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Review

Genetic Strategies to Reduce Enteric Methane Emission from Ruminants

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Abstract

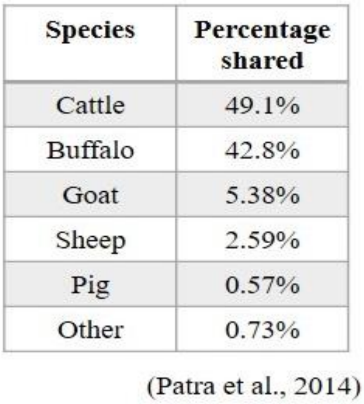
Measuring and controlling methane emissions from cattle is becoming extremely important for the environment and policymakers. Although ruminants have the advantage of being able to eat forages and graze on land that is not appropriate for arable cropping, they also produce methane which is a potent Greenhouse gas (GHG), 2% to 12% of the gross energy consumed is converted to enteric CH4 during ruminal digestion, and they produce around 6% of worldwide anthropogenic greenhouse gas emissions. As a result, ruminant producers must identify cost-effective solutions to minimize emissions while still fulfilling customer demand. Traditional strategies for reducing ruminant methane output have been successful, but only to a limited and frequently transient extent. When assessed in respiration chambers, individual animal emissions are somewhat heritable and repeatable across diets. To date, few of the suggested strategies have been implemented because the methane emissions currently have no direct or indirect economic value for farmers, with no financial incentive to change practices and secondly most strategies have limited, or no long-term effects. Potentially, the most sustainable way of reducing enteric CH4 emissions from ruminants is through breeding and genetic selection. There is a lot of potential in using genetics to reduce CH4 emissions from ruminants as the alterations made using genetics are permanent, cumulative, and far-reaching. New technologies, including genomic selection, microbiome-based breeding strategies, metagenomic investigations, and genetic selection of animals may be a sustainable way to reduce ruminant GHG emissions. These advanced genetic technologies also have the potential to give considerable long-term economic benefits and can also be used in grazing animals.

Keywords: Anthropogenic, Genomic selection, Greenhouse gas, Heritable, Methane.

Introduction

Due to the continuous expansion of the world population, a rising middle class, economic growth, and urbanization (Gerber *et al*., 2013), global demand for meat and milk is expected to increase by 73 percent and 58 percent, respectively, by 2050, compared to 2010 levels. These conditions are stressing the need for innovative strategies and approaches for food security and sustainable agricultural development. Ruminants are one of a kind in their capacity to digest cellulose-rich meals, due to the wide variety of microorganisms that break down feed in the rumen of the host animal. Microorganisms like bacteria, fungi, and protozoa use hydrolysis to break down complex substances into volatile fatty acids (VFA), primarily acetate, propionate, and butyrate. Fermentation produces different amounts of formic acid, hydrogen (H_2), and carbon dioxide (CO_2) as end products at the same time (Hook *et al*., 2010). In ruminants, the H_2 is used by the majority of methanogenic archaea in the rumen to decrease CO_2 and create methane (CH_4). Animal agricultural expansion is a source of concern since it contributes to rising greenhouse gas (GHG) concentrations in the atmosphere and, as a response, climate change occurs. Anthropomorphic production of greenhouse gases is boosting global temperatures to levels that are endangering the planet's ecosystems' long-term viability (Arora *et al*., 2018). Temperatures have risen by more than 1.2 degrees Celsius in some parts of the world during the 1960s, and yearly soil moisture in the farming districts of southern Australia is believed to have decreased by 20 to 40 percent (Manabe *et al*., 2019). Carbon dioxide and methane concentrations in the atmosphere have increased from 350 to 410 parts per million (28%) and 1100 to 1875 parts per billion (70%) since 1950, respectively (the United States Environmental Protection Agency). Methane is 28 times more potent as a greenhouse gas than carbon dioxide over 100 years, and 80 times more potent over 10–20 years (Pachauri *et al*., 2014). Enteric methane, which is produced by the microbial fermentation of plant material by ruminant animals, predominantly cattle, accounts for 30% of all methane released into the atmosphere, more than any other single source (www. globalmethane.org). Gerber *et al*. (2013) found that enteric methane is the greatest contributor (40%) to global greenhouse gas emissions from animal supply chains, accounting for 6% of total anthropogenic greenhouse gas emissions (Ripple *et al*., 2014; Beauchemin *et al*., 2020). Ruminants also create a significant quantity of carbon dioxide (CO_2), with a CH_4:CO_2 ratio of roughly 4:1 (Clauss *et al*., 2020), accounting for 8% of all anthropogenic greenhouse gas emissions. Concerns that bovine enteric CH4 contributes to anthropogenic GHG emissions have prompted new research into the mechanisms that influence methanogenesis in the rumen, as well as the investigation of a wide range of potential mitigation measures. Because India has a large livestock wealth of 536.76 million cattle, 149 million caprines, and 74 million ovines, as well as considerable numbers of pigs, camelids, and equines, the livestock industry is essential in India's fight against global warming and its consequences for food production economics (20th Livestock census, 2019). Although the cattle industry accounts for only 4.11 percent of total GDP, it accounts for 25.6 percent of agricultural GDP (Gross Domestic Product). Furthermore, in 2015-16, the sector employed 8.8% of the Indian population (Shanmathy *et al*., 2018). The enteric CH_4 emission is responsible for 44% of the GHG emissions from the total livestock group and 55% from the ruminants (FAO, 2019). Enteric methane emissions from Indian livestock contributed 15.1% of total global enteric methane emissions. In India, cattle ranked first in emitting enteric methane contributing about half (49.1%) of total enteric methane, followed by buffalo (42.8%), goat (5.38%), and sheep (2.59%), and other (0.73%) (Patra *et al*., 2014).

