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Livestock Farming and Methane: Emission and Mitigation approaches: A review

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Abstract
Ruminants, through ongoing ruminal methane emissions, substantially contribute to environmental pollution and global warming. Methane (CH_4) is 23 times more effective at trapping heat than carbon dioxide (CO_2) and is presently the second largest contributor to global warming. The rumen of ruminants is the primary site for methanogenesis. In environments without oxygen, microbes in the rumen break down food and produce gases like carbon dioxide (CO_2) and methane (CH_4), which are expelled when the animal burps, while substances like acetate, propionate, and butyrate provide energy. Improving accurate and dependable ways to measure methane is important for understanding how animal care, nutrition, and management affect methane emissions and for finding effective ways to reduce them. Methane emissions can be reduced through various methods, such as choosing specific animal breeds, using feed additives like fats, essential oils, and plant compounds, applying methane blockers, incorporating algae, and changing diets. This review aims to present an overview of ruminant methane production, several methodologies for quantifying its emissions, and strategies for reducing bovine methane emissions.

Keywords: Emissions, Methanogenesis, Methane, Rumen, Ruminants, Mitigation

Introduction
Methane (CH_4) is mostly produced by ruminants with a mean daily production capacity of 250-500 liters (Olijhoek and Lund 2017) [1]. The emissions from livestock account for about 73% of methane emissions in the agricultural sector (US EPA 2013) [2], with dairy and beef cattle accounting for 30% and small ruminant and buffalo accounting 15% (Islam and Lee 2019) [3]. According to UN estimates, there will be 9.8 billion people on Earth in 2050 and 11.2 billion in 2100 (Rate 2017) [4], and the demand for meat and milk products will increase proportionately. Global warming will unavoidably accelerate as the demand for ruminants rise and methane generation rise as well (Salter 2017) [5]. It is important to note that animal-derived greenhouse gas emissions and their effects on global climate change are serious global concerns (Martin *et al*., 2010) [6]. CH_4 is the second most prevalent greenhouse gas (GHG) with 23 times more potential than carbon dioxide worldwide (IPPC 2007) [7]. In addition to its detrimental effects on the environment, it results in energy loss ranging between 2-2.15% of gross energy intake by the animal, wasting dietary energy that could have been utilized to increase animal productivity (Kim *et al*. 2012) [8]. Methane, N_2O and CO_2 are three primary greenhouse gases contributing greatly to global warming. Compared to CO_2, it has a 23-fold greater potential for global warming (IPPC 2007) [7]. It is main ingredient in natural gas, is an odorless, colorless, tasteless and highly combustible. It has a specific gravity of 0.554 and is lighter than air. The density of methane gas is 0.717m^{-3}/kg, and it's melting and boiling points are -187 °C and -161 °C, respectively. It is soluble in organic solvents but insoluble in water. Methanogenesis is the primary process that produces naturally existing CH_4. Owing mostly to the production of food, the breakdown of waste and the exploitation of fossil fuels, the

making it the second most significant contributor to presumed human-induced global warming after CO_2 and significantly impacts the deterioration of ozone layer (Stackhouse *et al*. 2011) [9].

