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Panoply of microbial protein production in ruminants – A review

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ABSTRACT

Microbial protein (MBP) production plays a pivotal role in ruminant nutrition. Any improvement in the quantitative production of MBP in rumen can resolve simple to complex issues such as digestibility to methanogensis. Quantitative aspects of rumen microbial biomass are important than qualitative milieu present in rumen for functional expression of nutrition to the health and productivity of host animal. Functional biodiversity observed in the rumen microorganism is well orchestrated adaptation between various subpopulations. Magnificent array of biological, physical and chemical factors involved in MBP production and its efficiency are discussed in this review.

Key words: Biological, Chemical, Factors, Microbial protein, Physical, Rumen

The process involved in the establishment of microbial population (MBP) within the various compartments of the gastrointestinal tract is very complex, involving microbial succession as well as microbial and host interactions (Liu *et al*. 2008). Apart from their succession, the amount of bacterial mass produced in the rumen is as important as the energy produced during anaerobic fermentation. Their population dynamics are controlled by biological, physical and factors related to animal and dietary components (Krishnamoorthy and Srinivas 2010). Many reviews published in the past (Bergen 1977, Vériç *et al*. 1986, Karsli and Russel 2001, Verbic 2002, Russel 2007, Pathak 2008) dealt the subject restricting to a single factor or few factors in nutshell. Aim of the present review was to consolidate panoply of factors influencing the MBP production in rumen.

Microbial population in rumen: The archaeal bacterial microbial ecosystem of rumen (Fenchel and Finley 1995), comprises at least 30 predominant bacterial species at a total concentration of 10^{10} to 10^{11}, some 40 species of protozoa at a total concentration of 10^5 to 10^7 and 6 species of fungi at a total concentration of 10^5/ml of rumen liquor (Stewart *et al*. 1997, Williams and Coleman 1997, Nagpal *et al*. 2009). Bacterial species of the rumen are considered more important than protozoa and fungi in determining the extent and rate of particle reduction while fungi support in their adhesion of colonization (Lee *et al*. 2000).

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Functional biodiversity of rumen bacteria: Two diverse arguments needs highlight here where one argument considers that the species concept for bacteria is neither completely developed and applicability of the species concept to bacteria is contradicted (Cowan 1971) while others support the usefulness of characterizing and cataloguing microbial species based on molecular, genetic, environmental, ecological and metabolic end products data. A widely accepted bacterial species definition is 'a collection of strains showing a high degree of overall similarity, compared to other, related groups of strains' (Stainer *et al*. 1986) hence, we opine latter argument has more gravity. Rumen anaerobic bacteria are a source of extraordinary diversity with unlimited potential and variability in their end products of fermentative digestion in quantitative is important nutritionally, and often differ with their cataloguing because of variation in microbial composition and activity among animals fed the same diets which otherwise should have been similar as for the taxonomic and phylogenetic relationship (Weimer *et al*. 1999).

Bacteria were typically classified and aggregated into functional groups according to morphology and motility, growth factors required, substrates hydrolysis, and products produced in pure culture (Dehority 2003, Firkins and Yu 2006). Van Gulswyk (1990) classified rumen bacteria as (a) a wide range of species, (b) cellulolytic bacteria, (c) lactate-fermenting bacteria, and (d) non-fermentative bacteria and provided a clue that the entire microorganism present in the rumen does not indulge in fermentative digestion. Czerkavaski (1984) and Cheng and McAllister (1997) were described distinct subpopulations purely in nutritional point

of view as (*i*) those associated with the ruminal fluid and these are dynamic and move through the organ at a faster rate, (*ii*) those loosely attached to feed particles, (*iii*) those firmly attached to feed particles, and (*iv*) bacteria adhering to rumen epithelium. Weimer *et al*. (1999) reported that differences in cellulolytic populations in individual cows were larger and each cow maintains a unique assemblage of cellulolytic species. Though these studies present a overview of population biodiversity but less focused on quantitative aspects of their population of greater importance functional nutrition. Krause *et al*. (1999) quantified that the population size of major cellulolytic species in rumen, viz. *Ruminococus albus, R. flavefaciens* and *Fibrobactor succinogenes* was only 0.3 to 4%. and rest of the population can be manipulated in quantitative terms because they have cross-feeding relationship with the cellulolytic bacteria.

Metaphor of factors influencing rumen MBP production: Often quantitative estimates of MBP production could not drawn much attention as it required, due to practical problems associated in its estimation till an indirect, non-invasive methods were developed (Chen and Ørskov 2003). Different aspects of microbial nitrogen (MBN) or MBP (MBN × 6.25) production in rumen including the substantive findings as well as theoretical and methodological contributions were classified as animal, feed, biological, chemical and physical factors in rumen (Fig.1).

Animal factors and rumen MBP production

Species and age: MBN production varies with species, age and physiological stage (Russell and Rychlik 2001). Wanapat (2001) observed higher bacteria and fungal zoospores and lesser protozoa in the rumen of buffaloes than in cattle. MBP production rate in cattle fed straw alone or supplemented with concentrate supplement (CS) or urea-molasses-mineral block (UMMB) licks was 80, 269 and 251 g/d, respectively, as measured by ^{35}S Sodium sulphate (Srinivas and Gupta 1997). MBN production in sheep was 8 gN/d or 51 g of MBP when fed guar straw and CS (Srinivas *et al*. 2011). MBN production in lactating goats was 10.35 gN/d or 51 g of MBP when fed wheat straw and CS (Bala *et al*. 2009). Experiments of Fujihara *et al.* (2003) showed more allanotin excretion in kids than lambs when purine free diets were fed. These studies highlight that MBN production in goats is higher than sheep. Different genotypes (62.5, 50 and 37.5% blood of *Bos indicus*) of zebu crossbred cattle did not show any significant difference in purine metabolism and urinary excretion pattern (Ojeda *et al*. 2005). MBN production efficiency in cattle fed wheat straw and CS was 15 gN/kg DOMI (Srinivas and Gupta 1997) while it was 13 gN/kg DOMI in lactating goats fed similar diet (Bala *et al*. 2009). MBP production in sheep varies from 15 to 35 g N/kg of fermented OM (FOM) in the rumen and it was 18 gN/kg DOMI when sheep fed guar straw and CS (Srinivas *et al*. 2011). Such large variation within species was due to feeding regime, pattern and feed intake. Although extent of MBN production varies between species, its efficiency is diet dependent. Contribution of MBN to total nitrogen (N) in abomasal contents was similar to that of mature ruminants by 9 and 15 wk of age for calves depending on early (1 m) or late (2 m) weaning (Quigley *et al.* 1985). MBN reaching the duodenum also increases with age (Devant *et al*. 2000). However, studies based on excretion of purine derivatives (PD) in the urine showed, that their excretion was lesser in adult sheep than in the lambs or yearlings (IAEA 1997), because of reduced efficiency of MBP production (Verbic 2002) and also due to increase in proteolytic activity in rumen of the mature animals (Rotger *et al*. 2005). Srinivas *et al.* (2011) observed higher MBN production in lambs followed

