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Popular Article

Methanogenesis and its mitigation in ruminants

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Abstract

Rumen micro-organisms play a crucial role in all ruminants for efficient utilization of complex plant materials. As by-product of rumen anaerobic fermentation, methane is emitted which is considered to be a potent global warming agent and most abundance in concentration, after carbon dioxide. Improved economic states and increasing human population will inflate animal product demands, culminating in elevated levels of methane emissions from livestock component of agriculture sector. Methods to reduce enteric methane include genetic manipulations, use of feed additives, defaunation, plant secondary metabolites and dietary alterations. Some strategies are highly valuable in terms of methane reductions but more intensive in-vivo research studies are required for their practical use and on-farm implementations. Ideal method would reduce methane levels effectively without altering optimum productive capacity of animal and its overall performance.

Introduction

At present time scenario, two important aspects which are globally concerned are- providing food for burgeoning population and using strategies to lessen adverse climate changes. Livestock under agriculture sector have crucial role in both the major concerned areas. Livestock services including milk, meat, fabrics and other by-products, fulfill basic requirements of people and abatement of methane production from animals can significantly contribute to clean and safer environment. In order to bridge the gap created between demand and supply due to over burden of population, array of development methods and technologies have been applied for increasing animal productivity. As the result, substantial improvement in output per animal has been achieved. But in 21st century, apart from such gains, also there is shift in awareness regarding climate changes, which has been deteriorated, resulting in negative impacts on both human and animal population. Greenhouse gases are the cause of drastic damage to environment. In this paper, GHG, methanogenesis and their mitigation have been elaborated.

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Greenhouse gases (GHG)

GHG are potent contributors to global warming. They absorb solar heat energy and keep atmosphere suitably warm for survival of life. But due to their enhanced concentrations in atmosphere, excessive heat gets trapped and results in global increase in temperature. Carbon dioxide, methane and nitrous oxide are predominant gases under this category. Agriculture activities contribute to average 41% global methane emissions. Therein, 73% methane emission attributed solely by livestock system. Within livestock, ruminants significantly emit enteric methane. Beef and dairy cattle accredit major portion of emitted methane (average 65%) while small ruminants and buffaloes have smaller portions (average 15%). Hence, as the animal count increase, proportionate emitted CH_4 amount increases. CH_4 is said to be 28 times more powerful than CO_2 as global warming gas. After CO_2 , it stands on second position in terms of abundance in environment. There is two-way interaction between animal husbandry and climate change. Livestock contributes significantly in adverse climatic changes through CH_4 emission and resulting change in climatic conditions endangers animal productivity by affecting agriculture practices via drought and floods which culminate in disease outbreaks and animal life losses. Moreover, energy lost (2-13% of gross energy of feed) as CH_4 gas serves as additional tax on overall animal performance which otherwise could have been part of boosting animal productivity.

Why CH_4 mitigation?

Impact of GHG on climate changes is chiefly regulated by two key factors viz. length of time for which it remains in atmosphere and its ability to absorb heat energy. CH_4 atmospheric lifespan is shorter (around 12 years) in comparison to that of CO_2 (around centuries). Foremost, CH_4 has more potency to trap solar heat energy, so it is desirable to mitigate it first. Second, since CH_4 emission do not accumulates over centuries as that in case of CO_2 it is advantageous in terms of slight abatement in CH_4 emissions which could significantly stabilize global warming at approximately present levels. Findings suggest that methane can exacerbate climate changes 3 times more in comparison to carbon dioxide. So reverse is also true that mitigating methane can be 3 times more effective. Also, IPCC has indicated a global warming potential (GWP) for methane which means that 1 tonne of methane can be considered equivalent to 28 to 36 tonnes of carbon dioxide if looking at its impact over 100 years.

