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Approaches and ways for rumen manipulation for its beneficial effects in ruminant production systems

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INTRODUCTION

United Nations figures had projected global human population to be 9.5 billion by 2050 (UN, 2015). Livestock products provide one sixth of energy and more than one-third of the protein portion of human food on global basis.The ruminants (cattle, buffaloes, sheep and goats) are predominant for supply of human food.The rumen microbial ecosystem is efficient anaerobic fermentation system that have advantages over non-ruminants animals such as digestion of fibrous feeds efficiently, utilisation of non-protein nitrogen sources like urea, and detoxification of many toxic ingredients present in feeds of plant origin.

What is rumen manipulation?

Manipulation of rumen fermentation is an alteration (maximization and/or minimization) of fermentation process to improve animal productivity.

Why do we need rumen manipulation?

Anaerobic fermentation of feeds in the rumen is beneficial for the host animal. The co-existence of animal and its microbial eco-system has resulted in stable and the most favoured natural selection of microbes to perform the fermentation process optimally. 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. (Santra and Karim, 2001)

Objectives of rumen manipulation

1. 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 hemicelluloses.
2. Increase microbial protein synthesis: Major portions 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.
3. 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 amino acids in the rumen should be discouraged.
4. 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.
5. 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.
6. Shifting acetate to propionate production: In fattening beef/lambs the production of propionate in the rumen at the expense of acetate may be helpful.
7. Metabolism of plant toxins: Rumen fermentation can be manipulated for efficient utilization of feeds which contain anti nutritional factors viz. tannin, saponin, mimosine, etc.

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 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).

I. Genetic Rumen Manipulation

The potential of application of molecular techniques in achieving the goals of rumen manipulation are enormous (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 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 digestibility of feedstuffs and to improve animal production.

CONSTRAINTS OF GENETIC RUMEN MANIPULATION

1. The physiological conditions in the rumen are not favourable for most of the non-rumen microbes.
2. The genetically engineered microbes mostly have low competitive ability to survive in a mixed culture(genetically engineered *Prevotellaruminicola*has half-life 30 min)
3. 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.

II. Non Genetic Rumen Manipulation

1) Ionophore compounds
2) Organic acid
3) Probiotics
4) Defaunation
5) Plant secondary metabolites
6) Halogenated compounds
7) Forage to concentrate ratio

1. Ionophore compounds

Ionophores are highly lipophilic substances capable of interacting stoichiometrically with metal ions, thereby serving as a carrier by which these ions can be transported across a bimolecular lipid membrane(Ovochinniko, 1979). Ionophores are toxic to many bacteria, protozoa, fungi and higher organisms and thus fit the classical definition of antibiotics (Pressman, 1976).