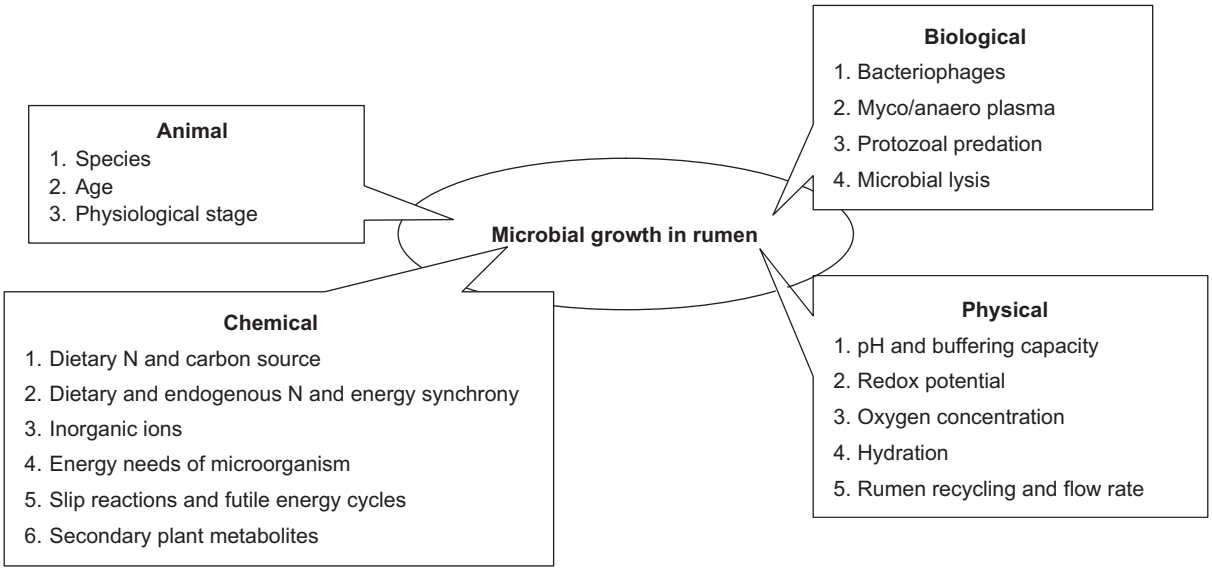

Fig. 1. Factors affecting the microbial growth yield in rumen.

by yearlings and lactating sheep with or without CS.

Physiological stage: Since the protein requirement varies with changing productive or physiological state, the rumen microorganism appears to provide sufficient protein for maintenance, slow growth and early pregnancy, but not for fast growth, late pregnancy or early lactation (Kempton *et al*. 1977). Among different physiological stages, MBN production was lesser during the late pregnancy followed by lactation and higher during growth (Srinivas *et al*. 2011). MBP production in spring-born (during May) nursing calves increased from 67 to 278 g/d right before weaning in September (Lamother *et al*. 2003). Lesser MBN production during pregnancy may perhaps relate to poor energetic efficiency of just 20% for fetal growth and heat production (Srinivas and Sankhyan 2010). Even influence of homoeorheotic factors on MBP production during pregnancy and lactation can not be overruled however, more research work required on these aspects.

Physical factors and rumen MBP production

Rumen pH and buffering capacity: Rumen microbes require stable pH environment ranging from 6.5 to 6.8. Functional performance of rumen will be greater when rumen pH is above 6.0 and pH above 5.7 is necessary for protein synthesis in rumen (Komisarczuk-Bony and Durand 1991). When rumen pH fell below 6, microbial enzymes in rumen do not function effectively and bacterial growth declines markedly. Fibrolytic bacteria failed to maintain pH inside their cells when rumen pH is low. This incapacitates the cell machinery making growth impossible (Russell and Wilson 1996). Calsamiglia *et al.* (2002) observed that infrequent shortfalls in pH had no effect but fluctuation of pH for more than 3 h from high to low had impact on microbial activity and fiber digestion declined by 14%.

Under normal conditions, Na^+, K^+, bicarbonate and short chain fatty acids are the main buffering components in the rumen. Forages encourage buffering through increased salivation and cation exchange of fibre (McBurney *et al.* 1986). Buffering capacity of forages in rumen will be greater when indigestible cell wall pool in rumen was lower (Srinivas and Singh 2010). MBP production of forage based diet is higher than even silages (Verbic 2002) because of greater buffering capacity on forage based diets or green fodder supplemented diets (Srinivas *et al.* 2002b). Interestingly, buffering capacity in legumes is due to high lignin which has a high capacity for cation exchange (McBurney *et al.* 1986). Low ion-exchange and buffering capacities in tropical grasses and straws, apart from unreliable nutritive value due to association between fiber components are resulting in greater fluctuations in MBP production (Van Soest 1988).

Redox-potential and dissolved oxygen in rumen: Anaerobes require a redox potential between +500 to –400 mV (Baldwin and Emery 1960). The redox potential (Eh) in the digestive tract of ruminants varies mostly within the ranges from –300 to +200 mV. Eh in the rumen medium ranges from -130 to –200 mV. Unlike aerobes which are capable of metabolizing in highly oxidative media, anaerobes depend largely upon enzymes for metabolism which do not operate normally until the media are partially reduced (Walden and Hentges 1975). Eh and pH changes are of a linear character. Enhanced fermentation moves Eh towards negative values and improves growth of microorganisms. Optimum Eh values for the most of the rumen bacteria e.g., *Megasphaera elsdenii, Streptococcus bovis* etc., are negative. More positive Eh values inhibit metabolism and growth rates of *Selenomonas ruminantium, Bacteroides amylophilus, Bacteroides succinogenes* and *Streptococcus bovis* to different degrees (Kalachniuk *et al*. 1994).

Although rumen environment is basically anerobic, dissolved oxygen is present in ruminal fluid at concentrations as high as 3 µM. Czerkawski (1986) calculated an entry of 10 to 20 l/d oxygen from capillaries through the mucosal lining but lesser amounts originate from saliva and drinking water. The contributions that bacterial and protozoal populations make to the overall utilization ability of dissolved oxygen by the ruminal microbiota were found to be approximately equal at physiological concentrations (Ellis *et al*. 1989). It is presumed that any inactivation of H_2 evolving system either directly by O_2 or possibly by a product of univalent O_2 reduction i.e., single O_2, hydroxyl radical, superoxide or peroxide may affect microbial growth. However, dissolved oxygen levels are not traceable after 25 min of feeding because hydrogen (H_2) production in rumen dominates. Effect of dissolved oxygen on microbial metabolism and growth is very negligible.