Ruminants

Ruminant is derived from Latin word 'ruminare' meaning chewing the cud. They are cloven footed foregut fermenters having specialized digestive chambers mainly rumen and reticulum. Inside these chambers, a consortium of micro-organisms resides having elegant and complex inter-relationship. By degrading complex plant materials through anaerobic fermentation process, microbes aid ruminants to thrive on plants as energy sources while in exchange they get favorable conditions for their growth and survival. Numerous communities of bacteria, protozoa, fungi, methanogenic archea and bacteriophage aids in breaking complex molecules of plant stuff, generating short chain VFAs (Volatile Fatty Acids) and microbial crude protein

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which are utilizable by host animal and by-products like methane, carbon dioxide, hydrogen sulphide gases which are eructed via mouth to the environment. About 80% of animal's total energy requirements are supplied by short chain VFAs.

Methanogenesis

Rumen fermentation is an oxidative process, under which reduced co-factors (NADH, NADPH and FADH) are re-oxidized (NAD^+, $NADP^+$ and FAD^+) resulting from dehydrogenation process and subsequent release of hydrogen in rumen. During fermentative changes, acetate, propionate and butyrate are chiefly formed VFAs. There is relative difference in terms of hydrogen produced along with them as by-product. There is net generation of H_2 with formation of acetate and butyrate while propionate itself acts as hydrogen sink causing removal of hydrogen from rumen. Continual removal of H_2 is crucial for uninterrupted fermentative pathways inside rumen otherwise excess of it will impair function of dehydrogenase enzyme required for further regeneration of oxidized factors and followed by cessation of VFA production. Methanogens removes this hydrogen from rumen.

Methanogens are archea bacteria which produce methane by utilizing CO_2 and H_2 produced during fermentation process, for their growth and energy requirements. They can be categorized into 3 groups on basis of type of substrate utilization *viz*. methane derivatives (methylotropic), H_2 / CO_2 (hydrogenotropic) and acetate (acetoclastic). Among these three, hydrogenotropic methanogens predominate and serves as main route of H_2 disposal. Under rumen conditions, following pathways occurs.

- Hydrogen and CO_2 combined to form CH_4 via Wolf cycle of methanogenesis, stoichiometrically represented as $4H_2 + CO_2 = CH_4 + 2H_2O$.
- Methylamines and methanol can also be oxidized to CO_2 releasing electrons which are used to reduce methyl groups of methane. During acetoclastic methanogenesis, acetate splitted to generate carbonyl compounds and are further oxidized to CO_2. But bacteria governing these reactions are naturally low in counts owing to their limited growth rate and subsequently wash out from rumen.
- In propionate production pathway, net hydrogen is consumed, so there is inverse relationship exists between propionate amount and CH_4 in rumen.
- Nitrate reduction is alternate pathway consuming either hydrogen by denitrification process or by assimilatory and dissimilatory processes. Sulphate is also known to be hydrogen sink due to virtue of its high reduction potential and affinity for H_2. Although, nitrate and sulphate reaction are thermodynamically more feasible but due to their limited concentrations in rumen, rate of electron flow in their reduction processes are rare.

Overall, methanogenesis can be said as the most effective route of hydrogen utilization inside rumen for sustained rumen fermentation process. This leads to adoption of various methane mitigation strategies for better outcomes.

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Methane abatement strategies

Genetics- Direct selection based on CH_4 traits and indirect selection correlated to CH_4 emissions have been explored and exploited for creating low methane emitting population of animals. Selective breeding is very effective method because changes brought are permanent in nature. Yet, requirement of large number of animals with methane records, prolong time consumption, cumbersome investigations, multiple data processing and enormous need of funds restricts practical applicability of selection process. As alternative to this, development of biomarkers which can suitably measure CH_4 production are reliable.

Feed additives- They may be organic or inorganic substances which act either by inhibiting methanogenic bacteria or diverting metabolic pathways which leads to lowered availability of substrates for methane production.