Methane originated from Ruminants

1. **Methane Produced by Enteric Fermentation:** Methanogenic microorganisms, Archaea, produce enteric CH_4 as a byproduct of ruminant digestion through a process known as fermentation or methanogenesis (Olijhoek and Lund 2017) [1]. Cattle produce around seven and nine times higher CH_4, respectively than lambs and goats. The rumen produces 87-90% of the enteric CH_4 while the large intestine produces remaining 13% to 10% (Dini *et al*. 2012) [10]. Only 11% of the CH_4 produced by hindgut is released through anus and 89% of it was found to be expelled through the breath, whereas 95%gas produced in the fore stomach by enteric fermentation was eliminated through eructation, indicating eructation as the primary method used to remove it (Murray *et al*. 1999) [11]. Breakdown of food by gut microbiota (bacteria, protozoa, and fungi) produces volatile fatty acids and the animal uses these acids, primarily butyrate, propionate and acetate, as source of energy. Creation of methane utilizes CO_2 and H_2, lowering their amount available for anabolic activities (Cassandro *et al*. 2013) [12].
2. **Methane produced from Manure:** Excreta is another major source for methane emissions mainly that is aerobically stored, in addition to the enteric source (Klevenhusen *et al*. 2011) [13]. Methane from manure due to livestock accounts 2% and 0.4% of CH_4 and GHG emission worldwide, respectively. A fraction of organic substances, such as proteins, carbs and lipids, are present in livestock manure used by anaerobic bacteria as a source of food and energy (Olijhoek and Lund 2017) [1]. The energy worth of the gas itself may obviously be a benefit of methane generation, but the key factor influencing manure gas production is the effectiveness of the mechanism that produces it. A specified quantity of gas produced per unit of materials broken down by anaerobic bacteria is known as the gas yield (Song *et al*. 2011) [14]. In the natural process of anaerobic digestion, organic matter is consumed by bacteria in an oxygen-restricted environment generating microbial biomass and greenhouse gases (Olijhoek and Lund 2017) [1]. The organic matter's anaerobic breakdown and the CH_4 formation are influenced greatly by the volatile content of manure (proteins, carbs and fatty acids) and of these, only a portion of it is readily biodegradable (Godbout *et al*. 2010) [15].

Methanogenesis: Ruminants belong to order Artiodactyla and 95% of the earth's ruminant population comprise of domesticated cattle, sheep and goats (Hackmann and Spain 2010) [16]. They feed themselves by grazing or browsing, utilizing their unique digestive systems (compartmentalized stomach) and an intricate symbiotic network of microbes to survive on plant matter (Clauss and Hofmann 2014) [17]. Enzymes that degrade complex macromolecules in feed are synthesized in the rumen by the complex community of bacteria (10^{10}-10^{11} cells/ml), ciliate protozoa (10^4-10^6 cells/ml), methanogenic archaea (10^6-10^8 cells/ml), and fungi (10^3-10^6 cells/ml) (Matthews *et al*. 2019) [18]. Rumen provides environment favorable for growth and survival of microbiota favouring fermentation process and yields short volatile fatty acids (SVFAs) and microbial crude protein which act as source for protein and energy for the hosts. Methane is subsequently produced as a byproduct of this anaerobic fermentation by methanogens in the rumen (McCann *et al*. 2014) [19].

The primary pathway for disposing hydrogen from the substrate is hydrogenotrophic methanogenesis H_2/CO_2, while in anaerobic environment CO_2 acts like hydrogen sink (Thauer *et al*. 2008) [20]. Similarly, as the nitrate/sulfate reduction pathway is more thermodynamically advantageous, nitrate and sulfate can potentially function as hydrogen sinks (Van Zijderveld *et al*. 2010) [21]. The low concentration of this substance in the rumen, however, restricts the sinking of electrons in the reduction of sulfates and nitrates, which directs most H_2 towards the synthesis of methane. Therefore, the usual method for getting rid of ruminal hydrogen and enabling the fermentation to continue is methanogenesis. Furthermore, the H_2 levels in the rumen are controlled by intercellular H_2 transfer between methane producing and fermentative bacteria, protozoa and fungi. This is because H_2 traces have been shown to limit hydrogenase activity, which has a detrimental effect on carbohydrate oxidation (Thauer *et al*. 2008) [20]. Overall, the internal flow of hydrogen into competing metabolic pathways and the interspecies transfer of hydrogen between bacteria, control the fermentation process in the rumen (Ungerfeld 2020) [22].

