Rumen Manipulation to Improve Animal Productivity

A. Santra* and S. A. Karim
Central Sheep and Wool Research Institute. Avikanagar - 304 501. Rajasthan. India

ABSTRACT : Anaerobic rumen microorganisms mainly bacteria, protozoa and fungi degrade ligno-cellulosic feeds consumed by the ruminants. The ruminants in developing countries are predominantly maintained on low grade roughage and grazing on degraded range land resulting in their poor nutrient utilization and productivity. Hence, manipulation of rumen fermentation was tried during last two decades to optimize ruminal fermentation for improving nutrient utilization and productivity of the animals. Modification of rumen microbial composition and their activity was attempted by using chemical additives those selectively effect rumen microbes, introduction of naturally occurring or genetically modified foreign microbes into the rumen and genetically manipulation of existing microbes in the rumen ecosystem. Accordingly, rumen protozoa were eliminated by defaunation for reducing ruminal methane production and increasing protein outflow in the intestine, resulting in improve growth and feed conversion efficiency of the animals. Further, Interspecies trans-inoculation of rumen microbes was also successfully used for annulment of dietary toxic factor. Additionally, probiotics of bacterial and yeast origin have been used in animal feeding to stabilize rumen fermentation, reduced incidence of diarrhoea and thus improving growth and feed conversion efficiency of young stalk. It is envisaged that genetic manipulation of rumen microorganisms has enormous research potential in developing countries. In view of feed resource availability more emphasis has to be given for manipulating rumen fermentation to increase cellulolytic activity for efficient utilization of low grade roughage. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 5 : 748-763)***

Key Words : Rumen Manipulation, Defaunation, Probiotics, Growth, Feed Conversion Efficiency, Nutrient Utilization

INTRODUCTION

Continuing increase in the human population consume an ever greater fraction of earth's food. among which proteins are extremely important. Some proteins formed in plants especially as reserve food in the seeds and meat. milk. wool/fur and hide of grazing mammals have long been important source of food protein and protective clothing for human. Among these grazing animals. the ruminants. particularly cattle, buffaloes. sheep and goats are predominant animal 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).

Ruminants are fore gut fermenters and their stomach has four distinct compartments consisting of rumen. reticulum. omasum and abomasum. The rumen, which is located at the beginning of the tract. plays a major role as at least 50% of the total digestion occurs there. Although a myriad of microorganisms are found throughout the digestive tract of ruminant. still only the microbiota inhabiting in the rumen have true symbiotic relationship with the host. Individual rumen microbial species have developed in a complex process of evolution extending over long period and provide nature's best example of microbial symbioses. These rumen microorganisms are predominantly bacteria. protozoa and phycomycete fungi (Imai, 1998). The mammalian system is devoid of enzymes to degrade structural carbohydrate and hence the symbiotic microbes inhabiting in rumen elaborate enzymes for fermentative digestion of large amounts of fibrous feed consumed by the ruminants. By providing a suitable habitat for these microorganisms, the ruminants are able to utilize the end products of microbial fermentation to meet their own nutritional need. Rumen is an open. self-contained ecosystem in which feed consumed by the ruminant is fermented by rumen microbes to volatile fatty acids and microbial biomass those serve as source of energy and protein for the host animals (Weimer. 1998). The rumen microbial ecosystem is an efficient anaerobic fermentation system that confers added advantages on ruminants over monogastric or non-ruminants animals and these are:

a. Ruminants can digest large amount of fibrous feeds (ligno-cellulosic materials) efficiently.

b. They can use non protein nitrogen sources like urea as a source of nitrogen to meet part of their protein requirement,

c. They can detoxify many toxic ingredients present in feeds of plant origin.

WHY WE NEED RUMEN MANIPULATION?

Anaerobic fermentation of feeds in the rumen is beneficial for the host animal. The co-existence of animal

** This paper was presented at an 2002 International Symposium on " Recent Advances in Animal Nutrition" held in New Delhi, India (September 22, 2002).

* Reprint request to: A. Santra. Central Sheep and Wool Research Institute, Avikanagar - 304 501, Rajasthan, India. Tel: +91-1437-20143, Fax: +91-1437-20163,
E-mail: santra@cswri.raj.nic.in

and its microbial eco-system has resulted in stable and the most favored natural selection of microbes to perform the fermentation process optimally. Therefore, do we really need the manipulation of the rumen ecosystem? The answer to this question is definitely yes. During last three decades high producing varieties of plant and livestock have been evolved world over by genetic manipulation using scientific selection and breeding and also by application of biotechnological tools. Likewise, there exist considerable scope for selection and improvement of rumen microbial strains for improved feed utilization, better feed conversion efficiency and production performance of the animals. The rumen microbial ecosystem is not so efficient for digestion of ingested feed as evident from the presence of sizable portion of undigested feeds in the faeces and production of large amount of methane gas in the rumen which could be otherwise utilized as source of energy by the animals.

Tropical/developing countries are poorest in the world on economic ground whereas richest area in terms of vegetation content. These countries import large quantity of plant protein to meet out the requirement/demand of growing human population. Ruminant animals act as important source of animal protein in the region. Ruminants of the region are mainly maintained/fed on poor quality roughage or lignocellulosic agroindustrial by products with or without concentrate supplementation resulting in poor productivity of the animals. Additionally tropical forages have some important limitation for animals feeding like:

1. Tropical forges have low energy value because their cell walls contain higher amount of lignin, silica and cutin resulting in lower fermentation of structural carbohydrate (Dominguez Bello and Escobar, 1997). Tropical forages in comparison to temperate produces less amount of VFA and microbial biomass (microbial protein) after ruminal fermentation.

2. Intake of tropical forages by the animals is low due to their poor ruminal digestion and prolonged retention time (Dominguez Bello and Escboar, 1997).

3. They are deficient of essential nutrients: contain lower amount of energy, protein (Egan et al., 1986) and minerals (Minson, 1980).

4. Feeding of tropical forages to the animals results in imbalance in digestive end products (high acetate and low propionate) which causes inefficient utilization of metabolizable energy (MacRae and Lobley, 1982).

5. Many plant species, particularly legumes and tree leaves contain anti nutritional compound (Jansen, 1975).

Therefore, considerable scope exist for manipulation of ruminal fermentation to improve the utilization of forages particularly in tropical as well as developing countries to maximize the productivity of animals by using available resources.

Research efforts have been devoted to the manipulation of rumen metabolism with the final aim of improving ruminant productivity. Manipulation of rumen fermentation can be considered as an optimization process, whereby optimal condition are sought by maximization and/or minimization of fermentation process, depending on factors such as kind and level of feeding and animal production. Some of the major objectives of rumen manipulation are:

a. Enhance fibrolytic activity: To increase the fibre degradation mainly through manipulation of lignocellulosic bonds in high lignocellulosic feeds as the rumen microbes are the only degraders of cellulose and hemicellulose.

b. Increase microbial protein synthesis: A major portion of the amino acid reaching the duodenum are of microbial protein origin. Therefore, attempts should be made to maximize microbial protein synthesis in the rumen.

c. Reduction in proteolysis: Hydrolysis of feed protein, deamination of amino acids and reutilization of ammonia for microbial protein synthesis are all energy consuming process, hence the degradation of protein and deamination of aminoacids in the rumen should be discouraged.

d. Reduction in methanogenesis: Methane generation in the rumen is a wasteful process as 5-10% of GE intake of ruminants is converted in to methane. The provision of an alternate hydrogen sink in the rumen may help in increasing digestible energy (DE) availability for production.

e. Prevention of acidosis: In high grain fed animals, the level of lactic acid can be controlled to avoid acidosis and inhibition of feed utilization due to lowered pH of the rumen liquor.

f. Shifting acetate to propionate production: In fattening beef/lambs the production of propionate in the rumen at the expense of acetate may be helpful,

g. Novel microbes: The quality of protein is important in high producing ruminants. Microbes, can be tailored to synthesize the amino acids in the form of the peptides and supply to the animals in the intestine.

h. Metabolism of plant toxins: Rumen fermentation can be manipulated for efficient utilization of feeds which contain anti nutritional factors viz. tannin, saponin, mimosine etc.

i. Synthesis of useful secondary metabolites.

METHODS OF RUMEN MANIPULATION

Several techniques of rumen manipulation have been tried in different laboratories of the world during the last two decades with varying results. Broadly the methods of rumen manipulation can be classified in two i.e., genetic manipulation and non genetic manipulation. In genetic manipulation, attempts were made to develop genetically engineered rumen microbes by gene transfer/manipulation technique to enhance the animal productivity. However success in the field of genetic manipulation of rumen

microbes is very poor/sporadic. Non genetic manipulation of the rumen can be done by physical methods (dietary manipulation) and by using suitable chemicals or feeding microbes (probiotics).

GENETIC RUMEN MANIPULATION

The potential of application of molecular techniques in achieving the goals of rumen manipulation are enormous (Forano, 1991; Flint, 1994; Wallace, 1994). These techniques could allow the introduction or increase of desired activities such as cellulolysis and detoxification or reduction of undesirable activities such as proteolysis, deamination and mthanogenesis. For this purpose, one approach would be to select the desirable gene and to express them in a predominant rumen bacteria. Naturally present microorganisms in the rumen can be genetically modified to enhance their capacity of defined functions or to add new functions (Chang, 1996). Introductions of diverse genes into gut microorganisms have been extensively explored (McSweeney et al., 1999). The genetically modified microorganisms are either able to digest fibrous components and lignins of forage, or degrade toxins, synthesize essential amino acids, reduce ruminal methane production and tolerate acids (Forsberg et al., 1993). The second approach would be to introduce new species or strains of microorganisms into the gut (Stewart et al., 1988). Application of the said two approaches has a great potential to increase digestibilities of feedstuffs and to improve animal production.

First step in the process of genetic modification of rumen microbes is the selection of desired gene which has to be engineered i.e., if cellulose degradation is to be improved, the bacteria which are deficient in cellulose degradation should be selected. *Fibrobacter succinogens* which form succinic acid as one of the end products of cellulose fermentation is one such example. This bacteria can be modified to contain a large number of genes for cellulose degradation so that cellulose degradation in the rumen become increased. After selecting the desired gene a suitable vector for carrying the gene to the recipient cell is required. One of the most important vector is plasmid, which has extra chromosomal genetic material unable to integrate with the chromosomal genetic material and remains autonomous and is dispensable. Some of the rumen bacteria also have been shown to harbor plasmids (Smith and Hespell, 1983). The plasmid of rumen bacterai can be recombined with the bacteria containing the desired gene. The recombined plasmid is then transferred back into rumen bacteria to facilitate the insertion of desired genetic property. It is always recommended to use a shuttle vector which has two replication origins and are able to replicate in two host species. Thus the genetic manipulation/engineering work can be done in bacteria, easy to handle e.g. *Escherichia coli* and then transfer the recombined genetic material into the rumen bacteria which can be used for practical application. The genes which have been cloned in *Escherichia coli* are endoglucanase, xylanase, β-glucosidase, amylase, glutamine synthetase from the donor source of *Bacteroides fibrisolvens*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Neocallimastix frontalis*, *Streptococcus bovis* etc.

