Recent Advances in Measurement and Dietary Mitigation of Enteric Methane Emissions in Ruminants

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Methane (\(\text{CH}_4\)) emission, which is mainly produced during normal fermentation of feeds by the rumen microorganisms, represents a major contributor to the greenhouse gas (GHG) emissions. Several enteric \(\text{CH}_4\) mitigation technologies have been explored recently. A number of new techniques have also been developed and existing techniques have been improved in order to evaluate \(\text{CH}_4\) mitigation technologies and prepare an inventory of GHG emissions precisely. The aim of this review is to discuss different \(\text{CH}_4\) measuring and mitigation technologies, which have been recently developed. Respiration chamber technique is still considered as a gold standard technique due to its greater precision and reproducibility in \(\text{CH}_4\) measurements. With the adoption of recent recommendations for improving the technique, the \(\text{SF}_6\) method can be used with a high level of precision similar to the chamber technique. Short-term measurement techniques of \(\text{CH}_4\) measurements generally invite considerable within- and between-animal variations. Among the short-term measuring techniques, Greenfeed and methane hood systems are likely more suitable for evaluation of \(\text{CH}_4\) mitigation studies, if measurements could be obtained at different times of the day relative to the diurnal cycle of the \(\text{CH}_4\) production. Carbon dioxide and \(\text{CH}_4\) ratio, sniffer, and other short-term breath analysis techniques are more suitable for on farm screening of large number of animals to generate the data of low \(\text{CH}_4\)-producing animals for genetic selection purposes. Different indirect measuring techniques are also investigated in recent years. Several new dietary \(\text{CH}_4\) mitigation technologies have been explored, but only a few of them are practical and cost-effective. Future research should be directed toward both the medium- and long-term mitigation strategies, which could be utilized on farms to accomplish substantial reductions of \(\text{CH}_4\) emissions and to profitably reduce carbon footprint of livestock production systems. This review presents recent developments and critical analysis on different measurements and dietary mitigation of enteric \(\text{CH}_4\) emissions technologies.

Keywords: methane, ruminants, measurement method, mitigation technology, diet

INTRODUCTION

Greenhouse gas (GHG) emissions, largely methane (\(\text{CH}_4\)) from the rumen and nitrous oxide from manure management, from livestock contribute considerably to the atmospheric GHG (1, 2). \(\text{CH}_4\) is normally produced during microbial fermentation of feeds, mainly structural carbohydrates, in the rumen by methanogenic archaea. Globally, about 95 million tones of \(\text{CH}_4\) are emitted from...
enteric fermentation of domestic animals in 2010 with an annual growth rate of 0.90% (2). Enteric \( \text{CH}_4 \) contributes 17 and 3.3% of global \( \text{CH}_4 \) and GHG emissions, respectively, which mostly arises from ruminant livestock (3). The contribution of GHG from livestock is expected to grow due to increasing populations of livestock animals triggered by an increasing demand of animal protein, especially in developing countries. Abatement of enteric \( \text{CH}_4 \) emission is required to minimize the liability of livestock production for GHG emission. Mitigation strategies of enteric \( \text{CH}_4 \) are considered to be less expensive than carbon dioxide (\( \text{CO}_2 \)) emissions (3, 4). Inhibition of \( \text{CH}_4 \) emission by some technologies generally does not cause much detrimental effects on rumen fermentation, but may improve rumen fermentation efficiency. Sometimes, \( \text{CH}_4 \) mitigation options are associated with improved efficiency of animal production, which is advantageous both environmentally and nutritionally. Several comprehensive reviews have been published recently, which describe a number of options and strategies to mitigate \( \text{GHG} \) from livestock production (3, 5–7). Generally, these review papers on \( \text{CH}_4 \) mitigation technologies have been focused on research conducted during the past 10–15 years.

An accurate, cost-effective and repeatable measurement technique of enteric \( \text{CH}_4 \) production from ruminants is required to evaluate a \( \text{CH}_4 \) mitigation technology, preparation of inventory of \( \text{CH}_4 \) gas emissions and assessment of carbon footprint of livestock products. Therefore, it has been an important area of research to develop new techniques, to improve accuracy of the existing methods and to compare among the methods of \( \text{CH}_4 \) measurements in recent years. A number of methods are used to measure \( \text{CH}_4 \) emission from ruminants, all of which differ in their application, cost, accuracy, precision, and repeatability depending on their conditions of use [e.g., Ref. (8–11)]. This paper primarily presents the latest advances in measurements and dietary mitigations of \( \text{CH}_4 \) emissions from ruminants.

**MEASUREMENT METHODS OF \( \text{CH}_4 \) EMISSIONS**

A number of methods have been developed and improved in recent years, which is employed for long-term and short-term measurements in individual animals or grouped animals directly as well as indirect prediction of \( \text{CH}_4 \) emissions (Figure 1). These methods are described here along with their advantages and disadvantages.

**Long-Term Measurement Methods**

**Respiratory Chamber Technique**

Respiration chamber (RC) technique was used for determining energy balance and gaseous exchange in animals for many years [e.g., Ref. (12, 13)]. The principle of this technique is to measure the concentrations of \( \text{CH}_4 \) (coming out through all avenues, i.e., mouth, nostrils, and rectum from enteric fermentation) in gas samples and total volume of air removed from the RC (10). An air pump continuously removes air from a RC through a flow meter in the open-circuit system to calculate volume of air removed. Outlet gas from the RC is continuously sampled for analysis through a duct system. The RC system is equipped with ventilation fans inside the chamber for proper mixing of expired gases and incoming air. Fresh air to the RC is directly drawn from outside or through an air conditioning system to control humidity and temperature. The RC is fitted with humidity, temperature, and barometric pressure meters to determine gas volume at standard temperature and pressure (STP) conditions (9).

The RC method for measurement of enteric \( \text{CH}_4 \) production is often considered as the “gold standard” technique due to high accuracy and repeatability, and low animal-to-animal variations of \( \text{CH}_4 \) measurement using this technique (14, 15). However, RC system is costly for establishment and labor intensive for operation. This method imposes restrictions on eating and other natural behaviors of the animals resulting in \( \text{CH}_4 \) production that

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**FIGURE 1** A schematic presentation of different \( \text{CH}_4 \) measurement techniques in ruminants using different approaches.
often differs from CH4 production in their normal environments. This technique also requires great technical expertise to generate accurate CH4 emission measurements. Their potential negative effects on feed intake and milk production of lactating animals while confined in the RC can be minimized with proper adaptation of the animals to the system, but must also be considered. Moreover, RC method has limited “throughput,” and thus is less suitable when CH4 measurements on large numbers of animals are required, such as screening of low CH4-producing animals for genetic selection.

The RC technique can be highly accurate and precise when used with rigor and it measures total CH4 emission, including losses from anus and rumen fistulas. This method has additional advantage of measurement of gas production or consumption of other gases (e.g., oxygen, CO2, hydrogen, ammonia). In RC, the design may also allow to investigate other nutritional evaluations, such as digestibility, nitrogen balance, and energy metabolism. The RC method can detect relatively small effects of diets and supplements on CH4 emission determined on a small number of animals (9). With repeated measurements over the course of daily CH4 production patterns, RC technique can be employed to characterize diurnal CH4 emission variations, which may provide insight into underlying mechanisms of enteric CH4 formation, including relationships with H2 production when antimethanogenic compounds are used. Despite the better accuracy and many nutritional advantages in the RC method, this method could invite significant errors in CH4 measurement unless proper calibration procedures are regularly followed. For example, in a recent ring test of calibration of RC in different laboratories, ducting system resulted in 15.3% variations, followed by errors in mixing of air in the RC by 3.4% and analyzer by 1.3% variations (16). In conclusion, although RC technique is considered as “gold standard” in determining CH4 emission due to high accuracy and low animal and day variation of measurements, low throughput, high cost, imposing restriction on natural animal behavior, and greater technical requirements for operation limit this technique for widespread use.

