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Assessing Enteric Methane Emissions in Ruminants: A Comparative Study of the Green Feed Technique

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ABSTRACT

ivestock-generated methane, particularly from cattle, was a significant contributor to climate change. Methane emissions I from ruminant animals, such as cows and sheep, are primarily caused by the microbial fermentation of food in their digestive systems, a process known as enteric fermentation by making this process a prime source of greenhouse gas emissions in animal production. Considerable knowledge gaps existed in animal agriculture regarding effective strategies for mitigating these emissions while maintaining productivity. A key factor was the uncertainty surrounding methods for estimating emission rates, each having inherent limitations. For example, the suitability of the green feed system varied based on specific experiment objectives. Compared to respiration chambers and the sulfur hexafluoride tracer method, the The GreenFeed system often required more time and a larger number of animals for treatment comparisons due to higher within-day variances. It measured numerous short-term methane emissions from individual animals at various times throughout the day over several days. Recent advancements focused on improving accuracy, ease of use, and cost-effectiveness, essential for better monitoring of greenhouse gases. Traditional methods, such as respiration chambers, while accurate, were costly and impractical for field measurements. The GreenFeed system's software facilitated control over feed availability timing and CH₄ measurement allocation. Therefore, careful planning was necessary to ensure accurate estimates of methane production. This review emphasized the need for effective measurement techniques to mitigate methane emissions from livestock.

KEYWORDS: Green feed, methane, heat stress, ruminants, feed additives

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1. INTRODUCTION

uminants are responsible for approximately one-third Kof global anthropogenic methane emissions. (Pachauri et al., 2014). Enteric CH₄ production from ruminants plays a significant role in greenhouse gas emissions, which are a major contributor to climate change. Additionally, methane emissions represent a loss of dietary energy, thereby diminishing the overall feed efficiency of livestock (Hristov et al., 2013). Efforts to mitigate these emissions include the use of feed additives such as 3-NOP (3-nitrooxypropanol), which has demonstrated the potential to inhibit the enzymes responsible for methane production in the rumen (Beauchemin et al., 2015). Cattle farming are one of the largest contributors to global methane emissions. As the demand for high-quality meat and dairy products continues to rise, methane emissions also increase, leading to a rise in global temperatures. (Tseten et al., 2022).

Recent research indicates that methane production is a heritable trait, suggesting that selective breeding could serve as an effective long-term strategy for reducing enteric methane emissions (Pinares-Patiño et al., 2013). However, integrating these strategies—dietary adjustments, genetic selection, and additive-based solutions—into practical livestock management systems present challenges. Moreover, Hammond et al. (2016) suggested that methane measurement techniques could be improved by enhancing the capacity to monitor a larger number of animals with minimal technical expertise and reduced human involvement. The GreenFeed (GF) system, for instance, estimates daily methane production (g day-1) by measuring gas concentrations and airflow for 3 to 7 minutes during each cow's visit to the GF unit. While gas emission monitoring (GEM) systems effectively capture CH₄ measurements at various times throughout the day, they rely on frequent animal visits (Ryan et al., 2022). Mol et al. (2024) reported that when assessing the effect of dietary treatment on CH and H, production, using a spot-sampling device such as a gastric fistula in a setting where measurements rely on voluntary visits from dairy cows to the GF.Methane mitigation options that provide both nutritional and environmental benefits are likely to be more readily adopted by farmers. For instance, fat supplementation can reduce CH₄ production while simultaneously enhancing animal productivity (Patra, 2016).

Another established method is the sulfur hexafluoride tracer technique, which estimates $\mathrm{CH_4}$ production; however, like the respiration chamber method, it is labor and equipmentintensive, making it impractical for evaluating large numbers of animals simultaneously. In response to these challenges, various non-invasive 'sniffer' methods have been developed, focusing on measuring $\mathrm{CH_4}$ concentrations or the $\mathrm{CH_4}$

to CO₂ ratio when cows visit automatic milking systems or concentrate feeders (Garnsworthy et al., 2012; Lassen et al., 2012). Similarly, Islam et al. (2023) introduced a non-contact exhalome-sampling technique utilizing the GreenFeed (GF) System from C-Lock Technology Inc. in Rapid City, SD.