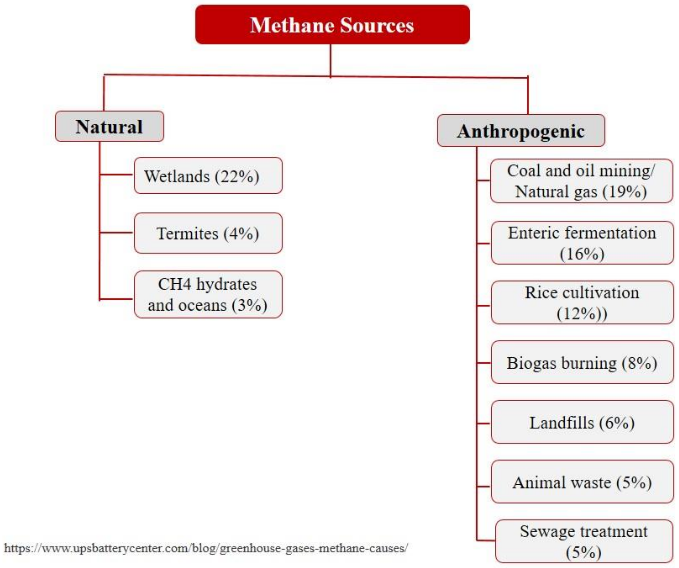
Table 1: Percentage share in total enteric methane emission



Species	Percentage shared
Cattle	49.1%
Buffalo	42.8%
Goat	5.38%
Sheep	2.59%
Pig	0.57%
Other	0.73%

(Patra et al., 2014)

Methane can be produced by natural and by various anthropogenic sources as shown in **Figure 1**.



https://www.upsbatterycenter.com/blog/greenhouse-gases-methane-causes/

Figure 1: Sources of methane emission

The Indian government is taking several initiatives to reduce methane emissions, including the "Gobar (Galvanizing Organic Bio-Agro Resources) – Dhan" scheme and the "Nepal Methane Action Plan." The Gobardhan scheme, for example, encourages the recovery of biodegradable trash and the conversion of waste into resources, as well as the reduction of GHG emissions. Despite massive multinational research efforts, finding ways to reduce methane emissions remains a problem to be solved. Nutrition, physiology, microbiology, and genetic selection are just a few of the areas in animal science that have concentrated on developing techniques for reducing methane generation in cattle. Common approaches to reduce CH_4 emissions in ruminants include dietary manipulation, drugs to reduce or control the quantity of methanogenic microorganisms in the gut, and/or vaccination. However, current strategies to inhibit methanogen activities in the rumen typically fail or have limited success due to low efficacy, poor selectivity, microorganism resistance, toxicity, or side effects of the compounds or drugs in the host species (Leahy *et al*., 2013). As a result, breeding low-methane-emitting animals is a realistic option. Low methane (CH4) emitting cows can be genetically selected, which can be an effective and long-term strategy for reducing GHG emissions from dairy cattle (Garnsworthy *et al*., 2012; Lassen and Difford, 2020). Because genetic changes are cumulative and irreversible, this strategy requires additive genetic variation as well as time to have an effect, as selection occurs across generations. Sustainable intensification has been proposed (Godfray *et al*., 2010) to address food security as well as the economic and environmental implications of food production, with genetic improvement of feed conversion efficiency in farm animals being of primary relevance. Through an exhaustive assessment of literature and case studies, the current review addresses the dynamics of global warming, and the implications of climate change on cattle production. There has been a wide array of CH_4-related publications, ranging from genome sequencing of rumen methanogens (Henderson *et al*., 2015, Henderson *et al*., 2018, Seshadri *et al*., 2018) to mitigation strategies (Beauchemin *et al*., 2009; Hristov *et al*., 2013; Eckard and Clark, 2018).

The Rumen Microbiome

The rumen is a complex, dynamic ecosystem that is mostly composed of anaerobic bacteria, protozoa, anaerobic fungi, methanogenic archaea, and phages. The protozoa can comprise up to half the rumen microbial biomass, the fungi about 7%, the archaea 1–4%, and the bacteria form the remainder and are normally the most abundant population. All contribute to methanogenesis directly or indirectly, but their role in and response to nitrate metabolism is much less clear (Yang *et al*., 2016). These microorganisms interact with one another and with the host, obtaining energy from the breakdown of plant cell wall polysaccharides (Mizrahi, 2013). It's also been suggested that these microorganisms have niche specialization in terms of nutrient utilization and that they engineer the rumen ecology in terms of following microbial colonization and nutrient usage (Pereira and Berry, 2017; Shaani *et al*., 2018). Ruminants supply human-edible nutritious food obtained from marginal land as a result of their highly evolved rumen microbiome, without competing with human food (Kingston-Smith *et al*., 2010). The hydrogenotrophic route, i.e. $4H_2 + CO_2 = CH_4 + 2H_2O$, is the most common route for methanogenesis in the rumen (Martin *et al*., 2010; Morgavi *et al*., 2010). Hydrogen gas is produced by ciliated protozoa, anaerobic bacteria, and fungus whereas methanogenesis is carried out by the archaea. As a result, it seems logical that methane emissions should be related to archaeal abundance in the rumen, which accounts for 87 percent of enteric emissions in the digestive system (Murray *et al*., 1976).

