Methane emission: strategies to reduce global warming in relation to animal husbandry units with emphasis on ruminants

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Methane Emission: Strategies to Reduce Global Warming in Relation to Animal Husbandry Units with Emphasis on Ruminants

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Abstract: Concerns about global warming and greenhouse gases have increased the interest of governments and the public sector to find solutions. To reduce the effects of global warming caused by greenhouse gases, especially methane, it is necessary to change animal production systems and adopt new strategic approaches. The reduction of enteric methane in livestock is a long-standing problem regarding the energy efficiency of consumed feed. In this review, the sources of production, dissemination, and introduction of accepted scientific and practical solutions in order to reduce methane gas in breeding and production units of dairy cows have been investigated. To carry out this research, a thorough search was conducted in articles published in valid databases between 1967 and 2022. A total of 213 articles were reviewed, and after screening, 159 were included in the study and analyzed using a PRISMA flow diagram. In general, low livestock efficiency, low-quality feed, a shortage of knowledge, and inadequate investment are the main causes of emission of these gases in poor or developing countries. On the other hand, developing countries may not always have access to the same methods that are utilized in industrialized countries to minimize the production of methane and other greenhouse gases like nitrous oxide. According to their conditions, developing countries should use the available tools to reduce methane production and emission, considering the costs, local knowledge, feasibility, and local laws. In future, there will be a greater need for interdisciplinary research to look for sustainable and acceptable methods for reducing methane emissions and other greenhouse gases from animal husbandry units, especially dairy cows. To change the population of rumen methanogens, as the main producers of methane, strategies such as feeding management, addition of inhibitors and vaccination are suggested. Also, there is a need for more applied research for reducing methane emissions.

Keywords: feed; inhibitor; livestock industry; methanogenic archaea; rumen fermentation; vaccination

1. Introduction

Today, one of the most pressing problems in the world is the rise in greenhouse gases (GHG), which is primarily causing climate change. The main GHG produced by human activity are carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). The movement of GHG into and out of the atmosphere causes Earth's surface to warm and cool, respectively. Although the world's increased production of GHG poses a threat to life, it has been found that their absence can cause the average temperature of the earth's surface to decrease by about $-18\ °C$ [1], demonstrating the crucial role of these gases in ensuring the survival of life on Earth. However, the rapid rise in the concentration of these gases causes climate change and global warming.

CH_4 is the second-most significant greenhouse gas (GHG) after carbon dioxide, which is a significant contributor to climate change and global warming (16–25% of total GHG) [2]. Currently, CH_4 accounts for 20% and carbon dioxide for 60% of the GHG resulting from anthropogenic sources, which include human-related activities such as factories, mines, and agriculture [2]. The largest share of CH_4 emissions is caused by the use of fossil fuels and agricultural activities [3]. Meanwhile, CH_4 has a shorter half-life than carbon dioxide (a half-life of 8.6 years versus 120 years) and is 28 times stronger in terms of global warming potential [4,5]. Due to this issue, research on emission reduction and carbon capture, use, and storage (CCUS) technology has been taken into consideration [6].

Of the total CH_4 emissions, 40% is caused by natural sources (wetlands, termites, and oceans) and 60% is caused by human activities [7]. The primary sources of GHG emissions in agriculture include crop production, livestock breeding, decomposition of biomass and plant residues, and the application of both organic and mineral fertilizers in the soil [8]. In the agricultural sector, livestock generates a major share of CH_4 production [95–109 Tg (i.e., 10^{12} g) CH_4 yr^{-1}], and this gas is primarily released into the atmosphere due to enteric fermentation (87–97 Tg CH_4 yr^{-1}) and manure management (smaller contribution) [9]. As farm species have been shown to be the major contributors of total GHG emissions from the livestock sector, the most attention has been focused on enteric CH_4 emissions from ruminants, particularly cattle. In all, 18.9% of these emissions come from dairy cattle, primarily in the form of enteric CH_4 emissions [9]. Also, animals' genetics, the type and amount of feed consumed, and the method of waste disposal all have an impact on how much CH_4 is produced.