The physiological conditions in the rumen are not favorable for most of the non rumen microbes. The genetically engineered microbes (rumen origin or non rumen origin) mostly have low competitive ability to survive in a mixed culture. *Prevotella ruminicola* in the rumen has a half life of less than thirty minutes, which has been attributed to bacteriocin like activity present in the rumen liquor (Attwood et al., 1988).

The rumen bacteria from one ruminant may not necessarily be established in the rumen of another animal. The best example of the successful introduction of a new organism (not genetically modified) in the rumen was the introduction of bacteria that was capable of degrading 3-hydroxy-4 (1H)-pyridone (DHP) into Australian ruminants (Wallace, 1994). These animals were unable to use *Leucaena leucocephala* which have a toxic factor mimosine which was degraded to the toxic goitrogen DHP by the rumen bacteria. Jones and Megarrity (1983) reported that Australian goats those consumed *Leucaena leucocephala* developed toxicosis, but Hawaiian goats consuming the same plant spcies did not develop toxicosis. Moreover, these workers demonstrated that inoculation of enriched cultures derived from ruminal content of Hawaiian goats into Australian ruminants conferred resistance to leucaena poisoning (Jones and Megarrity, 1986). Allison et al. (1992) isolated from resistant goats (against mimosine toxicity) a new bacterial species, *Synergistes jonesii*, that was capable of metabolizing DHP. Culture of this organisms was subsequently used as inoculant to protect ruminants in other part of the world from mimosine toxicity (Hammond et al., 1989).

The problems with the establishment of genetically engineered rumen bacteria are too many and very complex. In addition to the scientific and technical problems involved in the establishment of these bacteria in the rumen, the existing regulations about the release of genetically engineered microbes in the atmosphere is also a limitation. A more realistic approach will be to study as to whether the introduced genetic product can serve the purpose of improving rumen fermentation (Wallace, 1994). The success of this approach will depend upon the stability of these gene product against its degradation in the rumen.

NON GENETIC RUMEN MANIPULATION

Microbial feed additives (probiotics)

The digestion process in ruminant occurs by chemical reaction and by the fermentation provided by the rumen microbial flora. During the last decade, the rumen as well as intestinal microbial flora balance have been recognized as main factors to manipulate in order to obtain the best growth performance of the animals. These microbial flora are essential to the animal's health, whereas, their equilibrium is constantly threatened by proliferation of undesirable microbes, detrimental to the health and performance of the animals. Therefore, use of live microbial cultures (probiotics) is being tried now a days as natural feed additives for enhancing rumen metabolic activity and thereby overall animal production. Supplementation of different probiotics (fungi/yeast and bacteria) resulted in improved nutrient status and productivity of the ruminants under certain conditions.

The term "Probiotic" which was a Greek word and meaning for life was first of all used by the Parker (1974). He described it as the organisms or substances those positively contribute to intestinal microbial balance. Fuller (1989) defined probiotics as "A live microbial feed supplement which beneficially affects the host animals by improving its intestinal microbial balance." This definition encompasses single strain or a mixture of two or more species/strains of microbes, with or without growth medium. However, in 1989, US Food and Drug Administration (FDA) used the term direct fed microbes (DFM) instead of probiotic. The FDA defines DFM as a source of live (viable) naturally occurring microorganisms and this includes bacteria and yeast (Miles and Bootwalla, 1991). The commonly used probiotics for animal feeding are broadly divided into two categories i.e., bacterial origin and yeast origin. The primary micro-organisms currently used in animal feeding are:

Bacterial origin

Bacillus licheniformis
Bacillus subtilis
Bifadobacterium adolescentis
Bifadobacterium animalis
Bifidobacterium bifidus
Bifadobacterium infantis
Bifadibacterium longum
Bifidobacterium peudolongum
Bifidobacterium suis
Bifadobacterium hermophilum
Lactobacillus acidophilus
Lactobacillus bulgaricus
Lactobacillus brevis
Lactobacillus casei
Lactobacillus cellobiosus
Lactobacillus delbrueckii
Lactobacillus fermentum
Lactobacillus lactis
Lactobacillus plantarum
Lactobacillus salivarius
Lactobacillus sporogens
Lactobacillus reuteri
Streptococcus interndius
Streptococcus thermophilus

Yeast origin

Aspergilus oryzae
Saccharomyces cerevisiae

Nowadays scientists are trying to isolate the superior strain of rumen fungi for better cellulytic activity and their interspecies transinoculation. Lee et al. (2000) isolated a polycentric fungal strain (*Orpinomyces* strain KNGF2) from the rumen of Korean native goat and by feeding the isolated rumen fungi to sheep, observed better nutrient digestibility, nitrogen retention, increase ruminal bacterial and fungal number. The micro organisms which used as probiotics should possess the following properties:

- Resistance to low pH and bile salt.
- Production of lactate and other antimicrobial agents.
- A normal inhabitant of the gut in the target animal species.
- Able to survive, colonize and multiply at a faster rate in the gut.
- Viable product can be formed at industrial scale for its commercialization.
- Stable and viable during long storage and field conditions.
- Must produce beneficial effect in host animals.

The effects of probiotics are greatest in the fastest growing animals and diminish with age. This age effect is consistent with the capacity of the normal gut flora to resist change as the animal grows with the most stable situation occurring in the adult animals. The utilization of probiotics in farm animals may contribute in the following aspects:

- Growth promotion,
- Improved feed conversion efficiency,
- Better absorption of nutrients by control of gut epithelial cell proliferation and differentiation.
- Improved metabolism of carbohydrate, calcium and synthesis of vitamins,
- Neutralization of anti nutritional factors i.e., trypsin inhibitor, phytic acid etc.
- Microbial enzyme production, compensating for deficient intestinal enzyme activities of the host.
- Elimination or control of intestinal microorganisms producing sub clinical or clinical diseases.
- Stimulation of non specific and specific immunity at the intestinal level.

Administration of probiotics in livestock may be most effective under following conditions:

- After birth to encourage the early establishment of beneficial rumen microflora.
- Following antibiotic treatment.
- In the presence of enteric pathogen such as *E. coli*, *Salmonella*, Coccidia.
- During environmental or mangemental stress.

In calves, administration of probiotics may be most effective under the following circumstances:

- After birth,
- Before and after transportation.
- At weaning,
- Following over eating or antibiotic administration.

In adult cattle, administration of probiotics may become more effective under the situation of:

- Ketosis.
- Antibiotic treatment.
- Bloat.
- Difficult calving.

Recent studies indicated that the administration of probiotics had an impact on growth performance, disease resistance, improving animal production and providing a cost effective dietary supplement. Their widespread use as manipulating agents for rumen fermentation (so called direct fed microbials) is of recent origin and most of the published research papers on the topic have been periodically reviewed by Dawson (1990, 1992), Martin and Nisbet (1992), Wallace and Newbold (1992) and Walli (1994). The published data indicated that microbial feed additives may benefit ruminant nutrition in terms of live weight gain and milk production of the animals in the tune of 7 - 8% (Wallace and Newbold, 1993).

PROBITICS FOR NEONATAL RUMINANT

Application of microbial preparation for newborn animals includes dosing or drenching the animals soon after birth or inclusion of direct fed microbial products (DFM) in either milk or milk replacer. The goals of microbial supplementation of the neonatal ruminant are similar to those for non ruminants like rapid adaptation to solid feed by accelerating the development of a normal adult intestinal microflora, avoiding the establishment of enteropathogens and also to stimulate the early development of rumen. Lactic acid producing bacteria are administered to calf, lambs and kids soon after birth and/or in milk replacer. The primary goal of inoculating neonates with lactic acid bacteria is to establish a beneficial populations of bacteria in the GI tract capable of competing successfully with pathogens. There is evidence of rapid rumen development and faster growth by additions of certain Lactobacillus bacteria in the animal's feed.

PROBIOTIC FOR GROWING RUMINANT

For many years, ruminant nutritionists and microbiologists have been interested in the manipulation of microbial ecosystem of the rumen to improve production efficiency of domestic ruminants. Recently the use of yeast culture and lactobacillus bacteria in ruminant diets as a probiotics has received renewed attention. Production benefits due to feeding of live yeast or lactobacillus culture to the ruminant to be caused by changes in ruminal fermentation, in particularly by increase degradibility of forage and flow of microbial protein from the rumen (Wallace and Newbold, 1992).

Effect of probiotic feeding on rumen function

The yeast cells were able to maintain viability throughout the digestive tract. Using yeast culture in rumen simulating continuous fermentor culture, Dawson and Newman (1987) observed that yeast replicated in the *in vitro* system. However, the yeast culture was unable to maintain a productive population within the rumen ecosystem. The viable cell numbers of yeast showed a decline in rumen and the rate of decline being 0.17/h, similar to the likely rate of liquid outflow from the rumen (Newbold et al., 1990). The decline in viable cell numbers was due to extensive lysis (Burning and Yokoyama, 1988) and due to rumen outflow. Two type of yeast have been proposed to stabilize the rumen ecosystem: *Saccharomyces cervisiae* and *Aspergillus oryzae*. Both have been shown to stimulate VFA production *in vitro* by increasing the degradation of the dietary forage fraction in mixed diets (Jouany et al., 2000).

Rumen microbial population : Yeast feeding has been found to be increase the total number of rumen bacterial population along with higher proportion of cellulolytic bacteria (Newman and Dawson, 1987; Widmeier et al., 1987; Dawson et al., 1990; Kumar et. al., 1994; Saha et al., 1999) thus improved cellulose digestion. Dawson (1989) reported that different cellulolytic bacteria respond differently to yeast culture. The yeast strains that stimulate the growth of *Bacteroides succinogenes* in pure cultures did not stimulate the growth of *Ruminococcus albus* to the same extent. Nisbet and Martin (1990) and Newbold et al. (1998) reported that *Saccharomyces cervisiae* stimulated the growth of lactic acid utilizing bacteria *Selenomonas ruminantium* in pure culture. Jonecova et al., (1992) observed higher number of amylolytic, pectinolytic and xylanolytic bacteria in the rumen liquor of animals fed *Lactobacillus cellobiosus* along with yeast. The viable yeast culture itself (Dawson et al., 1990; Dawson, 1990) or some heat liable nutrients in the yeast (El Hassan et al., 1993; Kumar et al., 1994) may be responsible for stimulation of rumen bacterial growth. Newbold et al. (1993) suggested that the ability of yeast cells to stimulate bacterial numbers in the rumen may be related to their ability to decrease potentially inhibitory concentrations of oxygen in rumen. However, effect of direct fed yeast culture on rumen ciliate protozoal population is contradictory. Frumholtz et al. (1989) reported 45% reduction in ciliate protozoal number whereas Maurya et al., (1993), Panda (1994), Plata et al. (1994) and Mathieu et al. (1996) reported increased ciliate protozoal population due to feeding of yeast culture to the animals. On the contrary, Fondevila et al. (1990) and Kim et al. (1992) observed that ciliate protozoal number was not altered significantly due to feeding of yeast to the animals. The percentage of *Entodiniomorphid* protozoa decreased and *Dasytricha* increased in the rumen of yeast culture fed

animals (Arakaki et al., 2000). Oellermann et al. (1990) reported higher ruminal fungal number in the fungal culture (*Aspergillus orygea*) fed animals.