Hydration and flow rate of digesta: Moisture or sufficient hydration in the substrate is a common necessity for bacterial growth. Bacteria absorb food through cell walls which are not permeable to solids hence, rumen bacteria needs moisture to breakdown solid food to the point where they can absorb it and, any substrate that has a water activity value below 0.85 will not support the growth of the bacteria. Srinivas and Singh (2010) reported 14% dry matter in rumen with 8 to 9 kg solid and 48 to 57 kg of liquid in cows fed on early and late matured mixed grass hay diets. At any point of time during a circadian cycle, rumen dry matter content would be within the range of 11–14% on diets containing cell wall content ranging from 25 to 75% and fed once in a day (Srinivas *et al.* 2002a). Hydration of the substrate in the rumen will increase with postprandial time while minimum was soon after ingestion of the diet or 4 h of post feeding.

Rumen microbial population attached with the solid and liquid in the rumen are continuously flowing out from the rumen thus, making the rumen more dynamic. The bacteria and protozoa are found both free-floating and attached to particular matter and flow along with digesta (Faichney 1993). Quantitatively protozoa leaving the rumen are lesser than those in rumen fluid suggesting that their mean retention

time is substantially longer (Faichney *et al.* 1989). The proportion of microbial population that is free-floating in the rumen has been observed to range from 15 to 50%, depending on the diet, age of substrate and rumen turnover rate (Faichney and White 1988). The proportion of microbial mass flowing from the rumen was estimated to range from 30 to 80% out of which protozoa accounted for not more than 20 to 30% (Steinhour and Clark 1982). Harrison *et al.* (1976) noted that the infusion of mineral salts increased the fluid dilution rate and flow of amino acid N (AA-N) from the rumen, and concluded that it was due to increase in the efficiency of MBP synthesis. When the fluid dilution rate increases, greater opportunity exists for bacteria to pass out of the rumen prior to lysis and turnover. Rumen bacterial growth efficiency varies with the flow rate from 45% at the flow rate of 0.02/h to 85% of maximum efficiency at 0.12/h (Dijkstra *et al*. 1998a).

Microenvironment: Change in environment temperature and relative humidity can bring about variations in rumen profiles. These changes are evidential irrespective of physiological state of the animals (Marai and Haeeb 2010). Since a system is notable for its many organizational levels based on functional properties (Srinivas and Singh 1998), environment discomfort affects the animal system in top-down integration from organ to cellular levels while animal respond to it with temporary adaptations in bottom-up response from cellular level to animal as a whole (Srinivas 2010). Since homeotherms maintain a constant body temperature by regulating the metabolism, impact on the microbial population in the rumen is presumably indirect, such as reduced appetite in hot environment. Reduced appetite in hot environment is a consequence of reduced metabolic rate to minimize heat increment from the diet and hindered reflexive stimuli on the animal to secret saliva, gastric and pancreatic juices, enzymes and hormones associated with gastrointestinal tract (Bruce 2005). Jhonson *et al.* (1966) attempted to alter metabolic rate of cows during heat stress by forcibly maintaining the feed intake by putting refused feed directly into the rumen through fistula however, no significant impact was found on the reduced metabolic rate. Srinivas and Swain (2011) reported rumen MBN of 28, 17 and 18 g/kg DOMR ($P < 0.01$) during rainy, winter and summer seasons in sheep in semiarid regions of Rajasthan (India). Heat index (Fahrenheit) during rainy, winter and summer seasons reported in the forenoon and afternoon were 120 and 99 in monsoon, 84 and 79 in winter and 125 and 102 in summer, respectively. However, apart from heat stress, nutritional deficiency during summer was greatest among 3 seasons (Srinivas *et al*. 2008a). Hence, the impact of heat stress on MBN during different seasons was also due to inadequacy in nutrition. Based on the available literature, the impact of microenvironment on rumen MBN production may be an indirect effect resulting from changed appetite, metabolic rate and nutrition status of animal. Resilience in the rumen microflora quantitatively and qualitatively to different degrees of rise in temperature and relative humidity needs research focus.

Biological factors and rumen MBP production

A variable part of MBP produced in the rumen does not flow to the duodenum but is also recycled within the rumen. Many ruminal microorganisms may not survive for long periods of time, but the impact of microbial death on ruminant performance is questionable. Lysis and turnover are phenomena that appear to have a greater impact on animal performance. Theoretical calculations made by Dijkstra *et al*. (1998b) indicated that the synthesis of 1 g of MBP flowing to the duodenum at rates of 35 and 75% of recycling requires 4.7 and 12.1 g of organic matter. On variety of diets, the recycling of MBN in the rumen ranges from 20 to 90% of total MBN synthesized (Firkins *et al*. 1998).

Bacteriophages, mycoplasma and, anaeroplasma: In view of high bacterial population density normally present, it would not be surprise to find bacteriphages which are obligate pathogens of bacteria occurs in the dense populations in the rumen (Firkins *et al.* 1998). Bacteriophages have been found at 1×10^7 to 1.6×10^{10}/ml of ruminal fluid (Klieve and Swain 1993). Klieve *et al.* (1989) reported that out of 38 ruminal bacteria studied, nine organisms (23.7%) representing the 5 genera (*Eubacteria, Bacteroides, Butyrivibrio, Ruminococcus*, and *Streptococcus*) and Abmrozic *et al.* (2001) reported 2 organisms (*Prevotella bryanti*, *Prevotella brevis*) representing *Prevotella* genera were produced phage like particles. Bacteriophages have been reported associated with the facultative anaerobes *Streptococcus bovis*, *Prevotella bryanti*, *Prevotella brevis* etc., which occur in rumen and also attached to bacterial cell walls (Ambrozic *et al.* 2001). Klieve *et al*. (1989) concluded that lytic ruminal phages were of little importance, but 25% of the ruminal bacteria contained chromosomally stable lysogenic prophages. One bacterium in rumen can give rise to as many as 47 phages, but the polysaccharide coating of ruminal bacteria may protect them from phage infection (McAllister *et al.* 1994). The presence of viral genetic material in a significant percentage of the bacteria tested, as well as in a range of different genera, indicates that viral genetic material may be a normal constituent of the genome of appreciable numbers of ruminal bacteria. Phage populations differed markedly between animals and highly labile but also showed that the phage population at any given time is unique to that animal (Klieve and Swain 1993). The terminal step of phage-infection of rumen microbes was cell killing as observed in *Streptococcus durans* (Brailsford and Hartman 1968). In sheep fed once daily, a distinct diurnal variation in the phage population was observed (Firkins *et al.* 1998). Two hours post-feeding the total phage DNA dropped to its lowest level. The phage population then increased, reaching a maximal level 8 to 10 h post-feeding before declining over the next 4 h to reach a

stable concentration for the rest of the cycle. The general trend in phage DNA concentration appeared similar to previously recorded diurnal fluctuations in ruminal bacterial populations in cattle fed once daily (Swain *et al*. 1996).