Predictions state that the demand for meat and milk would rise globally by 73 and 58% by 2050 compared to 2010 levels, respectively, as a result of continuing population growth, formation of the middle class worldwide, rising incomes, and more urbanization [10]. However, throughout the same time, the number of livestock will increase by two times [11]. Livestock are the largest consumers of land resources in the world. The world's livestock industry consumes 8% of all water and occupies 80% of all agricultural land used for grazing or food production. Hence, one of the main causes of the atmosphere's GHG concentration rising and contributing to climate change is the expansion of animal husbandry. As a result, the amount of GHG emissions from sources related to livestock (including animals, manure, feed production, and agricultural expansion to forest areas) accounts for 14.5% of all anthropogenic factor emissions globally [12]. Forty percent of the total CH_4 emissions by livestock is produced in the rumen, which constitutes approximately 6% of the anthropogenic GHG emissions in the world [10]. In fact, the ruminant methanogens' final metabolic product after rumen fermentation is CH_4 [13]. An acceptable hypothesis regarding methanogens as the only producers of CH_4 is the increase in their abundance in the rumen, and, as a result, the release of more CH_4 from the animal [14,15]. This issue has caused research on the potential of rumen methanogenesis in animal performance and the environment to be considered.

In this study, CH_4 production routes, microbial populations that contribute to production, and scientific and practical CH_4 reduction strategies were identified and introduced. A step can be taken toward controlling and reducing global warming by researching and implementing CH_4 reduction techniques in agriculture and dairy farms.

2. Materials and Methods

To conduct this research, a complete search was conducted in the articles published between 1967 and 2022 on CH_4 production in dairy cows. This review was carried out by using the Scopus, Web of Science, American Chemical Society, Elsevier, and Springer databases, as well as the Google Scholar search engine. Keywords including environmental pollution, global warming, methanogens, dairy cows, and CH_4 emission were also used. There were 213 articles in the initial database. After revising them, 52 articles were removed, as they were unrelated to the purpose of the study, which included 18 unrelated Persian articles, 21 conference articles, and 13 encyclopedias. After screening, 160 articles were

included in the study. These articles were used in the sections of introduction (15 articles), GHG emissions (29 articles), CH_4 measurement (4 articles), herd management practices (11), enteric CH_4 (6 articles) and methane reduction strategies (97 articles). Finally, methods to regulate and reduce the amount of CH_4 in the atmosphere were proposed in order to reduce CH_4 production while identifying the sources of CH_4 production in dairy cows. The selected articles were of the research and review type, and there were no restrictions on the selection of journals. Figure 1 shows the PRISMA flow diagram from the method of collecting articles to their analysis.
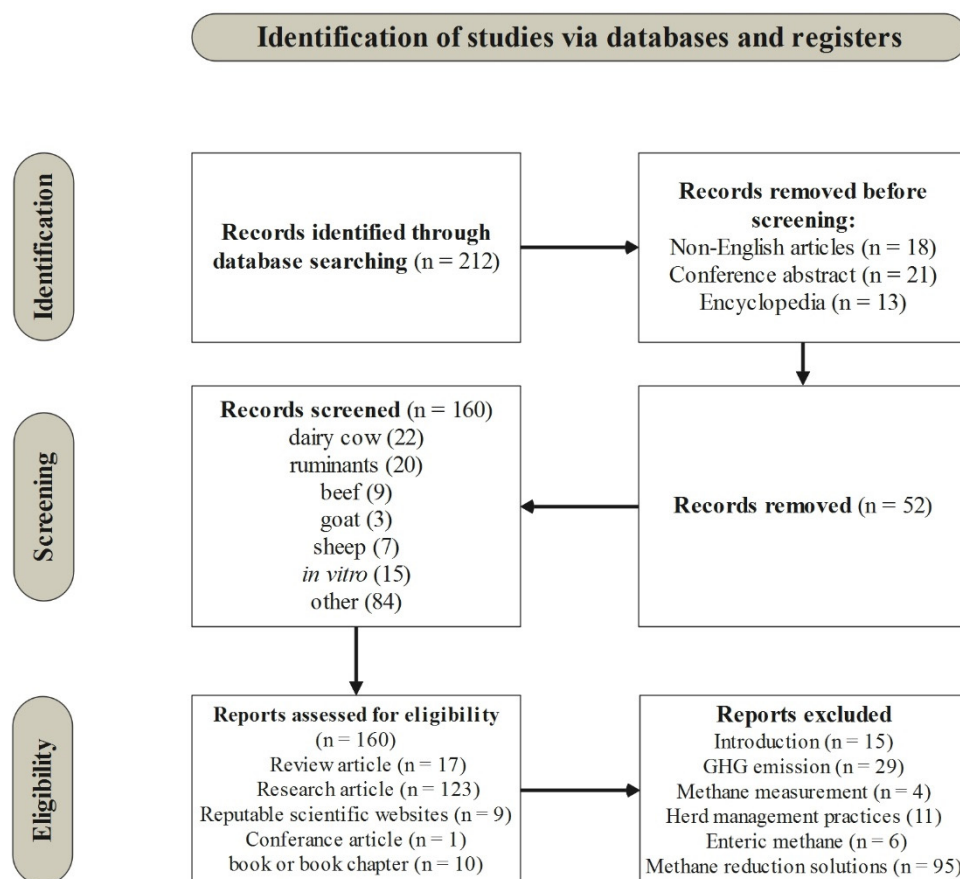