Rumen pH : Yeast have a buffering effect in the rumen medium and prevent sharp drops in rumen pH and thus stabilize the pH even in the high concentrate fed animals. Newman and Dawson (1987) reported a rise in rumen pH from 6.36 to 6.55 when *Saccharomyces cervisiae* was added in rumen fermenter fulled with roughage ration. The higher ruminal pH in yeast culture fed animals was associated with a lower lactate concentration (Martin and Nisbet, 1992; Walli, 1994) although, yeast itself, do not utilize lactate. Yeast culture inhibit the growth of lactic acid producing rumen bacteria (Girard et al., 1993; Wallace, 1996) by utilizing their substrate (soluble sugar) and thus decreasing lactic acid production and sudden fall in pH of the rumen medium. Moreover, yeast culture stimulate the growth of lactic acid utilizing rumen bacteria as well as uptake of lactate by them (Martin and Nisbet, 1992). Nisbet and Martin (1990) and Newbold et al. (1998) reported that *Saccharomyces cervisiae* stimulated the growth of lactic acid utilizing bacteria *Selenomonas ruminantium*. Jonecova et al. (1992) observed higher ruminal pH in sheep fed *Lactobacillus cellobiosus* along with yeast culture. In contrast, some workers reported no effect on rumen pH due to probiotic supplementation (Widmeier et al., 1987; Chademana and Offer, 1990 and Yoon and Stem, 1996).

Volatile fatty acid production : Published reports on the effect of yeast culture feeding to the animals on ruminal VFA concentration are variable: some workers reported yeast culture had no effect (Adams et al., 1981; Chademana and Offer, 1990), while others found a stimulation in VFA production as well as proportion propionate production at the expense of acetate (Harrison et al., 1988; Dawson et al., 1990; Newbold et al., 1990) or even an increase in the proportion of acetate (Mutsvangwa et al., 1992). Williams et al. (1990) observed lower ruminal TVFA's concentration in the yeast culture fed steers whereas Andrighetto et al. (1993), Kumar et al. (1994) and Dutta et al. (2001) reported that the mean molar concentration of VFA's was higher in the rumen liquor of yeast culture fed animals. However, Kopecny et al. (1989) did not found any significant effect on ruminal VFA production when *Streptococcus bovis*, *Butyrovibrio fibrosolven* along with *Lactobacillus acidophilus* was fed to the animals.

Ammonia nitrogen : Animals consuming yeast culture have lower ruminal ammonia nitrogen concentration and higher microbial protein synthesis (Harrison et al., 1988; Erasmus et al., 1992; Dutta et al., 2001). An increased in microbial protein synthesis with altered amino acid profile of duodenal digesta was observed in dairy cows (Erasmus et al., 1992). Greater number of total bacteria and cellulolytic bacteria may explain why ruminal ammonia concentration are lower and microbial protein synthesis higher in yeast culture fed animals. Ammonia is the preferred source of nitrogen for ruminal microbial population (Bryant and Robinson, 1963) for incorporation into ruminal bacteria (Mathison and Milligan, 1971). Lower concentrations of ammonia in the rumen of cows fed yeast culture may reflect increased transportation of ammonia into microbial protein. The increased bacterial biomass by feeding yeast culture result in increased in microbial protein flow from rumen to the duodenum (Williams et al., 1990; El Hassan et al., 1996). Feeding of *Lactobacillus acidophilus* also reduced rumen ammonia nitrogen concentration (Skrivonova and Machanova, 1990). However, Quionez et al. (1988) and Chademana and Offer (1990) reported no effect on ruminal ammonia concentration due to probiotic feeding to that animals.

Rumen enzyme profile : The yeast supplementation in the diet increased the activity of carboxymethyl cellulase enzyme in the rumen of animals (Maurya et al., 1993). Panda (1994) observed no effect on ruminal amylase and protease activity but a stimulatory effect on carboxymethyl cellulase activity by yeast feeding in goats. Similar results were also reported by Widmeier et al. (1987), Harrison et al. (1988) and Dawson et al. (1990). However, Agarwal (2002) reported that ruminal hydrolytic enzyme activity like carboxymethyl cellulase, amylase, xylanase, β-glucosidase and protease remained unchanged by the feeding of yeast to the calves. Increased ruminal amylase activity was also reported in *Lactobacillus cellobiosus* and Streptococcus fed calves (Bomba et al., 1992).

Rumen digesta flow : No change in rumen digesta flow was observed in yeast culture fed animals (Martin and Nisbet, 1992). Moloney and Drennan (1994) reported that rumen volume, liquid dilution rate and liquid outflow rate were not affected by the inclusion of yeast culture in animal's diet. However, in some studies it was observed that ruminal liquid dilution rate in creased (although statistically non significant) due to yeast culture inclusion in the diet (Widmeier et al., 1987; Harrison et al., 1988; Chademana and Offer, 1990; Malcolm and Kiesling, 1990).

Oxygen scavenger : Yeasts act as a oxygen scavenger in the rumen (Wallace, 1996). During feed ingestion, some amount of oxygen enter the rumen along with feed and its adversely effect the rumen environment as well as growth of the rumen microbes. There was increased oxygen disappearance (between 46-89%) by adding *Saccharomyces cervisiae* in rumen fluid *in vitro* and stimulate rumen bacterial growth (Newbold et al., 1996).

Effect of probiotic feeding on animal performances

Feed intake : Effect of probiotic supplementation on dry matter intake by the animals are inconsistent. Supplementation of yeast in the animal's feed improved it

palatability as glutamic acid produced by yeast is responsible for improvement in the taste of feed stuffs (Agarwal, 2002). However, Erdman and Sharma (1989) observed no change in daily dry matter intake whereas Putnam et al. (1997) reported higher daily dry matter intake in yeast culture fed/supplemented dairy cows. Increase in fed intake was also reported by Adams et al. (1981) in steers fed 50:50 roughage: concentrate based diet, supplemented with a yeast additives and Edwards et al. (1990) in bulls reared intensively and supplemented with *Saccahromyces cervisiae* at the rate of 1.5 kg/ton fresh feed.

Nutrient digestibility : Numerical significant improvements have been reported in digestibility of dry matter, organic matter, crude protein and fibre in yeast fed animals. Higher retention of nitrogen and energy have also been reported in yeast fed animals whereas, the effects have been variable and the response influenced by the type of diet, physiological state of the animals and microbial strain employed. Basal diet of the yeast culture fed animals influence the nutrient digestibility and productivity of the animals (Williams and Newbold, 1990; Moloney and Drennan, 1994). Fallon and Harte (1987) reported that yeast culture increased nutrient digestibility and growth performance of calves fed a starch-based concentrate (barley) but not in calves fed a non starch based concentrate (corn gluten). In addition, Williams et al. (1991) suggested that the effect of yeast culture is likely to be greatest in diets containing a high proportion of readily fermentable carbohydrate, such as barley based concentrate. Contrarily Harrison et al. (1988) and Williams et al. (1990) did not observed any effect of yeast culture supplementation to the animals on ruminal nutrient digestibility. The inclusion of yeast in animals diet might be affect by the site of digestion: increasing the ruminal digestion and reducing the hind gut digestion and so that the overall tract digestibility appears same as control (Williams et al., 1990). However, Widmeier et al. (1987), Panda et al. (1995) and Pandey et al. (2001) reported better nutrient digestibility in yeast fed animals. A significant improvement in the digestibility of crude protein and crude fibre had been also observed on supplementation of diets with *Lactobacillus acidophilus* and *Saccharomyces cervisiae* in goats (Sharma and Malik, 1992). Abu-Tarboush et al. (1996) also reported that there was no significant effect on apparent digestibility of DM, OM, CP, ADF and gross energy in Holstein calves fed diet containing culture of *Lactobacillus acidophilus*.

Growth and feed conversion efficiency : The published reports on live weight gain and feed conversion efficiency due to yeast feeding is variable from nil to positive effect. Adams et al. (1981), Edwards et al. (1990) did not find any significant increase in gain or feed conversion efficiency on yeast (*Saccharomyces cervisiae*) supplementation to the animals, whereas, Fallon and Harte (1987), Singh et al. (1998), Saha et al., 1999 and Pandey et al. (2001) reported that young calves respond well to dietary supplementation of yeast in the starter diet in terms of live weight gain and feed conversion efficiency. Supplementation of lactobacillus culture to the milk fed dairy calves also improved the growth rate of the animals (Gilliland et al., 1980; Schwab et al., 1980; Voronin et al., 1990, Abe et al., 1995; Abu-Tarboush et al., 1996; Prahalada et al., 2001). However, Jenney et al. (1991), Higginbotham and Bath (1993) and Cruywagen et al. (1996) reported no significant improvement in growth performance of the animals by feeding of either lactobacillus culture or lactobacillus along with other microorganisms.

Milk production : Positive response on milk production have been reported in yeast fed animals (Harris and Lobo, 1988; Gunter, 1989; Huber et al., 1990). Response have been greater in early compared to mid of lactation (Alarcon et al., 1991) and the response was greater with diets containing the higher proportion of concentrate (Williams et al., 1990).

Incidence of diarrhoea : Incidence of diarrhoea reduced due to feeding of probiotic to the young calves (Abe et al., 1995; Abu-Tarboush et al., 1996; Saha et al., 1999). There was considerable reduction in the number of total coliform bacteria in the rumen liquor as well as faeces of calves fed probiotic in their diet, irrespective of the chemical composition of the ration offered to the animals (Kamra et al., 1997).

DEFAUNATION

The process of making the rumen of animals free of rumen protozoa is called defaunation and the animal is called defaunated animal. The role of rumen ciliate protozoa on the performance of host animals became debatable issue when Becker and Everett (1930) demonstrated that rumen protozoa were non-essential for growth in lambs. Nevertheless, the reports of recent years reflect that though protozoa may be non essential for ruminant, still they have significant role to play in the rumen metabolism specially to stabilize the rumen pH (Santra et al., 1996; Santra and Karim, 2002a). Rumen protozoa are the largest in size among rumen microbes and contribute 40-50% of the total microbial biomass and enzyme activities in the rumen (Agarwal et al., 1991).

Methods of defaunation

There are several ways to defaunate the animals and to obtain a ruminant animal free from rumen ciliate protozoa. The different methods of defaunation are:

Isolation of new born animals : One of the method of producing defaunated animals is the separation of newborn animals from their dams after birth and preventing them

from any contact with the adult ruminant animals. The newborn animals should be separated 2 to 3 days after birth (Jouany, 1978). During this time the newborn animals gets contaminated with the native bacterial population but do not get rumen ciliate protozoa (Fonty et al., 1984). However, once the animal is separated, proper care should be taken so that the isolated animals do not come in contact with any adult animals as well as any contamination from the handlers who look after faunated and defaunated animals.

