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Review

Towards Sustainable Livestock Production: Estimation of Methane Emissions and Dietary Interventions for Mitigation

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Abstract: The increasing need for sustainable livestock production demands more research in the field of greenhouse gas (GHG), particularly methane (CH_4), measurement and mitigation. Dietary interventions, management, and biotechnological strategies to reduce the environmental impacts and economic implications of enteric CH_4 emissions are needed. While the use of biotechnological interventions and management strategies can be challenging on a routine basis, feed additive supplementation appears to be the most researched, developed, and ready to use strategy to mitigate enteric CH_4 emissions. This paper discusses various recently developed feeding strategies to reduce enteric CH_4 emissions in livestock. Additionally, the manuscript reviews various technologies developed for CH_4 estimation since the accurate and reliable estimation of CH_4 emissions can be a limiting step in the development and adoption of any mitigation strategy.

Keywords: climate change; livestock production; methane estimation; nutritional strategies; in vitro fermentation; gas chambers

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1. Introduction

The contribution of the agriculture sector to the climate crisis is typically underestimated due to numerous overlooked emission sources. According to the environmental protection agency, the agricultural sector alone accounts for 10–12% of total global anthropogenic greenhouse gas (GHG) emissions [1]. Of the net global emissions, this accounts for 13% of carbon dioxide (CO_2), 44% of methane (CH_4), and 82% of nitrous oxide emissions through anthropogenic activities [2]. Among the total agriculture GHG emissions, the ruminant supply chains alone release around 5.7 gigatonnes CO_2-equivalent GHGs in a year, contributing 80% of the total emission from the entire livestock sector [3,4]. The contribution of livestock towards the existing GHG pool is mainly in the form of enteric CH_4 (around 63%), followed by 25% from the use of manure as a fertiliser to the plants and pastures and 12% emissions from dung and urine management [5]. Therefore, the scientific communities are concentrating on research activities to reduce enteric CH_4 emission from livestock and substantial progress has been achieved during the last decade [6–8]. For example, Australia's agricultural GHG emissions have declined by 15.77% since 2005, now reaching 72.04 $MtCO_2e$ GHG emission from the total agricultural sector [9]. However, the drastic increase in animal population could offset the efforts to reduce CH_4 emission. Pertinently, there is an increased focus on sustainable livestock production along with suitable CH_4 mitigation measures.

Decreasing the production of CH_4 from ruminant animals is desirable both as a strategy to reduce global GHG emissions and as a way of improving feed conversion efficiency. Among the various approaches available to reduce CH_4 emission, feed manipulation is the most widely used strategy to target enteric CH_4 reduction in livestock since they are near market-ready products [10]. To help meet this goal, more reliable and repeatable CH_4 measurement strategies are required to ensure the effectiveness and reliability of these feed additives and other CH_4 abatement strategies. Currently, there are a range of techniques being used around the world for the measurement of CH_4 from the ruminants. Lately, researchers and manufacturers are considering this as a vital area of research to develop new technologies and to modify existing instrumentation [11]. There are several methods available for the quantification of CH_4 although these vary in cost, application, repeatability, precision, and reliability [12]. Therefore, it is the premise of this manuscript to review the recent advances in technologies for the measurement of enteric CH_4 emission from ruminants and discuss the potential nutritional interventions for CH_4 mitigation. Recently, a few authors have reviewed the effects of different nutritional supplements for their CH_4 mitigation potential [4,13–16] and the readers are redirected to those reviews for regional or species-specific CH_4 mitigation strategies or mitigation using a particular group of feed additives.

2. Global Climate Change and the Role of Methane

The Earth's climate has varied tremendously in the past few decades due to enhanced anthropogenic activity [17]. The effects of climate change are unequivocal, as is now evident from the rise in global temperature and sea level, the shift in rainfall patterns, glacial retreat, increased frequency of extreme events, and prolonged periods of dry spells and frost [17]. The global mean surface temperature has increased by around 0.9 °C from the late 19th century, a change driven mainly by augmented CH_4, CO_2, and other anthropogenic emissions to the global gas pool [18]. Likewise, since the last century, sea levels have risen by approximately 20 cm, but the rate of rise in sea levels has doubled alarmingly in the past two decades, as compared to the figures of the last century [19]. Similarly, the widespread availability and pattern of rainfall has changed across the globe. The regional climate patterns are also in a changing phase, with an increase in the frequency and duration of extreme weather events [18]. Over the period from 1971 to 2008, the Earth has witnessed greater numbers of heatwaves and hot days, with the hottest days during these heatwaves becoming even hotter. The increased frequency of heatwaves may have devastating effects on livestock production and other agricultural sectors [20]. Further, the chemical composition of the global atmosphere has changed greatly since the 1700s because of anthropogenic activities, including livestock production and farming [21]. Among the various GHGs emitted from the agriculture sector, CH_4 is the second most abundant and is a very potent GHG since it has 25 times more global warming potential than CO_2, making it a current target for action [22–24]. Further, due to the short atmospheric half-life of CH_4, efforts to mitigate CH_4 would achieve substantial and swift effects on the global warming potential [24]. The CH_4 emissions from the agriculture sector have doubled since the pre-industrial time. Change in atmospheric CH_4 can substantially increase water vapor concentration and it can affect stratospheric and tropospheric chemistry. Ultimately, an increased concentration of CH_4 in the atmosphere can increase the magnitude of the GHG effect and the Earth's temperature [21,25].

3. Mechanism of Methane Production in the Rumen

Methane is produced by ruminants as a product of the fermentation of ingested feed [26]. Ruminant animals emit CH_4 mainly through two pathways, via midgut fermentation and hindgut fermentation. Midgut fermentation or enteric fermentation solely accounts for 89% of total CH_4 emission from the animal. Apart from the deleterious effects on global warming, CH_4 is also a dietary energy loss and ruminants can lose between 2 and 12% of ingested energy in the form of CH_4 [27]. The ruminant forestomach or rumen is host to a large group of diverse microorganisms [28]. These microbes ferment feed materials consumed by the animal through the process of enteric fermentation [29]. The products derived from the enteric fermentation of plant materials provide the nutrients required for the animal's survival. Microorganisms present in the rumen, such as bacteria, fungi, archaea, and protozoa, hydrolyse the dietary polysaccharides present in the feed materials into simple sugars through their enzymatic activity and finally yield volatile fatty acids (VFA), primarily acetate, propionate, and butyrate [30]. In unison, varying amounts of hydrogen (H_2), formic acid, and CO_2 are produced as end-products of fermentation [26]. Most of the methanogenic archaea and some bacteria in the rumen use H_2 ions to reduce CO_2 to produce CH_4 since this process is thermodynamically favourable to microbes. This process keeps the partial pressure of H_2 low, which directs fermentation towards the production of less reduced end-products including acetate [31]. The abundance of H_2 ions is determined by the proportion of end-products from the ruminal fermentation of ingested feed. Formate, which is abundant in most of the ruminant archaea, is also considered to be a part of this hydrogenotrophic pathway. *Methanobrevibacter ruminantium* and *Methanobrevibacter gottschalkii* are the major hydrogenotrophic archaea that alone encompass 74% of the methanogenic archaeal community in the ruminant stomach [32]. Likewise, methyl groups present in the methanol and methylamines may serve as another category of substrates that favour methanogenesis [33]. The formation of acetate results in an increase in hydrogen ions, while the process that yields propionate consumes hydrogen ions [34]. Therefore, the greater the production of acetate, the more CH_4 can be expected, whilst an increase in propionate production is associated with lower production of CH_4 [13].