Figure 1. The PRISMA flow diagram of the systematic review from initial search and screening to final selection of publications.

3. Results

3.1. Emission of GHG Produced by Livestock

There are two types of GHG emissions caused by livestock: direct and indirect. Enteric fermentation results in direct emissions, but external activities such as feed production and deforestation caused by pasture growth are causes of indirect emissions. If all the emissions are considered for the livestock production chain in the farm, its global value along this chain will be equal to 7.1 Gt CO_2-eq, but for intestinal CH_4, this value is equal to 5.4 Gt CO_2-eq [16], and that amount of enteric CH_4 is different depending on livestock production sectors [17]. Reducing indirect emissions can be achieved by improving management techniques, but a bigger problem is reducing fermentation emissions [18]. CH_4 production and release by enteric fermentation of ruminants varies between animal species, microbial community from each animal, ration composition, genetics, forage to concentrate ratio and dry matter intake (DMI) [19]. In the study by Kuhla and Viereck [20], in a 30-year period, the amount of internal CH4 production in different animals was investigated. The highest values were related to cows, followed by horses, mules and asses, sheep, and goats, and the lowest values were related to pigs (Table 1). In other words, dairy cows are currently

producing CH_4 at the highest level [18,19,21]. Based on the type of production, cattle breeds are divided into beef cattle, dairy cattle, steer (castrated bulls), and heifers (young females), which each year can generate between 32 and 98.6 kg of CH_4 per head. Although there are many variables that affect ruminal CH_4 production, numerous methods have been suggested to reduce rumen fermentation. One of the key factors in reducing CH_4 emissions and enhancing animal growth and efficiency is feed management [18]. By modifying the ration or increasing the grain concentration in the ration, including lipids or other chemical feed additives, which will be discussed in more detail below, it is possible to reduce CH_4 emissions during fermentation [22,23].

Table 1. CH_4 emission in some animal species.

Animal Category	Number of Animals	Enteric CH_4 (kt $\times$ Year^{-1}) Total Studied Population	Enteric CH_4 (kt $\times$ Year^{-1}) Every Animal Was Studied
Horses	6600	118.19	0.017908
Mules and asses	19.14	0.19	0.009927
Pigs	20,837	2.83	0.000136
Cow	20,765	2056	0.099013
Sheep	35,246	176.2	0.004999
Goat	6749	33.5	0.004964