Methane Production

Anaerobic fermentation, in which bacteria break down organic matter to produce hydrogen (H_2), carbon dioxide (CO2), and methane, produces naturally occurring methane (CH_4) (Palangi *et al*., 2022). Figure 2 shows the process of enteric methane production from livestock. In the digestive systems of domesticated and wild ruminants, natural wetlands, and rice paddies, this process occurs naturally. Enteric fermentation occurs in the digestive system or rumen of ruminants, where bacteria degrade and ferment plant components such as celluloses, fiber, starches, and sugars. One of the by-products of this digestive process is enteric methane, which is released by the animal through burping.

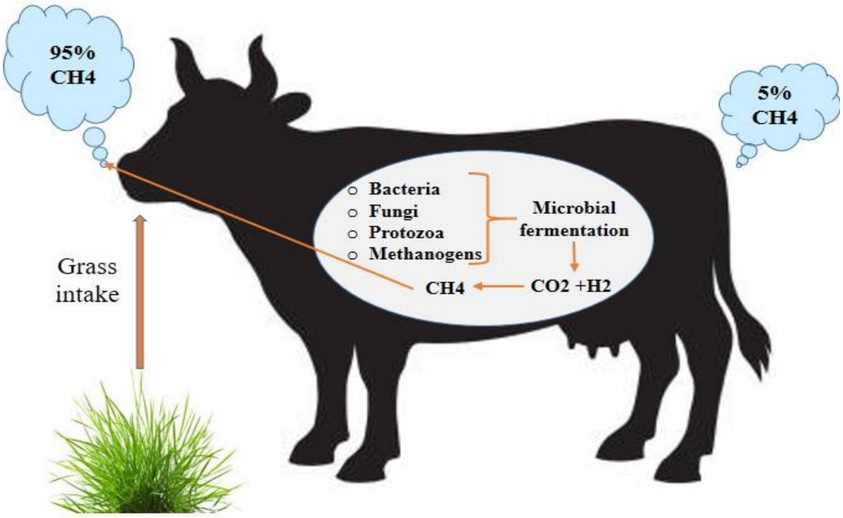


Figure 2: Shows the Process of enteric methane production from livestock (Palangi *et al*., 2022)

Other by-products (acetate, propionate, and butyrate) are absorbed by the animal and used as energy precursors to produce milk, meat, and wool. The level of intake, the kind and quality of feed, the amount of energy consumed, animal size, growth rate, degree of production, and environmental temperature all influence enteric methane output. The enteric fermentation process normally loses between 2 and 12 percent of a ruminant's energy intake (Johnson and Johnson, 1995). According to the literature, the most important issue to consider when creating strategies to decrease ruminant CH_4 emissions is the management of H_2 generation in the rumen. By suppressing H_2 liberating processes or boosting alternate H_2-using reactions or methods for disposing of H_2 during fermentation, it should be able to reduce CH_4 creation. Cattle can produce 250-500 liters of CH_4 per day, this level of production results in estimates of the contribution by cattle to global warming that may occur in the next 50 to 100 yr to be a little less than 2% (Johnson and Johnson, 1995). As a result, it is critical to reduce the contribution of CH_4 from ruminants to environmental pollution and to put this food energy to good use for the host animal. The primary methanogens in the bovine rumen use hydrogen and carbon dioxide to make methane, but there is a group of methanogens in the genus Methanosarcina that grow slowly on hydrogen and carbon dioxide and hence have their niche by producing methane using methanol and methylamines (Hungate *et al*., 1970; Patterson *et al*., 1979). Formate, which is produced during the synthesis of acetate, can be utilised as a methanogenesis substrate, albeit it is frequently transformed to hydrogen and carbon dioxide instead (Hungate *et al*., 1970; Archer *et al*., 1986). Because the conversion of volatile fatty acids (VFA) into carbon dioxide and hydrogen is a time-consuming process that is slowed by rumen turnover, they are not often used as methanogenesis substrates (Hobson and Stewart,1997). As a result, once VFAs are created, methanogenesis frequently consumes the hydrogen and carbon dioxide produced by carbohydrate fermentation (Hungate *et al*., 1970). Methanogens allow the microorganisms engaged in carbohydrate fermentation to function optimally and facilitate the complete oxidation of substrates by eliminating hydrogen from the ruminal environment as a final phase of carbohydrate fermentation (Sharp *et al*., 1998). When carbohydrates are fermented, hydrogen is produced, and if this end product is not eliminated, it might limit rumen microbial metabolism (Sharp *et al*., 1998).

Pre-Requisites for Studying Methane Emission by Using Genetics

A way to evaluate the features or phenotypes of interest is the first and most obvious necessity for selective breeding. Such data might be used to identify biological sources of phenotypic variances, such as non-genetic variables, which would need to be eliminated experimentally or statistically. As a result, it is crucial to figure out whether the phenotypic trait is considerably repeatable and heritable in the conditions in which animals are anticipated to perform. Heritability is determined by recording information about related individuals, but repeatability is determined by recording the same individuals many times during their productive lives. If the phenotype is heritable, genetic characteristics that are substantially associated (high confidence and low SE) with existing variables in the selection index must be established. Methods for enhancing the accuracy of estimated breeding values (EBVs) by including genetically connected indicator qualities, reviewing prediction methods and models, and examining how potential genotypes interact with the environment are all areas of genetic study. Once these genetic criteria have been determined, cost-benefit analyses can be used to estimate the economic value of recording and selecting for specific features (Lassen and Difford, 2020).

