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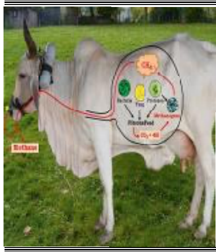

Methanogenesis: A global problem and its mitigation

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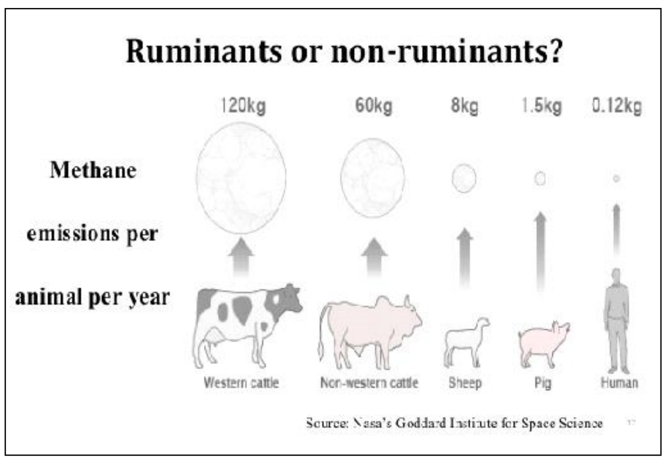
Global warming is the most critical problem of this century which is due to the uncontrolled emission of gases like CO_2, SO_2, CFC and CH_4.Methane is about 21 times more potent gas in global warming production. Most of the methane is introduces in environment by agriculture sector. In which livestock play a major role because it's produced in the ruminant in excess amount, which is directly exhaled by the animals in the environment. Large population of ruminant animals leading a huge methane production and release in atmosphere. There are many ways by which this methane production can be reduced and so lesser its contribution in global warming (Green house effect). Further it is less costly to control methanogenesis than to control its global warming effect. Some techniques of methane mitigation had positive impact on nutrient utilization and productivity of animals. This study was based on the methanogenesis and different methods of its mitigation.

Global climatic change in one of the most serious problem in front of the world since last few years. There are six major green house gases in our atmosphere *i.e.* carbon dioxide,methane, nitrous oxide, two fluorocarbons and sulphur hexafluoride. Methane is one of the most potent greenhouse gases, having 21 times greater global warming potential than the carbon dioxide (Sirohi *et al.*, 2013). Livestock are the major source of methane emittion contributing about 80 to 115 million tonnes methane per annum globally (IPCC, 2001). India has livestock wealth of 272.1 million cattle, 159.8 million buffaloes, 71.6 million sheep, 140.6 million goats and 13.1 million other ruminants which produce large amount of methane as a part of their normal digestive process (GOI, 2012).Among livestock's, methane production is greatest in ruminants, as methanogens are normal inhabitant of rumen. Space, PH and anaerobic environment of rumen provide favorable condition for multiplication and mathanogenesis by rumen microbs.

Ruminants and some other animals considered as pseudo-ruminantslike *Camelidae*, other animals like the bird Hoatzin have in addition large anaerobic fermentative chambers located at the beginning of the tract. Hydrogen (H_2) is one of the major endproducts of fermentation by protozoa, fungiand pure monocultures of some bacteria. This H_2 is utilized by the rumen microbes to produce methane (CH_4). In ruminants, feed is converted to short chain fatty acids in the rumen, which are used as a source of energy and the hydrogen generated as an intermediate, which is converted rapidly in to methane by the methanogens (Stewart *et al.*, 1997). In the rumen, formation of methane is the major way of hydrogen elimination through the following reaction:

$$CO_2 + 4\ H_2 \longrightarrow CH_4 + 2\ H_2O$$

Methane that produced in the rumen as a product of normal fermentation of feedstuffs, is exhaled into the atmosphere which contributes in global warming.