Methane Production Affected by Feeding: The type and quantity of feed have a major impact on the amount of enteric CH_4 **(**Shibata and Terada 2010) [23]. While dietary carbohydrate composition has only a minimal impact, gross energy (GE) is positively correlated with meal digestibility and adversely correlated with concentration of fat in diet and level of feeding. Although methane yield per kg of DMI declines with increase in feeding level, diet digestibility, feed composition or pasture quality, and inclusion level of lipids or concentrates in the diet; many studies state that dry matter intake (DMI) is the primary source of daily methane output (Beauchemin *et al*. 2020) [24]. The bacterial activity, which requires minerals, energy, and nitrogen, is what drives digestion in the rumen (Shibata and Terada 2010) [23]. As a result, the rumen's ability to produce CH_4 and the activity of its bacteria are both impacted by the quality of the feed. The types of forages, how they are processed, how much of them are consumed, and where the grain comes from, all affect the quantity of methane ruminants produce. Generally, methane generation tends to rise with feed's increased fiber content and digestibility of feed while falls with increase in protein and fat content of feed (Shibata and Terada 2010) [23]. Olijhoek and Lund (2017) [1] observed that consumption of 25% higher non-structural carbohydrates would produce upto 20% less methane, however, this could have other negative consequences as well, such as acidosis, laminitis, and issues with reproduction. The ration's forage to concentrate ratio affects the rumen fermentation process, which in turn affects the acetate to propionate ratio. Van Soest stated that feeds high in soluble carbohydrates caused a change in the fermentation process in rumen, which led to the unfavorable environment for the methane producing bacteria (Van Soest 1982) [25].

Mitigation Strategies

Researchers have been working on various approaches to reduce enteric methane emissions since the 1950s. Although a number of strategies have demonstrated remarkable efficacy in decreasing enteric methane emissions and enhancing animal yield, yet they come at a high cost and pose hazards to both human and environmental health. Therefore, it is essential to comprehend current methods and develop better ones in order to reduce methane emissions from ruminants.

(A) Mitigation through Feed Manipulation: The simplest and least expensive method of reducing intestinal methane levels is still diet modification through feed mix changes (Haque 2018) [26]. Depending on the form or manner of the nutritional intervention, this strategy alone could reduce ruminant methane emissions by up to 70% (Benchaar and Greathead 2011) [27]. Changing the kind or quality of forage or adjusting the feed's concentrate to forage ratio are the most common approaches. Greater-quality forage is compensated for by younger plants with reduced non-digestible fiber (NDF), greater fermentable carbohydrates, and a lower C:N ratio. This result in increased digestibility and passage rate, which can steer rumen fermentation toward propionate and less H_2 will be available for methanogenesis (Beauchemin *et al*. 2020) [24]. It has been noted that increased productivity is accompanied by a decrease in CH_4 generation when feed containing 35% or 60% concentrate is provided (McGuffey *et al*. 2001) [28]. The elevated concentrations of concentrates may increase the levels of lactic acid and volatile fatty acids (VFAs) in the rumen.

(B) Mitigation through Additives: Generally, feed additives are added in the form of direct-fed probiotics or inorganic or organic chemicals. These additives reduce the substrate for methanogenesis by either explicitly inhibiting methanogens or by changing the metabolic pathways (Haque 2018) [26]. It is used to manipulate ruminal fermentation and enhancing feed efficiency, as it has been shown to control the ratio of propionic to acetic acid generation, leading in body weight growth (Odongo *et al*. 2007) [29]. Furthermore, there is a noticeable decrease in the rumen's proteolysis, which lowers ammonia production as a byproduct and increases the overall amount of protein that enters the small intestine for absorption (Marques and Cooke 2021) [30]. Ionophores can also function as antimicrobials by upsetting the gradient of ions (Ca^{2+}, K^+, H^+ and Na^+) across particular microbial membranes, forcing the bacteria to enter a pointless cycle of ions and giving some a competitive edge over others (McGuffey *et al*. 2001) [28].
Methanogens have less access to hydrogen as a result of this carboxylic polyether compound's selective inhibition of gram-positive bacteria that yield hydrogen, formate, butyrate, acetate, and lactate as end products (Marques and Cooke 2021) [30]. Supplementation of feed with ionophores was associated with a nearly 80% reduction in the ciliate protozoal population and a decrease in methane emission as depicted in Angus yearling steers (Guan *et al*. 2006) [31]. Similar results were seen by Odongo *et al*. (2007) [29], who fed nursing dairy cows 24 mg of Rumensin Premix/kg of dry matter for six months, resulting in a reduction in methane output of over 9%. Ionophores have the ability to lower methane production, but they also appear to affect dairy cows' and beef steers' dry matter intake. The emergence of resistance in bacteria that produce propionate and succinate (Patra *et al*. 2017) [32] cause the impact of ionophores to diminish with time.