Methodologies for Measurement of CH4 from Ruminants

Respiration Chambers

The respiratory chamber (RC) system is frequently referred to as the "gold standard" for measuring emissions. This method, however, is expensive, complex, labour intensive, and has a low capacity for the number of animals that can be measured at one time, limiting its availability for large-scale applications. Furthermore, it does not reveal the animals' living conditions in production systems (Storm *et al*., 2012). Few believe that RC measurements accurately estimate CH_4 output over the typical measurement period of 1 to 3 days and that they do so by sampling emissions often. Respiration chambers are appropriate for small-scale investigations with a small number of animals and a high demand for precision and accuracy. However, RCs are not comparable across sites because they are not representative of all environmental variables, such as grazing systems. There are two major sources of measurement error in RC: (1) airflow rate or ducting efficiency, and (2) gas mixing within the chamber. The response time reflects both of these difficulties (Hammond *et al*., 2016). . A single chamber may record the CH_4 production of 120 cows over a year, assuming a single day of acclimatization and two consecutive days of recording (Garnsworthy *et al*., 2019). In actuality, as documented in the sole large-scale genetic investigation of CH_4 emissions of 1042 developing Angus steers and heifers, this number is likely to be much lower (30 to 50 cows) per year (Donoghue *et al*., 2016a). This herd of cattle demonstrated that CH_4 production is repeatable (t = 0.97) over days (Donoghue *et al*., 2016b), heritable (h2 = 0.27± 0.07) (Donoghue *et al*., 2016a), and has a moderate genomic prediction accuracy of 0.32± 0.04, Hayes *et al*., 2016).

Sulfur Hexafluoride Technique

Sulfur hexafluoride **(**SF6**)** is non-toxic, physiologically inert, and long-lasting (Lester and Greenberg, 1950; Johnson *et al*., 1992). Sulfur hexafluoride, like CH4, is capable of combining with rumen gas and is cheap to detect and analyze. The SF6 approach is one instrument that allows for longer-term field measurements, although it needs rumen boluses, daily animal handling, and laboratory gas analysis (McGinn *et al*., 2006). Furthermore, the sample processes produce an average CH_4 output for periods of generally 24 hours, but they can be repeated for up to 10 days, or until the rate of SF6 release from the permeation tube becomes unstable. While the reproducibility of daily CH_4 output is improving as the technology is enhanced (Deighton *et al*., 2013), SF6 remains a difficult method for obtaining precise emission measurements in individual animals over many days. The accuracy of the release rate calculation is necessary to determine CH_4, and tubes with high release rates represent higher CH_4 emission recordings, resulting in a high error rate (Storm *et al*., 2012).

Sniffer Method

Around 2010, the sniffer method for determining CH_4 concentrations (ppm) in individual cows was first detailed (Madsen *et al*. 2010). The early sniffer devices were designed to automatically screen livestock without interrupting animal behaviour or farm management. In contrast to other methods (Green Feed automated emissions monitoring (GF) system 1,200 2,250 L/min, respiration chambers e.g. 3,000 L/min), this method does not use active airflow and instead measures only gas concentrations in front of the cow's muzzle (in general with passive pumping of air to the sensor at 14 L/min). As a result, the sniffer system captures a small percentage of gases, and readings are impacted by external factors such as wind, breathing rate, and head movement. Researchers have explored using recovery rates (Garnsworthy *et al*. 2012a) or anticipated CO_2 production (g/d; as a tracer gas) based on other animal parameters such as weight, milk output, and feed intake to estimate CH_4 production (g/d) (Madsen *et al*. 2010). The majority of studies have used a sniffer system in an AMS feed bin at both research and commercial farms (Garnsworthy *et al*. 2012b; Lassen *et al*. 2012; Bell *et al*. 2014b; Difford *et al*. 2016, Bell *et al*. 2014a; Lassen *et al*. 2016; Pszczola *et al*. 2017; van Engelen *et al*. 2018). It works best when there is already equipment on the farm that can identify animals, allowing the sniffer to be installed.

Green Feed Machine

It is a CH_4 analyzer and automatic feeding system that helps to measure daily CH_4 and CO_2 mass fluxes from individual cow’s breath and eructation gas to rank them based on their daily measurements (Zimmerman *et al*., 2011; Hristov *et al*., 2015). While the animal is eating, automatic feeding systems can read ear tags or collars and record measurements for all cows. This approach can be used in traditional tie-stalls as well as for grazing animals fed supplements through feeders. This system has a limitation in that it can only measure CH_4 when the animal's head is in the feeder (Storm *et al*., 2012).