4. Estimates of Enteric Methane Production: How Much Does Each Species Contribute?

Livestock production is an integral component of global agriculture and also a significant contributor to anthropogenic GHG emissions. The enteric CH_4 emission is responsible for 44% of the GHG emission from the total livestock group and 55% from the ruminants [35]. Altogether, ruminants contribute 2,098,787.77 CO_2-eq of enteric CH_4 to the global GHG pool, of which 54.7% is from non-dairy cattle, 18.9% is from dairy cattle, 10.5% is from buffaloes, 7% is from sheep, 4.4% is from goats, 1.3% is from camels, 1.1 is from horses, and 0.5% is from donkeys [36]. As per FAOSTAT [36], on a yearly average, dairy cattle, non-dairy cattle, buffaloes, sheep, and goat emit 1419.1, 926.5, 1155, 117.2, 105 kg CO_2-eq of CH_4, respectively. There is a strong link between the quantity of enteric CH_4 emission and species differences in animals. Variations in the quantity of feed intake/body mass and the quality of the feed are the major reasons behind this drastic difference in enteric CH_4 emission between each species [3]. Within the species, there can be breed-wise variation in enteric CH_4 emission. Differences in the genetic potential and feed digestion efficiency among breeds better explain this variation [3,37]. Figure 1 shows the percentage of enteric CH_4 from total CH_4 emission from each species. Figure 2 represents species-wise contributions to the global enteric methane pool.

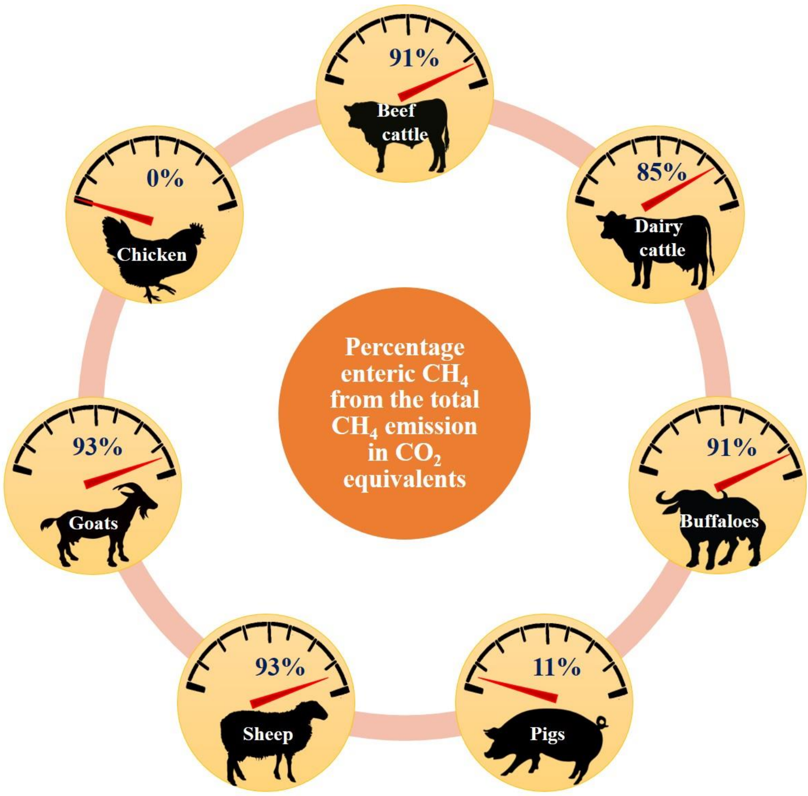

Figure 1. The proportion of enteric methane in the total methane emissions arising from different species; data adapted from Grossi et al. [38], FAO [39].

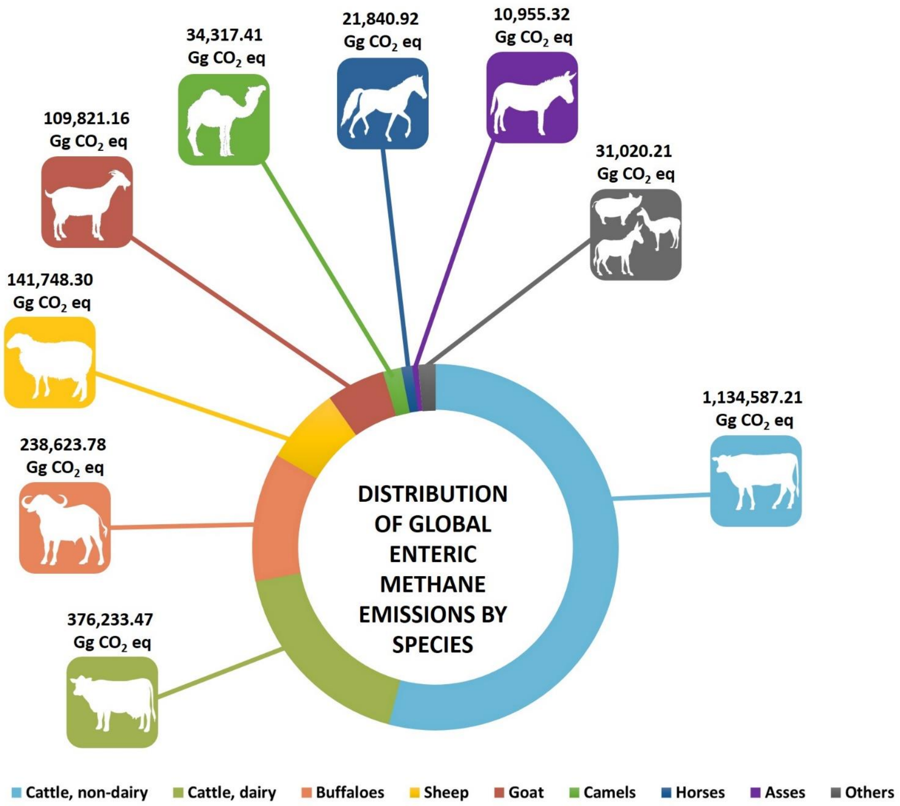

Figure 2. Distribution of enteric global methane emissions by species (CO_2 equivalents); data adapted from FAOSTAT [36].