GF and respiration chamber (RC) systems showed strong agreement for daily enteric CH_4 and hydrogen (H_2) emissions. However, the observed differences in H_2 estimation and moderate correlations for other gases indicate the need for careful consideration of methodological nuances and treatment effects. Further investigation may be necessary to fully understand these dynamics and enhance the reliability of both measurement systems for future research (Ma et al., 2024).

Interestingly, the higher O_2 consumption recorded by the GF system corresponded to cows spending more time standing-an activity that necessitates greater O_2 uptake due to an increased heart rate compared to lying down. This increased activity also results in elevated heat production and thermoregulatory changes (Talmón et al., 2023; Reiche et al., 2023).

This review aims to address the knowledge gaps in animal agriculture regarding greenhouse gas emissions by comparing methane and carbon dioxide emission rates obtained from the GreenFeed system with those measured using the respiratory chamber and other techniques.

2. TECHNIQUES USED FOR MEASUREMENT OF METHANE EMISSION IN RUMINANTS

2.1. Green feed system

GreenFeed, developed by C-Lock Inc. and based in Rapid City, South Dakota, USA, is an innovative system designed to monitor animal emissions by capturing breath samples when animals visit a specially designed bait station (Huhtanen et al., 2015). The GreenFeed Emission Monitoring (GEM) system is tailored to measure emissions from livestock in production environments. Similar to other monitoring systems, GreenFeed collects the breath of individual animal multiple times a day typically between 4 to 6 times over short durations ranging from 3 to 7 minutes. During these sampling intervals, a slight under-pressure is created to ensure the complete collection of the animal's breath for accurate flux measurement. The system records methane and carbon dioxide emissions during these brief sampling periods, which last between 3 to 10 minutes, as cattle approach an automated feeder equipped with a semienclosed head hood. Air is continuously drawn through an air-collection pipe, allowing for precise measurement of gas fluxes (Huhtanen et al., 2015; Hammond et al., 2016).

To accurately measure CH₄ and CO₂ concentrations, air samples are analyzed every second using non-dispersive infrared sensors. Gas fluxes are then calculated based on airflow and gas concentrations, adjusted for environmental factors such as temperature, humidity, and pressure, as well as corrected for background levels. A separate infrared sensor monitors the animal's head position within the feeder. If the animal's head is not properly aligned, gas flux calculations are halted until the head is relocated to ensure accurate airflow collection.

The Green Feed portable system is a self-contained unit, capable of operation in either barns or pasture environments, equipped with an extractor fan to maintain airflow and a head position sensing mechanism for precise breath sampling collection. The manufacturer processes the measurements beforehand, allowing for real-time access to the data through an online management system (Hammond et al., 2015).

GreenFeed effectively captures a substantial amount of emitted air and measures airflow, allowing for the calibration of tracer gases. This enables the estimation of methane emissions in grams per day based on the flux recorded during each animal's visit. When these visits are steady over a 24-hour cycle, methane emissions can be accurately calculated (Hammond et al., 2015; Huhtanen et al., 2015).

Importantly, the repeatability of $\mathrm{CH_4}$ measurements is crucial, and thus the duration of the measurement period is a significant factor (Huhtanen et al., 2015; Arbre et al., 2016). For example, a correlation coefficient (r) of 7 was observed after a measurement period of 17 days, which increased to r=.93 after 45 days (Arbre et al., 2016).

The GreenFeed system (GFS) is an innovative tool designed to measure methane emissions from ruminant livestock, such as cattle and sheep. By providing a means to assess methane output in both pen and pasture settings, GFS plays a critical role in understanding and managing the environmental impact of livestock farming.

The GreenFeed system (GFS) from C-Lock Inc. plays a crucial role in monitoring the gas emissions of livestock while they consume a specific pelleted supplement. Positioned centrally in a group pen, the system allows for unrestricted access for animals, though a narrow alley in front of the GFS hood ensures that only one animal can enter at a time. The operational mechanics of the GFS and the methodology for calculating gas-emission rates have been thoroughly described in the literature by researchers such as Hammond et al. (2015), Hristov et al. (2015) and Huhtanen et al. (2015). The system employs advanced non-dispersive infrared (NDIR) sensors for measuring methane (CH₄) and carbon dioxide (CO₂), alongside a paramagnetic oxygen (O₂) sensor. These sensors enable the real-time

monitoring of gas emissions as animals consume the feed within the GFS.