Using Proxies and Prediction Equations for Measurement

Proxies, such as feed consumption, milk production and composition, and faeces, can also be used to estimate CH_4. There is growing interest in using proxies as indicators of CH_4, such as milk fatty acid measurements, milk mid-infrared (MIR) spectroscopy, and rumen metabolites (Dehareng *et al*., 2012; Vanlierde *et al*., 2015; Negussie *et al*., 2017). Aside from these, prediction equations can also predict CH_4. Blaxter and Clapperton (1965) proposed a primary prediction for CH_4 measurement based on gross energy intake, apparent digestibility of dietary energy at maintenance (percent), and level of energy intake relative to that required for maintenance. However, due to significant changes in the genetic merit of the animals, which would necessitate new validation studies, this prediction equation is no longer used. Other prediction equations are also available, with the most accurate ones including dry matter intake, metabolizable energy intake, acid detergent fibre intake, and lignin intake (Pires Sobrinho *et al*., 2019). To summarize, there are several methods for measuring CH_4, each with its own set of advantages and disadvantages. As a result, before selecting an optimal method to use in future studies based on experimental design and strategies, careful consideration is required. Similarly, when examining individual animal CH_4 emissions, it is important to consider not only the amount of CH_4 produced but also the amount of feed consumed and milk/meat produced, as this can indicate animal efficiency.

Genetic Strategies to Reduce Methane Emission

Many methane emission reduction initiatives directly target methanogenic populations or methane itself. Methanogenesis inhibitors and treatments that directly influence methanogens or methane removal by methanotrophic bacteria are examples of these (Liu *et al*., 2017). Inhibitory substances, such as structural analogues (Yu *et al*., 2000; Wagner *et al*., 2017; Duval *et al*., 2018) or algae supplementation (Burreson *et al*., 1976; Roque *et al*., 1976; Machado *et al*., 2016), have been employed to block one or more enzymatic steps of the methanogenesis pathway. However, due to adaptation and resistance mechanisms, the impact of these substances is generally temporary (Knight *et al*., 2011; Ungerfeld *et al*., 2004). When it comes to methane mitigation techniques, understanding heritability, repeatability, and correlation is crucial. Methane production in ruminants is heritable, ranging from 0.12 to 0.52 (de Haas *et al*., 2011; Kandel *et al*., 2012; Pickering *et al*., 2015a; Brito *et al*., 2018), indicating that selective breeding can make genetic progress. Reducing CH_4 by improving the genetic merit of the herd is a cost-effective strategy to reduce CH_4, though measuring CH_4 remains difficult under practical conditions (de Haas *et al*., 2011, 2017). Based on these considerations, several mitigating measures are discussed below and represented in Figure 3, which shows various methane mitigation strategies.

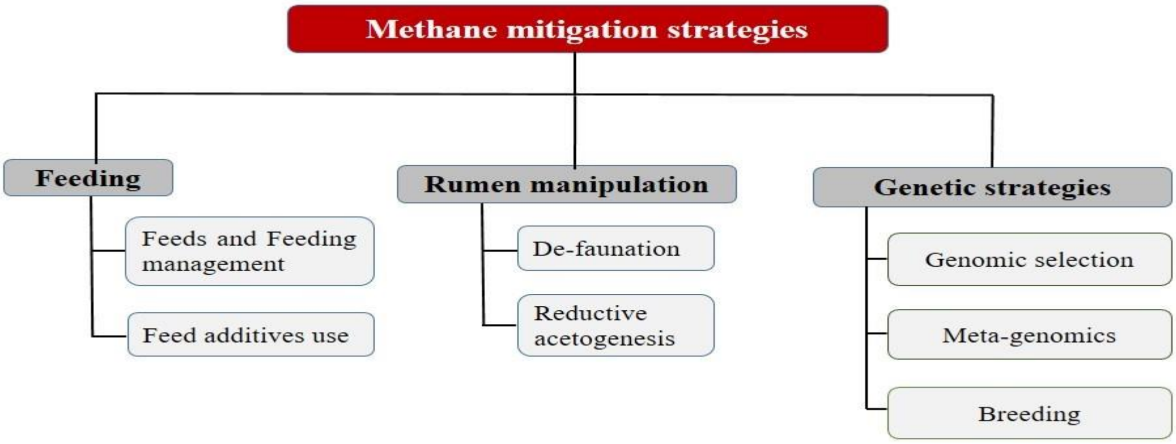


Figure 3: Shows various methane mitigation strategies

Breeding to Reduce CH4 Emissions from Livestock

Although measuring CH_4 remains difficult under realistic conditions, reducing CH_4 by improving the genetic quality of the herd is a cost-effective way to reduce CH_4 (de Haas *et al*., 2011, 2017). Genetic selection is a proven method for achieving long-term and cumulative decreases in quantitative features such as intestinal CH_4 emissions. A more recent study on 530 cattle animals (Donoghue *et al*., 2013) and 1225 sheep (Pinares-Patio *et al*., 2011, Pinares-Patio *et al*., 2013) supports the idea that CH_4 qualities are heritable and can be improved through direct selection. Using the complete 24 h measurement, estimated heritability and repeatability of CH_4 over days, rounds, and years were 0.29 0.05 and 0.13 0.03 for gross CH_4 production (g/day) and MY (g /kg DMI), respectively (Pinares-Patio *et al*., 2013). There were a lot of repeatabilities between days. The estimates of repeatability were lower across rounds and years than for consecutive days, but they were generally steady. Estimation of phenotypic and genetic correlations with some of the most important New Zealand production traits, including weaning weight at 3 months, live weight at 8 months, fleece weight at 12 months (FW12), eye muscle depth, and dag score (accumulation of feces on the perineum region) at 3 and 8 months of age, show that correlations with methane yield (MY) are low or close to zero, except for FW12. The negative genetic and phenotypic associations of FW12 with MY (0.32, 0.11, and 0.08, 0.03, respectively) suggest that selecting for increased hogget fleece weight will result in lower CH_4 output in part. Donoghue *et al*. (2013) found that the heritabilities of Australian Angus beef cattle were remarkably similar. Heritability estimations for gross CH_4 production (L/day) and MY (L/kg DMI) were 0.40, 0.11, and 0.19, 0.10, respectively, based on 530 pedigreed cattle fed at a proportion of maintenance (1.2). Eye muscle area and gross CH_4 output had genetic and phenotypic associations of 0.17, 0.29, and 0.01, 0.05, respectively. The genetic and phenotypic correlations for MY (Methane yield) were respectively 0.02, 0.30 and 0.03, 0.05. It is important to remember that phenotyping feed intake or DMI is difficult in commercial settings and is usually only done on experimental farms. In the long run, it may be able to incorporate genetic information into breeding schemes to estimate genomic breeding values (GEBVs) for CH_4 emissions (Meuwissen *et al*., 2013). To put GEBVs into practice, a large reference population of animals with the CH_4 phenotype accurately measured is required to provide preliminary estimates of each genomic region's contribution to the expression of the phenotype under investigation (Calus *et al*., 2013). Similarly, if directly detecting CH_4 on enough animals to form a reference population is impractical, the selection of GEBVs for linked indicator features can be used. Finally, for the CH_4 trait to be given the proper weighting in any breeding program, there must be an economic and/or societal incentive to breed animals with the trait that is included in the selection aim.