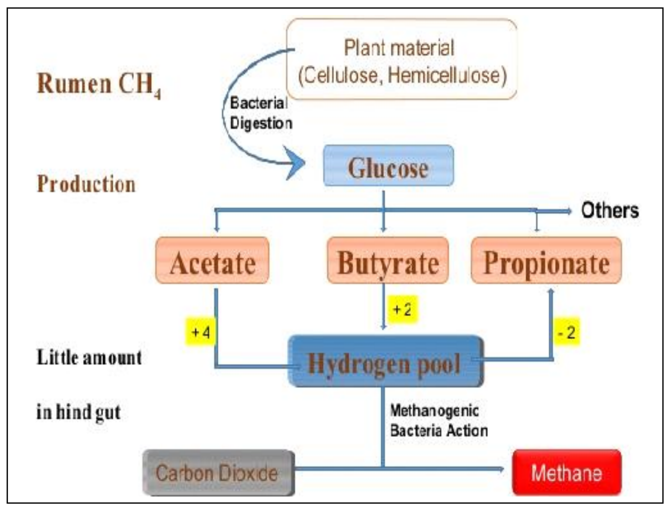
Methane production during ruminal fermentation as a result of methanogenesis by bacteria and protozoa is an unavoidable and inefficient product of rumen fermentation. CH_4 from enteric fermentation by ruminants is not only an important greenhouse gas associated with environmental problems, but it also represents a loss of feed energy intakes. 10-12 per cent of gross energy ingested is lost through methane (1g methane =13.34 kcal). Therefore, developing feeding strategies to minimize CH_4

emission is desirable in long-term mitigation of emission of greenhouse gases into the atmosphere and for short-term economic benefits.

Methanogens: Methanogens belong to the domain *Archaea* and the phylum *Euryarchaeota*. About 113 species of methanogens are recognized in the ecosystem but only few specieses sre found in rumen (Janseen and Kirs, 2008). The different genera and species of methanogens have various shapes and physiological characteristics like cocci, rods, spirilla and thermophylic and mesophylic species, motile and nonmotile cells (Woese *et al.*, 1990). Methanogens like *Methanobacterium formicicum, M. ruminantium, M. bryanti, Methanobrevibacter ruminantium, Methanosarcina barkeri, Methanomicrobium mobile and Methanoculleus olentangyi* are present in the rumen in a large number in rumen liquor depending upon the type of diet given to animals, especially the fibre content in the ration (Baker, 1999). *Methanbevibacter* spp. were initially colonized in the rumen and are only methanogen present after birth of animal (Skillman *et al.*, 2004). In the bovine rumen, *Methanobrevibacter ruminantium* are the largest group of methanogens found in lactating dairy cattle fed with totalmixed ration, followed by *Methanosphaera stadtmanae* (Whitford *et al.*, 2001). Isolation of methanogens from grazing cattle found *Methanomicrobium mobile* may be presentat 10^6 cells/ ml (Jarvis *et al.*, 2000). *Methanobacterium formicicum* was isolatedas the second most common methanogen, followed by anisolate phenotypically similar to *Methanosarcina barkeri* (Jarvis *et al.*, 2000). *Methanobrevibacter* spp. was not identified in grazing cattleal though it has been detected in cattle kept indoors andfed total mixed ration (Whitford *et al.*, 2001). *Methanobrevibacter ruminantium* is rod shaped with variable motility and is able to use hydrogen and carbon dioxide and formate as substrates for methane production whereas *Methanosarcina barkeri* is able to produce methane from hydrogen and carbon dioxide, acetate, methylamines and methanol, whereas *Methanosarcina mazeii can use the same* substrates except hydrogen and carbon dioxide.

Methanogenesis by methanogens: Methogens are strictly anaerobic in nature and grow only in environment having redox potential of -300mv (Shete and Tomar, 2010). The rumen temperature *i.e.* 39^0C, reducing medium of rumen and their pH provides suitable environment for development of microbes in rumen. Hydrogen is one of the major end products of fermentation by protozoa, fungi and pure monocultures of some bacteria, it does not accumulate in the rumen, because it is immediately used by other bacteria which are present in the mixed microbial ecosystem. The collaboration between fermenting species and H_2-utilising bacteria (e.g. methanogens) is called "interspecies hydrogen transfer".

The molar percentage of volatile fatty acids (VFAs) influences the production of methane in the rumen. Acetate and butyrate promote methane production, while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen. The production of acetate and butyrate leads to simultaneous production of H_2 whereas propionate production leads to production of O_2 in rumen. This O_2 react with H_2 molecule and produce water. But the excess of H_2 utilised by methanogenic microbes to reduce CO_2 to produce CH_4. Coenzyme M, HS-HTP, F_{420} and lipids like isopranyl glycerol ether acts as co-factor for the methanogenesis in rumen by rumen microbes.