Chemical treatment : Another method of defaunation is by use of chemicals and majority of researchers has used this method for obtaining animals free from rumen ciliate protozoa. The chemicals which have been widely used to defaunate the animals are copper sulphate (Ramprasad and Raghavan, 1981), manoxol (Chaudhary et al., 1995) and sodium lauryl sulphate (Santra et al., 1994a; Santra and Karim, 1999). Chemicals which are used as defaunating agent are introduced in the rumen of animals either orally by a stomach tube or through rumen fistula. However, these chemicals are not only toxic to the rumen protozoa but also kill the other rumen microorganism like bacteria. These chemicals are also toxic to the animals resulting in depressed feed intake, dehydration and some time mortality also reported (Jouany et al., 1988).

Dietary manipulation : The ciliate protozoa are very much sensitive to change in rumen pH. The activity of ciliate protozoa is adversely affected when the pH of the rumen fall below 5.8 and if the rumen pH fall below 5.0, the ciliate protozoa are be completely eliminated. Therefore, offering high energy feed (especially cereal grains like barley, maize etc) to the starved (for 24 hours) animals, creates acidic condition in the rumen and rumen pH fall below 5.0. This fall in rumen pH eliminate the ciliate protozoa completely and the animal become defaunated. However one serious disadvantage of this method is that chances of developing acidosis in treated animal is more. Once rumen acidosis develops the animals will suffer form various secondary complications. The drenching of vegetable oils eliminate ciliate protozoa and hence can be used as a defaunating agent (Newbold and Chamberlain, 1988; Nhan et al., 2001).

Effect of defaunation on the rumen ecosystem rumen microbes

Defaunation causes both qualitative and quantitative change in rumen bacterial population. After defaunation the bacterial population increased (Chaudhary et al., 1995) since rumen protozoa feed on the rumen bacteria to meet our their nitrogen requirement. A total of 4 to 45 g bacterial dry matter is engulfed by rumen protozoa per day per sheep (Coleman, 1975). Defaunation increase the number of amylolytic bacteria due to elimination of nutritional competition between bacteria and protozoa for using starch (Kurihara et al., 1978) whereas the cellulolytic bacterial population become decreased (Jouany et al., 1988). Fungal population in the rumen also increase due to defaunaton (Smet et al., 1992). Orpin and Letcher (1983/84) reported a predation of fungal zoospore by rumen protozoa but till today it is unclear whether the increase in fungal populatio following defaunation is a consequence of reduction in the predation by the protozoa or increased availability of nutrients for fungal growth in the absence of protozoa.

Rumen pH : The buffering capacity of the rumen seems to be better in presence of protozoa on a wide variety of diets. The rumen pH starts falling immediately after ingestion of feed, both in faunated and defaunated animals whereas, the drop in pH was much higher in defaunated than in faunated animals (Jouany et al. 1988; Nagaraja et al., 1992; Mendoza et al., 1993; Santra et al., 1996; Santra and Karim, 2002a). Rumen protozoa engulf the readily fermentable carbohydrate (starch) which is stored in their body as amylopectin (Williams and Coleman, 1992; Santra et al., 1996; Hristova et al., 2001; Santra and Karim, 2002a) and thus decrease the rate of carbohydrate (starch degradation) fermentation (Williams and Coleman, 1992), resulting in a lower pH in the rumen of defaunated compared to faunated animals.

Volatile fatty acid (VFA) production : The effect of defaunation on the production and composition of VFA is variable. The VFA production rate and its composition are greatly influenced by experimental diet. Increase in TVFA concentration in defaunated animals was reported by Punia et al. (1987), Santra et al. (1996) and Santra and Karim (2002a) while non-significant effect was recorded by Itabashi et al. (1982), and Ivan et al. (1992). However, lower VFA production in the rumen of defaunated animals reported by several workers because of diet effect (Jouany et al., 1981; Kayouli et al., 1983; Hegarty et al., 1991; Chaudhary et al., 1995). Higher VFA conecntration in the rumen of faunated animals may be due to higher hydrolytic enzyme activity in the rumen protozoa because about 40-60% of hydrolytic enzyme activity is found in the rumen protozoa (Agarwal et al., 1991) and also due to stimulatory effect of protozoa over bacteria (Onodera et al., 1988).

The ciliate protozoa engulfed the feed particle and degrade it to acetic acid and butyric acid during carbohydrate metabolism (Demeyer and Van Nevel, 1979; Ushida et al., 1986a,b). Defaunation should, therefore, increase the molar proportion of propionic acid (Williams and Withers, 1991; Mendoza et al., 1993; Chaudhary et al., 1995). Moreover higher propionate production in the rumen of defaunated animals may be due to increase and shift in ruminal bacterial population. It has been reported that in defaunated animals number of acetate producing species such as *Ruminococcus* spp. are not predominant while succinate producing bacteria such as *Bacteroides* spp. are

predominant (Kurihara et al., 1978). This changes in ruminal bacterial population may stimulate more propionate production in the rumen of ciliate free animals. However, the reported effect of defaunation on VFA composition are variable. Demeyer et al. (1982), Itabashi et al. (1984), Rowe et al. (1985) and Ushida and Jouany (1990) reported that the molar proportion of propionate in defaunated and fauntaed animals was similar whereas, Demeyer et al. (1982) and Ivan et al. (1992) reported lower proportion of propionate production in the rumen of defaunated animals.

Ammonia nitrogen concentration : Significant reduction in ammonia-N concentration in the rumen of defaunated animals was reported by several workers (Itabashi et al., 1984; Chaudhary et al., 1995; Santra et al., 1996; Nhan et al., 2001; Santra and Karim, 2002a). Ammonia is utilized by bacteria to meet their nitrogen requirement for body protein synthesis while ciliate protozoa does not use it. In defaunated animals, the number as well as activity of rumen bacteria increase (Eadie and Gill, 1971) resulting in more uptake/utilization of ammonia by bacteria and as a result, ruminal ammonia concentration is reduced. Further, low production of free amino acid from the degradation of protein or peptide in absence of ciliates and/or lower rate of recycling of microbial nitrogen in the rumen of defaunated animals (Demeyer and Van Nevel, 1979), could have contributed to lower ruminal ammonia nitrogen concentration. The recycling of bacterial nitrogen in the rumen is higher in presence of ciliate protozoa (Hristova et al., 2001) and the number of ruminal bacteria capable to utilize ammonia decrease with increased ruminal break down of dietary protein (Leng, 1982).

Microbial protein synthesis : Microbial protein synthesis in the rumen of defaunated animals was higher than faunated animals (Bird et al., 1994). It is now generally accepted that in absence of rumen ciliate protozoa, the efficiency of rumen bacterial growth is enhanced and more microbial protein flows from reticulo-rumen to duodenum (Bird and Leng, 1985). Although bacteria and protozoa are active in synthesis of microbial protein, outflow of microbial protein in to duodenum is primarily of rumen bacterial origin. About half of the microbial protein in the rumen can be of protozoal origin while as a proportion of the microbial protein leaving the rumen, protozoal protein is usually under 10% because of higher rate of bacterial was out from reticulo-rumen (Owens and Zinn, 1988). Additionally, the absence of rumen protozoa is known to increase the efficiency of net bacterial growth due to elimination of protozoal predation and increasing rumen bacterial turn over (Demeyer and Van Nevel, 1979). This could have resulted in more microbial protein flow to the duodenum in the defaunated animals.

Enzyme profile : Rumen ciliate protozoa secrete various hydrolytic enzyme which are responsible for break down of the plant cell wall poly saccharides (Williams and Coleman, 1988; Agarwal et al., 1991). The ciliate protozoa and fungi are most important microbial groups of the rumen organisms required for the ruminal digestion of plant fibre (Amos and Akin, 1978; Windham and Akin, 1984). Carboxymethyl cellulase enzyme activity was lower in the rumen of defaunated than faunated animals (Williams and Withers, 1991; Santra et al., 1996; Santra and Karim, 2002a). About 62% of the total rumen cellulase enzyme activity is associated with rumen protozoal population (Coleman, 1986). Hence elimination of ciliate protozoa decreases ruminal cellulase enzyme activity. The activity of other carbohydrate degrading enzymes like amylase, xylanase and β-glucosidase are not affected by the presence or absence of ciliate protozoa in the rumen (Santra et al., 1996; Santra and Karim, 2002a). Protease enzyme activity was lower in the rumen of faunated than defaunated animals (Ushida and Jouany, 1985; Santra et al., 1996). The specific activity of protease enzyme from bacterial fraction is 6-10 times higher than that from protozoal fraction (Brock et al., 1982). Defaunation, increases the number of ruminal bacterial population, resulting in higher ruminal protease enzyme activity. Bacteria are the only source of ruminal urease enzyme (Cook, 1976) while ciliate protozoa have no urease enzyme activity (Onodera et al., 1977) and hence ciliates can not utilize urea for their body protein synthesis. However, observed similar urease enzyme activity in the rumen of faunated and defaunated animals (Santra et al., 1996; Santra and Karim, 2002a) needs further studies.

Methane production : Defaunation is reported to considerably decrease the methane production compared with the normal faunated animals (Jouany et al., 1988; Williams and Coleman, 1992; Santra et al., 1994b). The reduction in methane production in absence of rumen protozoa has been attributed to various reasons. Rumen protozoa contribute hydrogen moiety for the production of methane by the methanogenic bacteria (Prins and Van Hoven, 1977; Van Hoven and Prins, 1977). Further, ectosymbiotic attachment methanogens have with ciliate protozoa and elimination of their symbiotic partner on defaunation results in reduced methane production. Reviewing the published literatures on the topic, Kreuzer (1986) calculated that defaunation decreased energy losses through methanogenesis by 5.5 to 7.9% of gross energy intake.

Rumen volume, flow rate of digesta and physical characteristics : The effect of defaunation on rumen physiological characteristics (e.g. motility, absorption) are lacking in literature. Contradictory reports have appeared on the effect of defaunation on rumen fluid volume and digesta flow rate. Lindsay and Hogan (1972), Faichney and Griffiths (1978) and Veira et al. (1983) reported no

significant difference in the rumen volume of faunated and defaunated sheep whereas. Orpin and Letcher (1983/84) observed higher rumen fluid volume and lower fractional outflow of liquid digesta from reticulo-rumen in defaunated sheep. Chaudhary et al. (1995) reported higher rumen volume in defaunated buffaloes whereas liquid outflow rate remained unchanged irrespective of presence or absence of ciliate protozoa. Kayouli et al. (1983/84) reported no difference in rumen volume and liquid fractional outflow rate in defaunated and faunated animals while. the particle outflow rate was significantly higher in the absence of ciliate protozoa. On the contrary, Punia et al. (1987) found lower rumen liquid volume in defaunated animals.

Animal performance

Feed intake and nutrient digestibility : The first and the most easily detectable influence of defaunation (by chemical method) on the animal is the loss of appetite and therefore decreased feed intake for a few days after defaunation of the animals. In case the animals does not regain its appetite even after 5 to 7 days of defaunation it can be induced by offering highly palatable feed. Once stabilized. daily dry matter intake in defaunated animals attend the level similar to that of faunated animals. Daily feed intake of the animals is not influence by the presence or absence of ciliate protozoa is a consistent finding in several studies (Chaudhary and Srivastava, 1995; Santra and Karim. 2000, 2002b).