Genomic Selection for Reduced Methane Emissions in Ruminant

Genomic selection has been used to select for difficult-to-measure traits like hoof lesions (Dhakal *et al*., 2015) and fertility in Holstein cattle (Guarini *et al*., 2019), and mastitis in meat sheep (McLaren *et al*., 2018), and could be used to reduce CH_4 emissions (Hayes *et al*., 2013). To be successful, genomic selection needs a large training population (i.e., animals with both genotypic data and CH_4 measurements). Measuring methane from individual farm animals necessitates costly equipment and extensive animal handling. The only genomic selection appears to be a viable option for selecting low methane-generating seed stock for the beef cattle and sheep industries (Hayes *et al.*, 2016). Selecting cows that emit less methane necessitates knowledge of the most physiologically relevant methane emission phenotypes that are near the breeding aim and can be reached through genomic selection. Past research on dairy cows has indicated that CH_4 generation is heritable, allowing for mitigation measures based on selective breeding. Genome-wide association studies (GWAS) is a supplemental method for discovering key genomic areas and candidate genes linked to phenotypes of interest. For example, a GWAS for CH_4 yield and gross CH_4 in sheep identified two significant markers containing the candidate genes Tetraspanin 14 (*TSPAN14*), (Jostins *et al*., 2012), and Peroxisomal Biogenesis Factor 2 (*PEX2*) that have been significantly associated with inflammatory bowel disease in human and this PEX2 is thought to be associated in lipid metabolism and fatty acid oxidation in cattle (Rowe *et al*., 2014). According to Rowe *et al*. (2014), rumen fermentation involving fatty acid metabolism and the complex microbial community of the human colon are both good physiological candidates for CH_4 yield. As a result, information can be incorporated into genomic prediction approaches using systems biology and GWAS to improve CH_4 selection strategies (Saleem *et al*., 2013; Ursell *et al*., 2014; Cánovas, 2016). Manzanilla-Pech *et al*., (2016) performed a genome-wide association study GWAS on direct measurements of CH_4 production in beef cattle, with validation in dairy cattle. Pszczola *et al*. (2018) carried out the first two GWAS on direct measurements of daily CH_4 production in dairy cattle (2018) and they used heritable variation to investigate the genetic architecture of CH_4 production and discovered genomic regions affecting CH_4 production. Potential QTL regions affecting CH_4 production were discovered in traits related to feed efficiency, milk-related traits, health status and body size. Five candidate genes were discovered to be involved in a variety of metabolic processes that are likely related to CH_4 synthesis on *CYP51A1, BTA 4, PPP1R16B* on BTA 13, and *NTHL1, TSC2*, and *PKD1* on BTA 25. One of the most promising candidate genes was linked to digestive system development (*PKD1*). The findings imply that CH_4 synthesis is a polygenic trait. Sarghale (2020) discovered that SNPs in GWAS were related to QTLs for milk yield, body weight, and RFI. For PME traits the existence of olfactory receptor activity genes was associated with feed intake and preferences, as well as high correlations of these traits with PME traits and overlap of our identified genomic regions with previously reported QTLs, which could confirm that these SNPs can be good candidates for methane emission. Findings have shown that MAS and genomic selection may be useful in improving difficult-to-measure traits like PME. Furthermore, using VFA indicators to forecast methane emissions could help expand the size of the reference population needed for GWAS and genetic selection (Sarghale *et al*., 2020). Alemu *et al*. (2011) found a substantial association between acetic and propionic acids (0.85). Based on the chromosome-wide Bonferroni correction threshold, Shargale (2020) discovered two SNPs significantly linked with isovaleric acid (BTA9 and 28) and 29 SNPs significantly connected with valeric acid (BTA5, 11, and 25). Isoacids (e.g., 2-methyl butyric, isobutyric, isovaleric acid, and straight-chain valeric acid) are primarily formed during the digestion in ruminants by the degradation products of the amino acids' valine, isoleucine, leucine, and proline (Andries *et al*., 1987). Because of the small number of CH_4 records in genotyped animals, research into this phenotype has been limited (de Haas *et al*., 2011, 2017; Hayes *et al*., 2013, Hayes *et al*., 2016, Rowe *et al*., 2014).