It's essential to note that while animals have the freedom to visit the GFS at any time, not every visit results in gas measurement or feed dispensation. Valid gas measurements and feed dispensations are contingent upon the animal visiting the GFS during a designated period when feed is dispensed as a reward. This ensures that only those visits that coincide with the feed drop are considered 'valid' for the purposes of emission measurements, thereby accurately reflecting the desired data. Hammond et al. (2015) conducted three experiments to compare methane (CH₄) emissions measured by the GreenFeed (GF) system with those obtained via respiration chambers (RC) and the sulfur hexafluoride (SF₆) tracer technique. Two of these experiments involved indoor Holstein heifers, while the third was conducted with grazing animals fed various diets. In both indoor experiments, daily CH₄ emissions (g day⁻¹) and CH₄ yield (g kg⁻¹ dry matter intake) were comparable between the GF and RC methods. For instance, in experiment 1, the GF system recorded 198 g day⁻¹ and 26.6 g kg⁻¹ DM intake, while the RC system recorded 218 g day⁻¹ and 28.3 g kg⁻¹ DM intake. In experiment 2, values were similarly close, with 208 g day⁻¹ and 27.8 g kg⁻¹ DM intake measured by GF, and 209 g day⁻¹ and 27.7 g kg⁻¹ DM intake by RC. However, in experiment 3, where grazing animals were studied, CH₄ emissions and yields obtained via the SF₆ technique were higher than those measured by the GF system, with values of 186 versus 164 g day⁻¹ and 21.6 versus 18.8 g kg⁻¹ DM intake, respectively. Furthermore, CH₄ production measured by the GF system showed little concordance (r=0.10) with the RC method and only moderate agreement (r=0.60) with the SF₄ technique. Significant differences in CH₄ emissions based on treatment and individual animal variation were observed using the RC and SF₆ methods, but not with the GF system. This discrepancy was attributed to the limited number of measurements obtained from the GF system in grazing animals and the timing of measurements, which may not have aligned with natural CH₄ emission patterns. These findings underscore the importance of collecting a sufficient number of observations when using the GF system, particularly in grazing settings.

The GF system yielded precise estimates of methane and carbon dioxide emissions from cattle over a short measurement duration. Yet, to effectively assess the daily emission rates for an individual animal, it is crucial to consider additional variables (McGinn et al., 2021). These factors might include the animal's diet, activity level, physiological state, and environmental conditions throughout the day, as they can significantly influence overall emissions.

2.1.1. Steps for animals to use green feed system

2.1.1.1. Initial placement of green feed system

Allow the cows to explore the system freely for at least two days without introducing feed, enabling them to acclimate at their own pace while you monitor their behavior for signs of curiosity, stress, or disinterest; also, ensure the system is set in a safe location free from hazards and away from extreme temperatures, excess moisture, and poor drainage that could impact the feed quality and the cows' willingness to use it.

2.1.1.2. Preparation of pelleted feed

To create a high-quality feed pellet, accurately measure 70% ground corn, 27% dry molasses, and 3% soybean oil, pre-mix the dry ingredients to ensure even distribution, gradually incorporate the soybean oil to enhance the binding and texture, and carefully control moisture levels to prevent clumping and dust, ensuring optimal palatability and health for the cows.

2.1.1.3. Familiarization with pelleted feed

Introducing new feed to animals requires careful consideration to ensure acceptance and minimize stress. Instead of simply placing the new feed on top of the usual feed

2.1.1.4. Gradual introduction of green feed system

Gradually relocate the AHCS unit to a distance of 1.5 meters from the animals while monitoring their comfort levels, and provide approximately 1 kg of pellets in a bucket to allow them to smell and sample the feed.

