confirms a similar report from Australia

(Watson and Norton, 1982), which was accompanied by a longer mean retention time. With high quality forages, it was concluded that there may be little differences between-species in partitioning of nutrients, digestion of dry matter, neutral detergent fiber and non-ammonia nitrogen (Alam *et al*, 1985). In the same study it was also reported that there were no major differences between species in protein digestion. In the study comparing sheep and goats on anatomical and physiological differences (Singh *et al*, 1980) reported that the gut length of sheep was more than goats while total retention time of digesta in the GI tract was more in goats than sheep.

Dry matter intake (DMI) and total water intake is generally higher in sheep than goats (Quick and Dehority, 1986). However in another study, goats shown greater digestible organic matter intake (DOMI) that sheep (Alam *et al*, 1985). Differences between browsers and grazers extend beyond diet selection: they include specialization within the digestive tract that may allow grazing and browsing herbivores to better extract nutrients from their preferred forage class. Grazers and browsers have measurable differences in the morphology of the foregut (rumen, reticulum, abomasums and omasum), the hindgut, salivary glands, mouth, teeth, liver, and body mass that may influence their ability to consume grasses and browses and its digestion. All fore gut fermenters have a pouch (rumen/reticulum) that lies before the true (acid-pepsin) stomach (abomasum) in which the bulk of fermentation occurs. The longer plant fiber is retained in the rumen, the more complete the digestion of cellulose and other structural carbohydrates (Demment and Soest, 1985).

As regards rumen contents, sheep are almost exclusively grazers. In goats, Thomson's gazelle and Impala, grass accounted for about 70 % of all plant parts identified. In Grant's gazelle, browse including *Acacia* seed which constituted 68 % of rumen ingesta. The preferred food selected from range land would in turn decide the types of microbial dominance in reticulo-rumen. Sheep band together and stay together when grazing for protection. This instinct is stronger in fine wooled sheep such as the Rambouillet and decreased in black faced sheep like the Suffolk, but it is there to some degree in all sheep (Cobb, 1999). Grazers tend to have larger, more muscular, subdivided rumen/reticulum, and a smaller opening between the reticulum and omasum than do browsers (Shipley, 1999). This adaptation may serve to retard the fractional passage rate of digesta to lower tract, giving more time for fermentation of plant fiber. Because a greater proportion of grass cell is cellulose hence this adaptation would presumably allow grazers to digest the cell wall more thoroughly and obtain more energy per unit of food. Moreover during higher water intake in terms of DMI result in faster rumino-reticulum wash out which is reflected in lower fiber digestibility (Odashima *et al*, 1991). Although the total length of GI tract is higher in sheep than goats, still greater fractional retention time beyond the pyloric end till ceacum in goats leads to higher total retention time in GI tract of goats than sheep. In contrast, grazers have fewer, uneven papillae that limit the absorptive capacity of the rumen. Browsers have a proportionately large abomasum, or true stomach, a larger hindgut (ceacum and colon), and the ventricular groove in the rumen/reticulum which allow some cell contents to escape inefficient rumen fermentation in favor of

direct digestion in the abomasum and lower digestive tract.

In contrast, most browses contain less cell wall and fiber within their cell wall are more lignified and indigestible, so the smaller rumen of browsing animals should allow indigestible food particles to flow more rapidly through the tract (Shipley, 1999). This rapid flow should promote a higher food intake. Browsers (goats) tend to have extensive dense papillae in all parts of the rumen, enlarging the surface area by 22 times, which may allow efficient absorption of VFA's from the rapidly-fermenting cell contents of the browse plants. Anatomical differences in sheep and goat digestive tract, digesta retention time and gut contractibility indicate that goats have greater ability to adapt to a wide range of conditions (Coblentz, 1977; Soest, 1982) but the causes of that ability are not clear. A higher digestive capacity has been found in goats in comparison with sheep when consuming roughages with low nitrogen and high lignin contents (Gihad *et al*, 1980; Doyle *et al*, 1984; Howe and Barry, 1988). These differences have been ascribed to special ability of goats for selecting the morphological parts of the plants with the highest nutritive value (Morand-Fehr *et al*, 1991), which become pronounced when food availability is scarce (Soest, 1982; Pfister and Malechek, 1986; Bato and Sevilla, 1988), greater retention time of the digesta in the rumen of goats (Watson and Norton, 1982; Domingue *et al*, 1991) and to interspecies differences in the rumen environment, such as a higher production of microbial protein in goats (Hadjipanayiotou and Antoniou, 1983) or a higher number of cellulolytic bacteria in goats than in sheep (Gihad *et al*, 1980).