5. Methods Used to Quantify Methane Production

Knowledge about different CH_4 measuring techniques is increasingly in demand due to the crucial role of CH_4 in global warming and several commitments to reduce global CH_4 emissions. There are several existing methods for estimating CH_4 production that include in vitro estimation and on-farm measuring techniques [40,41].

5.1. In Vitro Estimation

There are several methods for quantifying gas production through in vitro fermentation, with variations in complexity and sophistication. This is a relatively cheap method that is suitable for analyzing CH_4 emissions from a vast variety of feed additives and plant extracts without the error of animal to animal variation [42]. It is particularly useful for ranking different dietary interventions. The basic principle of every in vitro fermentation technique relies on the incubation of feed samples along with the rumen microbial inoculum and buffer solution in an anaerobic environment [43]. The anaerobic fermentation of feed samples can yield various gases in the container and the cumulative volume can be later recorded [44]. The typical gas compositions and CH_4 concentrations can be estimated using the gas samples harvested from the headspace of the container [43].

The ANKOM rumen fluid gas production system is one of the easiest to use and reliable gas production systems that is commercially available. The system is equipped with sample bottles and pressure sensor modules [45]. The gases produced in the headspace of bottles, filled with buffered rumen fluid and feed samples, can be aspirated through the vents using gas-tight syringes [46]. Rumen motility and temperature are simulated by placing the bottles in a shaking water bath maintained at 39 °C [47,48]. The in vitro gas production technique (IVGPT) is another routine method for evaluating feed samples and gas production. This system uses glass syringes, to incubate feed samples and rumen liquor, instead of bottles [49]. Rusitech is another type of artificial rumen apparatus that is currently available. The main advantage of this system is that it can simulate the rumen for several days and can maintain protozoal numbers [50].

5.2. Respiration Chamber

Respiratory chambers are one of the main in vivo CH_4 measuring technologies that have been used for more than 125 years, with varying degrees of complexity [11,51]. This equipment provides the user with an opportunity to measure enteric CH_4 and other gases emitted from the mouth, nostrils, and rectum [41]. Among the two models of respiration chambers available, open-circuit chambers are more widely used over closed-circuit chambers. The basic working principle of this technique is based on the first law of thermodynamics and measures the concentrations of CH_4 leaving the chamber. Gas samples are collected from the inflow and exhaust ducts during this period by creating a negative pressure inside the chamber [52]. Internal ventilation fans fitted inside the chamber ensure proper mixing of the incoming air and exhaled gas. The CH_4 produced by the animal is calculated by multiplying the air circulation through the system by the concentration difference between incoming and outgoing air. Animals can be fed and watered inside the chamber for 1–7 sequential days [11,53]; this gives an opportunity to measure CH_4 emission in terms of dry matter intake [54]. Further, open-circuit respiratory chambers are often referred to as the 'gold standard' for measuring accurate CH_4 emission measurements; hence, they account for the losses from rectum and rumen fistulas in addition to the losses through regurgitation [12,55]. However, CH_4 can still be lost if the chamber is imperfectly sealed [56].

5.3. Ventilated Hood Systems

Ventilated hood systems or head hood systems are a simplified version of respiratory chambers that only enclose the head of the animal [57]. The mobile head hood system used by Fernández Martínez et al. [58] consists of a headbox, rotameter, flow meter, air volume totaliser, adjustable and precise membrane pump, gas cooler, and gas analyser unit. The gas analyser associated with the instrument analyses the composition of gases drawn through the head hood. The head hood has blowers that can move air from the inlet to the exhaust. Further, the head hood, made up of clear polycarbonate material, allows a full range of vision to the animal during the sampling time [59]. The sufficiently large headbox allows the animal to move its head effortlessly to access feed and water and to lay down [11,60]. The ventilated hood systems can be used consecutively over 24 h or for longer periods to measure CH_4 emission. This can be useful for researchers trying to establish a link between CH_4 emission, feed consumption, and energy metabolism [60]. In addition to the CH_4 measurement, this equipment can measure ethanol, methanol, water vapor, nitrous oxide, acetic acid, CO_2, and oxygen (O_2) from the animal on a real-time basis [61]. As compared to respiration chambers, ventilated hood systems are less expensive and they require much less space [62]. The main demerit with this system is that it can only account for the CH_4 produced from midgut fermentation [63]. Furthermore, a sufficient amount of time and training is required to make the animals accustomed to the head hood apparatus. Like the respiratory chamber, this equipment also cannot be used in grazing conditions [64].

5.4. Sulfur Hexafluoride Tracer Gas

Another CH_4-measuring technique that can be used for grazing animals, as well as penned animals, is the sulphur hexafluoride (SF6) tracer technique [65]. In this basic system, animals are orally administered with a permeation tube that releases a known amount of SF6 into the reticulorumen junction of the animal [66]. The exhaled air from the animal is collected from a point near the nostrils and mouth by means of a tube with in-line flow restrictors connected to an evacuated canister connected to a halter and attached with a capillary tube around the neck or held in a harness on the back of the animal [67,68]. At the end of each day, gas accumulated in the canisters will be collected and subjected to gas chromatography. This method assumes that the rate of tracer gas emission is the same as the CH_4 emitted from the animal [59,64]. The enteric CH_4 production is determined by multiplying the CH_4 to SF6 ratio by the release rate of the permeation tube, corrected for the actual length of sample collection and the background CH_4 level [69]. As compared to the respiration chambers and ventilated hood systems, the SF6 tracer method is less costly and can be concurrently used in a greater number of animals. However, the release rate of the tracer can affect the CH_4 release and it can account for 6 to 13% variation in the CH_4 accountability [70]. Furthermore, among- and within-animal variation in CH_4 emission are greater when using the SF6 technique as compared to the respiratory chamber [71].