(C) Methanogenesis Inhibitors: In anaerobic methanogenesis, methyl-coenzyme M reductase (McR) is essential (Shima *et al*. 2012) [33]. As the electron donor coenzyme F430, which contains nickel (active: Ni^+ or inactive: Ni^{+2}), catalyzes the last stage of methane metabolism involving a methyl-transfer reaction to coenzyme M (HS-CoM or 2-mercaptoethanesulfonic acid). This reaction reduces the substrate methyl-CoM and releases methane in the process (Chen *et al*. 2020) [34]. The main mechanism of several halogenated and nitro-derivatives of alcohols, fatty acids and hydrocarbons, is to disrupt any one of these series of processes. *In-vitro* methane emissions can be reduced from 70% to 80% using halogenated, sulfonated compounds like bromoethane sulfonate (BES) and bromopropanesulfonic acid (BPS), which structurally mimic CoM (2-mercaptoethanesulfonic acid) without compromising organic matter digestibility and VFA concentrations (Hwang *et al*. 2012) [35]. When chloroform was fed to cattle at 6-7% w/w rate reduced the generation of methane by 30% (g/kg) and had a major impact on the Methanobrevibacter and Methanosphaera species (Martinez-Fernandez *et al*. 2018) [36]. As a structural analog of methyl-coenzyme M, 3-NOP (3-nitrooxypropanol) is a nitro derivative that binds to the active site of McR competitively, has the ability to oxidize the cofactor $Ni^+$and inactivates McR (Zhang *et al*. 2018) [37]. The amount of enteric methane emissions was reduced by 20% to 60%, contingent on the mode or length of supplementation.

(D) Essential Oils and Other Plant Extracts: Since the EU banned the use of antibiotics as growth promoters in 2006, new additives derived from biological sources have been studied for their potential to improve cattle performance and lower greenhouse gas emissions (Abbott *et al*. 2020) [38]. Essential oils (EOs) are ephemeral, aromatic, volatile liquids that are extracted from a variety of plant materials, including wood, fruits, twigs, flowers, seeds, buds, leaves and herbs. EOs are widely regarded as safe for ingestion by humans and animals due to their broad-spectrum antibacterial effects (Davoodi *et al*. 2019) [39]. Methanogens are one example of a type of microbe that responds differently to EOs, either by encouragement or suppression of certain groups of microorganisms (Benchaar and Greathead 2011) [27]. Some reduce the amount of hydrogen available for methanogens by bio hydrogenating unsaturated fatty acids and inhibits the proliferation of protozoa in an indirect manner. Guyader *et al*. (2017) [40] found that during an *in-vitro* batch culture, an increase in saponin dosage resulted in a 50% drop in the protozoal population and a 29% reduction in methane production. Garlic reduced CH_4 production (*in-vitro*) by 91%, eucalyptus reduced it by up to 85% (Wang *et al*. 2018) [41] while thyme and peppermint resulted in 30% less CH_4 production (Guyader *et al*. 2017) [40].

(E) Additional Organic Additives: Over the past ten years, biochar has gained popularity due to studies showing improvements in growth, egg yield, blood profiles, enteric methane emission regulation and also has inhibitory effects on the proliferation of rumen pathogens (Man *et al*. 2021) [42]. Due to their anti-methanogenic qualities, seaweeds—also referred to as macroalgae—such as brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) seaweeds, have gained popularity as feed additions (Vijn *et al*. 2020) [43]. Several *in-vitro* investigations with seaweed supplements demonstrated a negative link with methane generation especially using *Asparagopsis taxiformis* (Min *et al*. 2021), which may reduce *in-vivo* methane emission in dairy cattle from 50% to over 80% (Kinley *et al*. 2020) [44]. By altering the composition of the rumen bacterial community, prebiotics including chitosan, inulin and yeast products, can also reduce the amount of methane released during digestion (Tong *et al*. 2020) [45]. While chitosan causes methanogens to lose their capacity to pass through their cell walls and causing cell death, yeast products and inulin encourage the growth of other rumen bacteria that compete with methanogens for hydrogen (Zanferari *et al*. 2018) [46].