Protozoa play an important role in the digestion of plant cellwalls, especially cellulose and hemicellulose. The digestibility of fibre fractions like, NDF, ADF, hemicellulose and cellulose are decreased by defaunation (Demeyer. 1992; Uhida and Jouany. 1990; Chaudhary and Srivastava., 1995; Santra and Karim, 2000b). The reduced digestibility of fibre fraction in defaunated animals may be due to elimination of large Entodiniomorphid ciliates which have higher cellulolytic activity (Ushida and Jouany. 1990). Moreover. better digestibility of cell wall constituents in faunated animals might be due to increased retention time of feed particles in the rumen (Kayouli et al. 1983, 1984; Ushida and Jouany, 1990). stabilization of rumen environment favoring development of cellulolytic microbes (Hegarty et al., 1991) and stimulatory effect of rumen ciliate protozoa on rumen bacteria for cellulolysis (Onodera et al., 1988). It is also reported (Demeyer, 1981) that when rumen fibre digestion is impaired in absence of ciliate protozoa, it is often increased in hindgut resulting in similar total GI tract digestibility of fibre in presence or absence of ciliate protozoa. However. the effect of defaunation on the digestibility of starch and sugars is negligible (Jouany,

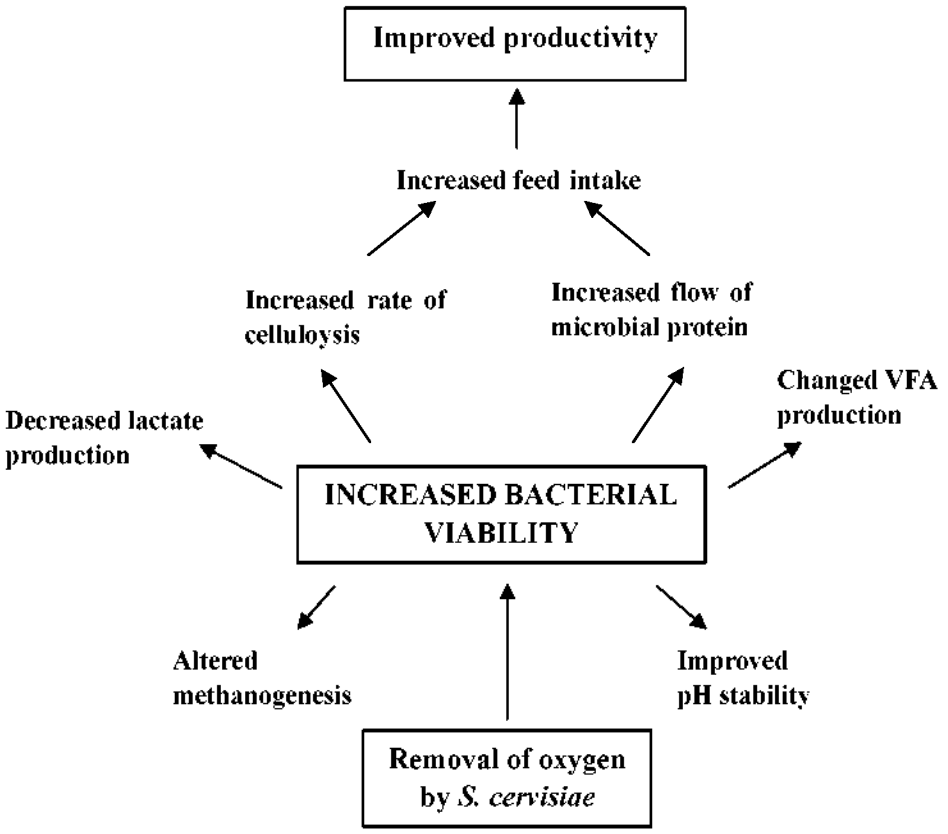

Figure 1. A schematic diagram describing the mode of action of yeast culture (Source: Wallace. 1994)

1978).

Bacteria and free amino acids may provide nitrogen to satisfy the requirement of protozoa for their body protein synthesis (Bonhomme, 1990). Bacteria play a dominant role in degradation of most soluble proteins while rumen protozoa help in ruminal degradation of relatively insoluble protein (Ushida and Jouany, 1985). Protozoa utilize bacterial and feed protein available in the rumen. Hence, on defaunation, the degradability of protein is decreased in the rumen (Jouany et al., 1988; Santra and Jakhmola, 1998). However, defaunation had no effect on apparent protein digestibility in the rumen (Santra et al., 1994a; Chaudhary and Srivastava, 1995; Santra and Karim, 2000, 2002b).

Degradation of toxic substances : Defaunated animals are more susceptible to the bloat than normal animals. Ochratoxin A produce more toxicity in the defaunated animals than in faunated animals (Jouany et al., 1988). Further, defaunated animals are more sensitive to copper toxicity. The rumen protozoa induce the complexion of the Cu^{+2} in sulphide form resulting the toxic copper become unavailable for absorption from the intestine.

Growth and feed conversion efficiency : The results of effect of defaunation on the animal performance are mainly related to animal feed composition and feeding schedule (nature of feed stuff, its presentation and distribution). The heart girth of ciliate free lambs was found to be larger than that of faunated lambs (Kamra et al., 1987). Bird and Leng (1984) observed increased wool growth in the defaunated lambs. The availability of metabolizable energy was higher in defaunated animals due to reduction of energy loss in methane production. Total heat production of animal was also significantly lower in absence of rumen ciliate protozoa (Kreuzer, 1986). Therfore, defaunation has a positive effect on the growth rate and feed conversion efficiency of the animals (Bird et al., 1979, Santra and Karim, 2000, 2002b). On the contrary, Osman et al. (1970) and Ramprasad and Raghavan (1981) observed reduced feed conversion efficiency in the absence of ciliate protozoa while Chaudhary and Srivastava (1995) reported that defaunation had no effect on feed conversion efficiency. The effect of defaunation on feed conversion efficiency seems to be diet dependent. On high roughage fed animals the protozoa do not seem to have any specific function to perform and the general function of feed degradation is taken over by the increased population of bacteria and fungi in the absence of ciliate protozoa. The reduction in methanogenesis results in better feed conversion efficiency on such feed. But when the animals are fed on high grain diet, the ciliate protozoa have a specific function to perform i.e., the pH stabilization by controlling the degradation of easily fermentable sugars by protecting them in their bodies as amylopectin. Thus in absence of ciliate protozoa, the pH drops below the optimum level required for various enzymes activity in the rumen. This results in poor feed utilization and decreased feed conversion efficiency on such feeds. Therefore, defaunation protocol can be used as an important tool to improve the productivity of animals in tropical countries, where majority of livestock are maintained on sole diet on low grade roughage. Sen et al. (2000) reported that carcass composition of defaunated, refaunated and normal finisher lambs was similar. Ramprasad and Raghavan (1981) reported that defaunation had no effect on the quantity of carcass fat in lambs.

AREA OF FUTURE RESEARCH

- Screening of non conventional animal feeds specially tree leaves for anti protozoal activity.
- Standardization of defaunation method for its implication at farmer's level.
- New species/ strains of microorganism should be screened to use as probiotic.
- Mechanism of action of probiotic should be studied thoroughly.
- Reduction in methanogenesis to improve availability of digestible energy and reduce environmental pollution.
- Production of suitable strain of recombinant microorganisms and their propagation in the rumen for efficient detoxification of plant toxins, reduction in methanogenesis, higher cellulolysis, reduced ruminal proteolysis (deamination).

CONCLUSIONS

Rumen is an natural fermentative anaerobic system which should be manipulated essentially by altering the composition of rumen microflora. There is ample scope to manipulate the rumen by feeding local plants or tree leaves or agro industrial by products to defaunate the animals for improving its productivity. Introduction of naturally occurring microorganism from digestive system of one species to another species for efficient degradation of plant toxins as well as for efficient utilization of nutrients will be one of the major thrust area in near future for rumen manipulation. Genetically manipulation of rumen microorganism for efficient ruminal fermentative digestion has an enormous biotechnological potential. However in tropical countries, more emphasis should be given for manipulating the rumen to increase cellulolytic activity for efficient utilization of low grade roughage.

REFERENCES

Abe, F., N. Ishibashi and S. Shimamura. 1995. Effect of

administration of Bifidobacteria and lactic acid bacteria to new born calves and piglets. J. Dairy Sci. 78:2838-2846.

Abu-Tarboush, H. M., M. Y. Al-Saiady and A. H. Keir El-Din. 1996. Evaluation of diet containing lactobacillus on performance, faecal coliform and lactobacillus of young dairy calves. Anim. Feed Sci. Technol. 57:39-49.

Adams, D. C., M. L. Galyean, H. E. Kiesling, J. D. Wallace and M. D. Finker. 1981. Influence of viable yeast culture, sodium bicarbonate and monensin on liquid dilution rate, rumen fermentation and feedlot performance of growing steers and digestibility in lambs. J. Anim. Sci. 53:780-789.

Agarwal, Neeta., N. Kewalramani, D. N. Kamra, D. K. Agrawal and K. Nath. 1991. Hydrolytic enzymes of buffalo rumen: Comparison of cell free rumen fluid, bacterial and protozoal fractions. Buff. J. 2:203-207.

Agarwal, Neeta. 2002. Microbial feed additives for ruminants. In: Recent Advances in Rumen Microbiology (Ed. D. N. Kamra, Neeta Agarwal, L. C. Chaudhary and D. K. Agrawal). IVRI Publication, Izatnagar, India, pp. 47-56.

Alarcon, R. A. G., J. T. Huber, G. E. Higginbotham, F. Wiersma, D. Amnon and B. Taylor. 1991. Influence of feeding *Aspergillus oryzae* fermentation extract on milk yield and early pattern of body temperature on lactating cows. J. Anim. Sci. 69:1733-1740.

Allison, M. J., W. R. Mayberry, C. S. McSweeney and D. A. Stah. 1992. Synergistes jonesii, gen. Nov., sp. nov.: A rumen bacterium that degrades toxic pyridinediols. Syst. Appl. Microbiol. 15:522-529.

Amos, H. E. and D. E. Akin. 1978. Rumen protozoal degradation of structurally intact forage tissue. Appl. Environ. Microbiol. 36:513-522.

Andrighetto, I., L. Bailoni, G. Cozzi and P. Berzaghi. 1993. Effect of yeast culture addition on digestion in sheep fed a high concentrate diet. Small Rum. Res. 12:27-34.

Arakaki, L. C., R. C. Stahringer, J. E. Garrett and B. A. Dehority. 2000. The effects of feeding monensin and yeast culture alone or in combination, on the concentration and generic composition of rumen protozoa in steers fed on low-quality pasture supplemented with increasing levels of concentrate. Anim. Feed Sci. Technol. 84:121-127.

Attwood, G. Y., D. L. R. Gordon, J. P. Ue and J. D. Brooker. 1988. Use of a unique gene sequence as a probe to enumerate a strain of *Bactericides ruminocola* introduced into rumen. Appl. Environ. Microbiol. 54:534-5441.