5.5. Open-Path Lasers

An open-path laser or tunable diode laser is another possible CH_4-measuring instrument that uses beams of infrared light to quantify CH_4 from a grazing herd [72]. The sensor associated with the instrument captures the reflected light and analyses the intensity of the received light as an indicator of CH_4 levels along the path [73]. The laser system used by Laubach and Kelliher [74] consists of a main unit that contains an infrared laser source and a reference cell, remote heads that contain a photodiode that converts the reflected light to an electric signal, and four retroreflector units. The efficiency of an open-path laser is highly dependent on the weather elements and the location of the animals. Sometimes, insufficient laser lighting and wind variation can create disruptions in the CH_4 measurements [75]. The CH_4 emission rates are usually calculated using a backward-Lagrangian stochastic model [76]. Usually, the laser path will be located at 0.5 m height and 1 to 1.5 m outside the perimeter of the pens. The instrument considers cattle herd as a surface source and the individual animals fitted with collars as a point source [77]. The main advantage of this technique is that it can cover a large area and large herd and it does not affect the normal grazing behaviour of the animals. The accuracy of the measurements depends heavily on the positioning of the animals. For example, in larger paddocks, animals may congregate around water tanks and feeders or away from the beam of infrared path of the instrument, resulting in inconsistent measurements [78].

5.6. GreenFeed Emission Monitoring System

GreenFeed is a short-term CH_4-measuring system that offers a small amount of pelleted bait to attract animals to the measuring unit [79]. The gas collection head of the instrument measures CH_4 in the exhaled air every time the animal approaches the equipment. This instrument is highly compatible with in-house conditions as well as in an extensive grazing condition [80]. The user has the freedom to change the type, frequency, and amount of the pellet. The user can adjust the pellet flow remotely to make the animal spend more time in the semi-enclosed hood in order to capture more eructations. The GreenFeed system automatically monitors the positioning of the muzzle in the hood and omits incorrect data due to incorrect head positioning [81]. The extractor fan inside the hood samples the eructed and exhaled air for analysis of various gases. Each animal has to be tagged using unique radio frequency identification (RFID) tags so that the instrument can identify each animal [82]. The instrument can restrict the excessive visit of animals for accessing bait with the aid of RFID tags. The instrument will not dispense the bait feed to the animals that visit the instrument more frequently than the interval set by the user [83]. The data generated from the GreenFeed equipment can be accessed on a real-time basis using a web-based data management system [84]. The main advantage of the GreenFeed system is that it does not require extensive labour or any other laboratory equipment and more animals can be monitored over a short span of time [79]. However, the main drawback associated with this instrument is the supply of an attractant that can modify the VFA concentration and overall digestibility of the diet. Furthermore, in the grazing paddocks, some animals might be reluctant to approach the instrument [82]. Animals must be trained thoroughly to use GreenFeed equipment before the experiment [85].

5.7. Portable Accumulation Chambers

Portable accumulation chambers are another short-term CH_4-measuring technique that shows resemblance to the respiratory chamber [86].The accumulation chambers are essentially a portable, airtight polycarbonate box that contains CH_4, CO_2, and O_2 analysers mounted on it [64,87]. The portable accumulation chamber captures all the exhaled and erected air from the animal during the sampling period and analyses it at the end [11]. The CH_4 emission is calculated as the airflow inside the chamber multiplied by the level of CH_4 inside the chamber that is corrected for the CH_4 concentration of the incoming air, pressure, and temperature in the chamber [12,88]. This method is suitable for measuring CH_4 from a large number of animals and to classify the animals based on their genetic potential to produce CH_4 gas [88]. Portable accumulation chambers can also be used to assess the impact of different types of feeds and feeding regimes on CH_4 production [89]. Moreover, this method is relatively inexpensive compared to many other pieces of commercially available CH_4 measuring equipment [90]. However, as compared to the respiration chambers, results from portable accumulation chambers seem to be less repeatable [91].

The recent advances in CH_4 estimation techniques and technologies have played an important role in the accurate quantification and mitigation of CH_4 emissions and in preparing the inventories. Furthermore, different types of measuring devices have helped researchers and producers to cover emissions from heterogenous farming systems to develop national inventories. Accurate quantification of CH_4 is not only critical to track our industry emissions but is equally important for the assessment of mitigation technologies that are highly needed to reduce global methane emissions. There are various mitigation technologies in addition to nutritional interventions, which are one of the main focuses of this manuscript and are reviewed in the next section. Each technology has certain merits and demerits and proper field configurations, which are outside the scope of this review but have been previously reviewed, and readers are directed to the review by Pragna et al. [13].

6. Nutritional Interventions as One of the Important Methane Mitigation Options

Among various CH_4 mitigation strategies, nutritional intervention or dietary manipulation is the most effective and increasingly used strategy to mitigate enteric CH_4 emission in ruminant livestock [92–94]. Table 1 summarises the effect of various feed additives on the CH_4 and other rumen fermentation characteristics.

6.1. Concentrate Supplementation

It is obvious that the use of concentrate feed can reduce enteric CH_4 production in ruminants. This is achieved mainly through shifting the fibre-based fermentation to starch fermentation [93,95]. The fermentation of starch creates an alternative hydrogen sink in the rumen by lowering the ruminal pH and inhibiting the growth of methanogens, thereby promoting more propionate production [96]. Nampoothiri et al. [97] investigated the effects of different levels of concentrate supplementation (20, 40, and 60%) on the CH_4 emission from Murrah buffalo calves housed in a well-ventilated shed and reported a reduction in daily CH_4 emission and yield while using the SF6 technique to measure CH_4. Jiao et al. [98] fed perennial ryegrass grazing Holstein Friesian dairy cows with different ranges of concentrate feeding levels (2 kg, 4 kg, 6 kg and 8 kg as-fed basis) and reported a decline in CH_4 emission (using SF6) with the increase in the level of concentrate supplementation when expressed in terms of emission per unit of feed intake and energy-corrected milk. Further, individually housed Charolais cross heifers showed a decline in enteric CH_4 production when they were supplemented with 80 and 90% concentrate, although the effect was not significant at 35 or 60% concentrate inclusions [99]. A recent study conducted in Alpine Grey and Brown Swiss cattle fed on different levels of concentrate diets (low and high) showed a decrease in emission of CH_4 biogenic compared to low concentrate when estimated using a life cycle assessment model [100]. Van Wyngaard et al. [101] tested three different levels of concentrate intake (0, 4, and 8 kg) in lactating Jersey cattle reared under medium-quality summer pasture and observed a decrease in the CH_4 yield and intensity with increasing concentrate level, though the CH_4 production peaked with the increase in concentrate supplementation. Holstein cows, tied in a modified respiratory chamber, were fed different forage to concentrate levels, 47:53, 54:46, 61:39, and 68:32, and showed 25.9, 28.2, 29.1, and 31.9 g/kg of DMI CH_4 production, respectively [102]. Moreover, a very low CH_4 production of around 2–3% of gross energy ingested was reported in cows supplemented with 90% concentrates [27]. In contrast, Muñoz et al. [103] observed an increase in CH_4 emission (measured using the SF6 technique) per unit of milk yield with an increase in the level of concentrate supplementation. This was plausibly due to the high digestibility of perennial ryegrass pasture ingested by the Holstein animals. However, concentrate feeding beyond a certain limit is not recommended as it can cause severe damage to the animal itself and its production performance because of acute or sub-acute acidosis. Furthermore, grains that may be used for concentrates are more valuable for human feeds in arid and semi-arid regions, where much of the global ruminant production is located.