Methods for reduction of methanogenesis in ruminants:

Feed processing technologies: Various feed processing techniques helps to increase the palatability of feed and total feed intake of animals. Chopping and grinding of straws, alkali/ammonia treatment of straws and feed residues, urea-molasses blocks treatments are the best example feed processing techniques. These processing techniques are reported to depress the methane emission from rumen by 10 per cent. Reduction in methane is associated with increased propionate production (Johnson and Johnson, 1995).

Type of ration: The major influence on the proportion of energy lost as methane in ruminants is quantity and quality of ration consumed by animals. Methane emission would be less when high grains are fed as a result of higher production of propionic acid. Methane emission fall down drastically to as low as 2-3 per cent (Johnson and Johnson, 1995). High carbohydrate containing diet with high digestibility has lower methane production whereas high fibre containing diet had higher methane producing tendency in rumen.

Protein supplementation in the diets increased the nutrient digestibility hence, there was significant decreased in methane production in rumen (Mehra *et al.,* 2006). Higher protein supplementation promotes the growth and population of rumen microbes which actively participate in rumen fermentation process and propionate production. The higher efficiency of energy utilization is cited by as the most efficient strategy to reduce methane emission per kilogram of milk or meat in ruminants (O'hara *et al.,* 2003).

Defaunation: The methanogenic bacteria are attached on outer surface of ciliated protozoa in the rumen liquer. This relationship is called as eco-symbiotic relationship. Protozoa in the rumen are responsible for a high proportion of H_2 production, and are closely associated with methanogens by providing a habitat for upto 20 per cent of rumen methanogens (Newbold *et al.,* 1995). Defaunation in the term to use the removal of protozoa from the rumen of animal. Removal of protozoa simultaneously reduce the population of methanogens in rumen liquor hence, there are reduced methane production. Copper sulphate, acids, surface-active chemicals, triazine, lipids, tannins, ionophores and saponins are the compounds which were commonly used as defaunating agents. It was also obseverd in several researches that reduction in methane production can be amplified by increase in concentrate diet to the treated animal. On defaunation the methane production is reduced by 20-50 per cent (Van Nevel and Demeyer, 1977).

Supplementation of unsaturated fatty acids: The fatty acids having two or more double or triple bonds in their chemical structure are called as polyunsaturated fatty acids (PUFA). They have great potential to be used as hydrogen sinks, because their bonds (double and triple) will get saturated by hydrogen and less hydrogen will be available for methane production. The saturation processes of polyunsaturated fatty acids were very efficient because of reducing environment of rumen which helps in the hydrogenation process. Adding fats to the diet can reduce methane emission by lowering ruminal fermentability and to a lesser degree, through hydrogenation of the unsaturated fats (Johnson and Johnson, 1995).

Organic acids: Organic acids are commonly used in the animal feed as feed acidifier. Thath helps to maintain the pH of rumen of the ruminant animals. Acidic medium helps to promote the growth of propionate producing bacteria. That leads to reduction in the methane production in the rumen. Addition of fumaric acid decreased methane emissions *in vitro* (Asanuma *et al.,* 1998) and *in vivo* (Bayaru *et al.,* 2001). Dietary supplementation of dicarboxylic organic acids such as malate, fumarate, aspartate etc. reduces methane production (Martin, 1998). Malate, a potent methane inhibitor is present in animal feeds like alfalfa (2.9-7.5% of DM) and bermuda grass (1.9-4.5%) but its level varies with variety and stage of maturity. These organic acids are converted to succcinate or propionate by reduction process and less hydrogen will be available for methane production.

Haloginated methane analogues: Various haloginated methane analogues so far tried as methane inhibitors are such as carbon tetrachloride, chloral hydrate, trichloroacetamide, DDT, trichloacetaldehyde, bromochloromethane, chloroform, methylene chloride, methylene bromide, nitrapyrin, hemiacetyl of chloral and starch etc. They generally inhibit methanogens (Haque, 2001). Positive impact of these had been reported only in those animals fed on high roughage diets, as common in indigenous livestock. Chloral hydrate is converted in the rumen to chloroform prior to inhibiting methanogens. Bromo-chloromethane is believed to inhibit methane production by reacting with reduced form of Vitamin B12 which inhibits methanogenesis.