Physiological differences: pH, water requirement and saliva secretion

An animal's anatomy and physiology clearly affect its food choices. Characteristics of food, in turn, are one of the primary forces that shape animal behavior, physiology and anatomy (Alam *et al*, 1964). Present evidence suggests that sheep are more selective, have a higher intake, rumen volume/gut fill, salivary secretion and rumen ammonia/urea recycling but lower water intake and turnover rate compared to goats. Increased salivary function and urea recycling may be associated with their ability to have a higher tolerance for tannins. On the other hand goats appear to digest fiber more efficiently than sheep (Gihad, 1976). Precise reasons for these and a much better understanding of the real differences merit more thorough investigations on particle size, salivary secretion and microbial activity. Rumen pH was not significantly different between species, with mean values ranging between 6·0 and 6·3. Besides differences in the structure of the gastrointestinal tract, grazers and browsers also differ in the relative size of the parotid salivary glands and composition of saliva. Parotid salivary gland weight increases linearly with body mass in both grazers and browsers, but averages 4 times larger in browsers than in grazers (Robbins *et al*, 1995). Although (Hofmann, 1989) suggested that larger parotid salivary glands yield greater flow of liquids to the digestive tract and buffer fermentation. Robbins *et al.* (1995) did not find differences in rate of saliva production between grazers and browsers. Cattle and sheep saliva is thin and watery compared to mule/deer saliva which is viscous and gelatinous. These observations suggest that the larger parotid salivary glands of browsers produce tannin binding

salivary proteins that may prevent tannins in browses from greatly reducing protein digestibility (Austin *et al,* 1989; Robbins *et al,* 1995). Hofmann (1989) also noticed that browsers have up to 100 % more liver tissue for their body size than grazers because plant secondary metabolites and toxic chemicals present in browses may be detoxified in the liver (Foley *et al,* 1995), a large liver might be an additional adaptation to the chemicals in browse species which do not commonly occur in grasses.

Microbial population in ruminants

The rumen is composed of several muscular sacs, the cranial sac, ventral sac, ventral blind sac and reticulum. The lining of the rumen wall is covered with small fingerlike projections called papillae. The reticulum (derived from the Latin for net) is lined with ridges that form a hexagonal honeycomb pattern. The hexagons in the reticulum increase the surface area of the rumino-reticulum wall, facilitating the absorption of VFAs. Despite the differences in the texture of the lining of the two parts of the reticulo-rumen, it represents one functional space. In rumino-reticulum digestion of feed is very complex process and it occurs through microbes by fermentation rather than enzymes which are secreted by animal system. Digestion of cellulose and other structural and non structural carbohydrates occurs in rumino-reticulum. Not only starch, sugar and pectin are the non structural carbohydrates which are digested here but also structural carbohydrate like cellulose, hemi-cellulose as well as nitrogen containing compounds like amino acids and polymer of amino acids (protein) are also well digested in it. Here microbial enzymes are used to hydrolyse monosaccharides and di-saccharides. Then these monosaccharides and di-saccharides are transported to microbes or fermented to VFAs like acetic acid, propionic acid, butyric acid, lactic acid and valeric acid to yield energy for the microbial cell. Rumino-reticulum wall absorbs most VFAs and transferred them to blood stream where these are used as substrate for energy production and biosynthesis and hydrolyzed product of protein are transferred across the microbial cell wall for assimilation into cell biomass. A little amount of nitrogen containing source like peptide, amino acid and ammonia are used directly by microorganism without hydrolyzing. If the nitrogen for microbial growth is in excess amount then protein and its derivatives can also be fermented to produce energy and yield ammonia.