Sulfur Hexafluoride Tracer Technique

The sulfur hexafluoride (SF6) tracer technique was developed by Zimmerman (17) and was first used for measurement of CH4 in grazing cattle by Johnson et al. (18). The SF6 method has been used extensively during the last two decades for measurement of CH4 emissions from ruminants. The principle behind this method is that CH4 production can be measured if SF6 gas production rate from the rumen is known (18). Small permeation tubes are filled with SF6, and are then placed into the rumen of animals. Test animals are fitted with gas sampling apparatus, which consists of a halter to support capillary tubing whose inlets to be placed close to the nose and an evacuated canister to collect gas samples (10). Representative gas samples containing respired and eructated gas are collected usually for 24 h through capillary tubing connected to an evacuated canister. The tubing regulates the gas sampling rate (19). The concentrations of SF6 and CH4 in the gas samples collected in canister are analyzed by gas chromatography. The CH4 emission is calculated from the SF6 release rate and concentrations of SF6 in the canister gas samples in excess of background concentrations in the air using the following equation (18).

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\text{CH}_4 (\text{g} / \text{day}) = \text{SF}_6 (\text{g} / \text{day}) \times \left[ \frac{[\text{CH}_4]_c - [\text{CH}_4]_b}{[\text{SF}_6]_c - [\text{SF}_6]_b} \right]
\]

where [CH4]c and [SF6]c are the concentrations of CH4 and SF6 in the canister, respectively; while [CH4]b and [SF6]b are the CH4 and SF6 concentrations in the background air, respectively.

Unlike the RC method, the SF6 method can be employed on large numbers of animals concurrently to measure CH4 emissions from both grazing and non-grazing animals in comparatively less time and cost. Also, SF6 method can impose lesser effect on animal behaviors under typical animal management conditions (18). However, it is labor intensive and requires great technical expertise to minimize experimental errors of measuring CH4. There are concerns over the variability and repeatability of measurements (9, 20). In addition, the long-term instability of release rate of the permeation tubes remains a concern for use in studies with long period of experiments (21, 22), and background atmospheric gas concentrations can impact markedly on the success of the SF6 technique (23). Thus, the day-to-day and animal-to-animal variations in CH4 emission estimates are greater with the SF6 technique compared with the estimates derived from RC (20, 24, 25). These greater variations would induce a negative impact on the power of statistical analyses using this technique as it will require more animals (replicates) to detect treatment differences in evaluation of CH4 mitigation studies (9). The CH4 emissions measured by SF6 technique were similar to the values quantified by RC and ventilated head hood systems in some studies (14, 18, 26), but were different in other studies (14, 25, 27). Johnson et al. (18) observed that CH4 emission using the SF6 technique agreed with CH4 emission using RC technique in grazing cattle (7.3 versus 7.2% of gross energy intake). In subsequent studies (28, 29), slightly lower emissions (5–10%) were observed with the SF6 method than with the RC method for both cattle and sheep, which can partly be due to the few percent of CH4 lost via rectum. By contrast, higher values of CH4 emission were observed using the SF6 technique than RC technique (20, 27, 30). Muñoz et al. (25) also noted significantly higher mean values of CH4 emissions (gram/day) and CH4 yield (gram/kilogram DM intake) for the SF6 technique than for the RC method (443 versus 396 g/day and 26.7 versus 24.2 g/kg DM intake). Although average CH4 emissions may not be different in some studies, within- and between-animal coefficients of variations were much greater for the SF6 technique than for the RC method (14, 20, 31). Pinares-Patiño et al. (20) utilized the same animals for measurement of CH4 using with the SF6 and RC technique and reported that the within coefficients of variations were 4.7, 13.5, and 11.7% in RC technique, SF6 technique, and with SF6 technique performed inside RC, respectively. The between-animal variations were also considerably higher with the SF6 technique than with the RC technique.

A number of factors, such as release rate of SF6 from permeation tubes, influence of SF6 release rate on CH4 emission rate, measurements of background concentrations of SF6, flow rate in the tubing during sample gas collection, inconsistency of CH4 measurements using RC and SF6 techniques, and within- and
between-animal variations, have been implicated for low accuracy in CH₄ emission measurements in the SF₆ technique (9, 22, 32). A considerable research effort has been undertaken to improve the accuracy, precision, and consistency of the results in the SF₆ technique in different studies [e.g., Ref. (22, 23, 33)]. The determination of SF₆ release rate over time from tubes is important, which affects emission estimates (19, 34). Permeation tubes that release SF₆ in greater rates may result in higher CH₄ production compared with the tubes with lower release rates (34). Hence, permeation tubes with similar SF₆ release rate are recommended to obtain better accuracy, especially, for the comparison of different mitigation treatments (34). Permeation tubes are regularly weighed over 1 month under laboratory conditions to obtain the release rates of SF₆ (9). Only highly linear permeation tubes are used for measuring experiments (19). The SF₆ release rates from permeation tubes are calculated applying zero-order kinetics (19). However, Lassey et al. (19) demonstrated that the SF₆ release rates from permeation tubes kept at 39°C in a dry laboratory incubator decreased with time. Thus, the application of zero-order kinetics to predict the SF₆ release rate from permeation tubes invites a considerable error in measurement of enteric CH₄ outputs, particularly when an experiment continues more than 30 days after the calibration of permeation tubes (22, 35). The inconsistency in measurements of CH₄ emissions using the RC and SF₆ techniques increased substantially when the permeation tubes were kept in the rumen for long time (21). Researchers have shown that the curves of the rate of SF₆ release from permeation tubes are curvilinear under laboratory conditions (22, 27). Therefore, Lassey et al. (19) suggested the use of quadratic equations to predict the rate of SF₆ release from permeation tubes with time. This approach was suggested to be certainly much better than using zero-order kinetics (22). Recently, Moate et al. (22) demonstrated that the use of Michaelis–Menten kinetics can more accurately describe the SF₆ release rate pattern from permeation tubes and this may improve the accuracy of the measurement of CH₄ emissions from ruminants. Using this kinetics, measurement of CH₄ emissions can be continued for up to 800 days after insertion of the tubes into the rumen compared with the typical period of 60–90 days. Another important factor is the design of capillary-tube flow rate restrictors that can also cause appreciable errors in measurement of CH₄ emissions of up to 15.6% (33). Orifice plate flow restrictors can control the gas sample collection rate into canisters and lower errors in measurement of CH₄ production (33). The SF₆ technique using these modifications resulted in measurements of CH₄ emission to be greatly accurate with measurements taken using RC (33). In this experiment, mean CH₄ yield (gram/kilogram DM intake) was 21.9 ± 1.65 and 22.3 ± 1.44 in lactating dairy cows when measured by the RC and SF₆ technique, respectively; and the between-animal coefficients of variation were 7.5 and 6.5% using the RC and SF₆ technique, respectively.

The background SF₆ and CH₄ concentrations should be corrected, but determination of representative background concentrations of these gases under field conditions may be difficult because direction and velocity of wind and other animals in the vicinity may influence the background concentrations (23). Besides, the SF₆ release from permeation tubes may affect the background concentration of SF₆ to which an adjacent animal is exposed (23). For this reason, Williams et al. (23) emphasized that recording of improper background concentrations of SF₆ can affect the extent of CH₄ emission measurements. From these observations, Lassey (36) concluded that the relationship between the estimated CH₄ emission rates and the SF₆ release rates from permeation tubes was merely an artifact as a result of inappropriate background SF₆ concentrations. Pinares-Patiño et al. (34) suggested that this concern could be ameliorated to a certain degree by using all permeation tubes with similar rate of SF₆ release in an experiment. In summary, the SF₆ method can be employed without imposing much effect on natural behavior of grazing animals and has a greater throughput, but it could result in greater variations in CH₄ emissions compared with the RC method. Nevertheless, the SF₆ method can be used with a high level of precision when recent recommendations of this technique are followed for measurement of CH₄ emissions.