(F) Mitigation through Direct-Fed Microbials (DFMs)/Probiotics: A single or mixed culture of living organisms that supports a favorable rumen microbiota and has positive effects on animals when fed, is known as a DFM (Krehbiel *et al*. 2003) [47]. Different rumen bacteria are hypothesized to promote propionogenesis, acetogenesis and nitrate/nitrite or sulfate reduction, which can operate as an alternative H_2 sink, in order to compete with methanogens for the hydrogen supply. This reroutes the rumen hydrogen's metabolic flux, which would have otherwise been used for methanogenesis, towards the synthesis of volatile fatty acids (Ungerfeld 2020) [22].

(G) Propionic Acid Bacteria (PAB): Gram-positive bacteria known as promibacteria make up around 4.3% of all rumen microbes and are found there naturally. Several PAB strains have been studied both*in-vitro* and *in-vivo* that may be essential in lowering methane emissions. *Propionibacterium acidipropionici, P. propionicus, P. jensenii, P. freudenreichii,* and *P. japonicas* are a few of them (Vyas *et al*. 2015) [48]. *In-vitro* use of rumen fluid from Norwegian dairy cows fed a grass silage-concentrate mixture on supplementation with *Propionibacterium thoenii* T159 has shown a 20% reduction in methane and a 21% rise in total VFA synthesis (Chen *et al*. 2020) [34].

(H) Methane Oxidizing Bacteria (MOB): A family of bacteria known as MOBs is capable of growing only on methane as a source of carbon and energy. It is common in situations that are aerobic or micro-oxic (Pandey *et al*. 2014) [49]. Methane monooxygenase (MMO), a specific enzyme used by these bacteria, oxidizes methane to methanol (Sazinsky and Lippard 2015) [50]. Methanol dehydrogenase then catalyzes the further oxidation of methanol to formaldehyde, which is subsequently incorporated into the serine or ribulose-5-monophosphate route (RuMP) for the production of biomass (Kalyuzhnaya *et al*. 2015) [51]. Additionally, MOB was found in the rumen epithelium and rumen fluid of non-lactating Holstein cows (Mitsumori *et al*. 2002) [52]. A *Ca. Methylobacter coli* BlB1, that is capable of using both methanol and methane was recently discovered by an Indian team from the excrement of an Indian antelope (Khatri *et al*. 2021) [53]. Studies employing MOBs as probiotics *in-vivo* are still rare and to fully explore MOB's probiotic potential in reducing methane emissions and improving animal nutrition, more isolation, screening and *in-vivo* researches are required.

(I) Vaccination: The idea behind developing vaccinations to reduce methanogenesis is to stimulate the production of salivary antibodies by the animal's immune system, which should inhibit the growth of methanogens when they enter the rumen (Subharat *et al*. 2016) [54]. Depending on the type of antibodies and the immunization strategy, all *in vitro* experiments demonstrated a reduction in the amount of CH_4 emitted, ranging from 7 to nearly 70% (Baca-González *et al*. 2020) [55]. Few other studies have used vaccinations to reduce the amount of CH_4 that ruminants produce during enteric fermentation (Wedlock *et al*. 2013) [56].

(J) Reducing Methane Emissions through Genetic Selection: Over the past ten years, a number of studies have demonstrated that while sheep had higher heritabilities of CH_4 yield (0.24-0.55), dairy cattle had moderate heritabilities ranging from 0.11 to 0.33 (Pickering *et al*. 2015) [57]. Numerous case studies from the industry demonstrate how increasing animal performance has gradually reduced the intensity of CH_4 emissions. On the other hand, CH_4 intensity falls with curvilinear increases in animal productivity. Therefore, raising the productivity of animals that produce less has a very large effect whereas raising the productivity of animals that produce more has a comparatively little influence (Beauchemin *et al*. 2020) [24]. Physiological alterations affecting the rumen, feeding behavior, rumen outputs, and body composition have been brought about via genetic selection.

Conclusion

Livestock farming is a major contributor to methane emissions worldwide. Methane emissions and global temperature rise in tandem with the growing demands for milk and meat products. Therefore, reducing ruminant methane emissions is one of the best ways to mitigate the effects of climate change. However, it is a challenging problem to reduce ruminal methane production in ruminants. Nevertheless, by improving animal productivity, creating superior pastures and forages, utilizing concentrate feeds and alternative forages, we can significantly curb the amount of methane contributed by livestock farming operations to the global emissions.

Conflict of Interest Not available

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