Bacteriolytic microorganism in the rumen similar to mycoplasma, and several anaeroplasma present in the rumen that are characterized by their small genome size (0.58 to 1.38 Mbp) and lack of cell wall, have limited capability to synthesize macromolecule essential for growth, and are completely dependent on host rumen bacteria and cause partial digestion of rumen bacteria (Robinson and Hungate 1973). However, rumen mycoplasma are sensitive to osmotic pressure and susceptible to cell lyses due to lack of cell walls. Anaerobic mycoplasma that were capable of infecting live, gram-negative bacteria were present at less than 10^4/ml of ruminal fluid (Wells and Russel 1996).

Protozoa predation: Protozoa extensively prey upon the bacteria and a higher proportion of protozoa than bacteria lyse within the rumen (Williams and Coleman 1992). Some 65 to 85% of protozoa lysis occurs within the rumen because of autolysis in rumen and their protein is then degraded by the bacteria and recycled within the rumen (Leng and Nolan 1984). Dijkstra (1994) derived an elaborate mechanism and estimated the protozoal lysis in rumen to be in the range of 54 to 80%. The following observations strengthen the protozoa predation on rumen bacteria (Leng 1982).

1. Protozoa mass is in some cases nearly as great as the bacterial mass
2. Protozoa can engulf bacteria rapidly
3. Little solubulization of bacterial protein in the absence of protozoa
4. Ruminal ammonia concentration is generally greater in the absence of protozoa
5. Defaunation can increase the performance of ruminants on low protein diets

Under *in vivo* conditions protozoa take up small feed particles as well as bacteria, and most of the bacteria are present in biofilms rather than as free cells (McAllister *et al*. 1994). Infusion of rumen ciliates into ciliate free rumen decreased the number of small bacteria from 36×10^9 to 14×10^9/ml (Hobson 1988) or vice versa from 4×10^9 to 34×10^9/ml (Whitelaw *et al*. 1984). Even increase in the population of anaerobic fungi in rumen was also reported in defaunated animals (Romulo *et al*. 1989). The effect of protozoa predation was more on small and unculturable bacteria whereas impact on culturable bacteria was lesser as evident from the increase in their population from 2.5 to 9.2% after defaunation (Whitelaw *et al*. 1984, Wells and Russel 1996). Teather *et al*. (1984) found constant protozoal and bacterial protein in 22 lactating cows fed on corn silage and concentrates with a standard deviation of 28%. However, there was massive variation between animals with protozoal protein varying from < 0.01 to 5.73 mg/ml and bacterial protein ranging from 0.58 to 5.76 mg/ml. However, because of negative correlation between bacterial and protozoal numbers the total microbial protein varied from 1.33 to 6.42 mg/ml.

Bacterial lysis: Krebs *et al*. (1989) estimated microbial turnover in defaunated sheep using ^{15}N and concluded autolysis of bacteria in rumen. In the surface stress model of Koch (1991) described gram positive rods first deposited peptidoglycan at the inner surface while their outer layers were cut by autolytic enzymes and stress is gradually transferred to more recently synthesized portions of the peptidoglycan. Rapidly growing bacteria would have high membrane potential and would accumulate protons at the cell surface (Jolliffe *et al*. 1981). This low pH, in turn would inhibit autolysis. Conversely starvation could dissipate the membrane potential, increase pH, and activate the autolysins. Compounds those decrease the membrane potential accelerate the lysis of rumen bacterium. However, bacterium e.g., *Fibrobacter succinogenes,* appeared to be regulating its autolysis via a mechanism involving the proteolytic degradation of autolysins (Wells and Russel 1996). Differential rates of death of bacteria could have an impact on rumen microbial ecology, but, as yet, there has been little information to suggest that the death of individual species would affect fermentation end products.

Feed factors and rumen MBP production

MBP production in rumen influenced by the feed in many ways and few among them are: the characteristics of the diet, the amount of potentially degradable nutrients, the food intake level, frequency of feeding, dry matter intake of animals, forage to concentrate ratio of diets, the food residence time in rumen, food exposure to the rumen microorganisms, rate and extent of digestion and temporary changes occurring in the physical environment in the rumen (Ørskov and Miller 1988). Srinivas and Krishnamoorthy (2005) identified that the extent of gas production and lag time of fermentation of feedstuffs were related to microbial characteristics in rumen while, rate constant of fermentation and t-half (50% of fermentation) time were dependent on the structural characteristics of feed (Srinivas and Swain 2009).

Types of diets: The efficiency of MBP synthesis greatly differs in animals fed different diets, even within similar diets based on the nature of forage (Dehority and Orphi 1988). The average efficiency of MBP synthesis is 13.0, ranging from 7.5 to 24.3 for forage based diets (based on 34 studies); 17.6, ranging from 9.1 to 27.9 for forage-concentrate mix diets (based on 36 studies), and 13.2, ranging from 7.0 to 23.7 g microbial CP/ 100 g for concentrate diets (based on 14 studies) of OM truly digested in the rumen. Overall, the average efficiency of MBP synthesis is 14.8, ranging from 7.0 to 27.9 g microbial CP/100 g of OM truly digested in the rumen (Karsli and Russel 2001). The efficiency of MBP synthesis was predicted to be around 13 g microbial CP/100

g of total digestible nutrient (TDN) for beef cows (NRC 1998). Whitelaw and coworkers (1984) were observed more MBP production in steers fed diet with concentrate to roughage ratio of 50: 50 rather than on 90: 10 or 100: 0. Verbic (2002) reported that the efficiency of MBP production varied widely between forages. In grass silages it varied from 115 to 158, in hay it was 126, in maize silages it varied from 165 to 217 and in green forage from 145 to 199 g of MBP/kg of fermentable OM. MBN production in rumen is not necessarily increase with the increasing quantities of CS as demonstrated by the Srinivas and Singh (2010). Marginal efficiency of MBN production was higher when CS to sheep was 12 g/kg body weight and diminished when it was 20 g/kg body weight and in cows it was more when fed 3 kg compared to 6 kg CS (Ruiz-Albarrán *et al*. 2008).

Quality of diet: The maximum potential of rumen microbes to produce MBP can be explored only by the provision of high-quality forage. MBP production in cattle fed wheat straw without CS was 3 to 4 times lesser than when supplemented CS or UMMB supplements (Srinivas and Gupta 1997). The problem of low MBP yield in diets containing low quality forages cannot simply be solved by supplementing diets with high amounts of CS. It has been shown that in diets containing high levels of CS the efficiency of MBP synthesis in the rumen is lower than in well-balanced forage based diets (Pathak 2008). Selection of fibrous feeds based on high efficiency of MBP synthesis in the rumen along with high dry matter and fiber digestibility and development of feeding strategies based on high efficiency as well as high MBP synthesis in rumen will lead to higher supply of protein post ruminally (Makkar 2010). Generally diets having higher rate constant of fermentation tended to increase rumen microbial growth efficiency (Sudha *et al*. 2009). Different grain sources would have varying impact on the MBP production in cows. MBP production on supplements consisted soyameal alone or with maize or sorghum or oats as source of grains and made isonitrogen, was 122, 133, 184 and 174 g/d, respectively (Singh 2011). Such variation in MBP between grains was mainly due to structural properties and physical modifications in the amylopectin of the starch in the grains. MBP flow to intestine with supplementation of maize grain with urea-N was 137 g/d which was 29 g/d higher than supplement with maize, copra meal and urea-N, although both supplements were isonitrogenous. When groundnut meal, cottonseed meal and mustard cake used in place of copra meal, MBP production was 165, 146 and, 143 g/d, respectively. These studies clearly indicated qualitative difference between oilseed meals on MBP production due to different types of proteins e.g., albumin, globulin etc., although diets were made isonitrogenous (Mohanavel 2012). Albumin fraction of OSM had a positive influence on MBP production in rumen (Mohanavel 2012). Apart from grains, OSM, the impact of brans and husk based supplements on MBP is under progress from the same laboratory (Rathode 2012).