6.2. Lipid Supplementation

The use of lipid compounds offers another possible strategy to decrease enteric CH_4 emission from ruminants. Addition of lipid compounds inhibits the methanogenic and ciliate protozoan population in the rumen [104,105]. Lipid addition also decreases organic matter and fibre degradability and reduces fermentable substrate in order to reduce CH_4 production [106]. Machmüller and Kreuzer [107] suggested coconut oil as an efficient natural additive to reduce CH_4 production without causing detrimental effects on the nutrient utilisation of the animals. On average, they observed 28 and 73% reductions in daily CH_4 emission/animal when the Swiss Brown Hill wethers housed in respiratory chambers are fed with a ration containing 3.5 and 7% coconut oil, respectively. The reduction in CH_4 release could be due to the suppressive effect of coconut oil on methanogens and ciliate protozoa populations. Further, Hereford × Friesian cross steers, fitted with

SF6 breath sample collection canisters, were reared on canola-oil-sprayed (Oil-spray, 12 L/strip) ryegrass pasture and showed reduced CH_4 production by 18% in terms of g per day [108]. Using soybean oil, Mao et al. [109] demonstrated around a 13.9% decrease in CH_4 production in Huzhou lambs when measured using a simple, open-circuit respiratory chamber. Similarly, Chuntrakort et al. [110] investigated the effect of different feeding oil plant diets on CH_4 emission using a headbox respiration chamber system from Thai native Brahman crossbred cattle and observed a reduction in CH_4 production with oil supplementation. Among the oil-plant-supplemented diets, the coconut kernel diet was most effective in mitigating enteric CH_4 emission, followed by the sunflower seed and cottonseed diets. Using open-circuit respiratory chambers, Machmüller et al. [111] reported decreased CH_4 production in lambs fed different types of lipids along with total mixed rations. Within a short span of 3 weeks, they observed a 26, 27, and 10% reduction in CH_4 production per kg LW when the lambs were supplemented with coconut oil, sunflower oil, and linseed oil, respectively. Conversely, Cosgrove et al. [112] reported no significant change in CH_4 production (measured using a SF6 marker) from penned ryegrass pasture fed sheep supplemented with different concentrations of linseed and sunflower oil mixture (0, 1.2, 2.5, 3.7, 5.0, and 6.2% of DMI). While using lipid supplementation, one caution has to be observed that fat supplementation should not exceed over 6–7% to prevent a possible decline in dry matter intake by animals due to the inconvenient odour [93].

6.3. Ionophore Supplementation

Ionophores are generally used in livestock feed to improve feed efficiency and to increase body weight [113]. Commonly available forms of ionophores include lasalocid, monensin, laidlomycin propionate, tetronasin, salinomycin, narasin, and lysocellin [113,114]. Ionophores act as a CH_4-inhibiting factor by shifting the fermentation acids from acetic acid and butyric acid to propionic acid by promoting the growth and proportion of Gram-positive bacteria in the rumen. Stall-fed Holstein cows treated with 18 mg/kg of dry matter monensin showed a 24.3% decline in CH_4 production when expressed in g/day. According to the authors, this reduction could be due to the positive effect of monensin on the Gram-negative bacteria that produce propionate and due to the negative effect of monensin on the acetate and hydrogen-producing bacteria such as Eubacterium, Lactobacillus, and Streptococcus [115]. Likewise, feedlot-type penned Angus steers fed with 33 mg/kg monensin showed a 30% reduction in enteric CH_4 production (measured using the SF6 technique) along with a numeric reduction in ciliated protozoan populations [116]. The CH_4-reducing effect of monensin is mainly due to changes in the production of ruminal volatile fatty acids. Li et al. [117] reported a 20.3 L/day reduction in CH_4 in goats supplemented with monensin and housed in a closed portable static environmental chamber. Additionally, stall-fed Murrah buffalo heifers supplemented with sodium monensin showed 8–9% reduced energy loss in the form of CH_4 when estimated using the SF6 tracer technique [118]. However, the excessive supplementation of ionophores can lead to toxicity in ruminants, and they also need to be screened for their residual level in the animal products [119,120].

6.4. Anti-Methanogenic Compounds

Anti-methanogenic compounds are another important nutritional intervention in the enteric CH_4 mitigation studies, though the usage of some chemical anti-methanogenic compounds is not allowed in some countries because of their anti-nutritional effects. Bromochloromethane (BCM) is one of the widely researched anti-methanogenic compounds that has the potential to reduce a considerable amount of CH_4 from the ruminants. When incorporated into the diets of stall-fed steers, BCM-Cyclodextrin (1 g/100 kg BW/day) has been reported to reduce CH_4 production by around 95% by hindering the cobamide-dependent methyltransferase step in the process of methanogenesis through its reaction with vitamin B12 [121]. Furthermore, Lalu et al. [122] reported a 90% reduction in CH_4 emission from penned rams supplemented with BCM. In addition, 3-nitrooxypropanol (3-NOP) is another possible anti-methanogenic compound and Romero-Perez et al. [123]

conducted a series of experiments in barn-tied beef cattle using 3-NOP. In the first set of experiments, the authors compared different dosages of 3-NOP (0, 0.75, 2.25, and 4.50 mg/kg BW) and observed a linear decline in CH_4 production with the level of dosage, with 33% CH_4 reduction from the highest dosage. The authors found a shift in VFA production from acetate to more propionate without hampering body weight gain or feed digestibility. In the next long-duration experiment (112 days) using 3-NOP at 2 g/day level, the author had observed a 60% reduction in the enteric CH_4 production without the microbial adaptation to 3-NOP when measured using closed-circuit respiratory chambers [123]. Likewise, Lopes et al. [124] also reported a 31% decline in CH_4 production (CH_4 estimated using GreenFeed system) from lactating Holstein cows fed 3-NOP at the rate of 60 mg/kg of feed dry matter. In another study using ethyl-3-nitrooxy propionate (E3NP) and 3-NOP, Martínez-Fernández et al. [125] demonstrated 14 and 25% decreases in enteric CH_4 production, respectively, from E3NP- and 3-NOP-fed Segureña sheep using respiration chambers.