E.g. monensin, lasalocid, salinomycin

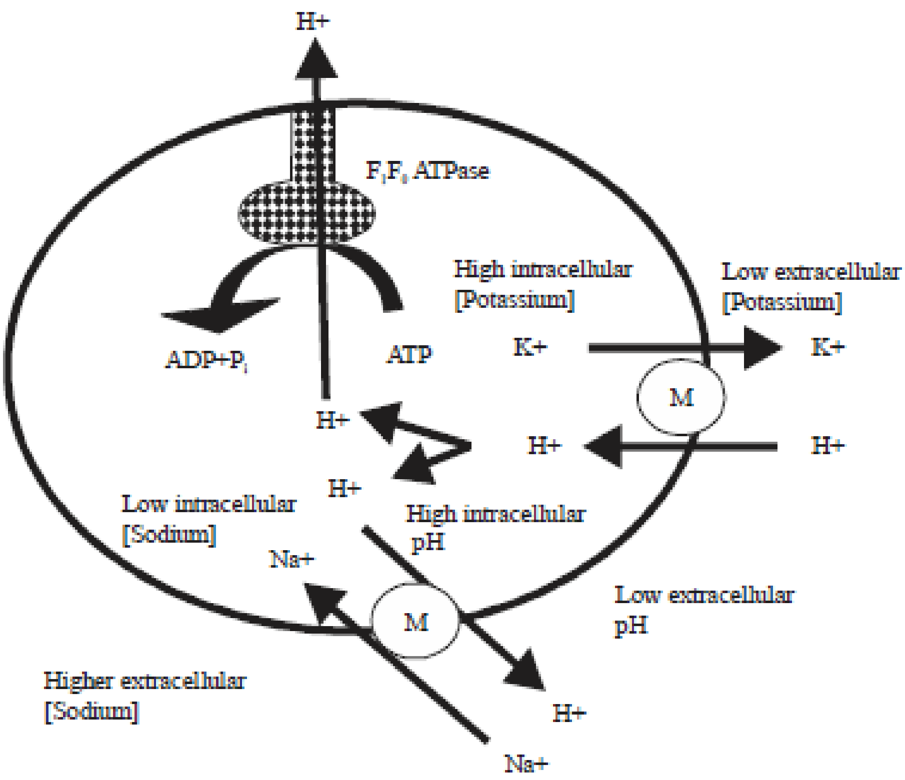

Fig. 1 Metabolic effects of ionophores on the rumen fermentation

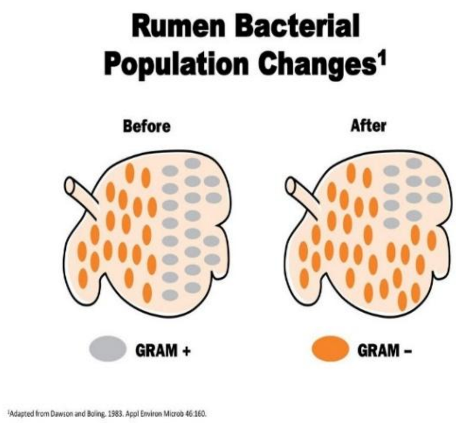

Fig. 2 Effects of ionophores on the Bacterial population

2. Organic acid/ acidifiers

Organic acids are compounds with specific antimicrobial activity and are short-chain acids (C1–C7) and the pKa value is from 3 to 5.Organic acids in the rumen aid in preventing the drop of ruminal pH and reducingmethanogenesis (Castillo *et al.,* 2004). Thus, in the rumen, these acids can stimulate ruminal growth of prominent bacteria and consequently change favorably ruminal fermentation, improving ruminant performance (Martin, 1998).The most common organic acids are the carboxylic acids, commonly found in biological tissues. Dicarboxylic acids such as malic acid are found naturally in forages at different levels. Dicarboxylic acids like aspartate, fumarate and especially malate stimulate lactate utilization in the rumen, moderating pH.

Mode of action for the organic acids is different than ionophores. Organic acids stimulate rather than inhibit some specific ruminal bacterial populations.

3. 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 has 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 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 ofprobiotic. The FDA defines DFM as a source of live(viable) naturally occurring microorganisms and thisincludes bacteria and yeast (Miles and Bootwalla, 1991). The commonly used probiotics for animal feeding arebroadly divided into two categories i.e., bacterial origin and yeast origin.

Improving intestinal health by stimulating the development of a healthy microbial ecosystem (Uyeno*et al.,* 2015). Increase digestive capacity and their bio-availability (Oyetayo and Oyetayo, 2005). Preventing enteric pathogens from colonizing the intestine (Casas and Dobrogosz, 2000).Restore the gut microflora, lower pH, and improve mucosal immunity and nutrient absorption (Uyeno*et al*., 2015).

i. Microorganisms used as probiotics

The major and frequently studied bacterial microorganisms used as probiotics in ruminant production include those derived from *Lactobacillus, Streptococcus, Entrococcus, Bacillus, Bifidobacterium* species, *Propionibacterium, Megasphaeraelsdenii* and *Prevotellabryantii* (Seo*et al.,* 2010). Yeasts and fungal probiotics such as *Saccharomyces* and *Asperillus*, respectively have given better results in adult rumen(Seo*et al*., 2010).

ii. The utilization of probiotics in farm animals may contribute in the following aspects:

1. Growth promotion,

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

4. 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 forruminant, still they have significant role to play in the rumen metabolism specially to stabilize the rumen pH (Santra and Karim, 2002). 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).

A. Methods of defaunation

i. 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.During this time the newborn animals gets contaminated with the native bacterial population but do not get rumen ciliate protozoa (Fonty*et al*., 1984).

ii. 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 sometime mortality also reported (Jouany*et al*., 1988).

iii. 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 falls 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 eliminates the ciliate protozoa completely and the animal becomes 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 from various secondary complications. The drenching of vegetable oils eliminate ciliate protozoa and hence can be used as a defaunating agent. (Nhan*et al*., 2001).

5. Plant secondary metabolites

Herbal plants produce secondary metabolites, which are biologically active by providing protection against attack from predators(Iason, 2005). These metabolites are referred to as phytochemical feed additives, phytobiotics, or herbal and botanical compounds(Kumar *et al*., 2014).