Frequency of feeding: MBN production also increases with forage processing, by natural or induced defaunation, feeding frequency and buffer and vitamin supplements (Vérité *et al*. 1986). Increasing the feeding frequency of dried grass meal from 2 to 8 time increased MBN synthesis from 36 to 46 gN/kg DOMI however, no significant effect was found on the diets containing rolled barley (McAllan *et al*. 1987) which indicated that the frequency of feeding lead to increase in MBN production mainly due to impact on the roughage diet. Feeds associated with lower outflow rates for e.g., processed-grain rations, have a higher total energy production but lower efficiency of MBP production and contrary was true with acid treated, high-moisture grains (Bergen 1977). Changing the feeding frequency of CS would improve the synchronization of protein and energy, particularly on low quality forages, which may have positive impact on the MBN production in rumen (Yang *et al*. 2010). Feeding an energy rich concentrate ingredient at the first feeding time in the morning stimulated microbial activity and efficiency of MBP production compared to energy and protein supplements together or protein source followed by energy source of supplements (Kaswari *et al*. 2006).

Chemical factors and rumen MBP production

Biological growth depends on the transfer of energy from catabolic to anabolic processes but the conversion or coupling is never complete and energy is dissipated into the environment as heat. Perusal of different factors enlisted by Bergen (1977) indicated that microbial cell synthesis is dependent on cellular energy catabolism and anabolism.

Nitrogen and carbon source: MBP synthesis is dependent on the relationship between the amount of soluble and degradable N or protein and amino acids, their rate of degradation, the amount of digestible OM fermented in rumen or carbon chains available to rumen microorganisms. Sources of N and degradable carbon chains in diet must be synchronized, both in quantity and rate of degradation, to improve the MBP synthesis (Andrade-Montemayor *et al.* 2009). Any imbalance between the ruminal degradation of feed nutrients will result in loss of ruminal N and affect MBP synthesis. The rumen microorganisms play a determining activity in digestion and metabolism of N compounds in ruminants. Bacteria in the rumen possess high proteolytic activity and intracellular events in the peptides and amino acids degradation (Broderick 2011).

A major energy source of OM is CHO for MBP synthesis. Some researchers have suggested that it would be more appropriate if the efficiency of MBP synthesis is expressed as a function of CHO digested rather than OM digested in the rumen (Russell *et al.* 1990). Basic biological processes that have ATP expenditures are protein synthesis, lipid synthesis, RNA, DNA synthesis, polysaccharide synthesis and transport gradient processes. The primary function of

the microbial CHO metabolism is to release the ATP required for microbial growth. Thus, patterns and rates of MBN metabolism are dependent upon the rates of CHO fermentation (Hoover and Stokes 1991). Excess CHO in the absence of sufficient N and other nutrients can be toxic, for example, *Fibrobacter succinogenes* cultures that have excess cellobiose secrete glucose and cellotriose into the culture medium, and *Prevotella ruminicola* produces methylglyoxal, a toxic substance that causes a dramatic decrease in viability. Though excess CHO is converted into intracellular polysaccharides, it can be saturated quickly (Russel 1998). CHO degradation kinetics in rumen is very complex, and modern methods of assessment need a precise knowledge of the characteristics of CHO and proteins degradation of seeds and grains those are used in animal rations. CHO and proteins were subdivided into 4 fractions based on their physical properties (Sniffen *et al.* 1992, Van Soest *et al*. 1991). Broadly these fractions are associated with cell and cell walls and could be grouped as non fibrous and fibrous CHO. Non-fiber carbohydrates (NFC) are those compounds that are not associated with the cell wall, with the exception of neutral detergent soluble fiber (NDSF) compounds (pectic substances, fructosans, beta-glucans). NFC is a very heterogeneous group of CHO that includes organic acids, sugars, starch, and NDSF. A subset of NFC is nonstructural CHO (NSC), which are primarily sugars and starches that are very rapidly fermented in the rumen (Kotarski *et al.* 1992) and provide large amounts of energy for MBP production (Hall 2003). Organism that ferments soluble sugars could contribute approximately 18% more of the MBP synthesis than the organism that ferments starch (NRC 1998).

Cabrita *et al*. (2005) summarized the positive impact of more degradable starch on MBN production from 2 studies and no effect from 5 studies. These generally small responses may be attributed to several factors. More degradable starch sources may, on the one hand, increase starch fermented in the rumen, but, on the other hand, lower ruminal fibre digestion. Diets with more degradable starch sources can negate the beneficial effects of the higher ruminal starch digestibility, resulting in a similar amount of OM fermented in the rumen (Beauchemin *et al*. 1999). Sources of quickly fermentable starch decrease rumen pH, allowing the maintenance of a greater pool of ammonia for MBP synthesis that was evident from feeding 5% molasses along with 2% urea in the diets (Cabrita *et al*. 2005). Since microbial cells contain 20 to 33% of non amino acid N, conversion of good quality proteins into MBP may actually impair N utilization. Non-protein N (NPN) can replace only part of the dietary (rumen degradable protein) RDP because RDP from peptides and amino acids stimulate MBP synthesis (Broderick *et al.* 2011). Kozloski *et al*. (2000) observed a linear decline in MBP as more and more soybean meal CP was replaced by urea CP. Sarwar *et al*. (2010) studied bacterial cell count in the rumen of buffaloes on isonitrogenous and isoenergy diet containing 50, 66, 82 and 100% RDP. The maximum bacterial cell count was 91.74, 150.0, 183.45 and 60.49×10^{10} cells/ml and observed after 9 h of feeding the respective diets. These observations clearly indicated that better response in bacterial count was obtained when RDP was 82% of total protein and feeding 100% of RDP in the form of urea was not effective. ARC (1998) suggested a maximum of 33% of urea N in the total N of the diet. Stanton and Whittier (2011) suggested no more than 20 to 33% of total N in supplement from urea when feeding harvested roughages or low quality roughages or, otherwise only 10% on high protein supplements. 12 g rumen degradable nitrogen (RDN)/kg of digestible OM from groundnut cake was reported optimum for MBP synthesis (Chandrasekharaiah *et al*. 2011). Ali *et al*. (2008) compared different sources of NPN and inferred that significantly higher bacterial count was observed with urea-starch compared to urea, biuret, biuret-starch and cotton seed meal however, no significant difference in bacterial count in rumen was observed among the latter sources. The efficiency of conversion of urea to MBN will vary according to method of feeding and nature of basal feeds. Feeding urea little and often in conjunction with rapidly fermentable starchy diets can give high rates of utilization. However, to cover the less than ideal situation an apparent efficiency of conversion of urea N to MBN is 0.80 against 1.0 used for RDP. This can be possible only under optimum conditions of availability of carbon source from CHO. Even urea N conversion to MBN may fell below 0.80 when rapidly released ammonia could not be trapped efficiently by rumen microorganism. A stimulatory effect of protein source as against the NPN source could be the result of supply of preformed monomers from protein degradation such as amino acids, peptides or the branched chain fatty acids (Krishnamoorthy *et al.* 1990).