If glycerol is present in lipids, than it is fermented otherwise it is partly hydrolyzed and hydrogenated. Proteins and some carbohydrates may be used for de novo synthesis of microbial lipid. Unsaturated lipids are poisonous for microbes present in the rumen and these are also suppressing fermentation activity. Fungi play an important role to solublise phenolic compounds like lignin. Minerals which are necessary for growth are also absorbed by microbes. Microbes also synthesize many vitamins like cyanocobalamine (Vit. B_{12}) in large quantity which is often enough to sustain the ruminant even when vitamins are highly deficient in the diet.

Ruminants, the fore gut fermenters, have developed a microbial symbiosis to digest fiber in ingested feeds (Dehority, 1997). Rumen flora and fauna belonging to diverse families from bacteria, protozoa, fungi and phages (Kamra, 2005). Among the ruminal micro biota, members of the

domain Archea, which occupy < 4 % of the entire microbial population, play a vital role in microbial fermentation (Lin *et al*, 1997; Sharp *et al*, 1998; Ziemer *et al*, 2000). The majority of the Archaea in the rumen are methanogens which utilize H_2 as the energy source to reduce CO_2 to CH_4 and provide oxidized reducing factors (e.g., NAD+) to other microbial metabolic pathways (Hungate *et al*, 1970; Wolin, 1979). Methanogenesis provides thermodynamically favorable conditions for ruminal microbial fiber degradation (Zinder, 1993). However, the released CH_4 results in loss of dietary energy (Johnson and Johnson, 1995) and contributes to agricultural greenhouse gas emissions (Enviornment Canada, 2002; International Panel on Climate Change, 2001). Consequently, a better understanding of methanogens may facilitate mitigating production of enteric CH_4: a significant contributor to greenhouse gases. The methanogenic archaea are a morphologically diverse group of strict anaerobes that can be extremely thermophilic, moderately thermophilic, or mesophilic. Although they resemble bacteria, existing as cocci, spirillum, and rods, methanogenic archaea are phylogenetically and physiologically distinct from bacteria. Methanogens use hydrogen to reduce carbon dioxide to methane gas, hence their common name "methanogens". However, some methanogens use methyl compounds or acetic acid instead as alternatives to hydrogen and carbon dioxide for methanogenesis. Several species of methanogens have been isolated from the gastrointestinal tract of ruminants and other vertebrates, as well as from invertebrates, marine and bog sediments, lakes rich in decaying vegetation, sewage sludge and hydrothermal vents (Garcia, 1990; Wright and Pimm, 2003; Eecke *et al*, 2012). These microbes play significant role in the biological breakdown of organic matter in the anaerobic environments to methane which is produced by domesticated ruminants. However, methane is a very potent greenhouse gas that is estimated to be 23 times more potent than carbon dioxide. Hence, the growing interest in isolating and identifying methanogens from various environments, especially those from ruminants would be helpful in developing strategy to reduce GHG emission. Studies are required to delineate differences in microbial population between sheep and goat which might be helpful in enhancing productivity of sheep and goats reared under changing climatic conditions.

Green House Gases (GHG) and ruminants

As per 1994 estimates of GHG, all together 1228 m MT CO_2 equivalent (CH_4 21 and N_2O 310 times) emission took place from all anthropogenic sources in India which amounted to 3 % of global emission. Out of this about 794 m MT i.e. 63 % of CO_2 equivalent was emitted as CO_2 while 33 % of emission (18 m MT) was in form of CH_4 and the rest 4 % (178 m MT) was N_2O. The higher contribution of CO_2 was due to transformation activities, fuel combustion in transport, cement and steel production. Likewise the enhanced CH_4 production was due to emission from enteric fermentation from livestock and rice cultivation. The major contribution of N_2O came from agriculture due to fertilizer application. In summary CO_2 contributed 61 %, agriculture 28 % and 21 % was contributed by other sources viz. industrial processes, waste generation, land use system, forestry etc. Although the compound annual growth rate of CO_2 equivalent emission is relatively higher in