3.1.1. Fermentation of Carbohydrates by Rumen Microbiome

Ruminants' main source of energy comes from carbohydrates. When polysaccharides enter the rumen, which are mainly cellulose, hemicellulose, and starch, these compounds are hydrolyzed into glucose and other hexoses and pentoses (Figure 2). Additionally, carbon dioxide and volatile fatty acids are the main products of monosaccharide metabolism. During the metabolism of monosaccharides into volatile fatty acids, metabolic hydrogen is released and causes the reduction of intracellular co-factors, because the continuation of fermentation depends on the reoxidation of co-factors. Hydrogenase activity and the production of dihydrogen (that is, molecular hydrogen) play a significant role in this issue. Hydrogen exists in the rumen in two forms, soluble and gaseous, and only soluble hydrogen is available for microorganisms [24]. Dihydrogen is transferred from the fermentation community of bacteria, protozoa, and fungi to the methanogenic archaea (methanogens), preventing it from accumulating in the rumen. It is used by methanogens in the hydrogenotrophic pathway to transform carbon dioxide and other mono carbon compounds into CH_4. Most methanogens can use the formate produced in the formation of acetyl-coenzyme A from pyruvate as a hydrogen donor for rumen methanogenesis [25]. Unused formate quickly breaks down into carbon dioxide and hydrogen. Also, much less CH_4 is produced in the rumen through the use of methyl groups (methylotrophic pathway) [15]. On the other hand, most of the metabolic hydrogen created by microbes is transferred to methanogens as dissolved hydrogen and used in the methanogenesis process [26]. Also, there are other important pathways for the use of metabolic hydrogen that can direct metabolic hydrogen as a substrate to a chemical pathway and lead to the production of a secondary compound, such as propionate [27] (Figure 2). Additionally, there is an extremely strong positive correlation between the concentration of dissolved hydrogen and the production of propionate, demonstrating that an increase in dissolved hydrogen in the rumen can facilitate the reactions of the metabolic hydrogen compound in the production of propionate [28]. On the other hand, simple and complex carbohydrates are hydrolyzed into pentose and hexose sugars by the activity of microbial enzymes in the rumen and the posterior part of the intestine.
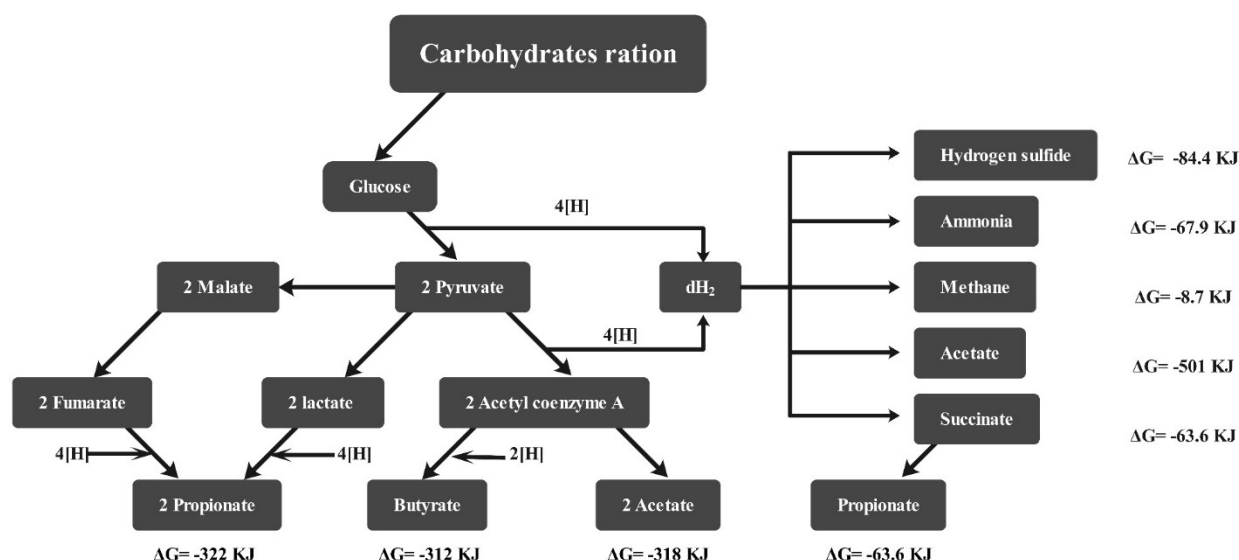
Figure 2. The main pathways of rumen fermentation in dairy cows (adapted from [29]). The schematic of the main pathways of rumen fermentation in dairy cows, including the production and synthesis of metabolic hydrogen [H] and dihydrogen [H$_2$], is shown in Figure 2. Also, the Gibbs energy changes are expressed without considering ATP generation.