Apart from the synthetic or artificial anti-methanogenic derivatives, some of the naturally occurring red algae, seaweeds, fungus, and lichens can produce haloforms, dihalomethanes, and some other organobromine compounds that have an anti-methanogenic effect [126,127]. Li et al. [128] demonstrated an 80% reduction in enteric CH_4 emission from penned Merino-cross wethers when they were supplemented with 3% *Asparagopsis taxiformis* organic matter; here, the authors used open-circuit respiration chambers for the measurement of CH_4. Kinley et al. [129] reported the anti-methanogenic effect of red macroalgae, *Asparagopsis taxiformis*, in vitro when fermented with a high-quality Rhodes grass. This work demonstrated a significant enteric CH_4 reduction with the supplementation of 1% *Asparagopsis*. Further, Roque et al. [130] reported a 26.4% reduction in CH_4 production (estimated using the GreenFeed Large Animal System) without compromising feed intake or milk yield when the freestall barn-housed cattle were supplemented with 0.5% level *Asparagopsis armata* (organic matter basis). However, when they increased the inclusion level to 1%, it resulted in a 67.2% enteric CH_4 reduction but with negative effects on feed intake and milk yield. Martínez et al. [131] found an anti-methanogenic effect of garlic-derived compound propyl propane thiosulfinate. In order to test the anti-methanogenic potential of allyl disulphide and lovastatin, Klevenhusen et al. [132] conducted a study with caged swiss Black-Brown Mountain sheep. Briefly, the sheep were randomly allocated to a diet supplemented with 4 g diallyl disulphide and a diet supplemented with 80 mg lovastatin per kg of total dietary dry matter for 23 days and the animals of the experiment were kept inside the open-circuit respiratory chambers for 4 days for measuring CH_4 emission. In summary, they could not find any significant influence of dietary supplements on daily CH_4 production. However, diallyl disulphide showed a reduction in CH_4 production when expressed in per kg NDF digested. Therefore, this study revealed the potential of diallyl disulphide, a garlic oil derivative, to improve fibre digestion and to limit energy loss in the form of CH_4.

6.5. Probiotic Feeding

Probiotics are potential feed additives that have many beneficial properties, including immunity stimulation, stabilisation of the microbes in the digestive tract, production of anti-microbial substances, prevention of feed-related allergies, improved dry matter intake and fibre digestibility, and CH_4 mitigation [133,134]. Some of the direct-fed ruminant specific probiotics include *Saccharomyces cerevisiae*, *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, *Prevotellabryantii*, *Bacillus*, and *Megasphaeraelsdenii* [8,135]. Recently, Hassan et al. [134] studied the effect of a *Ruminococcus flavefaciens*-based probiotic supplement on CH_4 production in Barki lambs kept in caged conditions. Their results showed a significant reduction in CH_4 production as compared to controls; this change could be attributed to variation in the rumen microflora. Likewise, in another CH_4 estimation study using head hood systems conducted in *Bacillus licheniformis*-supplemented

Dorper × thin-tailed Han wethers, Deng et al. [136] reported a 6% reduction in daily CH_4 production. Additionally, Latham et al. [137] found the possibility of using *Paenibacillus 79R4* as a probiotic supplement in order to reduce nitrate toxification and CH_4 production in nitrate-treated steers grazing on Bermuda grass pasture. Suryani et al. [138] found that *Saccharomyces cerevisiae* and combination of *Saccharomyces cerevisiae* with *Bacillus amyloliquefaciens* could reduce the CH_4 production from Bali cattle kept in individual pens by stimulating the acetogens in the rumen to compete with methanogenic bacteria. Recently, Chen et al. [139] conducted an in vitro experiment using a cluster of different propionic acid bacterial strains and reported the ability of *Propionibacterium jensenii LMGT2826* and *Propionibacterium thoenii LMGT2827* and *Propionibacterium thoenii T159* bacterial strains to mitigate CH_4 emission by 18, 8, and 20%, respectively, compared to the control. However, the application of *Propionibacterium acidipropionici* as a feed additive did not affect the CH_4 production (measured using open-circuit respiration chambers) from Merino wethers [140].

6.6. Essential Oils

Generally, essential oils are volatile aromatic substances extracted from herbs and spices [141]. Essential oils contain a variety of chemical substances, such as isoprenes, terpenes, diterpenes, triterpenes, hemiterpenes, sesquiterpenes, and tetraterpenes, etc. Essential oils possess antimicrobial properties against ruminal inhabitants such as bacteria, fungi, and protozoa [142]. Additionally, essential oils have shown promising potential in improving the production potential and in mitigating enteric CH_4 emission [141]. The use of essential oils as a CH_4 mitigation strategy has been greatly tested by several authors over the last decade and extracts from citrus, oregano, garlic, thyme, and cinnamon have given consistent results [141,143]. For example, Wu et al. [144] suggested intermittent feeding of citrus essential oil as a potential CH_4 reduction strategy in Hu sheep housed in individual cages by reducing microbial adaptation to additives. Further, Hart et al. [145] demonstrated 6% less CH_4 production per day in cows kept in freestall barns with the supplementation of a commercial essential oil blend (Agolin Ruminant Liquid Formulation); this was measured using a GreenFeed large animal monitor. Cows fed with a feed additive rich in thyme essential oil have also shown a significant reduction in CH_4 produced (CH_4 was measured using an indirect calorimetry facemask system) [146]. Soltan et al. [147] conducted an essential oil feeding experiment in Santa Inês sheep. Briefly, sheep were fed with a microencapsulated blend consisting of cinnamaldehyde, carvacrol, capsicum oleoresin, and eugenol. Sheep were kept inside the respiratory chamber for measuring CH_4 emission. The sheep fed with the essential oil bled had significantly lower CH_4 production, without any antagonistic effect on nutrient digestibility. In another experiment, Sallama et al. [148] supplemented sheep kept in open-circuit respiration chambers with 10 mL and 20 mL/day eucalyptus essential oil and reported 31 and 22% reductions in CH_4 production, respectively.