India still it is 1/6 of USA. Moreover during the year 2000 per capita CO_2 equivalent emission was 15.3 % higher in USA than India. Agriculture contributes about 21- 25, 60 and 65- 80 % of total anthropogenic emission of CO_2, CH_4 and N_2O, respectively. Agriculture is thought to contribute 95 % NH_3, 50 % of CO and 35 % N_2O released in to atmosphere as a result of human activity. An emission of total 205-245 m MT CH_4 from agricultural sector is contributed from enteric fermentation (80), paddy rice production (60-100), biomass burning (40) and animal waste (25).

Methane emission by livestock species

Global warming and climate change is unequivocal as evident from increase in average global and ocean temperature, melting of polar ice cap and rise in sea level(IPCC, 2007;). Such climate change has lead to droughts/excessive rain in certain pockets damaging crop cycles and livestock production. Man made global Green House Gases (GHG) emission has increased since pre industrial times with an increase of 70 % between 1970 and 2004. The global concentrations of CO_2, CH_4 and N_2O have increased markedly as a result of human activity since 1970 and now far exceed the pre industrial values. Global increase of CO_2 is primarily due to extensive use of fossil fuel while land use changes also contribute marginally to the phenomenon.

Methane emission is characteristic of fore gut fermenters. In adult ruminants, the expanded fore gut (reticulo-rumen generally termed as rumen) represents 85 % of total GI tract volume amounting to 10-20 % of animal weight. In mono gastric animals, the fore gut digestion is effected by enzymes elaborated by the system while in ruminants the fore gut digestion is effected by symbiotic microbes inhabiting in rumen. In order to facilitate microbial degradation of feed consumed, the digesta is retained in foregut for longer period. In mono gastric digestion the end product is a monomer which is absorbed and assimilated in the body while in ruminants the monomers are further degraded to VFAs and fermentation gases. The VFA diffuse in to the system and the fermentation gases primarily CH_4 and CO_2 are eructed in to environment. It is established that under Indian conditions this loss will amount of 8-28 g CH_4/kg dry matter intake depending on species, production level, physiological state and type of feed consumed by the animals. Hind gut fermentation is another source of CH_4 accounting for 10-30 % which is mainly absorbed in to the system excreted through lungs and a fraction of it is passed off per rectum as flatus. Depending on the level of feeding, composition of the diet and digestibility of nutrients it is estimated that 2-15 % of gross energy of diet is lost as CH_4.

Rumen methanogenic bacteria utilize H, CO_2 or formate, acetate, methylamine and methanol for production of methane (Zeikus, 1977). However the major substrate for formation of methane in rumen is H and CO_2 while minor substrate is formate and acetate. The major factor affecting rumen fermentation of CH_4 are rumen pH and turnover rate of digesta while both in turn are affected by diet and, level of feed intake, feeding strategy, R: C ratio and quality. Cattle and buffaloes are the most important source of CH_4 from enteric fermentation in India because of the large population, large body size, predominantly roughage bases feeding system and ruminant digestion. Methane is also produced from decomposition of

animal excreta under aerobic conditions particularly where the animals reared under intensive system and under confinement. Methane emission from manure management is relatively smaller in quantity than enteric emission and of significance in intensive production system. The principal factors affecting CH_4 from animal manure are amount of manure produced and proportion of manure allowed to decompose an aerobically and climate of the location. One of the mitigating factors in manure management under Indian condition is the *in vogue* production system. Majority of livestock are reared under extensive production system where the feces voided are dispersed in grazing area which is rapidly desiccation under hot sun without further decomposition.