Ionophores**:** Antimicrobial agents, as indicated by name, acts by disrupting ion equilibrium maintained across microbial membrane, inducing futile ion cycle in microbes and causing their termination. Rather than directly inhibiting methanogens, they mostly alter acetate: propionate ratio. By damaging gram positive bacteria, ionophores indirectly reduces hydrogen availability to methanogens. As the pathways were shifted towards propionate production, acetate: propionate ratio is lowered and eventually methane production declines. In addition, ciliate protozoa counts were also decreased. Commonly supplemented ionophores includes monensin, lasolacid and salinomycin. Furthermore, they are also known for improved feed efficiency, energy metabolism, decreased rumen proteolysis and overall enhancement in animal productivity. However, as with other antibiotics, rumen microflora adapts and becomes resistant towards long term used iononphores and consequently their ability to diminish methane production becomes futile.

Organic acids**:** Fumarate, malate and acrylate are regarded as potent feed additives in methane abatement. They act by provoking propionate production in rumen and subsequently shifting hydrogen towards propionic acid generation. Among these, fumarate was observed to be more fruitful. In contrast to in-vitro trials, in-vivo studies yield inconsistent and variable results with use of organic acids. This arise question against their effectiveness as anti-methanogenic substance. Further research trails in animals are needed to assess their potency.

Probiotics**:** these are direct fed microbials having modulating effect on gastrointestinal microflora of host as beneficial in terms of improving animal health and overall enhanced performance. Acetogen group of bacteria are capable of producing acetate through reductive acetogenesis. Hydrogen is utilized in acetate formation instead of methane generation and additionally acetate serves as source of energy to the animals. But studies revealed that acetogens are relatively less efficient in comparison to methanogens. Propionic acid bacteria are gram positive in nature and they divert hydrogen towards propionate formation. However, their rumen survivability is doubtful. Yeasts were found to be methane reducers by provoking reductive acetogenesis, yet discrepancies in various studies presented them less effective. Methane oxidizing bacteria embodies methane

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monooxygenase enzyme, making them capable to thrive on methane solely. They could be used as potent alternate to curb methane but further in-vivo studies are imperative for evaluating their efficacy.

***Chemicals*:** Final step in methanogenesis involves methyl-coenzyme M reductase enzyme which aids in transfer of methyl group to coenzyme M and subsequently methane is generated. Blocking this enzyme could help to cease methane formation. Bromoethane sulphonate, bromopropanesulphonic acid, nitroethane and 3-nitrooxypropanol were observed to inhibit final step conversion. However, microbial resistance, rumen instability, economics and safety issues hampers their practical applications.

***Macroalgae*:** commonly called as seaweeds, sustaining their life cycle by growing on fresh or marine water. In several species of seaweeds, halogenated analogues of methane are present such as bromoform, having antimicrobial properties and tend to reduce methane emission by blocking cobamide dependent methyl transferase step of methanogenesis. Nevertheless, safety concerns arose since bromoform found to be carcinogenic in nature and a potent ozone layer depleting agent, limiting its use in animals.

Defaunation- Simply it means removal of protozoa from rumen**.** Ciliate protozoa are closely integrated with methanogens and protect them from oxygen injury. Protozoa are attributed to be the major hydrogen producers in rumen and it was revealed that on an average 37% of rumen CH_4 produced by protozoa associated methanogens. However, practical methods of defaunation do not exist still. Also, removal of protozoa from rumen environment could result in disturbed rumen fermentation process observed as lowered dry matter intake and organic matter digestibility. Moreover, protozoa are rapidly transferred from normal animal to defaunated animal when kept together.

Plant secondary metabolites- They are produced by plants for self-defense purpose against predators and stressful situations. Tannins, saponins, flavonoids and essential oils have been ascribed for their role as reducing methane emission.

Tannins are chemically polyphenols which generally binds to proteins and forms insoluble complexes rendering them from digestion. Hydrolysable tannins recognized to be toxic in animals so condensed tannins were more evaluated. They directly or indirectly abate methane emission by inhibiting methanogens or protozoa population.