**Ventilated Hood Chambers or Head Box System**

This system also can be used for measurement of daily CH₄ emissions using the same principles as the RC method [e.g., Ref. (37–40)]. In this technique, a box or hood is fabricated to accommodate the head of animals, and air samples drawn through the hoods are analyzed for CH₄ concentrations in incoming and exhaust air (8). Unlike RC, this method does not quantify CH₄ arising from the hindgut. Airflow is measured and used to calculate CH₄ emission. Head chambers are typically large enough to allow the animal to move its head in an unrestricted manner and obtain feed and water. Like RC, they can be used to obtain continuous measurements over successive 24-h periods. Ventilated head hoods with different designs and sizes for small and large animals have been installed in different countries for nutritional studies [e.g., Ref. (37–40)]. The flow rate of exhaust air is important for the accuracy of gas analysis and comfort of the animals in head box (37). This system could also be employed for nutritional evaluation and energy metabolism of feeds [e.g., Ref. (40)]. Boadi et al. (26) compared ventilated head hoods with the SF₆ technique. The average daily CH₄ production was similar for both the methods (130 and 137 L/day for head hood and SF₆ method, respectively). Animal-to-animal variation was, however, significant with the SF₆ method (11.7%), but not with the head hood technique (0.1%) for production. Troy et al. (41) obtained simultaneous measurements of CH₄ outputs from cattle using RC and feeder-mounted hoods located within RC. It was reported that increases in concentrations of CH₄ in hoods over RC background were positively correlated ($r = 0.67$) with daily CH₄ emissions, but there was substantial variability. This technique measures CH₄ emissions reliably similar to the RC method and involves low cost compared with RC, but animals are required an extensive training to become adapted to the head hood, which restricts its extensive use for screening of large numbers of animals for genetic selection purpose.

**Short-Term Measurement Methods**

**Face Mask Method**

Face-masks for “spot-sampling” of respiratory exchange and CH₄ emission have been used in cattle, sheep, and goats for many years.
[e.g., Ref. (42)]. In this method, animals are needed to train to stay in sternal recumbency for the measurement periods (e.g., 30 min) repeated over the course of 24-h periods (8). This method presents a greater animal and day variation and only provides a short-term emission rate (43). The CH4 emission values using this technique are highly dependent upon the number and timing of respiratory exchange measurements taken with respect to diurnal patterns of feeding cycle and CH4 emission. However, if sufficient data are collected from several animals with greater regularity in sample collection throughout the measurement period of 24 h, a typical CH4 emission pattern can be calculated (11). Face mask can be useful for short-term measurements of CH4 emission rate for screening of large numbers of animals, but may cause marked discomfort and distress and change behaviors of the animals, and consequently, can affect the gas measurements.

Portable Accumulation Chamber
Portable accumulation chamber (PAC) system is essentially a RC without airflow. In this technique, PAC acts to trap all exhaled gases (CH4, CO2, and other gases), while oxygen depletes during the collection period of 1–2 h, and a single CH4 or other gas measurement is taken at the end of the collection period (44, 45). Emission of CH4 is calculated as the concentration of CH4 (corrected for background) multiplied by net chamber volume, adjusted for STP, divided by time of measurement (44). The time period of use should be restricted to avoid negative effects of increased chamber CO2 concentration, and accordingly the PAC is essentially a short-term respiration measurement. Moderate repeatability (correlation of 0.33–0.43) of measurements of CH4 emission by individual sheep using PAC was reported in studies at different sites (46). This technique could be useful for screening of low CH4-producing animals from large herds for genetic improvement purpose.

CH4/CO2 Ratio Technique
The CH4:CO2 ratio method, which was conceptualized by Madsen et al. (47), determines CH4 emission from individual animals based on the calculated CO2 emission and CH4 and CO2 concentrations measured using a gas analyzer. Their method relies on analyzing air samples for CH4 and CO2 simultaneously with a gas analyzer that is based on Fourier transform infrared (FTIR) detection, and uses CO2 from the breath of animals as the tracer gas. Emission of CO2 can be predicted based on estimates of energy metabolism, heat production, and respiratory quotient (RQ), or carbon balance (47).

$$\text{CH}_4 (g/d) = \text{CO}_2 \times (g/day) \times \left( \frac{[\text{CH}_4]_{\text{BS}} - [\text{CH}_4]_{\text{BG}}}{[\text{CO}_2]_{\text{BS}} - [\text{CO}_2]_{\text{BG}}} \right)$$

where $[\text{CH}_4]_{\text{BS}}$ and $[\text{CO}_2]_{\text{BS}}$ are the CH4 and CO2 concentrations in the breath samples, respectively; while $[\text{CH}_4]_{\text{BG}}$ and $[\text{CO}_2]_{\text{BG}}$ are the CH4 and CO2 concentrations in the background air, respectively.

The repeatability of the CH4:CO2 ratio was reported to be 0.39 for Holsteins and 0.34 for Jerseys (48). Hellwing et al. (49) compared predicted CH4 emissions in lactating dairy cows using the CH4:CO2 ratio technique with CH4 emissions in the RC method and reported a positive relationship ($r = 0.55$) between the two methods, but the CH4:CO2 ratio technique significantly underestimated the CH4 production (412 versus 345 g/day). This difference may be due to an error in the prediction of within-day variation in CO2 emission, which needs to be improved to obtain better individual animal CH4 emission estimates (49). Several factors, such as diurnal variations in the CH4:CO2 ratio resulted from differences in digestive and metabolic activity and rumen fermentation pattern associated with feed intake and feeding frequency, and source of gas samples (e.g., exhaled air, flatus and fermentation of manure or bedding) could affect CH4 emission measurement (47, 50). Therefore, adequate numbers of measurements in different times of the days should be considered to account for diurnal and postprandial variation in CH4 and CO2 emissions in animals. Nonetheless, this technique could be employed to generate large-scale data for genetic evaluation of CH4 production.

GreenFeed System
GreenFeed (GF) system (C-lock Inc., Rapid City, SD, USA) has recently been patented by Zimmerman (51) for measurement of CH4, CO2, and H2 production from animals. This system includes an automatic baiting system, measurements of air flow and gas concentration systems, electronics and communication devices, a gas tracer device, and an animal detection system during visit of an animal to the unit. A detailed description and visualization of the system is provided in Hristov et al. (52). In GF system, CH4 emission is measured for a short period when animals visit to the system to consume feeds that are used as enticement. The concept of the GF system is that numerous short-term CH4 emission values from an individual animal measured in different times within a day for many days can be aggregated to estimate an average daily CH4 emission from the animal. Software function allows investigators to control the timing of feed availability and to allocate CH4 measurements across various times of the day. The animals entering an automatic feeding system are recognized and concentrations of CH4 are measured at that particular time. Air is constantly pumped out through the automatic feeding system to measure flow rate and thereby CH4 emission during feeding period. Daily CH4 emission is calculated using the same principle as in RC method, whereby CH4 emission rate is calculated using volumetric air flow rate adjusted to STP and corrected for capture rate, as detailed by Hultanan et al. (50):

$$\text{CH}_4 (L/min) = C_{p(i)} \times \left( \frac{[\text{CH}_4]_{\text{i}} - [\text{CH}_4]_{\text{BG}}}{F_{\text{air}(i)}} \right) / 10^3$$

where $C_{p(i)}$ is the fractional capture rate of air at time $i$; $[\text{CH}_4]_{\text{i}}$ and $[\text{CH}_4]_{\text{BG}}$ are the concentrations of captured gas (ppm) and background gas of CH4 (ppm), respectively, time $i$; and $F_{\text{air}(i)}$ is the volumetric air flow rate (L/min) measured on a dry-gas basis at time $i$.