During the last century global domestic animal population have increased by 0.5 to 2.0 % and one of the consequences of population growth is the increase in emission of CH_4. Cattle and buffalo on an average consume 4.0 kg DM/day while it is 0.8 kg/day in sheep and goats emitting an average of 78.4 and 19.6 g CH_4/day, respectively. Accordingly among the livestock species cattle, buffalo, goat and sheep contribute 54.72, 30.37, 9.70 and 5.38 % of total 9.31 Tg amounting to 12.4 % of total world emission of 75 Tg contributed by animals. It is generally agreed that domestic animals currently account for 15 % of annual anthropogenic CH_4 emission (Crutzen *et al*, 1986). However, CH_4 emission levels and sources widely vary among the countries depending on climatic conditions, industrial and agricultural production, energy types and its usage and waste management practices.

Rumen methane production

A wide variety of methanogenic Archea inhabit in rumen medium (Kim, 2012) out of which only 10 % have been characterized employing gene sequencing. Until recently it was thought that only three genera of methanogens are present in the rumen (*Methanobravibacter*, *Methanomicrobium and Methanosercina*) while of late, the total known genera of methanogenic Archea has increased to 22 (Leahy *et al*, 2010) hence it is likely that more genera especially more sub species of rumen methanogens will be identified. Modulation of rumen microbes by development of vaccines may be effective on some methanogens but not others (Wilams *et al*, 2009). Moreover development of vaccines has inherent limitation that it cannot be effective without knowing the exact identity of target organism. Lack of information on predominant species of rumen methanogens in Indian ruminants is a serious limitation for developing biological approach to emission control.

The biochemical pathways and the avenues for interventions in rumen methanogenesis are well defined and the pathways and enzyme system which enables methanogens to generate ATP from CO_2 and H have been intensively studied. The biochemical pathways for methanogenesis are not the limitation in development of protocol for emission reduction but understanding the chemistry of methanogens particularly glycoprotein S-layers and their intensive study will favor development of methanogen specific antibiotics (Deppenmeier, 2002). The methanogenesis serve as process of exhaust system in rumen fermentation to remove hydrogen gas which would otherwise inhibit the activity of bulk of other organism those do not contribute to

methane production. Hydrogen is considered as currency of rumen fermentation as transfer of hydrogen between organisms and its eventual disposal is fundamental to freeing the primary fermenting organism to ferment fresh feed. The principal source of hydrogen is the bacteria and protozoa which produce acetic acid hence the ruminants maintained on high fibrous diet usually have higher protozoa population and generally have higher methane emission (Prins *et al*, 1977). Hence provision of alternate hydrogen sink in the rumen or reduction of hydrogen production by manipulation of other hydrogen contributing microbes or defaunation will increase digestible energy availability, reduce emission of methane and improve product (Santra *et al*, 2003).

Normally about 12-14 % digestible energy is wasted in rumen fermentation as methane wherein the rumen protozoa contribute H^+ which is utilized by methanogens to convert it to methane. About 37 % methanogenesis is due to rumen methanogens or rumen protozoa (Newbold *et al*, 1995). Therefore elimination of rumen protozoa by defaunating agents will cut off the source of substrate for methanogenesis thereby improving utilization of digestible energy to the tune of 12-14 % with proportionate improvement in livestock production (Santra *et al*, 2003). Hence defaunation not only reduce emission in ruminant digestion but also improve energy availability for productive purpose.

Based on the fore going observations it could be inferred that there are large differences in the pattern of rumen fermentation between wild and domestic mixed-feeding ruminants which has evolved due to their respective food preferences. The basis for this conclusion is due to relatively large number of cases in which goats raised in harsh environments were found to be superior to other ruminants. Whenever goats were found superior to other ruminants, the digestive physiology was under reference viz. goats had an extended retention time of digesta in the gut (Devendra, 1990), and higher cellulolytic activity in the rumen which could be partially related to a more efficient recycling of urea from blood to the rumen (Devendra, 1990; Tisserand *et al*, 1991). The greater secretion of saliva in goats in comparison to sheep (Dominigue *et al*, 1991; Seth *et al*, 1976), and the larger surface area for absorption from the rumen due to broad leaf like papillae compared to narrow tongue like papillae in sheep (Bhattacharya, 1980) is a general characteristic of intermediate feeders like goats, in comparison to grass eaters, like sheep. These characteristics may explain more efficient urea recycling to the rumen because urea recycling is mediated via saliva secretion and via diffusion through the rumen walls. The process prevent fall in rumen pH even at peak fermentation due to buffering by higher salivary flow which is the major contribution to rumen buffer capacity and relatively efficient absorption of VFAs through the rumen wall also enhance the buffer capacity of the rumen (Silanikove *et al*, 1993). A capacity to maintain a spacious rumen helped the Bedouin goats to quench against reduction in the quality of the diet (Silanikove *et al*, 1993) and to maintain sufficient food intake under infrequent watering regimen (Silanikove, 1992; Silanikoe, 1994). The Mediterranean spring green vegetation has high protein content (>14%) and high *in vitro* and *in vivo* (Cattle, sheep) digestibility (70/80 %). Unlike sheep and cattle with abundant grazing on leafy material during spring,