Becker, E. R. and R. C. Everett. 1930. Comparative growth of normal and infusoria free lambs. Am. J. Hyj. 11:362-370.

Bird, S. H., M. K. Hill and R. A. Leng. 1979. The effects of defaunation of the rumen on the growth of lambs on low protein high energy diets. Br. J. Nut. 42:81-87.

Bird, S. and R. A. Leng. 1984. Further studies of the effects of the presence or absence of protozoa in the rumen on live weight gain and wool growth of sheep. Br. J. Nut. 52:607-611.

Bird, S. H. and R. A. Leng. 1985. Productivity response to eliminating protozoa from the rumen of sheep. Rev. Rural Sci. 6:109-117.

Bird, S. H., B. Rommulo and R. A. Leng. 1994. Effects of lucerne supplementation and defaunation on feed intake, digestibilty, N retention and productivity of sheep fed straw based diets. Anim. Feed Sci. Technol. 45:119-129.

Bomba, A., G. I. Kalacnjuk, J. Lenart, O. G. Savka, R. Zitnan and M. G. Gerasimiv. 1992. Diet and microbial stimulation of rumen digestion. Zivocisna-Vyroba. 37:747-754.

Bonhomme, A. 1990. Rumen ciliates: their metabolism and relationships with bacteria and their hosts. Anim. Feed Sci. Technol. 30:203-266.

Bradford, G. E. 1999. Contribution of animal agriculture to meeting global human food demand. Livest. Prod. Sci. 59:95-112.

Brock, F. M., C. W. Forsberg and S. J. G. Buchman. 1982. Proteolytic activity of rumen micro-organisms and effect of proteinase inhibitors. Appl. Environ. Microbiol. 44:561-569.

Bryant, M. P. and I. M. Robinson. 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of rumen bacteria. J. Dairy Sci. 46:150-154.

Burning, C. L. and M. T. Yokoyama. 1988. Characteristics of live and killed brewer's yeast slurries and intoxication by intraruminal administration to cattle. J. Anim. Sci. 66:585-591.

Chademana, L. and N. W. Offer. 1990. The effect of dietary inclusion of yeast culture on digestion in the sheep. Anim. Prod. 50:483-489.

Chang, H. 1996. Genetic engineering to enhance microbial interference and related therapeutic applications. Nature Biotecnol. 14:423-431.

Chaudhary, L. C. and A. Srivastava. 1995. Performance of growing Murrah buffalo calves as affected by treatment with Manoxol and the presence of ciliate protozoa in the rumen. Anim. Feed Sci. Technol. 51:281-286.

Chaudhary, L. C., A. Srivastava and K. K. Singh. 1995. Rumen fermentation pattern and digestion of structural carbohydrate in buffalo (*Bubalus bubalis*) calves as affected by ciliate protozoa. Anim. Feed. Sci. Technol. 56:111-117.

Coleman, G. S. 1975. The inter relationship between rumen ciliate protozoa and bacteria. In: Digestion and Metabolism in the Ruminant (Ed. W. Mc Donald and A. C. I. Warner). The University of New England Publishing Unit, pp. 149-164.

Coleman, G. S. 1986. The distribution of carboxymethyl cellulase between fractions taken from the rumen of sheep containing no protozoa or one of five different protozoal population. J. Agric. Sci. 106:121-127.

Cook, R. A. 1976. Urease activity in the rumen of sheep and distribution of ureolytic bacteria. J. Gen. Microbiol. 92:32-49.

Cruywagen, C. W., I. Jordan and L. Venter. 1996. Effect of *Lactobacillus acidophilus* supplementation of milk replacer on pre weaning performance of calves. J. Dairy Sci. 79:483-486.

Dawson, K. A. 1989. Modification of rumen function and animal production using live microbial cultures as feed supplements. In: Proceeding of California Animal Nutrition Conference, Centre Plaza, Holiday Inn, Fresno, California, pp. 25-43.

Dawson, K. A. 1990. Designing the yeast culture of tomorrow: Mode of action of yeast culture for ruminants and non ruminants. In: Biotechnology in the Feed Industry (Ed. T. P. Lyons). Alltech Technical Publications, Nicholasville, KY.

Dawson, K. A. 1992. Current and future role of yeast culture in animal production: A review of research over the last six years. In: Biotechnology in the Feed Industry (Ed. T. P. Lyons), Alltech Technical Publications, Nicholasville, KY.

Dawson, K. A. and K. E. Newman. 1987. Fermentation in rumen simulating continuous culture receiving probiotics

supplements. J. Anim. Sci. 66 (Supp. 1) 500 (Abstr.).

Dawson, K. A., K. E. Newman and J. A. Boling. 1990. Effect of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. J. Anim. Sci. 68:3392-3398.

Demeyer, D. I. 1981. Rumen microbes and digestion of plant cell walls. Agric. Environ. 6:295-337.

Demeyer, D. I. 1992. Biotechnology and the quality of animal products in sustainable agriculture. J. Appl. Anim. Res. 1:65-80.

Demeyer, D. I. and C. J. Van Nevel. 1979. Effect of defaunation on the metabolism of rumen microorganisms. Br. J. Nutr. 42:515-524.

Demeyer, D. I., C. J. Van Nevel and G. Van de Voorde. 1982. The effect of defaunation on the growth of lambs fed three urea containing diets. Arch. Tierernaehr. Tierz. 32:595-603.

Dominguez Bello, M. G. and A. Escobar. 1997. Rumen manipulation for the improved utilization of tropical forages. Anim. Feed Sci. Technol. 69:91-102.

Dutta, T. K., S. S. Kundu and D. D. Sharma. 2001. Potential of probiotic supplementation on *in vitro* rumen fermentation and ^{35}S incorporation in microbial protein. Indian J. Anim. Nutr. 18: 227-234.

Eadie, J. M. and J. C. Gill. 1971. The effect of the absence of rumen ciliate protozoa on growing lambs fed on roughage and concentrate diet. Br. J. Nutr. 26:155-167.

Edwards, I. E., T. Mutsvanga, J. H. Topps and G. F. M. Patterson. 1990. The effect of supplemental yeast culture (YEA-SACC) on patterns of rumen fermentation and growth performance of intensively fed bulls. Anim. Prod. Abstr. 579A

Egan, A. R., F. Frederick and R. M. Dixon. 1986. Improving efficiency of use supplements by manipulation of management procedures. In: Ruminal Feeding System Utilizing Fibrous Agricultural Residues (Ed. R. M. Dixon). International Development Program of Australian Universities and Colleges (IDP), Canberra, Australia, pp. 69-81.

El Hassan, S. M., C. J. Newbold and R. J. Wallace. 1993. The effect of yeast culture on rumen fermentation:growth of the yeast in the rumen and the requirement of viable yeast cells. Anim. Prod. 56:463 (Abstr.).

El Hassan, S. M., C. J. Newbold, I. E. Edwards, J. H. Topps and R. J. Wallace. 1996. Effect of yeast culture on rumen fermentation, microbial protein flow from the rumen and live weight gain in bulls given high cereal diets. Anim. Sci. 62:43-48.

Erasmus, L. J., P. M. Botha and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Erdman, R. A. and B. K. Sharma. 1989. Effect of yeast culture and sodium bicarbonate on milk yield and composition in dairy cows. J. Dairy Sci. 72:1929-1932.

Fallon, R. J. and F. J. Harte. 1987. The effect of yeast culture inclusion in the concentrate diet on calf performances. J. Dairy Sci. 70 (Supp. 1) 126 (Abstr.).

Faichney, G. J. and D. A. Griffiths. 1978. Behaviour of solute and particle markers in the stomach of sheep given a concentrate diet. Br. J. Nutr. 40:71-82.

Flint, H. J. 1994. Molecular genetics of obligate anaerobes from the rumen. FEMS Microbiol. Lett. 121: 259-267.

Fondevila, M., C. J. Newbold, P. M. Hotten and E. R. Orskov. 1990. A note on the effect of *Aspergillus oryzae* fermentation extract on the rumen fermentation of sheep given straw. Anim. Prod. 51:422-425.

Fonty, G., J. P. Jouany, J. Senaud, Ph. Gouet and J. Grain. 1984. The evolution of microflora, microfauna and digestion in the rumen of lambs from birth to four months. Can. J. Anim. Sci. Supp.:165-169.

Forano, E. 1991. Recent progress in genetic manipulation of rumen microbes. In: Ruminant Microbial Metabolism and Ruminant Digestion (Ed. J. P. Jouany). INRA Editions, France, pp. 89-103.

Forsberg, C. W., K. J. Cheng, P. J. Krell and J. P. Phillips. 1993. Establishment of rumen microbial gene pools and their manipulation to benefit digestion by domestic animals. In: Proceeding of the Seventh World Conference on Animal Production, Edmonton, Alberta, Vol. 1. pp. 281-316.

Frumholtz, P. P., C. J. Newbold and R. J. Wallace. 1989. Influence of *Aspergillus orygae* extract on fermentation of basal ration in the rumen simulation technique (Rusitec). J. Agric. Sci. 113:169-172.

Fuller, R. 1989. A Review: Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Gilliland, F. L., B. B. Bruce, L. J. Bush and T. E. Staley. 1980. Comparison of two strain of *Lactobacillus acidophillus* as dietary adjuncts for young calves. J. Dairy Sci. 63:964-974.

Girard, D., C. R. Jones and K. A. Dowson. 1993. Lactic acid utilization in rumen simulating cultures receiving a yeast culture supplement. J. Anim. Sci. 71 (Supp. 1) 288 (Abstr.).

Gunter, K. D. 1989. European association of Animal production, 40 th Meeting, Dublin, Vol. 1, pp. 372.

Hammond, A. D., M. J. Allison, M. J. Williams, G. M. Prine and D. B. Bates. 1989. Prevention of leucaena toxicosis of cattle in Florida by ruminal inoculation with 3-hydroxy-4(1H)-pyridone degrading bacteria. Am. J. Vet. Res. 50:2176-2180.

Harris, B. and R. Lobo. 1988. Feeding yeast culture to lactating dairy cows. J. Dairy Sci. 71 (Supp. 1) 276 (Abstr.).

Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon and K. B. Barker. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967-2975.

Hegarty, R. S., J. V. Nolan and R. A. Leng. 1991. Sulphur availability and microbial fermentation in fauna free rumen. Arch. Anim. Nutr. 41:725-736.

Higginbotham, G. E. and D. L. Bath. 1993. Evaluation of lactobacillus fermentation cultures in calf feeding system. J. Dairy Sci. 76:615-620.

Hristova, A. N., M. Ivan, L. M. Rode and T. A. McAllister. 2001. Fermentation characteristics and rumen ciliate protozoal populations in cattle fed medium or high barley based diet. J. Anim. Sci. 79:515-524.

Huber, J. T., J. Sullivan, B. Taylor, A. Burgos and S. Gramer. 1990. In: Biotechnology in the Feed Industry (Ed. T. P. Lyons), Alltech Technical Publications, Nicholasville, KY. pp. 35-38.