6.7. Organic Acid Supplementation

Predominantly, organic acids fed to the animals are of natural origin, with low potential for toxicity, as they naturally occur in the cell metabolism. Organic acid supplementation helps the animals to prevent their ruminal pH from falling; at the same time, it also helps to reduce the methanogenesis in the rumen [149]. Among the various organics acids, aspartate, malate, and fumarate are known for their ability to act as an alternative hydrogen sink to promote more propionate production [150]. Dietary supplementation of fumaric acid (2% of the diet dry matter) with a roughage-based diet has been reported to decrease CH_4 production (measured using the head hood system) by 23% in stall-fed Holstein steers, changes that were also accompanied by increased total VFA production and propionic acid production. However, the potential of organic acids to lower CH_4 may depend on the level of organic acid supplementation and the dietary condition [151]. To omit acidity-related issues due to organic acid supplementation, Wallace RJ [152] encapsulated fumaric acid with a shell of hydrogenated vegetable oil and observed a greater reduction (75%) in CH_4 production from Welsh Mule Cross lambs. CH_4 was estimated with the help of a polythene tunnel system. Further, Dorper × Thin-tailed Han crossbred ewes showed a decrease in the daily enteric CH_4 output from 66.1 L/kg digestible organic matter (DOP) to 61.01 L/kg/DOP when supplemented with allicin and this was measured using a headbox system [153]. Additionally, Charolais cross heifers supplemented with DL-malic acid also showed a 16% reduction in daily total CH_4 emission (measured using the SF6 technique) [154]. When tested using in vitro batch fermentation with a mixed diet of meadow hay, barley, and sugar beet molasses, sodium aspartate gained a 21.56% reduction in CH_4 production. Furthermore, supplementation of aspartate increased the production of propionate without reducing acetate production [155]. However, the high cost of organic acid makes its commercial usage a somewhat economically unviable option [150].

6.8. Exogenous Enzymes

Exogenous enzymes are widely used to remove the anti-nutritional factors in livestock feed and to improve digestibility [156]. The enzymes are generally sourced from bacteria such as *Lactobacillus acidophilus*, *5 Streptococcus faecium*, spp. L. *plantarum*, *and Bacillus subtilis*, and fungi like *Trichoderma reesei*, *Aspergillus oryzae*, and *6 Saccharomyces cerevisiae.* The studies linking CH_4 production and exogenous enzymes are very limited and equivocal. Some studies showed that enzyme addition decreased CH_4 production by ruminant animals but others did not [157]. Arriola et al. [158] tested the effect of a fibrolytic enzyme on CH_4 production from two groups of Holstein cows fed low- and high-concentrate diets, respectively, and they observed a reduction in CH_4 production when the animals were supplemented with the fibrolytic enzyme; these animals were housed in a freestall, open-sided barn. Further, these effects were more prominent in the high-concentrate-based diet. In a review of the nutritional management for enteric CH_4 abatement, evidence was presented to support a role for exogenous enzymes in the mitigation of enteric CH_4 produced from ruminants [93]. Zhao et al. [159] demonstrated a reduction in CH_4 production from feed substrates supplemented with cellulose and xylanase enzymes and tested in vitro. Contrastingly, negative effects of exogenous enzyme supplementation have also been reported in cattle [160,161] and goats [162].

6.9. Plant Secondary Metabolites

Plant secondary metabolites are the secondary group of molecules that help the plants to adapt to their micro and macro environment. Protease inhibitors, lectins, alkaloids, nonprotein amino acids, cyanogenic glycosides, terpenes, saponins, and tannins are some of the key plant secondary metabolites [163,164]. Secondary metabolites such as condensed tannins and saponins have an anti-methanogenic and anti-protozoal effect [165]. There are a plethora of studies citing the ability of secondary metabolites to mitigate CH_4 production from the ruminant animals [166,167]. SF6 canister-fitted Santa Inês lambs fed with *Leucaena leucocephala* showed a 25.7% enteric CH_4 reduction [168]. Similarly, hydrolysed tannins from *Castanea sativa* wood have also shown enteric CH_4 depressing activity (20% reduction) with significant anti-protozoal effects when tested with Swiss White Hill lambs kept in respiratory chambers [169]. Further, Baruah et al. [167] reported a 19–21% reduction in enteric CH_4 emission in penned, SF6-equipped Mandya lambs when they were supplemented with *Syzygium cumini* and *Machilus bomycina* leaves containing phyto-sources. The presence of condensed tannins in *Tamarindus indica* seed husk was found to be inhibitory to methanogenic activity in the rumen. Condensed tannins found in the *Tamarindus indica* seed husk could affect enzymatic activity, cell membrane composition, and metallic ion exchange in methanogens. Additionally, most of the tanniferous compounds present in the plants can increase the duodenal protein flow by reducing the rate of protein breakdown in the rumen when supplemented at a moderate dosage to ruminants [164,170]. Malik et al. [171] reported a 10–50% reduction in methanogenic activity following *Tamarindus indica* seed husk supplementation to penned crossbred cattle that were equipped with SF6 canisters. Furthermore, penned Thai native beef cattle fed Bamboo-Cass that contains 2.8% and 1.3% condensed tannins and crude saponins, respectively, showed a reduction in CH_4 production by suppressing protozoal populations [172]. Dietary supplementation of chestnut tannins was shown to reduce CH_4 production in Rideau Arcott sheep, without any negative effect on their growth performance. However, the sheep showed a reduction in methanogen and protozoa populations, and the authors used respiratory chambers to measure CH_4 emission [173]. Albores-Moreno et al. [174] supplemented caged Pelibuey × Katahdin lambs with saponin-rich ground pods of *Enterolobium cyclocarpum* and observed a 36% reduction in CH_4 production. Moreover, Váradyová et al. [175] reported that asphonins help the animal nutrients to bypass the rumen, thereby lowering the methanogenesis as a result of reductive acetogenesis. Plant secondary metabolites have been observed to have differing effects on the rumen methanogenesis depending on the plant sources and dosages; however, their mode of action depends on their direct or indirect effect on the microbes responsible for CH_4 production. It is important to consider the risk associated with anti-nutritional factors while feeding plant secondary metabolites, which might cause detrimental effects on animal health and feed palatability; in particular, plant saponins could cause haemolysis in animals [92].

Table 1. Effect of various feed additives on CH_4 and other rumen fermentation characteristics—a summary.