Defining phenotypes and biological indicators of CH4 poses a significant challenge. They must be independent of other physiological and nutritional factors, easily measurable, and ideally obtained at a low cost for breeders (Rowe *et al*., 2014). A moderate genetic correlation (0.72) has been found between residual feed intake (RFI) and predicted CH4 emissions (PME) (de Haas *et al*., 2011). This underscores the importance of studying feed efficiency (FE) alongside CH4 traits. RFI heritability varies from low to moderate in beef (0.07 to 0.62) (Berry and Crowley, 2013) and dairy cattle (0.04 to 0.36) (Jensen *et al*., 1995; Hurley *et al*., 2016). This presents an opportunity for genetic enhancement in RFI in conjunction with CH4 reduction efforts (Berry and Crowley, 2013). Thus, the cows with lower RFI have lower PME. Hence, it is possible to decrease the methane production of a cow by selecting more efficient cows.

Meta-Genomics to Mitigate Methane Emissions in Ruminants

The use of metagenomic and metabolomic approaches to understand variation in microbial genomes and microbial profiles has provided important insights into the contribution of rumen microbial structure and metabolite profiles, as well as the regulation of complex phenotypes such as FE (Asselstine *et al*., 2021), CH_4, and nitrogen retention (Attwood *et al*., 2008; Bath *et al*., 2013; Artegoitia *et al*., 2017; Goldansaz *et al*., 2017; Meale *et al*., 2017). Metagenomics enables the identification of the entire microbial community's makeup as well as the number of their genes. It could be used to provide novel selection criteria for hard-to-measure features or to better understand the relationship between host genetics, the microbiome, and the microbiome's activities. Rumen metagenomic profiling has been used in recent years to look for correlations between methane emission and microbial genes. It has previously been hypothesised that archaeal abundance in ruminal digesta is genetically controlled and can be used to genetically select animals without directly measuring methane. The host has an additive genetic influence on its microbiome, and the ideal host can be chosen based on the abundance of a set of genes associated with the attribute in the ruminal metagenome. Individual or collective gene abundances can now be used as proxies for methane emissions in genetic screening studies. In 2018, the first research on potential connections between host-microbial profiles and CH_4 was conducted in cattle using metagenomics to characterize microbiomes (Difford *et al*., 2018). Metagenomics has since been used to investigate a variety of aspects of rumen microbiology, including methane emissions in cattle (Wallace *et al*., 2015) and sheep (Shi *et al*., 2014), biomarkers to predict ruminal methanogenesis (Auffret *et al*., 2017), the effect of feed-conversion-ratio, and breed and host genetics on the composition of the rumen microbiome (Roehe *et al*., 2016). The rumen remains a valuable source of bioactive agents for the biotechnology industry, and metagenomics is a key tool for bioprospecting (Oyama *et al*., 2017; Roumpeka *et al*., 2017). Experiments in rodents reveal a genetically encoded host-driven modulation of the gut microbiota (Benson *et al*., 2010; Nguyen *et al*., 2015). According to human research, the abundance of microbial taxa, particularly the Christensenellaceae family, which create a co-occurrence network with other bacteria and methanogenic archaea and affects metabolism, has a host genetic influence on the rumen microbiota (Goodrich *et al*., 2014). Using metagenomics, Roehe (2016) discovered that the genes related to methanogenesis (e.g. *mcrA* and *fmdB*) were linked to methane emissions, while host-microbiome cross-talk genes (e.g. *FucI* and *TSTA3*) were linked to feed conversion efficiency. They also discovered that based on methane production or relative archaeal richness the ranking of sire progeny groups was consistent overall and within the diet. Lower emissions were related to higher Succino-vibrionaceae abundance as well as alterations in acetate and hydrogen synthesis, resulting in reduced methanogenesis, according to comparative metagenomics. The discovery that the metabolism of pyruvate and acetate differs between low and high emitters, as well as the number of Succini-vibrionaceae, provide insight into how metabolic pathways and the microbial community can be manipulated to reduce methane emissions and thus the environmental footprint of ruminant livestock production (Wallace *et al*., 2015).

Microbial profiles, which are linked to methane emissions and feed consumption, are heritable (Rowe *et al*., 2020). Seshadri *et al.* (2018) identified 410 cultured archaea and bacteria species, expanding our understanding of rumen microbes. Studies are increasingly focusing on host-microbiome metagenomic sequencing and microbial genomic sequencing of various sample types, including the rumen microbiome (Kim *et al*., 2017; Stewart *et al*., 2018; Martínez-Álvaro *et al*., 2020).

For instance, Xue *et al*. (2020) found that high milk protein cows have a lower relative abundance of organisms with methanogen and methanogenesis functions in their rumen microbiome, suggesting reduced CH4 production. A recent study across Europe identified 39 heritable microorganisms, seven of which were strongly associated with methane production, including members from different genera and orders along with protozoal species.

Exploring the biological nature of these heritability associations to host genetics and methane emissions is of great interest. These heritable microorganisms appear more interconnected in interaction networks, indicating a potential pleiotropic effect on microbiome composition that could lower methane emissions if manipulated through breeding programs. Bacteria and archaea collectively contribute to 13% of CH4 production, while host genetics (heritability) account for 21%, largely independently. Given the independence of cow genetic effects on the rumen microbiome from CH4 emission variation, breeding low methane-emitting cows and researching strategies to combat rumen microbiome changes are viable approaches to reducing CH4 emissions in the livestock industry (Difford *et al*., 2018).