Imai, S. 1998. Phylogenetic taxonomy of rumen ciliate protozoa based on their morphology and distribution. J. Appl. Anim. Res. 13:17-36.

Itabashi, H., T. Kobayashi, R. Morii and S. Okamoto. 1982. Effect of ciliate protozoa on the concentration of rumen and duodenal

volatile fatty acid and plasma glucose and insulin after feeding. Bull. Nat. Inst. Anim. Ind. (Japan), 39:21-32.

Itabashi, H., T. Koyabashi and Matsumoto, M. 1984. The effects of rumen ciliate protozoa on energy metabolism and some constituents in rumen fluid and blood plasma of goats. Jap. J. Zootech. Sci. 55:248-255.

Ivan, M., D. des. Dayrell, S. Mahadevan and M. Hiderolou. 1992. Effect of bentonite on wool growth and nitrogen metabolism in fauna free and faunated sheep. J. Anim. Sci. 70:3192-3202.

Jansen, D. H. 1975. Ecology of plants in the tropics. In: Studies in Biology (Ed. E. Arnold). No. 58, Uk, pp. 66.

Jenney, B. F., H. J. Vandijk and J. A. Collins. 1991. Performance of faecal flora of calves fed *Lactobacillus subtilis* concentrate. J. Dairy Sci. 74:1968-1973.

Jonecova, Z., R. Nemcova and V. Kmet. 1992. Effect of yeast cells and lactobacilli on rumen fermentation in sheep. Zivocsna Vypeoba. 37:771-776.

Jones, R. J. and R. G. Megarrity. 1983. Comparative toxicity responses of goat fed on *Leucaena leucocephala* in Australian and Hawaii (USA). Aust. J. Agric. Res. 34:781-790.

Jones, R. J. and R. G. Megarrity. 1986. Sucessful transfer of dihydroxypyridine-degading bacteria from Hawaiian (USA) goats to Australain ruminants to overcome the toxicity of leucaena. Aust. Vet. J. 63:259-262.

Jouany, J. P. 1978. Contribution a l'etude des protozoaries cilies de rumen: leur dynamique, leur role dans la digestion et leur interet pour le ruminant. These de Doctorat, Universite de Clermont II, no d'Ordre 256, Vol. 2, pp. 195.

Jouany, J. P., B. Zainab, J. Senaud, C. A. Groliere, J. Grain and P. Thivend. 1981. Role of the rumen ciliate protozoa *Polyplastron multivesiculatum*, *Entodinium spp.* and *Isotricha prostoma* in the digestion of mixed diet in sheep. Reprod. Nutr. Dev. 21:871-884.

Jouany, J. P., D. I. Demeyer and J. Grain. 1988. Effect of defaunating the rumen. Anim. Feed Sci. Technol. 21:229-265.

Jouany, J. P., B. Michalet-Dorea and M. Doreau. 2000. Manipulation of rumen ecosystem to support high performance beef cattle. Asian-Aus. J. Anim. Sci. 13:96-114.

Kamra, D. N., N. N. Pathak and R. Singh. 1987. Role of ciliate protozoa on digestion in ruminants. Livestock Adviser. 12:9-12.

Kamra, D. N., N. Agarwal, L. C. Chaudhary, A. Sahoo and N. N. Pathak. 1997. Effect of feeding probiotic (Lactic acid producing bacteria) on the growth of coliform bacteria in the grastointestinal tract of crossbred calves. In: Proceeding of VIII Animal Nutrition Research Worker's Conference, Chennai, India, pp. 130-131.

Kayouli, C., D. I. Demeyer and R. Dendooven. 1983/84. Effect of defaunation on straw digestion *in sacco* and on particle retention in the rumen. Anim. Feed Sci. Technol. 10:165-172.

Kayouli, C., C. J. Van Nevel and D. I. Demeyer. 1983. Effect de la defaunation du rumen sur la de-gradibilite des proteins de saja mesure in sacco. In: IVth Interantional Symposium on Protein Metabolism and Nutrition, Clermont-Ferrand, France. Vol. II. Lee Colloques de l'INRA16, INRA edn., pp. 251-254.

Kim, D. Y., B. A. Kent., M. R. Figueroa, D. P. Dawson, C. E. Battalas, M. J. Aramel and J. L. Walters. 1992. Effect of added yeast culture with or without *Aspergillus oryzae* in rumen fermentation and nutrient digestibility when fed to non lactating Holstein cows. J. Dairy Sci. 75 (Supp. 1) 206 (Abstr.).

Kopecny, J., J. Simunek, G. I. Kalacnjuk, O. G. Savka, M. G. Gerasimiv and B. Leskovic. 1989. Testing the probiotic effect of selected rumen bacteria. Zivocisna Vyroba. 34:205-214.

Kreuzer, H. 1986. Methods and application of defaunation in the growing ruminant. J. Vet. Med. Sci. 33:723-745.

Kumar, U., V. K. Sareen and S. Singh. 1994. Effect of *Saccharomyces cervisiae* yeast culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet. Anim. Prod. 59:209-215.

Kurihara, Y., T. Takechi and F. Shibata. 1978. Relationship between bacteria and ciliate protozoa in the rumen of sheep fed purified diet. J. Agric. Sci. 90:373-381.

Lee, S. S., J. K. Ha and K. J. Cheng. 2000. Influence of an anaerobic fungal culture administration on in ruminal fermentation and nutrient digestion. Anim. Feed Sci. Technol. 88:201-217.

Leng, R. A. 1982. Dynamics of protozoa in the rumen of sheep. Br. J. Nutr. 48:399-415.

Lindsay, J. R. and J. P. Hogan. 1972. Digestion of two legumes and rumen bacterial growth in defaunated sheep. Aust. J. Agric. Res. 23:321-330.

MacRae, J. C. and G. E. Lobley. 1982. Some factors which influence thermal energy loss during the metabolism of ruminants. Livest. Prod. Sci. 9:477-479.

Malcolm, K. J. and H. E. Kiesling. 1990. Effects of whole cottonseed and live yeast culture on ruminal fermentation and fluid passage rate in steers. J. Anim. Sci. 68:1965-1970.

Martin, S. A. and D. J. Nisbet. 1992. Effect of direct fed microbials on rumen microbial fermentation. J. Dairy Sci. 75: 1736-1744.

Mathieu, F., J. P. Jouany, J. Senaud, J. Bohatier, G. Bertin and M. Mercier. 1996. The effect of *Saccharomyces cervisiae* and *Aspergillus oryzae* on fermentation in the rumen of faunated and defaunated sheep: protozoal and probiotic interaction. Reprod. Nutr. Dev. 36:271-287.

Mathison, G. W. and L. P. Milligan. 1971. Nitrogen metabolism in sheep. Br. J. Nutr. 25:351-366.

Maurya, M. S., R., Singh, N. N. Pathak and D. N. Kamra. 1993. Effect of feeding live yeast (*Saccharomyces cervisiae*) on nutrient digestibility in goats. In: Proceeding of Sixth Animal nutrition Research Workers Conference, Bhubaneswar, India, pp. 142.

McSweeney, C. S., B. P. Dalrymple, K. S. Gobius, P. M. Kennedy, D. O. Krause, R. I. Mackie and G. P. Xue. 1999. The application of rumen biotechnology to improve the nutritive value of fibrous feed stuffs: pre and post ingestion. Livest. Prod. Sci. 59:265-283.

Mendoza, M. G. D., R. A. Britton and R. A. Stock. 1993. Influence of ruminal protozoa on site and extent of starch digestion and ruminal fermentation. J. Anim. Sci. 71:1572-1578.

Miles, R. D. and S. M. Bootwalla. 1991. Direct fed microbials in animal production. In: Direct Fed Microbials in Animal Production. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Minson, D. J. 1980. Nutritional differences between tropical and temperate pasture. In: Grazing Animals (Ed. F. H. W. Marely). Elsevier, Amesterdam, pp. 143-157.

Moloney, A. P. and M. J. Drennan. 1994. The influence of the basal diet on the effects of yeast culture on ruminal fermentation and digestibility in steers. Anim. Feed Sci. Technol. 50:55-73.

Mutsvangwa, T., I. E. Edwards, J. H. Topps and G. F. M. Paterson.

1992. The effect of dietary inclusion of yeast culture (Yea-Sac) on pattern of rumen fermentation, food intake and growth of intensively fed bulls. Anim. Prod. 55:35-40.

Nagaraja, T. G., G. Towne and A. A. Beharka. 1992. Moderation of ruminal fermentation by ciliate protozoa in cattle fed a high grain diet. Appl. Env. Microbiol. 58:2410-2414.

Newbold, C. J. and D. G. Chamberlain. 1988. Lipid as rumen defaunating agent. Pproc. Nutr. Soc. UK. 47:154A

Newbold, C. J., P. E. V. Williams, A. Walker and R. J. Wallace. 1990. The effect of yeast culture on yeast numbers and fermentation in the rumen ecosystem. Proc. Nutr. Soc. UK, 49:47A.

Newbold, C. J., R. J. Wallace and F. M. McIntosh. 1993. The stimulation of rumen bacteria by *Saccharomyces cervisiae* is dependent on the respiratory activity of the years. J. Anim. Sci. 71 (Supp. 1) 280 (Abstr.).

Newbold, C. J., R. J. Wallace and F. M. McIntosh. 1996. Mode of action of the yeast, *Saccharomyces cervisiae* as a feed additives for ruminants. Br. J. Nutr. 76:249-261.

Newbold, C. J., F. M. McIntosh and R. J. Wallace. 1998. Changes in the microbial population of a rumen simulating fermenter in response to yeast culture. Can. J. Anim. Sci. 78:241-244.

Newman, K. E. and K. A. Dawson. 1987. Associative effects of probiotics and diet on ruminal fermentation. In: 19th Biennial Conference on Rumen Function, Chicago.

Nhan, N. T. H., N. V. Hon, M. T. Ngu, N. T. Von, T. R. Preston and R. A. Leng. 2001. Practical application of defaunation of cattle on farms in Vietnam: Response of young cattle fed rice straw and grass to a single drench of ground nut oil. Aisan-Aus. J. Anim. Sci. 14:485-490.

Nisbet, D. J. and S. A. Martin. 1990. Effect of Yea-sacc1026 on lactate utilization by the ruminal bacterium Selenomonas ruminantium. In: Biotechnology in feed industry (Ed. T. P. Lyons). Alltech Technical Publications, Nicholasville, Kentucky, pp. 563-567.

Oellermann, S. O., M. J. Arambel, B. A. Kent and J. L. Walters. 1990. Effect of graded amounts of *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility in cattle. J. Dairy Sci. 73:2412-2416.

Onodera, R., Y. Nakagawa and M. Kandatsu. 1977. Ureolytic activity of the washed suspension of rumen ciliate protozoa. Agric. Biol. Chem. 41:2177-2182.

Onodera, R., N. Yamasaki and K. Murakami. 1988. Effect of inhabitation by ciliate protozoa on the digestion of fibrous materials *in vivo* in the rumen of goats and *in vitro* rumen microbial ecosystem. Agric. Biol. Chem. 52:2635-2637.