Saponins are surface active glycoside compounds found naturally in numerous plant species. They are observed to be anti-protozoal in nature and also limit hydrogen availability in rumen for methanogenesis. Although, their effects were seems to be temporary because bacteria degrades them into sapogenins which are ineffective against protozoa.

Flavonoids are polyphenols like tannins and found mainly in seeds and vegetables, having a broad range of biological functions including anti-microbial properties. This group consists of numerous compounds viz. flavanol, flavandiol, isoflavone, etc. They primarily inhibit gram positive bacteria, decrease protozoa population and methanogens thereby impeding H_2 utilization for methane synthesis. In addition, they possess rumen pH stabilizing property making them useful in sub-acute rumen acidosis conditions.

Essential oils are volatile aromatic compounds obtained from flowers, seeds, fruits, leaves, stem, barks and twigs of plants like garlic, onion, clove, fenugreek, ginger, thyme and rosemary. Numerous activities are found to be related with them *viz*. anti-microbial, anti-inflammatory, anti-oxidant and immunomodulation. Research data points towards their anti-methanogenic nature too.

Conclusively, plant secondary metabolites are effective natural harmless alternate to antibiotics but their extraction methods, cost, methanogen adaptability and undetermined dose in animals make them less feasible as methane reducing agents.

Dietary manipulations

Rumen bacterial and protozoa population greatly depends on composition of diet provided to the host animal. Animals fed on high-forage diets have considerably more gram negative bacteria whereas diet rich in grains resulted in more gram positive bacteria. Also, there is proportionate relation exist between fiber content of diet and methane production. In general, rumen microbiome (cellulolytic, amylolytic, pectinolytic) is governed by carbohydrate nature present in diet like more insoluble structures (cellulose, hemicellulose and lignin) or more soluble components (starch, pectin). Altering dietary feed composition fed to animals remains most practical and simple way to abate enteric CH_4 emissions. By nutritional changes alone, upto 70% of methane release could be reduced.

***Forage quality*:** as plant matures, amount of fiber composition increases. Lignin inherently enhances and forms cross-linkages with hemicellulose and cellulose of plant cell wall, which renders microbial fermentation in rumen of animals. Amount of soluble carbohydrates, protein and fat decreases as plant matures. With feeding poor-quality roughages, rumen retention time increases and consequently rumen methane emissions also. It is better to cultivate fodder in younger stages and used for feeding purpose.

Concentrate: Several studies revealed decreased rumen CH_4 emission due to increased concentrate proportion in diet which resulted because of alteration in rumen fermentation patterns and relative amounts of VFAs produced. Enhanced feed intake, passage rate and reduced rumen retention time of feed favors more propionate production which itself acts as H_2 sink. Mostly it was observed that feeding high dietary concentrate produced better results when fed with poor-quality roughages. But higher concentration feeding cost and likelihood of sub-acute and acute rumen acidosis conditions alarms to use of their elevated amounts in rumen diets. Moreover, adequate quantity of roughage is also needed in ruminant diets for proper rumen-reticular contractions and motility.

***Lipids*:** lipids decreases enteric methane emissions through several mechanisms including direct toxicity to ciliate protozoa, archaea methanogens, shifting VFA productions towards propionate and making bio-hydrogenation process as H_2 sink for saturation of fats. However, high dietary lipids can lower dry matter intake, fiber digestibility and organic matter fermentation which potentially results in enhanced nutrient losses

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in feces and elevated levels of methane from manure.

Conclusion

In above all described methods, each one has its own pros and cons. No single method seems to be adequate and effective in terms of abatement of methane emissions. Several barriers exist to their practical executions. Genetic manipulations require long time period, feed additives show inconsistent results in in-vivo studies, practical limitation of defaunation with adverse side-effects and adaptation of microbes to plant secondary metabolites. Among all, dietary intervention appeared to be immediate, straight forward and inexpensive method with on-field implementations. It can be replicated at farm levels easily. To summarize, developing cost-effective, practical and efficient methane reducing strategies are imperative in achieving sustainable animal production system with minimum environment deterioration.

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