browse constitutes at least 50 % of the forage selected by goats (Kakabya, 1994; Kakabya *et al*, 1998; Lu, 1988; Mill, 1990).

Rumen microflora of sheep and goat containing a large no. of bacteria from which three strains of tannin tolrent bacteria have been isolated in medium containing high concentration of crude tannin extract (Odenyo and Osuji, 1988). *Selenomonas ruminantium* is capable of growing on tannic acid as a sole energy source and has been isolated from feral goats browsing on high tannin containing acacia species (Skene and Brooker, 1995). Transferring these micro-organisms from feral goats to domestic goats and sheep fed tannin-rich foliage (*Acacia aneura*) increased feed intake and nitrogen retention in inoculated animals compared with uninoculated ones. Inoculation also improved N digestibility and improved rate of live weight gain in sheep and domestic goats (Miller *et al*, 1995). It is shown that acclimatization of the microbial system in the rumen of goats adapted to the Mediterranean scrubland forms a very important element in the capacity of these goats to utilize efficiently high-tannin tree leaves (Gilboa, 1996). Spring in the Mediterranean is very short, and after three months, the nutritional quality of the grass diminishes at an accelerated rate. Thus, much of the short-term advantage from switching the grazing habits can be lost during the time necessary to regain the capacity required for digesting high-tannin browse sources. It seems that although goats take advantage from the abundance of highly digestible grass (increasing its proportion from approximately 10 % in winter to 40-50 % in spring), they maintain the intake of browse sufficiently high to preserve their acclimatization to tannin-rich food. This maintains their specific advantage in digesting the food that is available to them in large amounts all the year around. On the other hand, the ability of goats to survive prolong periods of water deprivation allows them to graze far from the watering site and to exploit desert pasture evenly and efficiently. As a result, cattle are much more susceptible to changes than sheep and goats to malnutrition, disease and death during severe droughts. Goats in the tropics thrive well on diets composed of tree-leaves and shrubs (browse), which ensure a reliable and steady supply of food all year around, albeit, a low to medium quality food. This grazing strategy in combination with the anatomical and physiological adaptations renders the goat most efficient desert-dwelling species among domestic ruminants. The most remarkable feature of the intermediate selector ruminant is characterized by short-term or seasonal anatomical acclimatization to changes in forage quality (Hofmann, 1989). The corresponding morpho-physiological adaptations are larger salivary glands, higher surface area of absorptive mucosa than in grass and roughage eaters and capacity to increase substantially the volume of the foregut when fed high-fibrous food. The results discussed suggest that the general characteristics of intermediate feeders are probably important for the development of superior digestion and nitrogen conservation capacities and for the efficient use of water in goats.