Orpin, C. G. and Letcher, A. J. 1983/84. Effect of absence of ciliate protozoa on rumen fluid volume, flow rate and microbial population in sheep. Anim. Feed Sci. Technol. 10:145-153.

Osman, H., A. R. Abou Akkada and K. A. Agabawi. 1970. Influence of rumen ciliate protozoa on conversion of food and growth rate in early weaned Zebu calves. Anim. Prod. 12:267-271.

Owens, N. F. and R. Zinn. 1988. Protein metabolism of ruminant animals. In: The Ruminant Animal Digestive Physiology and Nutrition (Ed. D. C. Chruch), Prentice-Hall, Englewood Cliffs, NJ, pp. 227-249.

Panda, A. K. 1994. Effect of yeast culture supplementation on performance and rumen fermentation in crossbred calves. M. V. Sc Thesis. Indian Veterinary Research Institute (Deemed University), Izatnagar, India.

Panda, A. K., R. Singh and N. N. Pathak. 1995. Effect of dietary inclusion of *Saccharomyces cervisiae* on growth performance of crossbred calves. J. Appl. Anim. Res. 7:195-200.

Pandey, Poonam. and I. S. Agarwal. 2001. Nutrient utilization and growth response in crossbred calves fed antibiotic and probiotic supplement diet. Indian J. Anim. Nutr. 18:15-18.

Parker, R. B. 1974. Probiotics in other half of the antibiotics story. Animal Nutrition and Health (December) :4-8.

Plata, F., G. D. Mendoza, J. R. Barcena-Gama and S. Gonzalez. 1994. Effect of yeast culture (*Saccharomyces cervisiae*) on neutral detergent fibre digestion in steers fed oat straw based diets. Anim. Feed Sci. Technol. 49:203-210.

Prahalada, H. K., D. N. Kamra and N. N. Pathak. 2001. Effect of feeding *Saccharomyces cervisiae* and *Lactobacillus acidophilus* on nutrient utilization and performance of crossbred cattle calves. International J. Anim. Sci. 16:103-107.

Prins, R. A. and W. Van Hoven. 1977. Carbohydrate fermentation by the rumen cilia *Isotricha prostoma*. Protistologica. 13:549-556.

Punia, B. S., J. Leibholtz and G. J. Faichney. 1987. The role of rumen protozoa in the utilization of paspalum hay by cattle. Br. J. Nutr. 57:395-406.

Putnam, D. E., C. G. Schwab, M. T. Socha, N. L. Whitehouse, N. A. Kierstead and B. D. Garthwaite. 1997. Effect of yeast culture in the diet of early lactation dairy cows on ruminal fermentation and passage of nitrogen fraction and amino acid to small intestine. J. Dairy Sci. 80:374-384.

Quionez, J. A., L. J. Bush, T. Nalsen and G. D. Adam. 1988. Effect of yeast culture on intake and production of dairy cows fed high wheat ration. J. Dairy Sci. 71 (Supp. 1) 275 (Abstr.).

Ramprasad, J. and G. V. Raghavan. 1981. Note on the growth rate and body composition of faunated and defaunated lambs. Indian J. Anim. Sci. 51:570-572.

Rowe, J. B., A. Davies and A. W. J. Broome. 1985. Quantitative effect of defaunation on rumen fermentation and digestion in sheep. Br. J. Nutr. 54:105-119.

Saha, S. K., S. Senani, M. K. Padhi, B. R. Shome, Rajeswari. Shome and S. P. S. Ahlawal. 1999. Microbial manipulation of rumen fermentation using *Saccharomyces cervisiae* as probiotics. Current Sciences. 77:696-697.

Santra, A., Kamra, D. N. and Pathak, N. N. 1994a. Effect of defaunation on nutrient digestibilty and growth of Murrah buffalo (*Bubalus bubalis*) calves. International J. Anim. Sci. 9:185-187.

Santra, A., D. N. Kamra, N. N. Pathak and M. Y. Khan. 1994b. Effect of protozoa on the loss of energy in murrah buffalo (*Bubalus bubalis*) calves. Buffalo J. 3:249-253.

Santra, A., D. N. Kamra and N. N. Pathak. 1996. Influence of ciliate protozoa on biochemical changes and hydrolytic enzymes in the rumen of Murrah buffalo (*Bubalus bubalis*). Buffalo J. 1:95-100.

Santra. A. and R. C. Jakhmola. 1998. Effect of defaunation on animal productivity. J. Appl. Anim. Res. 14:103-116.

Santra, A. and S. A. Karim. 1999. Efficacy of sodium laurel sulfate as defaunating agent in sheep and goats. International J. Anim. Sci. 14:167-171.

Santra, A. and S. A. Karim. 2000. Growth performance of faunated and defaunated Malpura weaner lambs. Anim. Feed Sci. Technol. 86:251-260.

Santra., A and S. A. Karim. 2002a. Influence of ciliate protozoa on biochemical changes and hydrolytic enzyme profile in the rumen ecosystem. J. Appl. Microbiol. 92:801-811.

Santra., A and S. A. Karim. 2002b. Nutrient utilization and growth performance of defaunated and faunated lambs maintained on complete diets containing varying proportion of roughage and concentrate. Anim. Feed Sci. Technol. (In press).

Schwab, C. G., J. J. Moore, P. M. Hoyt and J. H. Prentice. 1980. Performance and faecal flora of calves fed a nonviable *Lactobacillus bulgaricus* fermentation product. J. Dairy Sci. 63:1412-1423.

Sen. A. R., S. A. Karim and A. Santra. 2000. Effect of defaunation on carcass and meat characteristics of finisher lambs. Indian J. Anim. Sci. 70:659-661.

Sharma, D. D. and R. Malik. 1992. Probiotic supplementation in animal feed. In: Proceeding of National Symposium on Role of Amino Acid and Feed Supplementation in Animal Feeds, Trivendum, India.

Singh, R., L. C. Chaudhary, D. N. Kamra and N. N. Pathak. 1998. Effect of dietary supplementation with yeast cell suspension (*Saccharomyces cervisiae*) on nutrient utilization and growth response in crossbred calves. Asian-Aust. J. Anim. Sci. 11:268-271.

Skrivonova, V. and L. Machanova. 1990. The influence of *Lactobacillus acidophilus* on differently developed ruminant stomach of bovine and buffalo calves. Indian J. Anim. Sci. 41:922-924.

Smet, De. S., D. I. Demeyer and C. J. Van Nevel. 1992. Effect of defaunation and hay:concentrate ratio on fermentation, fibre digestion and passage in the rumen of sheep. Anim. Feed Sci. Technol. 37:333-344.

Smith, C.J. and R. B. Hespell. 1983. Prospects for the development and use of recombinant deoxyribonucleic acid techniques with ruminal bacteria. J. Dairy Sci. 66:1536-1546.

Stewart, C. S., G. Fonty and Ph. Gouet. 1988. The establishment of rumen microbial communities. Anim. Feed Sci. Technol. 21:69-97.

Ushida, K. and J. P. Jouany. 1985. Effect of protozoa on rumen protein degradation in sheep. Reprod. Nutr. Dev. 25:1075-1081.

Ushida, K. and J. P. Jouany. 1990. Effect of defaunation on fibre digestion in sheep given two isonitrogenous diets. Anim. Feed Sci. Technol. 29:153-158.

Ushida, K., J. P. Jouany and P. Thivend. 1986a. Role of rumen protozoa on nitrogen digestion in sheep given two isonitrogenous diets. Br. J. Nutr. 56:407-419.

Ushida, K., A. Miyazaki and R. Kawashima. 1986b. Effect of defaunation on ruminal gas and VFA production *in vitro*. Jap. J. Zootech Sci. 57:71-77.

Van Hoven, W. and R. A. Prins. 1977. Carbohydrate fermentation by the rumen ciliate *Dasytricha ruminantium*. Protistologiaca. 13:599-606.

Veira, D. M., M. Ivan and P. Y. Jui. 1983. Rumen ciliate protozoa: effects on digestion in the stomach of sheep. J. Dairy Sci. 66:1015-1022.

Voronin, E. S., L-Ya. Stavtseva and T. N. Gryazneva. 1990. Prevention and treatment of diarrhoea in newborn calves. Veterinariya Moskkva. 3:35-37.

Wallace, R. J. 1994. Rumen microbiology, biotechnology and ruminant nutrition: prospects and problems. J. Anim. Sci. 72:2992-3003.

Wallace, R. J. 1996. The mode of action of yeast culture in modifying rumen fermentation. In: Biotechnology in the Feed Industry (Ed. T. P. Lyons). Alltech Technical Publications, Nicholasville, Kentucky, pp. 217-231.

Wallace, R. J. and C. J. Newbold. 1992. Probiotics for Ruminants. In: Probiotics:The Scientific Basis (Ed. R. Fuller). Chapman and Hall, London. pp. 317.

Wallace, R. J. and C. J. Newbold. 1993. Rumen fermentation and its manipulation: The development of yeast culture as feed additives. In: Biotechnology in the Feed industry (Ed. R. Fuller). Chapman and Hall, London. pp. 173.

Wallace, R. J., J. W. Czerkawski and G. Breckenridge. 1981. Effect of monensin on the fermentation of basal ration in rumen simulation technique (Rusitec). Br. J. Nutr. 46:131-148.

Walli, T. K. 1994. Role of yeast culture in rumen ecosystem and animal performance. International J. Anim. Sci. 9:117-121.

Weimer, Paul J. 1998. Manipulating ruminal fermentation: A microbial ecological perspective. J. Anim. Sci. 76:3114-3122.

Widmeier, R. D., M. J. Arambel and J. A. Walter. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy. Sci. 70:2063-2068.

Williams, A. G. and G. S. Coleman. 1988. The rumen protozoa. In: The Rumen Microbial Ecosystem (Ed. P. N. Hobson). London, Elsevier Applied Science, pp. 77-128.

Williams, A. G. and G. S. Coleman. 1992. The rumen protozoa. New York, Springer-Verlag.

Williams, P. E. V. and C. J. Newbold. 1990. Rumen probiosis: The effect of novel microorganisms on ruminal fermentation and ruminant productivity. In: Recent advances in Animal Nutrition (Ed. W. Haresign and D. J. A. Cole). Butterworths, London, England.

Williams, P. E. V., C. A. G. Tair, G. M. Innes and J. M. Newbold. 1990. Effects of inclusion of yeast culture (*Saccharomyces cervisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Williams, P. E. V., C. A. G. Tait, G. M. Innes and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cervisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Williams, A. G. and E. Susan. Withers. 1991. Effect of ciliate protozoa on the activity of poysaccharide-degrading enzymes and fibre breakdown in the rumen ecosystem. J. Appl. Bacteriol. 70:144-145.

Windham, W. R. and D. E. Akin. 1984. Rumen fungi and forage fibre degradation. Appl. Environ. Microbiol. 48:473-476.

Yoon, I. K. and M. D. Stem. 1996. Effect of *Saccharomyces cervisiae* and *Aspergillus oryzae* culture on ruminal fermentation in dairy cows. J. Dairy Sci. 79:411-417.