Ionophores: They are highly lipophilic ion carriers. They have ability to pass through the peptidoglycone layer of gram positive bacteria and destroy their ionic gradient. They may impaired the cell division of these micro-organisms that leads to death of the microbe. Ionophores are generally used as feed additives in order to improve the efficiency of digestion in ruminants, such as tetronasin, monensin, lasalocid, salinomycin, narasin, lysocellin etc. These ionophores antibiotics are produced by various strains of Streptomyces eg. Monensin by *S. cinnamonensis* and lasalocid by *S. soliensis.* Monensin is moderately active against gram positive bacteria, certainmyco bacteria and coccidian, while lasalocid is specifically active against hydrogen producing bacteria and results in higher propionate production which in turn is related with low methane production (Kobayashi *et al.,* 1992).

Microbial feed additives, probiotics and prebiotics:

Some acetogenic bacteria produce acetic acid by the redction of carbon dioxide with hydrogen and thus, depress methane production when added in rumen.The probiotics have been shown to stabilize rumen pH, increase propionate levels and decrease the amount of acetate, methane and ammonia production. Addition of *Sacchromyces cerevisiae* reduced methane production *in vitro* (Mutsvangwa *et al.,* 1992).

Sulphate supplementation: In the rumen fermentation, three H_2 utilizing microbes are the, sulphate reducing bacteria, methanogens and carbon dioxide reducing acetogens. It appears that sulphate-reducing bacteria have the highest affinity to utilize hydrogen in the rumen, even better than methanogens. The availability of sulphate in the rumen appears to be a limitation. It's observed that sulphate supplementation helps in increasing the production of fibre degrading enzymes and fibre degradation in the rumen (Kamra *et al.,* 2004). As sulphate/sulphite have high affinity for utilization of hydrogen for its reduction to sulphide, therefore, with fibre diet, supplementation of sulphate / sulphite can be a good mode of rumen amelioration for improving fibre degradability and inhibiting methanogensis, but a proper dose will have to be optimized, keeping in view the toxic levels of sulphide generated on sulphate reduction.

Vaccine: Another methane reduction strategy that is being investigated is the development of a vaccine that would stimulate the ruminant's immune system to produce antibodies against methane-producing methanogens. Two vaccines were developed, named VF3 (based on three methanogen strains) and VF7 (based on seven methanogen strains), which produced a 7.7 per cent methane reduction (Wright *et al.,* 2004). The vaccine targeted 20 per cent of the methanogens present in the rumen of the animals.

Herbal extracts in reducing methane production: The use of plant extracts appears as one of the best natural alternatives to the antibiotic use in animal nutrition. Plant extracts offer a unique opportunity in this regard, as many plants produce secondary metabolites, such as saponins and tannins, which have antimicrobial properties. These compounds have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Hristov *et al.,* 1999).

The presence of tannins in plants might be responsible for reduction inmethane emission. Phenolic acids such as coumaric acids, ferulic acids and some monomeric phenolics have been found to decrease methane (Asiegbu *et al.,* 1995). Garlic oil is a complex mixture of many secondary plant products including allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan. The decrease in methane production observed in garlic oil and its compounds confirms their ability to inhibit methanogenesis (Busquet *et al.,* 2005). Many plant extracts have high content of flavonoids which decreased methane production (Broudiscou *et al.,* 2002).

The tropical plants containing high amount of saponins have been found to have antiprotozoal and antimethanogenic activity. There are some feeds or forages plants, which contain saponins such as Alfalfa Acacia, Emblica officinalis etc. caused a decrease in methane production from 20-60 per cent. Tannins have been found to be toxic for many of the rumen microbes, especially ciliate protozoa, fibre degrading microbes and methanogenic bacteria. As a result of this property the methanogenesis in the rumen is also reduced (Kamra, 2006). Tannins reduce H_2 availability to lessen methanogenesis (Carulla *et al.,* 2005).

Conclusion: Methanogenesis in the livestock (mainly ruminants and pseudoruminants) is a majour contributor in the global warming. It is difficult to reduce their population because of their need for various animal products. But it's possible to control the methanogenesis in ruminants. Further methane production is a type of energy loses in animal. There are many ways to control the methane production in ruminants like by dietary manipulation, addition of ionophores, organic acids, VFAs, Halogen analogs of methane etc in the ration of animals or by defaunation. There are various herbal *i.e.* plant secondary compounds that helpful in the methane mitigation.

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