1. **Biotechnology tools for assessment of rumen microbial diversity**

2. Delineating the diversity of rumen microbes are of prime importance to intervene and manipulate rumen fermentation with ultimate goal to enhance

productivity by improving utilization of easily available low grade roughage diets. However, it would be difficult to study diverse population of rumen flora and fauna primarily due to requirement of stringent conditions for *in vitro* culturing and isolation in pure culture. The recent advancement in molecular biology tools have brought boom in this field of study and made this task possible. Biotechnology based tools helps in studies of complexities of microbial communities function and interaction among these microbes. Furthermore, molecular tools proved effective in culture independent analysis of the rumen ecosystem (Ghazanfar and Azim, 2009). Molecular ecological studies proved better in determining the metabolic role of uncultivated species of rumen (Edwards *et al*, 2004; Wright *et al*, 2004, 2006 and 2007). Advances in DNA extraction from rumen fluid and low cost sequencing technology has rendered analysis of microbial diversity within the reach of the small set up biotechnology laboratories worldwide including India. The conventional culture based techniques for estimating rumen microbial diversity is being rapidly replaced by modern molecular techniques. High quality DNA extraction from rumen liquor/contents, PCR amplification, cloning and advancement in sequencing protocols made this task possible. Sharma *et al.* (2003) described the DNA extraction protocols from rumen contents and several other workers have also mentioned the improved protocols for the same. Total DNA representing the complete diversity of rumen microbial communities is necessary to determine the composition of the microbial community and monitoring changes in population size. Advances in DNA extraction methods from rumen contents are instrumental in this field of study. Kang *et al* (2009) described the RNA extraction method for estimating gut microbial diversity. Popova *et al.* (2010*)* were the first to report a protocol for high-quality DNA/RNA co-extraction from rumen ingesta, thus improving nucleotide extraction efficiency. The success of applying molecular approaches strongly relies on the DNA/RNA quality, and proper nucleic acid isolation methods must be applied when studying different types of samples. Previously, DNA/RNA extractions were mostly conducted separately for rumen samples, because of the varied requirements of these two types of molecules (Guan *et al*, 2008).

For molecular identification, PCR amplification is the first essential step to enrich the DNA of microbial cells that are present in low numbers. PCR-cloning-sequencing methods are best suited for direct examination of the ruminal diversity of the microbiomes.

PCR detection and enumeration of rumen microbes has been successfully applied to study the molecular diversity of rumen microbiota (Koike and Kobayashi, 2001; Koike *et al*, 2003). Quantitative PCR chemistries have also been used with success to monitor and enumerate the microbial populations in the rumen (Tajima *et al*, 2001a; Sylvester *et al*, 2004; Skillman *et al*, 2006b; Koike *et al*, 2007). Real time PCR is important tool in quantitative analysis of different ruminal flora and fauna however sequencing of conserved genes like ribosomal RNA or other conserved gene like mcrA in methanogenes (Luton *et al*, 2002; Tatsuoka *et al*, 2004) will give better opportunity to identify species specificity. Pillard *et al.* (2007) has used molecular beacon chemistries for quantification of ruminal microbial population and

monitored diet associated changes in the population. Stivension *et al* (2011) has used this technique to quantify the uncultured bacterial population from sheep rumen fed on different diets. Abundance and population dynamics of uncultured bacteria was quantified in the study. Real-time PCR results complement the results of rRNA clone libraries. In another study, Zhou *et al* (2012) has used real time PCR to enumerate rumen methanogenes, bacteria, protozoa and fungi. In this study small subunit ribosomal DNA (SSU) i.e. 16S rDNA gene for methanogenes and bacteria and 18S rDNA gene for protozoa and fungi were used to quantify the microbes. In contrast, RT-PCR is an approach developed to provide quantitative measurement of a target from the early phase of PCR amplification. The detection threshold of RT-PCR is termed the threshold cycle (Ct) which is the point where the amplification curve surpasses the threshold line and enters an exponential phase. As a result, RT-PCR can measure the relative density of target molecules by comparing the Ct value with a reference, or measure the absolute quantity of the targeted fragments by reference to an external standard. This method has been utilized to quantify the abundance of the archaeal community (Ohene-Adjei *et al*, 2008; Hook *et al*, 2009; Zhou *et al*, 2009 and 2010) and to compare specific members among cattle fed different diets, and with different feed efficiencies (Zhou *et al*, 2009).