Feed Additives	Dosage	Species	Impact on CH_4	Current Feasibility	Reference
Concentrate feed containing vitamins and mineral supplement	6 kg/day	Cow	↑ CH_4 (g/day)	feasible	[176]
Ground corn	3.2 kg/day	Cow	↓ CH_4 yield by 7.3 g/kg DMI	feasible	[177]
Concentrate feed containing maize, rapeseed meal, soybean, Molaferm, and Megalac, etc.	6 kg/day	Cow	No effect (g/kgDMI) but ↓ CH_4 (g/kg Energy corrected milk)	feasible	[178]
Concentrate feed containing barley, beet pulp, soybean meal, maize meal, molasses, vitamins, and minerals	0.5 kg/day	Lamb	No effect (CH_4 g/kg DMI)	feasible	[179]
Concentrate feed mixture	18.1% of DM	Cow	↓ CH_4 (g/day and g/kg DMI)	feasible	[180]
Concentrate feed containing maize, deoiled mustard cake, soybean meal, wheat bran, rice bran, mineral mixture, and salt etc.	15% high ME content (2.82 Mcal/kg)	buffalo	↓ CH_4 g/day, g/kg DM intake)	feasible	[181]
Coconut oil	4%	Goat	34% ↓ CH_4 emission	Somewhat feasible	[182]
Coconut oil	2%	Goat	More than 50% ↓ CH_4 emission	Somewhat feasible	[183]
Soybean oil	4%	Goat	32% ↓ CH_4 emission	Somewhat feasible	[182]
Soybean oil	50 g/kg DM	Sheep	35.8% ↓ CH_4 emission	Somewhat feasible	[184]
Corn oil	30 g/kg DM	Goat	15.1% ↓ CH_4 emission (g/kg DMI)	feasible	[185]
Corn oil	5%	Cattle	~30% ↓ CH_4 emission	feasible	[186]
Lasalocid	200 mg/hd/d	Cattle	30.91% ↓ CH_4 production (g/kg DMI)	feasible	[187]
Monensin	30 mg/kg	Steer	16.67% ↓ CH_4 (MJ/100 MJ GE intake)	Currently not feasible	[188]

Table 1. *Cont.*

Feed Additives	Dosage	Species	Impact on CH_4	Current Feasibility	Reference
Monensin	22 mg/kg	Goat	28% ↓ CH_4 emission	Currently not feasible	[182]
Monensin	0.6 mg/kg of body weight	Buffalo	8–9%↓ CH_4 emission	Currently not feasible	[118]
Nitrate	11 g/kg DM	Cow	↓ CH_4 by 8%	Not permitted in some countries	[101]
Nitrate	23 g/kg DM	Cow	↓ CH_4 by 15%	Not permitted in some countries	[101]
Ethyl-3-NOP	50 and 500 mg/animal per day	Sheep	↓ CH_4 by 29% (L/kg of DMI)	Not permitted in some countries	[125]
3NOP	60 mg of 3NOP/kg DM	Cow	↓ CH_4 by 31%	Not permitted in some countries	[124]
Bacillus licheniformis	2.5×10^8 colony forming units (CFU)	Sheep	↓ CH_4 by 6%	Currently not economically feasible	[136]
Bacillus licheniformis	2.5×10^9 CFUs	Sheep	↓ CH_4 by 12%	Currently not economically feasible	[136]
Saccharomyces cerevisiae	$(1.2–2.3) \times 10^7$ CFU/g	Sheep	↓ CH_4 by 10% (L/day)	Currently not economically feasible	[189]
Leuconostoc mesenteroides	$(1.5–1.8) \times 10^9$ CFU/g	Sheep	No effect	Currently not economically feasible	[189]
Orange leaves	TMR	Goat	↓ CH_4 by 32% (g/day)	feasible	[190]
Citrus essential oil	0. 0.8 and 1.6 mL/L	sheep	↓ CH_4	feasible	[144]
Commercial essential oil blend	1 g/day	Cow	↓ CH_4 by 6% (g/day)	feasible	[145]
Encapsulated fumaric acid	117 g EFA/kg	Lamb	↓ CH_4 by 76% (L/day)	feasible	[191]
Fumaric acid	100 g FA and 17 g partially hydrogenated vegetable oil/kg	Lamb	↓ CH_4 by 62% (L/day)	Currently not economically feasible	[191]
Dl-malic acid	7.5% on a DM basis	Beef cattle	↓ CH_4 by 9% (L/day)	Currently not economically feasible	[154]
Cellulase	10,000 IU/g	Goat	No effect	Currently not economically possible	[192]
Cellulose/xylanase	7000 IU/g of cellulase and 5000 IU/g of xylanase	Goat	No effect	Currently not economically feasible	[192]
Leucaena leucocephala	350 g/kg DM	sheep	↓ CH_4 by 14.1% g/kg DMI		[193]
White grape marc	5.0 kg DM	Cow	↓ CH_4 by 15% g/kg DMI	feasible	[194]
Red grape marc	5.0 kg DM	Cow	↓ CH_4 by 15% g/kg DMI	feasible	[194]
Willow fodder (*Salix* spp.)	12 g CT kg/DMI	Sheep	↓ CH_4 by 19% (g/kg $BW^{0.75}$/day)	feasible	[195]

7. Conclusions and Future Perspectives

Most of the CH_4 emitted from the livestock production systems is mainly in the form of enteric CH_4. With the changing climate and global warming, it is very important to develop strategies to reduce or mitigate CH_4 emissions from livestock production systems. However, it is equally important to develop methods and technologies to measure CH_4 emissions efficiently and accurately. There are several methods and equipment available for the estimation of CH_4 emission from ruminants. However, most of these techniques have certain advantages and disadvantages and therefore a careful selection of methods is needed for specific production systems. For example, SF6 is more suitable for grazing studies while respiration chambers and hood systems are only useful for indoor studies. Likewise, a plethora of feed supplements for CH_4 mitigation from ruminants have been developed but some of these feed additives may not be feasible for farm usage because of their toxic levels, accessibility, and cost. However, some of the strategies, such as adjusting the roughage to concentrate ratio and using feeding additives such as lipids, essential oils, and plant secondary metabolites, can be used on the farm level to achieve CH_4 mitigation.

Mitigation strategies that do not hamper production and are able to reduce CH_4 emissions in ruminants have better acceptance among farmers and the industry. In practice, farmers are less likely to adopt any of the mitigation technologies that do not attain a minimum sustainable production level or are not economically viable, while reducing methane emissions. Therefore, during the initial phase of transitioning and adoption, the provision of rewards or some incentives might encourage farmers to adopt these mitigation strategies. Furthermore, most of the nutritional interventions have been developed and assessed under the intensive system or in the in-house conditions; therefore, further research is necessary to evaluate the long-term effectiveness of these feeding strategies in grazing farm systems, which are contributing a high proportion of livestock methane emissions.

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