To improve production efficiency, genomic information from integrative "-omics" data and systems biology analysis on desirable traits can be incorporated into breeding strategies (Cánovas, 2016; Suravajhala *et al*., 2016; Fleming *et al*., 2018). Integrated "omics" data and systems biology information can then be disseminated and applied to industry to reveal more biological and genetic information to improve understanding of complex and difficult-to-measure performance traits. This will result in improved selection accuracy, a shorter generation interval, and a faster rate of genetic improvement of difficult-to-measure traits (Cánovas, 2016; Suravajhala *et al*., 2016). Overall, this could lead to increased ruminant production industry competitiveness by improving economically and environmentally important traits like CH_4 using integrated "-omics" technologies and a systems biology approach. Integrative approaches are currently being used to uncover information associated with different levels of omics, and more research into multi-level analysis, such as integrating the genome, transcriptome, and metabolome, is required. Because the genome and transcriptome directly affect metabolite levels resulting in changes to metabolite profiles, evaluating these levels as a system is critical, as specific metabolites are greatly affected by specific genetic features, and approaches to connect genomics with metabolomics are emerging. The metabolite genome-wide association study (mGWAS) uses GWAS to identify QTLs and significant SNPs associated with a metabolite are included under integrative analysis. This enables the discovery of SNP-Metabolite trait associations, also known as mQTL (Wang and Kadarmideen, 2020). Table 2, is showing the various methane reduction strategies with their advantages and limitations.

Table 2: Various strategies with their advantages and limitations

Strategy	Advantages	Limitations	References
Animal breeding for low-CH_4 production	A cost-effective strategy to decrease CH_4 produces permanent changes	Need robust ways of measuring CH_4 in large numbers of individual animals. Relationships between CH_4 production and economically important traits are unknown. Need to know the long-term persistency of different diets and their effects on animal health	Pickering *et al*. (2015b); Løvendahl *et al*. (2018)
Animal breeding for feed efficiency and residual feed intake	Changes produced are permanent and cumulative	The existence of genotype × environment interactions need to be determined. Relationship to productivity-related traits at pasture unknown. Lack of information on the biological regulation of the trait	Basarab *et al*., (2013); Kenny *et al*., (2018)
Genomic selection for reduced methane emissions in ruminants	Can select for difficult-to-measure traits	Genomic selection requires a sizeable training population (i.e., animals that have both genotypic data and measurements for CH4) to be successfully implemented, which trait to select	deHaas *et al*., (2011); de Haas *et al*., (2017)
Metagenomics	Provide insight into the associations between the host-microbiome interactions and host production efficiency, overall health, and nutrient metabolism.	lack of sequence and culture information on specific rumen microbes for reference databases	Roehe *et al*., (2016)

Machine Learning

In addition to the approaches described above, machine learning algorithms have been used for clustering analysis. This includes multivariate analyses like Random Forest, a supervised machine learning algorithm that works in conjunction with decision trees to classify specific features (i.e., clustering multiple metabolites based on commonalities). Melzer *et al*. (2013) used Random Forest and Partial Least Squares (PLS) to find correlations between milk characteristics and metabolite profiles. Genetic correlations of metabolite profiles with traits such as CH_4 emissions can be performed to further integrate this approach with other -omics data. Other machine learning approaches, such as penalized regression, support vector machine, Random Forest, and artificial neural networks, have been used in studies aimed at identifying relationships between the microbiome and host phenotypes to integrate rumen microbiome features (Namkung, 2020). Even though these analyses are already used in human studies, there is a lack of studies that use these approaches to first integrate -omics levels in livestock research, and then to use the latter to better understand CH_4 emissions at a system level.

Conclusion

There is great potential for genetic selection to reduce methane emissions from ruminants sustainably and efficiently, providing a long-term solution to the environmental issues related to livestock production.

The development of cost-effective methods to reduce CH_4 emissions has risen to the top of the global priority list, and this can be accomplished by improving breeding strategies for more economically and environmentally efficient animals. Most current strategies are also clearly better suited to intensive animal production systems, with far fewer available for mixed crop-livestock feeding systems. The CH_4 mitigation strategy should be cost-effective while also addressing socioeconomic issues in Indian feeding and management conditions, where most farmers use traditional feeding practices with locally available feed resources. Dietary management protocols combined with breeding programmes- genetic as well as genomic selection may be more effective to achieving lifelong success since both are heritable and repeatable.

Large-scale phenotyping initiatives combined with the adoption of genomic selection tools and technology can quicken the genetic progress toward low-emission ruminant production, increasing the effectiveness and efficiency of breeding programs.

Genetic strategies for methane mitigation should be complemented with other management practices, including dietary interventions, improved feeding regimes, and enhanced animal husbandry practices, to achieve synergistic effects and maximize methane reduction potential.

More research is required to develop strategies for CH_4 mitigation as well as improved animal performance, which should include a mitigation strategy for extensive and zero grazing systems integrated with genetic and genomic selection to target the reduction of enteric CH_4 emissions feasible.

Genetic techniques provide a possible route to achieve considerable reductions in methane emissions from ruminant production systems, supporting global efforts to mitigate climate change and promote environmental sustainability by combining the most recent scientific discoveries and insights.

Contribution by Authors

Equal contribution. All authors declared that ‘written informed’ consent was obtained from the approved parties for the publication of this article and accompanying images.

Conflict of Interests

There is no conflict of interest.

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