Maize appeared to support more efficient synthesis of rumen MBP than molasses, as the rates of excretion of purine derivatives and of live weight gains were higher for maize (Luc *et al.* 2009). The degradation of soluble sugars is almost immediate, and the starch of cereals in most ruminant rations is complete (Andrade-Montemayor *et al*. 2009). There are differences among different kinds of cereals, in the proportion of soluble starch, fermented in the rumen and digested in subsequent sections of the digestive system (Andrade-Montemayor *et al.* 2009). Based on the starch fermentation rate grains are ranked wheat and barley> rye and oat > sorghum and maize (Krishnamoorthy *et al*. 1991). Most of the grains contain non-reducing soluble sugars 1 to 2 % while maize may contain even 3%. Sorghum only contains reducing sugars 0.1% while these are not traceable in oats, finger-millet and maize (Singh 2011). Starch from tapioca, wheat and barley are characterized by a faster and complete fermentation in the rumen than starch of corn or sorghum that can lead to more energy available to rumen microorganisms, favoring the synthesis of MBP, but also can cause problems of ruminal acidosis (Sauvant *et al*. 1994). *In*

vivo experiments have found that inclusion of moderate amounts of non-structural CHO in forage-based diets stimulates the synthesis of MBP synthesis and its efficiency (Archimede *et al*. 1997). This effect appears related to the inclusion of high levels of non-structural CHO in the diet, and may cause changes in the fermentation pattern by altering microbial growth. However, there is also evidence from studies *in vivo*, which shows how high levels of non-structural CHO in the diet may adversely affect the MBP synthesis and production efficiency (Van Nevel and Demeyer 1988). Efficiency of MBN production tends to increase when readily fermentable CHO is supplemented at less than 30% of the total diet, but decreased when the supplementation level was greater than 70% (Huber and Kung 1981).

Dietary N and energy synchrony: The major nutrients both in quantity and simultaneously ruminal degradability, required by rumen microbes are protein and carbohydrates. When designing a ruminant feeding program, concepts of rate of fermentation need to be synchronized to make sure simultaneous availability of energy and N for optimum MBP production. Yang *et al.* (2010) opined that theoretically, synchronization of energy and N supply in the rumen should allow more efficient use of nutrients by rumen microbes, increased MBP and fermentation end products, and thus increase in available nutrients in the small intestine. Manipulating synchrony by changing dietary ingredients presents some problems, since it is not possible to discount the possibility that apparent effects of synchrony are associated with the manipulation of the ingredients (level and type) themselves. These problems can be overcome, at least partially, by altering the feeding patterns (Srinivas 1999). However, a number of studies reviewed by Cabrita *et al*. (2005) and Yang *et al*. (2010) showed contradictory results in MBP synthesis, and suggested that the concept of the synchronization needs assertion prior to application in the field. Kasawari *et al*. (2006) calculated synchronization index (SI) based on the relationship between the sum of degraded N and sum of degraded OM from the different diets and observed that MBN synthesis was not consistent with the SI parameters. Different SI was simulated in one experiment by feeding energy at first and third feeding time and protein at second and fourth feeding time and in the second experiment simply reversing the order of feeding. Only in second experiment increase in MBP was observed (Kaswari *et al.* 2006).

According to the Dutch protein system (Tamminga *et al*. 1994), the optimal RDP balance (OEB value) of a diet is close to zero and corresponds to a RDN: FOM ratio equal to 25 g of N/kg of FOM, which reflects a well-balanced availability of energy and N to rumen microbes. When the OEB value is positive for a diet, N losses from the rumen occur, whereas a negative value indicates a shortage of RDP, and, consequently, the microbial activity may be impaired. Valkeners *et al.* (2006) suggested that the imbalance between dietary energy and N created over a 24 h interval was not detrimental to rumen microbial growth for the animal as long as the level of imbalance did not exceed 40 g of OEB/kg of DM. Similarly N losses in urine were lesser when OEB was –23 g/kg dietary DM (Valkeners *et al.* 2008). Fiems *et al*. (1999) suggested that negative OEB vale of –16 g/kg dietary DM was acceptable when animals were overfed true protein digestible at small intestine. Least impact of either positive or negative OEB on microbial growth may be due to N recycling in the rumen that could allow a continuous synchronization of N and energy-yielding substrates for the microorganisms (Valkeners *et al.* 2008). In meeting our goal of maximum MBP production, we need to match CHO and protein rates of degradation. This allows for somewhat equivalent amounts of rumen energy and N availability promoting efficient MBP yield and overall dietary protein incorporation (Van Saun 2007). If we can reduce the amount of protein used in the ration and yet maintain or improve milk yield, the animal becomes much more efficient, profitable and environmentally friendly.

Blümmel *et al*. (1997) proposed 'partition factor (PF)' based on the truly degraded OM/ml of fermentative gases produced to demarcate distribution of truly degraded substrate between microbial biomass and fermentation waste. When less gas is produced per unit weight of substrate truly degraded, proportionally more substrate is converted into microbial biomass, which mean that a higher PF would reflect higher conversion of truly degraded substrate into microbial biomass, and vice versa (Blümmel *et al*. 2003). When attempts are made to formulate feedstuffs on the basis of PF (Kiran and Krishnamoorthy 2007) and fed, no such impact on rumen MBP was observed (Thirumalesh and Krishnamoorthy 2008). Feeding of diets differing in PF of 3.01, 3.17, 3.33 and 3.49 to ram lambs resulted in MBP production of 140, 135, 145 and 175 mg without any impact on microbial N flow to the duodenum (Thirumalesh and Krishnamoorthy 2009). In nutshell, synchronization, Dutch protein system and PF emphasizes ensuring energy provisions simultaneously for N assimilation by microbial cells but at the most synchronization attempts were partially successful because microbial energetic is complex (Russel 2007).