The GF system was compared with the RC and SF5 techniques to assess the accuracy and suitability of the GF system for measurement and detection of treatment difference for CH4 emissions in different studies (53, 54). The mean value of CH4
The use of GF system requires a feed supplement, which treatments, due to higher within-day and within-animal variance using both RC and SF6 techniques, but were unable to detect using the SF6 technique and 17-day periods for the GF system to achieve the repeatability of 0.70 for CH4 yield (gram/kilogram DM intake). The repeatability did not increase after 4-day periods for the SF6 method (repeatability = 0.73), but increased for the GF method until 45-day periods (repeatability = 0.90), Hammond et al. (53) conducted three experiments (two experiments on indoor animals and one experiment on grazing animals) in which Holstein heifers were fed various diets to compare the GF system with the RC or SF6 method. Daily CH4 emissions (gram/day) and CH4 yield (gram/kilogram DM intake) were similar between the GF (198 g/day and 26.6 g/kg DM intake in experiment 1; and 208 g/day and 27.8 g/kg DM intake in experiment 2) and RC (218 g/day and 28.3 g/kg DM intake in experiment 1; and 209 g/day and 27.7 g/kg DM intake in experiment 2) methods in both indoor experiments. In experiment 3, CH4 emissions and yields determined using the SF6 technique were, however, greater than the values measured using the GF system during grazing (186 versus 164 g/day and 21.6 versus 18.8 g/kg DM intake). Moreover, CH4 production quantified by the GF technique was not concordant (r = 0.10) with CH4 production determined by the RC method, but was only in moderate agreement (r = 0.60) with CH4 production measured by the SF6 technique. Significant treatment and individual animal differences in CH4 emission were detected using both RC and SF6 techniques, but were unable to detect using the GF method (53). This was attributed to a limited number of measurements obtained with the GF system in grazing animals and the timing of the measurements relative to daily patterns of CH4 emission, highlighting the importance of obtaining sufficient numbers of observations using the GF system. However, Velasco et al. (56) reported that GF and RC methods produced similar CH4 emissions (209.7 versus 215.1 g CH4/day) and also CH4 yield (22.7 versus 23.7 g CH4/kg of DM intake).

In principle, this technique requires sufficient numbers of measurements over time to obtain accurate estimates of daily emission, and relies on animals voluntarily visiting the unit (56). Some animals may not visit the GF unit for sufficient times despite feed restriction (57). Compared with the RC and SF6 techniques, GF system requires more time and animals, when a study is planned for comparison of CH4 production among the treatments, due to higher within-day and within-animal variance (58). The use of GF system requires a feed supplement, which may also introduce between-day variation with respect to supplement consumption and interact with the actual treatments.

Sniffer Method
This technique was first conceptualized by Garnsworthy et al. (59). In this technique, a sampling inlet is placed in the feed manger of an automatic milking system to collect air eructed by cattle during milking (often called the “sniffer” technique). As described by Garnsworthy et al. (59), air in the manger is constantly sampled, analyzed, and logged at 1-s intervals using data loggers to measure CH4 and CO2 concentrations in close proximity to the muzzle of the animal. Information on eructation frequency and CH4 released per eructation are used to estimate CH4 emission rate by individual animal during milking. Garnsworthy et al. (59) reported a good relationship (r = 0.79) between the measurements of CH4 emission using the RC technique and CH4 emission rate using this method. By contrast, Huhtanen et al. (50) compared the measurements of eructated CH4 concentration with CH4 emissions determined using the GF system in lactating dairy cows in two experiments. They found between-cow coefficient of variation (11.0–17.6 versus 17.5–28.0%) was smaller for the GF system compared with the sniffer method. There was weak relationship (R2 = 0.09) between the CH4 measurements (gram/day) using GF system and concentrations of CH4 recorded by the sniffer method, which may be attributed to the inconsistent air-mixing conditions within the feeding troughs influenced by the geometry of feed troughs, muzzle movement, and muzzle position (50). Thus, further research is needed if this type of sniffer method could be employed for quantification of CH4 with some consistency.

Hand Laser CH4 Detector
The hand laser CH4 detector (LMD) technique (60, 61) measures exhaled CH4 concentrations in the air near the nose or mouth of an animal in normal environment. The data consist of a series of peaks representing the animal’s respiratory cycle. Only peaks reflecting the increase in CH4 concentrations due to exhalation or eructation are used in the analysis (61). As the measurements are made in the air close to the animal’s nostrils, and measurements may not be affected by head position unlike sniffer method of Garnsworthy et al. (59). In a study with dairy cattle, a relatively strong correlation between CH4 measurements using the LMD with those determined in the RC (r = 0.80) was noted (62). The LMD can also strongly detect periods of high-enteric CH4 concentration and avoid misclassifying periods of low-enteric CH4 concentration (60). However, in a subsequent study, weak relationships (r = 0.22–0.28) between RC and LMD methods in CH4 measurements (61) have been reported. This technique allows measurements of CH4 in same animal repeatedly in their normal environments, while measurements are restricted during milking and feeding periods for the sniffer and GF techniques. However, the LMD system is labor intensive and meteorological factors, such as wind speed and
direction, temperature, humidity, and atmospheric pressure, may influence the accuracy and precision of the measurements, with wind speed being a major factor for grazing studies and outdoor measurements (63). Like the sniffer technique, this technique also requires further improvements if LMD could be suitable for quantification of CH\(_4\) from large numbers of animals in normal management conditions for screening of animals on farms.

**Methane Hood System**

A novel method to quantify CH\(_4\) emissions during feeding has been designed recently by Troy et al. (64). This method can be used to measure CH\(_4\) output from individual animals in a group housed environment. In principle, this method is similar to GF system except that there is no requirement to provide extra feed supplements for enticement to visit an animal into the measurement area as required for GF system. Methane hood system measures CH\(_4\) concentrations in a hood designed to partially enclose the volume above a feed bin. Number of visits could be higher in methane hood than GF system, which may provide high accuracy in methane hood system. Troy et al. (64) compared this system with RC for measurement of CH\(_4\) emission employing nitrate as a CH\(_4\) mitigation option and found a comparable results between the RC method and this technique and detected significant treatment differences in CH\(_4\) production. Preliminary results suggest that this system could be a better alternative choice to quantify CH\(_4\) emissions from individual animals housed in a group in “natural” environments.

**Herd Scale Measurement Technique**

**Polytunnel Method**

Polytunnel method requires a large tunnel made up of polyethylene fitted with end wall and large diameter ports (43). As described by Lockyer and Jarvis (43), measure volume of air is continuously blown into and drawn from the large tunnel containing grazing animals, concentration of CH\(_4\) between the incoming and outgoing air is regularly measured, and temperature and humidity are monitored. Lockyer and Jarvis (43) and Lockyer (65) conducted two experiments using a polytunnel system of 4.3 m wide \(\times\) 9.9 m long \(\times\) 2.1 m height with an approximate volume of 66 m\(^3\) in which different numbers of sheep and calves were enclosed for up to 10 days. The recovery percentage of added CH\(_4\) in the tunnel was 104%. Average CH\(_4\) production was 13–14 and 74.5 g/day for sheep and calves, respectively. However, CH\(_4\) emissions decreased with increasing time of grazing, perhaps due to declining in available forage mass in the pasture as a result of very high stocking rates. Murray et al. (66) carried out two experiments to evaluate polytunnel system in comparison with the RC system for CH\(_4\) emissions from sheep. In both the system, the sheep were fed at maintenance levels of either fresh cut grass or dried grass pelleted diets. The results showed that CH\(_4\) production using the RC technique was greater (31.7 L/kg DM intake) than the tunnel technique (26.9 L/kg DM intake). The recoveries of the added CH\(_4\) in both the systems were similar (95.5–97.9% for tunnel versus 89.2–96.7% for RC). This system is suitable for measuring CH\(_4\) in semi-normal grazing conditions in individual or small group of animals. The operation of this method is simpler and portable, but there is difficulty in controlling temperature and humidity inside the tunnel.

**Micrometeorological Techniques**

During the last 10–15 years, a number of CH\(_4\) emission measurement techniques based on micrometeorological variables from whole farms, feedlots, and paddocks have been developed (67, 68). Micrometeorological methods involve measuring fluxes and concentrations of gases in the free atmosphere of a large area containing animals, and relating these fluxes and concentrations to calculate gas emissions from animals. Micrometeorological dispersion methods cannot measure emissions from individual animals as well as indoor housed animals. Furthermore, the scale of micrometeorological techniques makes their use difficult for testing mitigation options (69).