2.1.1.5. Encouragement to use AHCS

Slowly move the bucket closer to the AHCS feeding trough to encourage the cow to stretch and reach for it, and as you pour some bait feed into the trough, slowly adjust the unit closer to the animal; if it shows any signs of apprehension or fear, return the unit to a comfortable distance and try the introduction again later.

2.1.1.6. Repetition and positive reinforcement

Continue the training process over several days, closely monitoring the cows for indications of comfort and enthusiasm around the AHCS. If an animal does not adapt to the AHCS, consider replacing it with another animal and repeating the training procedure.

2.1.1.7. Patience is key

Provide sufficient time for the animals to acclimate to the AHCS, as hurrying the process could lead to anxiety or reluctance.

2.1.1.8. Observe body language

Observe the cows' body language for signs of stress, which may include backing away, emitting loud vocalizations, or showing reluctance to approach the AHCS.

2.1.1.9. Positive reinforcement

Consider using soft vocal praise or gentle petting to encourage and reinforce positive behavior when the cows interact with the AHCS.

2.1.1.10. Environment control

Make sure the training environment is peaceful and devoid of any sudden loud noises or disturbances that could startle the animals.

2.1.1.11. Record progress

Maintain comprehensive records of each animal's progress to identify those that may require more time or alternative strategies.

By following these structured steps and considerations, animals can be effectively trained to use the AHCS, leading to a smoother integration into the feeding and health management system.

2.2. Respiratory chamber technique

The Respiratory Chamber technique has been widely used for many years to assess energy balance and measure gaseous exchanges in animals, including methane (CH₄) emissions (Armsby (1903), Kellner and Goodwin (1913). This method relies on measuring the concentration of methane that is exhaled through all potential avenues like mouth, nostrils, and rectum resulting from enteric fermentation.

In the RC system, an air pump continuously draws air from the chamber, passing it through a flow meter in an open-circuit system. This setup allows for the calculation of the total volume of air removed from the chamber. Simultaneously, the outlet gas from the RC is continuously sampled and analyzed through a duct system.

To ensure accurate measurement, the respiratory chamber is equipped with ventilation fans that promote the even mixing of expired gases and incoming air. Fresh air is supplied to the respiratory chamber directly from the external environment or through an air conditioning system, which helps regulate both humidity and temperature. Furthermore, the respiratory chamber is outfitted with precision instruments to continuously monitor key environmental parameters, including humidity, temperature, and barometric pressure. These measurements enable the determination of gas volume under standard temperature and pressure conditions (Storm et al., 2012). The respiration chamber method is regarded as the gold standard for measuring methane emissions, although it requires the confinement of animals for duration of 2 to 4 days (Hellwing et al., 2012). This method is characterized by significant investment and labor costs, which can be a barrier to widespread application. The respiration chamber (RC) method is widely regarded as the "gold standard" for measuring enteric methane

(CH₄) emissions due to its high accuracy, repeatability, and minimal animal-to-animal variation (Grainger et al., 2007, Williams et al., 2013). Despite these advantages, the RC system has several limitations. It is costly to establish and requires intensive labor to operate. The system also restricts animals' natural behaviors, including eating, which can result in CH₄ emissions that may not fully represent those in typical environmental conditions. van Lingen et al. (2023) concluded that when applying a twice daily ad libitum feeding regime, three spot samples taken at 8-hour intervals, starting 2 hours after feeding, were sufficient for their analysis using respiration chamber data. This suggests that a limited number of time points can adequately represent the overall respiration measurements under these specific feeding conditions Lee et al. (2022) utilized respiration chamber data to simulate spot sampling for evaluating the accuracy of methane production estimates. They concluded that at least eight spot samples (i.e., every three hours within a 24-hour cycle) are necessary to accurately estimate daily CH₄ production and detect dietary effects on CH₄ emissions."

2.3. The sulfur hexafluoride tracer technique

Additionally, the method demands a high level of technical expertise to achieve accurate measurements. While it is possible to decrease the negative impact on feed intake and milk production in lactating animals through proper adaptation, this concern must still be considered. Furthermore, the RC method has limited throughput, making it less suitable for large-scale measurements, such as screening animals for low CH₄ emissions in genetic selection programs.