Fat is not an efficient energy source for rumen microbial growth (Dewhrust *et al.* 2000), their addition in moderate proportions can improve the efficiency of MBP synthesis (Klusmeyer *et al*. 1991), as fat addition appears to reduce the number of protozoa, which decreases the predation of bacteria in the rumen. However, when fat intake in the diet is high (e.g.> 8% of the ration DM), can lead to negative effects such as reduction in fiber digestion due to a toxic effect on cellulolytic microorganism and adversely affecting the efficiency of MBP synthesis (Oldick and Firkins 2000). Many rumen microbes are very sensitive to the presence of dietary polyunsaturated fats. Rumen microbes will attempt to reduce the metabolic toxicity of polyunsaturated fats by

saturating double bonds through a process of biohydrogenation. Recent research has identified *trans*-10, *cis*-12 conjugated linoleic acid (CLA), a product of incomplete microbial biohydrogenation, to be associated with milk fat depression in dairy cows (Bauman *et al.* 1999). The presence of *trans*-10 CLA inhibits or reduces mammary gland *de novo* fatty acid synthesis. The presence of large amounts of polyunsaturated fats in the rumen or small amounts with high grain feeding seems to promote the production of *trans*-10 CLA and produces milk fat depression syndrome.

Inorganic ions: In addition to N and CHO supply, microbial yield is affected by the concentrations of trace minerals and vitamins (Sniffen and Robinson 1987). Since no ionic species can pass into cells by passive leakage hence bacterial cells have specific transportation systems for each cation and anion required for the growth. In normal environmental conditions in rumen, the macro-mineral transportation systems have high *Km* and *Vmax* values (low affinity) and micro-mineral transportation systems have low kinetic parameters (high affinity). However microbial cells are able to adapt to nutrient limitation or excess in many cases by using competitive scavenging systems, repression/induction of feedback inhibition which protect the cells from a drastic mineral depletion or from accumulating toxic levels when external abundance is high. In spite of the quite efficient regulation control systems existing in the most bacteria, it is difficult to give precise values of mineral content in microbial cells principally because the amount can vary widely according to the strain considered, the growth phase and the extracellular concentration. Furthermore minerals, especially cations, adsorb non-specifically to the microbial surface by simple ionic interactions (Mackie and Therion 1984).

Calcium's main catalytic functions are related to the activation of extracellular enzymes such as proteases, lipases, α-amylases and cellulases. Potassium (K^+) and sodium (Na^+) are both required by rumen microorganisms. K^+ is used for protein synthesis, probably by stabilizing the ribosomes and it activates some enzymes (Caldwell *et al*. 1973). Some rumen bacteria, e.g., *Fibrobacter succinogenes* are slightly halophylic and Na^+ is involved in transport and energetic process. Phosphorus is another mineral required for the synthesis of ATP and protein by rumen microbes. MBP synthesis can be limited by an insufficient supply of P for microbial growth. Magnesium activates many bacterial enzymes including phosphohydrolases, phsophotransferases and pathways involving ATP and thiamine pyrophosphate reactions. Its concentration in the ribosomes makes it essential for the protein synthesis process but it can be partly replaced by manganese (Komisarczuk-Bony and Durand 1991). Dietary sulphur concentration affects MBP synthesis (Sniffen and Robinson 1987). The amount of sulphur required by rumen microorganisms for synthesis of methionine and cysteine ranges from 0.11 to 0.20% of the total diet, depending on the status of the cattle (NRC 1998). Limited intake of sulphur may restrict MBP synthesis when large amounts of NPN are fed to ruminant animals, such as urea. Sulphur sources differ in their ability to enhance ruminant microbial activities. Sodium sulphate and methionine have been shown to stimulate riboflavin and B_{12} vitamin synthesis by rumen micro-organisms to a greater extent than cysteine or elemental sulphur (Fron *et al.* 1990). It is essential in the synthesis of sulphur containing amino acids those are needed in the elaboration of the MBP. Sulphur is also involved in the synthesis of thiamin, biotin and coenzymes and also required specifically for endoglucanase activity (Stevani 2002).

Dietary supplementation of zinc (Zn) to maintain a rumen Zn concentration of 7 ppm was shown to inhibit ureolysis and increase molar proportion of propionate (Arelovich *et al*. 2000). High rumen Zn concentrations (14 ppm) reduced fiber digestibility. Zn is involved in the synthesis of many metalloenzymes including DNA and RNA polymerases, alkaline phosphatase, amylase and neutral protease. Supplementing manganese (Mn) at 100 ppm in the rumen resulted in increased *in vitro* dry matter digestibility. Manganese operates during glycolysis in the decarboxylation reactions and was shown to stimulate carbondioxide (CO_2) fixation in the production of succinic acid by *Ruminococcus flavefaciens* (Caldwell *et al*. 1973). Iron is also needed in the synthesis of many bacterial cellular proteins. Nikolic *et al.* (1983) concluded that copper and molybdenum are unlikely to have a negative influence on the utilization of urea and sulphate for MBP synthesis when they are present in the diet in the amounts recommended in practice. Cobalt is integrated in the structure of vitamin B_{12} and its coenzymes such as methyl and adenosylcobalamin, interact in many circumstances during microbial metabolism which include proton transfer in riboneucleotide reduction, hydrogen transfer, methyl transfer, acetate, propionate and methionine synthesis. It is also required for the growth of rumen ciliates (Bonhomme *et al*. 1982).

Energy needs of rumen microbial protein synthesis: The ruminal fermentation is a coupled process between carbohydrate degradation, volatile fatty acid (VFA) production and concomitant ATP generation and the process of microbial cell synthesis from nitrogenous precursors (mainly NH_3-N) and other needed substrates, such as carbon skeletons, sulphur and others. Factors impeding ruminal OM digestion or a lack of nitrogenous precursors will both lower VFA production and cell yield (Bergen 1977). MBP synthesis is dependent on total ATP availability as well as the efficiency of ATP use for biomass production. ATP is used for maintenance and growth. During the process of substrate level phosphorylation or a chemostatic mechanism there would be some energy spill.

Microbiologists have related ATP generation from substrate breakdown and cellular growth. These efforts resulted in a term for yield, Y, defined as: $Y_{substrate}$ = [weight

of bacteria formed × weight of substrate used]. In energy limiting batch cultures these Ys values are generally found to be constant and derived another term Y_{ATP} defined as: Y_{ATP} = g cells formed/mole ATP. The basic biological processes that have ATP expenditures are protein synthesis, lipid synthesis, RNA, DNA synthesis, polysaccharide synthesis and transport gradient processes. The various factors that strongly influence Y_{ATP} of bacteria are: (i) Maintenance needs of the bacteria, (ii) specific growth rate of bacteria, (iii) nature of N source, (iv) nature of carbon source and energy yielding compounds, (v) presence of energy requiring process other than formation of new cell material, (vi) futile cycles, and (vii) energy coupling (Bergen 1977). Mixed ruminal bacterial culture grown in continuous culture at 2%/h dilution rate, the fraction of ATP spent for maintenance was about 60%; whereas at 12%/h dilution rate the fraction of ATP spent for maintenance was about 15–20%. Generally, the physiological range of digesta (DM) flow from the rumen is from 2%/h to about 8%/h. Russel (1991) also observed reduction in heat of production also with increasing dilution rate. Fermentation rates of soluble sugars and starches are very high up to 2 h post feeding, but decrease almost completely approximately 4 h post feeding. Soluble sugars and starch provide higher levels of ATP than structural CHO up to 4 h post feeding, but they provide almost no ATP for microbial growth after 4 h post feeding. Approximately 3 to 4 h post feeding, cellulose and hemi cellulose degradation start and continue for a long period (up to 96 h) post feeding, providing ATP for later microbial growth.