The micrometeorological methods involve measurements of CH\(_4\) concentration and wind speed, but the number of points of measurement and the assumption utilized to compute emission rates vary depending upon the methods. In the external tracer ratio technique, a tracer gas is released in the paddock or barn area, and the tracer gas and CH\(_4\) concentrations are measured in the surrounding areas (68). This category of methods also includes a mass balance technique in enclosed barns, where CH\(_4\) emissions are determined from ventilation rate and concentrations in inlet and outlet air (10). While it is relatively easy to estimate emission rates from mechanically ventilated closed barns, naturally ventilated buildings are problematic because of difficulties with measuring air exchange rates (10, 70). Air exchange rates in the naturally ventilated buildings depend upon the temperature gradient, temperature humidity index, and the wind speed. The release rates of gases may also depend upon outside environment, such as wind speed, humidity, and the other parameters (10).

A considerable development in micrometeorological techniques has improved CH\(_4\) measurement accuracy using inverse dispersion method (71). Inverse dispersion technique has been employed with success in many feedlot gas emission studies (69, 71, 72). This method has some advantages, such as non-interference of natural behaviors of animals and estimation of carbon footprint over large areas (73). However, there are also many limitations of inverse dispersion method, including wind conditions and the need for source homogeneity (73).

Emissions of CH\(_4\) from grazing animals are measured in field experiments using paddock-scale micrometeorological methods (74). The paddock-scale techniques analyze the patterns of transportation and dispersion of CH\(_4\) emitted from animals by the wind (74). Consequently, the CH\(_4\) emission rates are computed from measurements of wind speed, wind direction and characteristics of turbulent airflow, and CH\(_4\) concentrations in the direction and against the direction of wind (74). The paddock-scale methods estimate CH\(_4\) emissions using flux-gradient method, mass-budget approach, and gas dispersion models (9, 74). A comparison between RC and this method show similar CH\(_4\) yield, i.e., 30.1 versus 29.7 g/kg DM intake (75). Accuracy in measurement is dependent upon certain meteorological and landscape conditions, such as wind velocity and direction, topography of the land, and location of animals in the paddock (74).
micrometeorological methods are expensive and require sensitive instruments to analyze CH₄ concentration (9, 11). Because several meteorological factors influence the accuracy of CH₄ outputs, further developments and documentations for obtaining consistent results are needed, but the methods are valuable in evaluating CH₄ emissions and carbon footprint in whole farm systems and interactions between animals and landscape.

**Indirect Measurements**

**In vitro Measurements**

The in vitro rumen fermentation techniques have been extensively used for assessment of nutritive value of feeds for many years (76) and the techniques have been improved to simulate the rumen conditions. In this technique, feeds are fermented for long term [rumen simulation technique (77)] and short term [gas production methods (78)] under controlled laboratory conditions by rumen microbial activities. The volume of total gas production during incubation is determined and CH₄ concentration in the gas is analyzed to obtain volume of in vitro CH₄ production. With this system, the maximum level of total gas production and CH₄ production can be determined, as well as the kinetics of gas production. Gas volumes are measured in different techniques (8) either directly by determining its volume at atmospheric pressure, e.g., Hohenheim gas production method or Menke's method (78) and liquid displacement system (79) or by determining pressure changes due to accumulation of gas in a fixed volume container using a manometric device (80), a pressure transducer device with computerized (81) and manual (82) recordings, and a combination of pressure transducer and gas release device (83). Factors affecting the gas production in in vitro rumen fermentation system have been described in details by Rymer et al. (84).

Recently, it has been shown that several other factors, such as biconcave concentrations in media and headspace gas composition (85), closed versus vented rumen batch culture system (86), and substrate dispersed in the medium versus kept in filter bags (87), influence the CH₄ production in this technique. For diets containing different fiber concentrations and digestibility, CH₄ production was close to that measured in RC method (88). Although several factors affect gas and CH₄ production in the in vitro techniques, a fast screening of feedstuffs and additives for CH₄ production is possible using these cost-effective simple techniques.

**Modeling Enteric CH₄ Production**

Measurement of CH₄ emissions in animals is difficult and labor intensive, and requires sophisticated and expensive equipments. Mathematical models predict CH₄ emissions from ruminants without undertaking extensive and costly experiments. Therefore, prediction models are widely used for estimating national or global emissions from animals. The models used can be categorized as statistical models, which estimate CH₄ production from nutrient intake directly [e.g., Ref. (2, 89)], or dynamic mechanistic models, which predict CH₄ emissions using mathematical descriptions of rumen fermentation biology [e.g., COWPOL model (90); MOLLY model (91)].

Mechanistic models (e.g., MOLLY and COWPOL) have advantages over the empirical statistical models in that CH₄ mitigation technologies adopted at a farm or national level can be evaluated for their efficacy. Empirical models can evaluate the changes in CH₄ emissions only in relation to changes in numbers of animals and feed intake. Diet-specific mechanistic models can more accurately predict CH₄ emissions in ruminants (92). However, due to complexities of the mechanistic models, preparation of national inventory of CH₄ estimates may not be straightforward. The Intergovernmental Panel on Climate Change (93) and Food and Agricultural Organization (1) publishes guidelines that are usually employed for official estimates of CH₄ emissions in different countries. However, accuracy of these models to predict CH₄ emissions has been challenged in different studies with cattle, buffaloes, sheep, and goats (2, 89, 94–96). The IPCC (93) developed methodologies to estimate enteric CH₄ emissions with the use of CH₄ conversion factor (Ym). However, Ym does not directly represent variations in CH₄ emissions resulted from the ruminal fermentation characteristics affected by different carbohydrates, dietary nutrient composition, and feeding levels. Thus, the utility of Ym-based models in predicting enteric CH₄ emissions and assessing the dietary CH₄ mitigation strategies has been criticized (94). The low predictive ability of the Ym approach may invite substantial inaccuracy in preparation of enteric CH₄ emission inventory (89, 94). Moreover, the IPCC and FAO models are generally developed based on the inputs from cattle. There was no model for predicting CH₄ emission from buffaloes, goats, tropical cattle, and sheep. Recently, several statistical models have been developed for buffaloes, goats, sheep, and tropical cattle (Table 1). These newly fitted models performed better than the IPCC (93) and FAO (1) models as the recently developed equations had lower RMSPE values compared with these extant models (95). These new models should be considered for accurate preparation of enteric CH₄ emission inventories for buffaloes, goats, sheep, and tropical cattle. For example, Patra and Lallhratpuii (95) showed that the estimates of CH₄ emission by goats were 5.23 and 5.15 kg/goat annually (actual CH₄ production was 5.22 kg/goat/year) using the equations based on gross energy intake and digestible energy intake for goats, respectively. The estimate of CH₄ emission using FAO (1) was 6.78 kg/goat/year, which was substantially greater than actual CH₄ production. IPCC (93) suggested a CH₄ emission factor of 5 kg/goat/year, which underestimated emissions. Similarly, based on the IPCC (93) tier II model, total enteric CH₄ emission from buffaloes in India was estimated to be 4584 Gg/year in 2007 (5, 97). However, the estimate of enteric CH₄ production from buffaloes using the equation based on DM intake (2, 89) was 4203 Gg/year, which was 8.3% lower than IPCC (93) model-based estimate (2, 89).