Initially developed by Zimmerman (1993) and later adapted by Johnson et al. (1994) for measuring methane (CH₄) emissions in grazing cattle, the sulfur hexafluoride (SF₆) tracer technique has gained widespread use over the past two decades. The method relies on the principle that CH₄ emissions can be calculated if the SF₆ release rate from the rumen is known (Johnson et al., 1994). In this procedure, small permeation tubes filled with SF₆ are placed inside the animals' rumens. The animals are then fitted with a gas-sampling apparatus, which includes a halter supporting capillary tubing positioned near the nostrils and an evacuated canister to collect gas samples (Broucek, 2014). Over a typical 24-hour sampling period, the gas samples containing both respired and eructated gas are collected through the tubing into the canister, with the tubing regulating the sampling rate (Lassey et al., 2001).

The levels of SF_6 and CH_4 in these samples are then analyzed by gas chromatography. Using the known SF_6 release rate and the measured SF_6 and CH_4 concentrations in the collected samples and CH_4 emissions are calculated with

a standard equation (Johnson et al., 1994). When methane (CH₄) emissions are expressed unit⁻¹ of dry matter intake (DMI), the proportion emitted from the rectum accounts for 3% of total CH₄ production. This proportion is slightly higher than the findings of Murray et al. (1976), which reported less than 2% for sheep using isotopic techniques. This result aligns with the expectation that estimates of CH₄ emissions derived from SF6 tracer techniques are generally lower than actual total emissions. The proportion of CH₄ emissions from the rectum is likely related to the degree of enteric fermentation occurring in the hindgut, which is influenced by fiber availability. Furthermore, evidence suggests that the agreement between CH₄ emission estimates from SF6 and respiration chamber techniques improves when cattle are fed at a restricted intake level (McGinn et al., 2006).

2.4. The sniffer method

Initially noted by Garnsworthy et al. (2012), this method involves placing a sampling inlet within the feed manager of an automatic milking system to collect air eructed by cattle during milking. This technique facilitates real-time sampling of methane (CH_1) and carbon dioxide (CO_2) concentrations near the animal's muzzle. As described by Garnsworthy et al. (2012), air is continuously sampled, analyzed, and recorded at one-second intervals using data loggers. The frequency of eructations and the CH₄ released eructation⁻¹ are then utilized to estimate the CH₄ emission rate for each animal during milking. Garnsworthy et al. (2012) found a strong correlation (r=0.79) between CH₄ emissions measured by the remote control (RC) method and those estimated using the sniffer method, indicating that this approach can provide a reliable estimate of individual animals' CH emissions. The potential of the sniffer analyzer as a practical, non-invasive tool for measuring enteric methane emissions in dairy cows under commercial conditions is promising. The method successfully identified variations in methane emissions between cows, providing insights for strategies to enhance environmental sustainability (Boutes et al., 2024).

It has also been reported that the sniffer technique is less accurate in estimating CH₄ production compared to the GF system. The accuracy of the sniffer technique is influenced by uncertainties related to dairy cow head movements at the feed trough, the various designs of feed troughs, and the positions of sampling points. All of these factors can lead to different air-mixing conditions and varying dilution effects of ambient air on the gas concentration in eructations (Wu et al., 2018).

2.5. The face mask method

This method involves "spot sampling" of respiratory exchange and methane emissions, which has been extensively utilized in the study of sheep, goats and cattle over the years.

(Washburn and Brody, 1937). In this method, animals must be trained to remain in sternal recumbency during the measurement periods (30 minutes), which are repeated, over a 24 hr. period (Bhatta et al., 2007). However, this approach is influenced by significant variability among different animals and across various days, yielding only a temporary emission measurement. (Lockyer and Jarvis, 1995). Accurate measurements of CH₄ emissions require careful consideration of sampling frequency and timing, which should be synchronized with the animals' daily feeding routines and natural methane emission patterns, and can be achieved through consistent and comprehensive sampling across multiple animals over a 24-hour period. The face mask method can be used to estimate a typical CH₄ emission pattern (Hill et al., 2016). This method is especially effective for quick CH₄ assessments when evaluating many animals; however, it may induce discomfort and stress, potentially influencing animal behavior and affecting the accuracy of gas measurements. Oss et al. (2016) noted that the facemask method may also present limitations in evaluating enteric CH₄ mitigation strategies that involve short duration and small numbers of animals due to significant variability among animals and across different days. Compared to the RC and ventilated hood methods, it requires greater cooperation from the animal and restricts the animal from eating and drinking while the facemask is on during the measurement period. Furthermore, animals may feel uncomfortable when confined in a squeeze chute, which can disrupt the measurement procedure (Zhao et al., 2020).