A) Essential oils

Essential oils, also known as volatile oils, are aromatic components found in many edible, medicinal, and herbal plants. e.g. Cinnamon, eucalyptus, coriander(Greathead, 2003).EOs and their components are hydrophobic cause's disruption of the membrane integrity and the extensive loss of cell contents leads to cell death . Essential oils modify cell permeability in microbes and their toxicity to Gram-negative microbes, and thus can be promising natural alternatives to antibiotics and ionosphores for manipulating ruminal fermentation and and improving feed efficiency and nutrient utilization by ruminants. (Calsamiglia*et al*., 2007) It was observed that better final weight, average daily gain, feed efficiency and hot carcass weight for bulls supplemented with 3 grams to animal day-1 of essential oils (cashew and castor oils) than for bulls fed control diet. (Valero *et al.,* 2014) Supplementing the essential oils @ 2 g/day increased the concentration of conjugated linoleic acid (CLA), a health promoting fatty acid, in milk fat (Benchaar*et al*., 2007). Essential oils also shows anti methanogenic properties and cashew nut shell liquid when added to batch cultures at a the rate of 200 µg/mL of incubated volume.

B) Tannins

Tannins are a complex water-soluble group of polyphenolic compounds found in a wide range of plant species commonly consumed by ruminants. They have the ability to form complexes with proteins. They can be used to prevent protein degradation and to form protein by-pass in the rumen, and it will increase protein supply and utilization in the small intestine thereby, improving ruminant performance.

Table No.1 Effect of tannin supplementation on rumen fermentation and animal performance

Type	Dosage	Host	Reports	Reference

Condensed tannin (Quebracho,)	0, 1% and 2% CT/kg DMI	Steers	Increased Average Daily Weight Gain Reduced rate of *in vitro* gas production was also reported. The decrease in ruminal methane production at increasing CT supplementation.	Min *et al.* (2016)
Condensed tannin (Silvateam, Ontario)	0, 0.2%, 0.4%, and 0.6% of dry matter basis	Holstein steers	ADG increased by 6.5%, Feed conversion efficiency increased by 5.5% and dietary NE by 3.2% at levels of 0.4 % and above	Rivera-Mendez *et al.* (2017)

C. Saponins

Saponins are naturally occurring surface-active glycosides produced primarily by plants and the name was derived from their ability to form stable soap-like foams in aqueous solutions. (Das *et al.*, 2012)Saponin reduce rumen protozoa population(Goel *et al.*,2008). It increases nitrogen utilization and directly leads to improved ruminant performance.

Table no.2 Effect of saponin supplementation on rumen fermentation and animal performance

Type	Dosage	Host	Reports	Reference
Quillajasaponaria (extract)	60 g/head per day	*In vivo* (cattle)	Decreased protozoa count by 61% when Compared with the control.	Goel *et al.* (2008)
Triterpenoid Saponi Alfalfa	2% and 4% of DMI	*In vivo* (Sheep)	Reduced protozoa population in the rumen by 34% and 66% at 2% and 4% level of inclusion Rates, respectively.	Lu *et al.* (1987)

6. Halogenated compounds

Halogenated methane analogues (e.g. bromochloromethane) can reduce methane production from ruminants (Morgavi*et al.*, 2010).Bromochloromethane is one of the most effective inhibitors and apparently reduces methane production by interfering with the cobamide-dependent methyl transferase step of methanogenesis (Chalupa, 1977). About 54% reduction in CH_4 output was reported in cattle when fed with bromochloromethane complexed with cyclodextrin twice daily over 8 weeks (McCrabb*et al.*, 1997).

7. Altering forage: concentrate ratio

Reducing plant fiber with starch shifts VFA synthesis from acetate toPropionate.CH4 production can be reduced to around 3% from 6.5% on mixed concentrate and forage rations as compared to ruminants fed primarily on forage(Beauchemin and McGinn, 2005). An experiment was conducted to evaluate the growth performance and cost per gain of Brahman local crossbred bull calves receiving three diets with varying concentrate to roughage ratio (C:R) of 75:25, 65:35 and 55:45 on dry matter (DM) basis. Improved growth performance and feed efficiency was observed at the ratio of 55: 45 (Rashid *et al*., 2015).

CONCLUSION

Ruminants plays predominant role for supply of food to ever increasing human population. In tropical country like India, main source of feed to ruminants is high fibrous crop residues causes low production levels which can be overcome by manipulation of rumenDifferent manipulation approaches consists of genetic and non-genetic methods like feeding of ionophores, organic acids, probiotics, defaunation, plant secondary metabolites, halogenated compounds and maintenance of forage: concentrate ratio. All these approaches aimed at inhibiting the numbers and activity of harmful/ undesirable microbes (e.g. methane producing bacteia) and increasing the numbers of benificial gut microbes so as to improve the utilisation of low quality feed and animal productivity.Plant secondary metabolites is found to be the most promising method amongst all approaches which can replace antibiotic/ionophore feeding in ruminants.

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