Static empirical models have advantages in that they are usually based on a small number of variables (e.g., DM intake, feeding level, dietary lipid%, dietary fiber%, etc.), they can be performed in a simple spreadsheet, and they are transparent and can be easily tested on a variety of datasets (35). The disadvantage of static models is that they do not rely on an understanding of the biology and biochemistry of methanogenesis in the rumen, and they are, therefore, of limited use to study new CH₄ mitigation strategies. Static empirical models are also restricted in predicting CH₄ production beyond the data used for their development. The national GHG inventory in many countries uses a static
TABLE 1 | List of developed linear and non-linear statistical models used to predict CH$_4$ production (MJ/day) from buffaloes, sheep, goats, and tropical cattle.

| Species            | Equation: CH$_4$ (MJ/day)                                                                                                                                                                                                                                                                                                                                 | RMSE   | $R^2$ | RMSPE% |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------|-------|
| Sheep (96)         | Eq. 1: $0.208_{(0.025)} + 0.049_{(0.026)} \times \text{GE intake (MJ/day)}$                                                                                                                                                                                                                          | 0.24   | 0.86  | 22.7  |
|                    | Eq. 2: $0.550_{(0.217)} + 1.299_{(0.120)} \times \text{DMI intake (kg/day)} - 0.266_{(0.021)} \times \text{FL} - 0.00093_{(0.020)} \times \text{NDF (g/kg)}$                                                                                                                              | 0.22   | 0.82  | 22.4  |
|                    | Eq. 3: $-0.784_{(0.103)} + 0.138_{(0.041)} \times \text{ME intake (MJ/day)} - 0.378_{(0.021)} \times \text{FL} + 0.00294_{(0.004)} \times \text{OMDm}$                                                                                                                                    | 0.21   | 0.94  | 24.5  |
|                    | Eq. 4: $5.699_{(0.145)} - [5.699_{(0.145)} - 0.133_{(0.041)} \times \exp(-0.021_{(0.017)} \times \text{ME intake (MJ/day)})]$                                                                                                                                                     | 0.14   | 0.91  | 20.7  |
| IPCC (93)a          | Eq. 5: $0.066 \times \text{GE intake (MJ/day)}$                                                                                                                                                                                                                                           | –      | –     | 23.1  |
| FAO (1)b            | Eq. 6: $[9.75 - 0.005 \times \text{DM digestibility (g/kg)}/100] \times \text{GE intake (MJ/day)}$                                                                                                                                                                                      | –      | –     | 30.6  |
| Goat (95)           | Eq. 7: $0.242_{(0.078)} + 0.051_{(0.022)} \times \text{DE intake (kg/day)}$                                                                                                                                                                                                                 | 0.31   | 0.83  | 30.3  |
|                    | Eq. 8: $-1.042_{(0.217)} + 2.205_{(0.149)} \times \text{NDF intake (kg/day)} - 2.417_{(0.110)} \times \text{EE intake (kg/day)} + 1.456_{(0.329)} \times \text{NFC intake (kg/day)} + 0.0208_{(0.092)} \times \text{OMDm (g/kg)} - 0.513_{(0.133)} \times \text{FL}$ | 0.14   | 0.82  | 30.3  |
|                    | Eq. 9: $0.885_{(0.154)} + 0.809_{(0.087)} \times \text{DM intake (kg/day)} - 0.397_{(0.042)} \times \text{FL} + 0.0198_{(0.022)} \times \text{OMDm (g/kg)} + 2.04_{(0.034)} \times \text{ADF intake (kg/day)} - 8.54_{(0.156)} \times \text{EE intake (kg/day)}$ | 0.24   | 0.88  | 36.3  |
|                    | Eq. 10: $1.721_{(0.151)} \times [1 - \exp(-0.072_{(0.0002)} \times \text{ME intake (kg/day)})]$                                                                                                                                                                                    | 0.17   | 0.79  | 38.0  |
|                    | Eq. 11: $1.239_{(0.158)} + 0.788_{(0.038)} \times \text{DE intake (kg/day)}$                                                                                                                                                                                                                 | –      | 0.81  | 19.4  |
| Cattle (94)         | Eq. 12: $9.311_{(0.093)} + 0.042_{(0.020)} \times \text{GE intake} + 0.094_{(0.041)} \times \text{NDF} - 0.381_{(0.102)} \times \text{EE} + 0.008_{(0.010)} \times \text{BW} + 1.62_{(0.118)} \times \text{MF}$                                                                 | 2.59   | –     | 15.6  |
|                    | Eq. 13: $-2.880_{(0.222)} + 0.053_{(0.050)} \times \text{GE intake} - 0.190_{(0.054)} \times \text{EE}$ for non-lactating cattle                                                                                                                                                                           | 1.29   | –     | 14.4  |
|                    | Eq. 14: $1.487_{(0.318)} + 0.040_{(0.020)} \times \text{GE intake} + 0.033_{(0.022)} \times \text{NDF} + 0.006_{(0.023)} \times \text{BW};$ for heifer cattle                                                                                                    | 1.23   | –     | 18.6  |
|                    | Eq. 15: $0.221_{(0.048)} \times \text{GE intake} + 0.048_{(0.031)} \times \text{OMDm}$ for steers                                                                                                                                                                                   | 0.92   | –     | 15.1  |
| Tropical cattle (98)| Eq. 16: $1.294_{(0.060)} + 0.878_{(0.076)} \times \text{DMI intake}$                                                                                                                                                                                                                 | 5.49   | 0.70  | 31.0  |
|                    | Eq. 17: $0.910_{(0.245)} + 1.472_{(0.164)} \times \text{DMI intake} - 1.388_{(0.415)} \times \text{FL} - 0.669_{(0.339)} \times \text{ADF intake}$                                                                                                                                 | 4.22   | 0.84  | 22.2  |
|                    | Eq. 18: $7.14_{(0.145)} \times [1 - \exp(-0.0156_{(0.002)} \times \text{DMI intake})]$                                                                                                                                                                                                                                                   | 3.56   | 0.83  | 30.3  |

*IPCC (93) had RMSPE% of 52.4, 27.2, 18.6–30.5, and 32.5 for the database of goat, buffalo, cattle, and tropical cattle, respectively.

*FAO (1) had RMSPE% of 51.0 and 19.2–29.7% for the database of goat and cattle, respectively.

GE, gross energy; DE, digestible energy; DM, dry matter; NDF, neutral detergent fiber; FL, feeding level; ADF, acid detergent fiber; ME, metabolizable energy; EE, ether extract; NFC, non-fiber carbohydrate; OMDm, organic matter digestibility at maintenance level of feed intake; MF, milk fat (%); BW, body weight (kg); RMSE, root mean square error; RMSPE, root mean square prediction error.

Empirical model to estimate emissions of CH$_4$ from ruminant livestock. Nevertheless, prediction models are the strong base for estimating national or global emissions from animals.

**Proxy Measures of CH$_4$ Emissions**

A considerable research effort has been directed toward development of proxy measures for predicting enteric CH$_4$ production from composition of milk and feces. Several studies examined the concentrations of specific fatty acids in milk as predictors of CH$_4$ production from dairy animals (99, 100). The assumption in this approach is that specific fatty acids in milk or feces are correlated with the composition of feeds or the amount of ruminant methanogenic archaea, which would influence CH$_4$ emissions in the rumen (100). Williams et al. (101), however, observed weak correlations between CH$_4$ production and the concentrations of specific fatty acids in milk fat. Few studies (100, 102) indicated some correlations between milk fatty acid profiles and CH$_4$ emissions. Milk mid-infrared spectra (influenced by milk fatty acid composition) measured using FTIR analysis apparatus could directly better predict CH4 emission (103) compared with fatty acid composition.

The use of archeol (2,3-diphytanyl-O-sn-glycerol), which is a membrane lipid ubiquitous in methanogens has been explored as a potential molecular proxy for methanogenesis in cattle (104). A significant correlation between fecal archeol concentration and CH$_4$ production measured using the SF$_6$ and RC technique in cattle fed either a grass- or concentrate-based diet was observed, but relationships between individual measurements within dietary treatments were weak to moderate, possibly due to selective retention of archaea in the rumen and degradation of the archeol during gut transit, differences in the CH$_4$ producing capability per cell and post excretion (104, 105). These methods could be useful to predict the individual CH$_4$ production to identify low-CH$_4$-emitting animals, but further research is needed to improve the predictability of CH$_4$ emissions using these proxy methods.

**Other Potential Technology**

**Intra-Ruminal Gas Sensor**

An intra-ruminal device, which measures the concentrations of CH$_4$ and CO$_2$ dissolved in rumen fluid, but does not measure flux (emission), has recently been fabricated (106). The rumen environmental conditions may be specifically unfavorable for an electronic device, which may cause corrosion of electrical circuits. In addition, the dissolved gases in rumen fluid must permeate quickly through the membrane of the intra-ruminal device in order to dynamically analyze the concentrations of
gases (35). Information on internal rumen pressure, rumen size, and eructation pattern can be integrated to estimate the gas production rates (11). Thus, further research would be required to develop an approach to measure CH₄ production from individual animals from the in situ measurements of gas concentrations in the rumen. The measurement of CO₂ and CH₄ concentrations in rumen and breath (respiratory and eructated) at the same time would be advantageous to assess the feasibility of using CO₂ as a tracer gas and this could guide to the use of low-cost handheld systems to estimate CH₄ production (11).