Since maintenance is the amount of energy that cannot be used for growth, it is apparent that maintenance energy must contribute to heat production. Heat is the direct product of maintenance functions, and it seemed that measurements of heat production might give a more straight forward interpretation of maintenance energy. Maintenance was assumed to be a time-dependent function that was proportional to cell mass and independent of growth rate. When growth rate is high, maintenance makes up a relatively small proportion of total energy use. Within the rumen, the concentration of soluble energy sources is usually very low, cell density is high, and average growth rate is low, so that maintenance exerts a significant impact on cell yield (Russel 1986). Growth would also give rise to heat; however, the amount of heat resulting from growth should be reasonably constant as long as the data are expressed per unit of cell protein. Heat due to growth would be directly proportional to the amount of cell protein and not necessarily a simple function of growth rate (Russel 2007). Rumen bacteria for example, *Butyrivibrio fibrisolvens* evolve a mechanism to utilize certain sugars for example glucose against arabinose, on priority to avoid high maintenance energy (Strobel and Dawson 1993).

Energy spilling: Simple growth studies with bacteria indicated that the efficiency of biomass production was often at least 3-fold lower than the amount that would be predicted from standard biosynthetic pathways. The utilization of energy for maintenance could only explain a small portion of this discrepancy particularly when the growth rate was high. These ideas and thermodynamic arguments indicated that cells might have another avenue of energy utilization. This phenomenon has also been called 'uncoupling', 'spillage' and 'overflow metabolism', but 'energy spilling' is probably the most descriptive term (Russel 2007). The term 'energy spilling' will be used to describe non-growth dissipation of excess ATP. Energy spilling can be caused by futile cycles of potassium, ammonium, or protons through the cell membrane (Tedeschi *et al.* 2000). Continuous culture studies with N-limited mixed ruminal bacteria indicated NFC bacteria fermented abnormally large amounts of glucose or starch, but fibrous CHO bacteria could not spill energy. NFC bacteria can "spill energy" without affecting the NFC digestion when N is limiting, but N-limitation has a negative effect on fibrous CHO digestion (Russel 1998). ATP requirement for synthesis of protein, lipid, polysaccharide, RNA and DNA are 36.4, 2.0, 12.6, 15.3 and 18.8 mmol/g, respectively (Russel 2007). Protein was the most costly polymer to synthesize, and it accounts for approximately half of the total ATP requirement, whereas polysaccharide was relatively inexpensive to synthesize. Many bacteria are accumulating polysaccharide that can be an extracellular material and helps protect the cell or intracellular glycogen which is source of energy during periods of starvation. When the mass of the polymer and the ATP requirement for biosynthesis were compared, it became apparent that the Y_{ATP} of bacteria should be 32 g cells/mole ATP (Stouthamer and Bettenhaussen 1977), a value 3 times greater than estimated by Bauchop and Elsden (1960). Probably the strongest argument that energy spilling is a common feature of bacteria is derived from growth experiments with the methanogen (Schönheit *et al*. 1980). Liu *et al*. (2001) used a microcalorimeter to study the acetotrophic methanogen, *Methanosarcina barkeri,* and concluded that microbial growth was characterized by 'enthalpy increase and correspondingly by a large increase in entropy may be called enthalpy-retarded growth'. Energy spilling is one of the main reasons for heat of fermentation in rumen and it may also vary widely on different diets, 140 kJ to 360 kJ/mol of hexoses fermented (Arieli 1986).

Secondary plant metabolites: Recent studies have shown that plant secondary metabolites (PSM) at lower concentrations could be used to manipulate rumen fermentation favourably. At appropriate dose, saponins or saponins containing plants have been shown to suppress protozoal population, increase bacteria and fungi population, propionate production, partitioning factor, yield and efficiency of MBP synthesis and decrease methanogenesis hence improve performance in ruminants. Tannins especially condensed tannins (CT) also decrease methane production

and increase efficiency of MBP synthesis (Makkar *et al*. 1994). Plant extracts rich in flavonoids increase degradation of cell wall constituents, yield and efficiency of MBP synthesis (Menke *et al*. 1988). Different herbal extracts showed varying level of effect on growth inhibition of gram positive or negative bacteria and only selected herbal extract could inhibit methanogenesis in rumen (Sirohi *et al*. 2009). Kumar *et al*. (2011) observed no adverse impact of secondary plant metabolites from mixture of leaves: mango (*Mangifera indica*), *jamun* (*Eugenia jambolana*), guava (*Psidium guajava*), seed pulp of *harad* (*Terminalia chebula*) and fennel (*Foeniculum vulgare*) fed @ 40 g/100 kg body weight of buffaloes on rumen fermentation pattern, MBP synthesis and nutrient digestibility in buffaloes. Intake of secondary plant metabolites at lower doses of intake appeared to have positive impact on the MBN production in rumen while rumen microbes have inherent ability to detoxify some of the secondary plant metabolites. Many of the secondary plant metabolites are inactivated by simple sun drying. Some of the grains like oats and soya contain lipase (Lehtinen *et al*. 2003) and trypsin inhibitor, respectively in their seeds. However, trypsin inhibitor in soya meal inactivated to large extent compared to it in the soya seeds. No adverse effect of lipase on ruminal fermentation was reported till now. Often exogenous enzyme source may act competitively or complimentarily with microbial enzymes (Srinivas *et al*. 2008b). Broderick and Reynal (2009) observed better MBN production, milk yield and composition when RDN was supplied by soy meal than urea. Efficiency of MBP synthesis was 19% greater when soya meal was supplemented with the high roughage diet (Weakley *et al*. 1982). Although numerous secondary plant metabolites were described in soya seeds, their adverse effect on MBN production or unsuitability to use in the ration of dairy animals were seldom found when soya meal was fed.

MBP production in rumen is focal point and the only solution for all functional issues related to nutrition of ruminant animals. Factors which influenced were related to feed, animal and microbiota. Enormous information is available on different factors that how they influence the rumen environment but direct measure of their influence on MBP production is few. For a pragmatic understanding of functional nutrition, it is necessary to conjoin different factors influencing the MBP production and productivity in rumen. Quantitative improvement of MBP can solve improving the innate immunity, environment stress tolerance, futile energy losses including methane production, overall animal productivity etc.

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