2.6. Intra-ruminal gas sensor

A recent innovation in the study of rumen gas measurement involves the creation of an intra-ruminal device that accurately monitors and quantifies the concentration levels of methane (CH₄) and carbon dioxide (CO₂) dissolved in rumen fluid. However, it is important to note that this device does not directly measure gas flux or emissions. The primary challenge for electronic devices placed within the rumen lies in the potentially harsh environmental conditions, which can lead to corrosion of electrical components over time. To overcome this, the device must allow dissolved gases to quickly permeate through a membrane, enabling real-time monitoring of gas concentrations (Moate et al., 2015). Furthermore, integrating information about rumen size, internal rumen pressure and the pattern of eructation can enhance the estimation of gas production rates. Although this method yields valuable data, Additional investigation is needed to enhance the methodology for precisely assessing methane production in individual animals by conducting in situ measurements of gas concentrations in the rumen. A key focus for future research is the simultaneous assessment of CO₂ and CH₄ levels in the rumen as well as in exhaled breath, encompassing both respiratory and eructated gases.

This combined assessment could aid in assessing CO₂ as a tracer gas, which may facilitate the creation of affordable, portable devices for estimating methane emissions from living animals (Hill et al., 2016).

3. MITIGATION STRATEGIES FOR ENTERIC METHANE PRODUCTION

3.1. Dietary

Enhancing Feed Quality: Utilizing higher-quality forages and grains can improve digestion and contribute to the reduction of methane emissions.

Incorporating Oils and Fats: The addition of unsaturated fats to the diet has been shown to inhibit the microbial pathways responsible for methane production.

Utilizing Tannins and Saponins: Certain plant compounds, like tannins found in tree leaves and saponins present in legumes, have the potential to reduce methane emissions by modifying the fermentation processes occurring in the rumen.

Implementing High-Starch Diets: Diets that are higher in starch, such as those that incorporate corn, tend to produce less methane compared to those rich in fiber.

3.2. Feed additives

Seaweed (Asparagopsis taxiformis): Studies have demonstrated that incorporating specific seaweeds into ruminant diets can cut methane emissions by up to 80% thanks to their bioactive compounds.

Nitrate: Nitrates can function as a feed additive that decreases methane emissions by facilitating the conversion of methane to nitrogen gas in the rumen.

Probiotics and Prebiotics: Certain microbial and nonmicrobial additives can promote gut health and improve fermentation efficiency, leading to reduced methane production.

3.3. Management practices

Grazing Management: Implementing rotational grazing can enhance pasture quality while also helping to decrease methane emissions.

Breeding and Genetics: Selectively breeding animals for lower methane emissions and improved feed efficiency can yield significant long-term benefits.

Manure Management: Effective handling and treatment of manure can mitigate methane emissions, as anaerobic decomposition is a primary source of methane production.

3.4. Technological innovations

Rumen Monitoring Technologies: Advancements in rumen monitoring technologies can enhance the assessment of feed efficiency and enable the prediction of methane emissions.

4. CONCLUSION

The green feed pasture system provided a reliable solution for monitoring methane emissions from cattle and other large animals. By allowing individual access to pelletized feed and using spot sampling, it enabled accurate measurement of enteric CH₄ in real farm conditions. Supporting around 20 animals day⁻¹, with customizable feed, the system advanced understanding of livestock greenhouse gas emissions and promoted sustainable farming practices. This technology proved vital for mitigating livestock's environmental impact and improving farm management strategies.

5. FURTHER RESEARCH

Further studies on genetic and microbial aspects are required to control methanogenic bacteria. Additionally, there is a need for simpler methods to estimate methane emissions and disseminate this information among farmers, promoting the use of these methods to help mitigate global warming.

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