**Dietary Strategies to Mitigate CH₄ Emissions**

Several mitigation options and strategies have been explored, which involve intervention at the animal level, dietary composition of animals, modulation of rumen fermentation, and inhibition of methanogenic archaea (Figure 2). Methanogen-specific inhibitors could be potentially effective mitigation agents if they utilize the evolutionary distinctiveness of methanogenic archaea (35). Archaea are evolutionarily distinct from other rumen microorganisms (bacteria, protozoa, fungi, and viruses), and all methanogenic archaea share a similar biochemical pathway of methanogenesis (107). Therefore, the inhibitors of this methanogenesis pathway may distinctively inhibit only methanogens without directly influencing other beneficial microorganisms in the rumen (35, 108). Several reviews on CH₄ mitigation strategies and options have been published recently (3, 6, 97). In this section, further recent advances in dietary CH₄ mitigation technologies are described here.

**Lipid Supplementation**

Several studies have confirmed that addition of fats or fatty acid to the diets of ruminants can decrease enteric CH₄ emissions [e.g., Ref. (2, 89, 101, 109)]. Each percentage increase in supplemental dietary fat decreases CH₄ emission by 4.30% (109). Fat concentrations of up to 6% of diet DM may also increase milk production and lower enteric CH₄ emissions appreciably (15%) in cattle (109), which is a win–win situation. Fat concentration beyond this concentration may decrease production efficiency due to adverse effect on rumen fermentation. Among fatty acids, C12:0, C18:3, and poly unsaturated fatty acids have more marked CH₄ suppressing effects, whereas saturated long-chain fatty acids are less effective for decreasing CH₄ production in cattle (109, 110). The by-products containing high concentration of lipids (e.g., brewers grains, grape marc, hominy meal, etc.) appear to be promising to mitigate CH₄ emissions cost-effectively (101). Grainger et al. (24) showed that supplementary feeding of whole cottonseed to dairy cows could cause a substantial decrease in CH₄ emissions without adversely affecting milk production. Moate et al. (111) compared brewers grains, cold-pressed canola, and hominy meal for their CH₄ mitigation potential and found

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**Figure 2** A schematic presentation of the potential targets of decreasing CH₄ emissions from ruminants. Boxes without dark could be the targets for suppressing CH₄ emissions and boxes with dark shade are the options that have been studied in vitro or in vivo to decrease CH₄ production [adapted with modification from Patra (6, 97)].
that all three by-products could substantially reduce enteric CH₄ emissions from dairy cows. The feeding of red grape marc to dairy cows decreased CH₄ emissions and CH₄ yield by 20% (32). In addition to the presence of fat, grape marc also contains many secondary compounds such as tannins, p-coumaric acid, and resveratrol, which may also inhibit enteric methanogenesis (32).

**Plant Secondary Compounds**

Several plant secondary metabolites present in forages and plant extracts have been identified to be potential for CH₄ inhibition in the rumen (108). Many forage plants rich in tannins and saponins have shown to be promising to reduce CH₄ production in ruminants (112, 113). Tannins may decrease CH₄ directly by inhibiting methanogenic bacteria, and indirectly by decreasing hydrogen production as a result of decreased fiber digestion and protozoal population in the rumen (108).

A recent investigation with different forage brassicas have shown that most of the brassicas species fed to sheep resulted in similar CH₄ yields. However, swedes and forage rape significantly decreased CH₄ yield (~20%) compared with ryegrass and other brassicas, such as turnips and kale (114). Although the mechanism of CH₄ inhibition is not clearly known, these forages may contain S-containing plant metabolites, which may be responsible for inhibition of CH₄ production (108). Additional research is required to examine the CH₄ mitigation effects of these forage brassicas fed to ruminants for long time.

The studies on flavonoid compounds on rumen methanogenesis are limited although other metabolites, such as tannins, saponins, and essential oils, have extensively been studied (108). A recent study reported that inclusion of flavone, myricetin, naringin, rutin, quercetin, and kaempferol significantly decreased in vitro CH₄ production from 8.6 to 5.7, 4.9, 6.3, 7.2, 6.2, and 5.3 mL/g DM, respectively. The inhibitory activities of flavonoids used in this experiment toward methanogenesis were in the following descending order as follows: myricetin ≥ kaempferol ≥ flavone > quercetin ≥ naringin > rutin ≥ catechin (115). Catechin decreased CH₄ production both in vitro (116) and in vivo (117). Catechin causes direct inhibition of methanogens (115) as well as may act as hydrogen sinks during degradation by rumen microbes via cleavage of ring structures and reductive dehydroxylation reactions (116).

**Nitrate Supplementation**

Nitrate decreases methanogenesis acting as electron sinks and directly inhibiting the methanogens (85, 118). Use of nitrate has two advantages – (1) it decreases CH₄ production and (2) it provides ammonia to the rumen microbial growth resulting in decreased dietary protein requirement. Nitrate as a dietary supplement fed to dairy cows in low nitrogen containing diet have been shown to reduce CH₄ emissions (119). However, dietary nitrate supplementation may increase the risk of nitrite toxicity, particularly for the forages containing high concentrations of nitrate and crude protein.

**Halogenated Compounds**

Halogenated compounds had been investigated as inhibitors of CH₄ production in the rumen over 40 years ago (5, 97). Some marine plants, such as the red seaweed, algae, and fungi, contain bromoform and other halogenated compounds at high concentrations (120), which have been exploited recently to inhibit CH₄ production. In a recent in vitro experiment, red seaweed, Asparagopsis taxiformis, was shown to reduce CH₄ production by 99% when it was used at 2% of organic matter substrate (121). Thus, the supplementation of ruminant diets with red sea weed may offer a natural means of CH₄ mitigation. In vivo experiments are required to decide optimum dose levels and to study the toxic effect of the weed, if any.

**Nitrooxy Compounds**

Novel inhibitors 3-nitrooxypropanol (NOP) and ethyl-3NOP have been shown to have specific anti-methanogenic properties. NOP interferes with the methyl-coenzyme M reductase of the methanogens, which is the last step in the formation of CH₄, thus lowering CH₄ production and inhibition of the growth of methanogens (122, 123). Reynolds et al. (124) noted a 7–10% lower in CH₄ production when NOP was administered directly into the rumen of cattle through a rumen cannula at a daily dose of 0.50 or 2.5 g per cow (i.e., 25 or 125 mg/kg DM). Digestibility decreased at high dose in this study. However, in subsequent studies, Haisan et al. (125) noted 60% decrease in CH₄ emission in cattle fed NOP at a dose of 2.5 g/day mixing with the feed to ensure continuous intake of this compound throughout the day. Feeding of NOP at 40–80 mg/kg diet in dairy cattle was also associated with decreased CH₄ production by 30% (126). In beef cattle diet also, 3NOP added to the diets at 2.0 g/day decreased CH₄ yield by 59% up to 112 days of experiment without much affecting feed intake, nutrient digestibility, and total volatile fatty acid (VFA) concentrations (127). In this study, the numbers of methanogens were reduced, but protozoal numbers were increased by 3NOP. These studies suggested that 3NOP needs to be continuously supplied to the diet to get optimal inhibitor effect of CH₄ production. The results have been confirmed in other study (128) where CH₄ yield was lowered by 37% due to feeding of 3NOP at 2.5 g/day in dairy cow. In sheep also, 3NOP at 0.5 g/animal per day decreased CH₄ production by 29% without adversely affecting digestion and rumen fermentation (123). It appears that 3NOP is a CH₄ inhibitor, which has potential for reducing carbon footprint of livestock products without affecting nutrient utilization and performance in ruminants.