Downstream to PCR like PCR-restriction fragment length polymorphism (RFLP), PCR- denaturing gradient gel electrophoresis (DGGE) and PCR-sequencing techniques have also been used successfully to characterize rumen microbe diversity (Hiraishi *et al*, 1995; Withford *et al*, 1997 & 1998; Wood *et al*, 1998; Kocherginskaya *et al*, 2001; Krause *et al*, 1999; Neufield *et al*, 2004; Regensbogenova *et al*, 2004a & 2004b). These techniques have been used to monitor succession of microbes in rumen with age and diet and also impact of a particular feed/feed additive on microbial diversity. DGGE results had shown greater microbial diversity as compared to 16S clone library (Rattray and Craig, 2007). Several other studies have shown the effectiveness of these techniques in study and identification of culturable and nonculturable microbes using ruminal fluid DNA/RNA (Ivey *et al*, 2009). Molecular diversity of rumen has been investigated by 16S rRNA gene library (Tajima *et al*, 1999; Tajima *et al*, 2001b; Wright and Pimm, 2003; Wright *et al*, 2004; Shin *et al*, 2004). Sequencing study provide better ecological information about rumen microbes and functional information on uncultured group of rumen microorganism could be obtained by providing required stringent conditions for culturing to characterize its enzyme secreting ability for feed digestion. 16S rRNA gene markers were used to quantify changes in the microbial population in the rumen (Skilman *et al*, 2006a; Perumbakkam *et al*, 2011). Since methanogens possess unique 16S rRNA gene sequences, and produce CH_4, both16S rRNA genes and genes coding enzymes that are unique to methanogens have been utilized to distinguish them from other microorganisms. Early experiments to obtain PCR amplicons of methanogen specific genes from different environments included amplification of the methyl-coenzyme M reductase (mcrI) gene of the family *Methano sarcinaceae* (Springer *et al*, 1995) and the 16S rRNA gene from methanogens isolated from blanket bog peat samples (Hales *et al*., 1996). Presently, the 16S rRNA gene and

methyl-coenzyme M reductase A-subunit (mcrA) gene are the principal targets that are amplified and used to characterize methanogens from environmental samples. An alternative to the 16S rRNA gene for phylogenic analysis is the mcrA gene. Methanogens have few morphological traits thus making them difficult to identify. They also have limited physiological diversity and some are either difficult to grow, or grow very slowly. With the advent of molecular technology, the methanogens were one of the first groups to have their taxonomy based upon 16SrRNA gene comparisons (Balch *et al*, 1979). Thus, many species can be easily identified by their 16S gene sequence. Consequently, a number of methanogen-specific fingerprinting assays have been developed with the aim of being simpler and rapid than conventional phenotypic characterizations.

Several other molecular methods like hybridization- and array-based techniques have been successfully applied to study the microbial diversity in different ruminants (Dore *et al*, 1993; Lin *et al*, 1997; Stahl *et al*, 1988; Dennis *et al*, 2000; Manefield *et al*, 2002; Ziemer *et al*, 2003; Koike *et al*, 2010). 16S rRNA-targeting probes have been used to compare the rumen microbial ecosystem (Doreay *et al*, 2002). Fluorescent in situ hybridization (FISH) probes targeting 16S rRNA genes are used to study the rumen microbial diversity of both cultured and uncultured populations.

DNA microarray platform offers the possibility to analyze biodiversity of microbial communities without cultivation. DNA microarray technology has ability to detect and measure thousands of distinct DNA sequences present in DNA samples extracted from rumen fluids. Microarray technology has been recognized as potentially valuable tool for high throughput, quantitative and systematic studies of microbial communities (Palmer *et al*, 2006). Flow cytometry (FCM) based analysis has also been successfully used in determining the phylogenetic position of uncultured rumen microbes (Yanagita *et al*, 2003).

Advent in sequencing technologies made possible the preparation of rumen microbiome profile domesticated ruminants. *De novo* assemblies of microbial genomes have been accomplished with new generation sequencing (NGS) technologies. Currently, 6 genome sequences belonging to 4 species are available for the family *Methanobacteriaceae*. *M. ruminantium* is the first methanogen from the rumen to have a completely assembled genome sequence.

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