**Fungal Metabolites**

Lovastatin is a secondary metabolite of idiophase of the fungi, which inhibits the key enzyme of cholesterol biosynthesis, such as enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase (129). Aspergillus terreus fungi fermented rice straw extract containing lovastatin significantly reduced CH₄ production and number of methanogens, but increased few fiber degrading bacteria (129). Ether-linked long-chain isoprenoid alcohol is a central component in arachal cell membrane lipid, which is produced from a key precursor, mevalonate. Mevalonate is synthesized by reduction of HMG-CoA catalyzed by HMG-CoA reductase and it is an intermediate rate limiting reaction in synthesis of cholesterol in human (108). Lovastatin is an inhibitor
of HMG-CoA reductase, which inhibits isoprenoid alcohol synthesis, and consequently arachidonic acid formation and growth of methanogens could be retarded (129). Strains of saprophytic fungi Mortierella wolffii were also promising as an inhibitor of methanogenesis without also reducing overall fermentation (130). In another study in sheep fed Monascus-fermented rice, CH4 emission was decreased by 30% by fungal metabolites (possibly pravastatin and mevastatin) produced by Monascus spp., which was associated with lower ruminal acetate to propionate ratio and decreased numbers of methanogens in the rumen (131).

Microalgae
An effort has been started to screen microalgae to inhibit rumen methanogenesis. Machado et al. (121) screened several marine microalgae in vitro and reported that Asparagopsis was not only strongly decreased CH4 production by 99% at a dose of as low as 2% of total substrate organic matter, but also decreased the production of VFA. Oedogonium was less effective with doses ≥50% OM significantly decreasing the production of CH4. A combination of Asparagopsis (2% OM) and Oedogonium (25 and 50% OM) continued to suppress the production of CH4 independent of the inclusion rate of Oedogonium. The brown algae (Cystoseira trinodis and Dictyota bartayresii) were also identified as a potent CH4-inhibiting agent in vitro (132). One algal meal containing 20% docosahexaenoic acid fed to cows up to 375 g/cow per day of algal meal corresponding up to 75 g of docosahexaenoic acid/cow per day did not affect CH4 production in vivo, which was probably due to the low concentration of this fatty acid to inhibit CH4 production (133).

Use of Combination of CH4 Inhibitors
A number of CH4 inhibitors have been frequently evaluated, primarily individually, to lower CH4 production in ruminants. However, they generally exert detrimental effects on digestion of feeds and rumen fermentation when they are added at high concentration in order to obtain substantial effect on CH4 inhibition (5, 97, 134, 135). Some of these inhibitors also cause toxicity to animals when used at large doses (97). These adverse effects on rumen fermentation and toxicity problems to animals can be overcome at low doses, but substantial effect on inhibition to methanogenesis is not noted at low doses. Combinations of inhibitors with complementary modes of actions may synergistically or additively lower CH4 emission without exerting any detrimental effects on digestion of feeds or rumen fermentation at low doses (134). Indeed, this hypothesis has been confirmed in some studies (134–138). In a study, a binary combination of nitrate and quillaja saponin inhibited methanogenesis additively in an in vitro rumen culture by [32% at 5 mM nitrate and 0.6 g/L saponins, and by 58% at 10 mM nitrate and 1.2 g/L saponins (134)]. Binary combination of nitrate and saponins might act additively to decrease methanogenesis in a multipronged manner: (1) saponins inhibit rumen protozoa, lowering hydrogen production by protozoa and reducing the abundance of protozoa-associated methanogens (139), (2) nitrate functions as a strong electron sink that outcompetes CO2 for electrons, and (3) nitrite, the first intermediate of nitrate reduction, exerts direct toxicity to methanogens. However, binary combination of high doses of nitrate and saponins decreased fiber degradability (85). Garlic oil is directly inhibitory to rumen methanogens acting through impairment of lipid synthesis (140). Combination of saponin + nitrate + sulfate, garlic oil + nitrate, and garlic oil + nitrate + saponin resulted in additive effects on methanogenesis (118, 136, 140). Hops extract (Humulus lupulus; containing β- and α-acids) and yucca saponin decreased CH4 in an additive manner when applied in combination (138). Additive effects of combinations of CH4 inhibitors have been tested a little in vivo. A recent in vivo study demonstrated that nitrate (3% calcium nitrate; 22% reduction) and linseed oil (4% of the diet; 17% reduction) in combination (32% reduction) for decreased methanogenesis additively in cows without altering diet digestibility (141). It appear that combinations of CH4 inhibitors with complementary mechanism of actions could be useful technology to substantially decrease CH4 production without adverse effect on nutrient utilization, but more in vivo research is required to identify the suitable dose combinations for practical on farm application.

CONCLUSION AND FUTURE RESEARCH CHALLENGES
Many existing methods have been employed to measure CH4 production with different purposes, such as nutritional evaluation of feeds and feed additives and screening of animals for genetic selection, and have been investigated to improve accuracy of measurements. Many new techniques are being developed to overcome the constraints of the existing methods. However, no method is suitable in all conditions for reliable measurement of CH4 emissions. Every method has its advantages and limitations, and a method is useful in particular conditions of a study for CH4 measurement and mitigation. Most consistent RC method is only a “gold standard” when this is used with adequate rigor and technical expertise. The SF6 method can be employed with lesser effect on animal behavior and has a higher throughput relative to time and cost. With recent recommendations for the technique, the SF6 method can be used with a high level of precision in grazing animal studies for long time.

The short-term measurement techniques have advantages as it is relatively cheaper, simpler, and mobile compared to other techniques, such as SF6 or RC. All short-term measurements invite variations at several points, such as measurement time relative to feed intake and level of activity before measurement (45). The higher within and between animal-to-animal and day-to-day variations in CH4 emissions would require more number of animals and measurement days to obtain significant differences of CH4 emissions between treatments using short-term measurement techniques. Daily CH4 emission patterns of ruminants generally show a diurnal pattern in relation to the feeding time and level of feed intake (58, 142). The diurnal profiles associated with feed intake exhibit a continuous rise to a peak followed by a period of a linear decline. Therefore, for short-term measurements of CH4 emissions, such as sniffer method (59) taken twice during the daily feeding and milking time, estimation of daily CH4 production based on measurement of short-term emission rates will be diet dependent (45). For systems such as GF and methane hood where short-term emission rates are measured...
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Throughout 24 h, daily CH₄ emission may be estimated without scaling up (45). For systems such as for PACs and LMD where small numbers of measurements for emission rates are taken and feed intake information of an animal is not known, scaling up to daily CH₄ emission is not currently possible (45). Short-term measures of CH₄ emission may strongly correlate with daily CH₄ emission depending on the time after feeding at which the short-term measurements are made. Thus, short-term measuring CH₄ emission depending on the time after feeding at which the short-term measurements are made is not currently possible (45). Short-term measurements are made. Therefore, short-term measuring CH₄ emission depending on the time after feeding at which the short-term measurements are made is not currently possible (45).

Several new CH₄ mitigation technologies have been explored, but only a few of them are practical and cost-effective, which can be adopted on farms to achieve substantial mitigation of total CH₄ emissions. A combination of different CH₄ mitigation strategies should be adopted in farm levels to substantially decrease CH₄ emission from ruminants. The CH₄ mitigation options that show both nutritional and environmental advantages would likely to be better adopted by the farmers. For example, fat supplementation could decrease CH₄ production as well as improve productivity of animals. Similarly, nitrate supplementation could reduce the expensive protein meals in diets. If some mitigation technologies could be employed to improving the nutritional values of forages, they have immense practical importance in tropical feeding situations. For example, if saprophyte fungi, which produce metabolites against methanogens, could be grown on low-quality forages, such as straws, this technology would be feasible for decreasing CH₄ production in ruminants. Toxicities and residues in milk and meat associated with CH₄ inhibitors must be assessed with long-term trials in animals. Many fungi, for example, may produce toxic metabolites in some growth conditions, which must be avoided for practical feeding of animals (143). Nitrate supplementation may cause toxicities in animals if it is not used in proper doses with relation to the nitrate content in forages. Future mitigation research should focus for the developments of both the short-term strategies, such as dietary strategies and animal management as well as long-term strategies focusing on plant and animal breeding, and rumen microbial modulation in order to profitably decrease carbon footprint and strengthen the future sustainable and environment friendly livestock production systems.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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