3.1.2. Effect of Rumen Microbiome on CH$_4$ Emission

The amount of maintenance, growth, and production of ruminant animals depends on the anaerobic microbial ecosystem of the rumen. Through the fermentation process, the rumen's microbiome enables the animal to transform indigestible plant materials for humans into high-quality products like milk and meat. The rumen microbiome consists of bacteria, archaea, protozoa, bacteriophages, and fungi that have evolved over millions of years and have a symbiotic relationship with their host [15,30,31]. Methanogens, which are bacteria from the archaea branch, can nevertheless produce CH$_4$ gas in anaerobic conditions. The number and diversity of methanogens in the rumen is much less than its bacteria, so arches constitute only 6.8% of the total rRNA small subunit of the rumen. So far, 13 species of methanogens have been isolated and identified by pure cultures, including *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, *Methanoculleus olentangyi*, *Methanosarcina barkeri* [32], *Methanobrevibacter boviskoreani* [33], *Methanobacterium beijingense*, *Methanoculleus marisnigri*, *Methanoculleus bourgensis*, and *Methanosarcina mazei* [14].

However, it seems that the population composition of methanogens is more related to CH$_4$ production than to their abundance in the rumen [13]. For example, in a study on dairy cattle regarding CH$_4$ production, no difference in the total relative frequency of archaea was observed between dairy cows with high and low CH$_4$ emissions [34]. An increase in the relative abundance of *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium*, which were connected to high and low CH$_4$ emissions, was noted by the authors of the same study, respectively. Studies in sheep indicate that certain methanogens, rather than the general abundance of archaea, are important for CH$_4$ excretion [35,36].

The most dominant member in the rumen arches belongs to the genus *Methanobrevibacter* [13]. The change in the abundance of members of this genus is correlated with CH$_4$ production, and the reason for this, is the difference in the expression of different forms of methyl-coenzyme M reductase (MCR) [13]. This enzyme is the catalyst of the rate-limiting step of methanogenesis [37]. Clades (a group of organisms that have evolved from a common ancestor based on the principles of homology) can be divided into two subgroups: *Methanobrevibacter smithii-gottschalkii-millerae-thaurei* (SGMT) and *M. ruminantinum-olleyae*

(RO). The expression of MCR, whose production is regulated by the amount of hydrogen in the rumen, can be synthesized by these clades [37].

Interactions between ruminal fungal populations and CH_4 emissions are also of great importance. However, research on dairy cows has not been able to reveal a relationship between rumen fungal populations and CH_4 emissions. Until today, no clear difference has been discovered in the diversity of fungal animal populations and their relationship with low or high CH_4 emissions [35]. In one study, 73.19% of unclassified fungal samples were identified, and therefore, poor identification could contribute to the lack of correlation reported. However, anaerobic fungi, which typically attach to feed particles, have been observed to affect identification [38]. The number of fungi and CH_4 output were found to be positively correlated in another study on anaerobic digesters inoculated with fungi isolated from rumen-fistulated cows [39]. The rumen's protozoa affect existing bacteria, fungus, and archaea populations as well. Additionally, rumen protozoa, particularly those in the *Vestibuliferida* order, and methanogen arcs are closely related. Furthermore, it is well known that some protozoa have hydrogenosomes, which can compartmentalize the final energy metabolism reactions and cause the release of hydrogen. This process allows methanogens to use hydrogen more efficiently to form CH_4 [37]. Also, many studies have shown a correlation between host genotype and CH_4 emission, which is probably related to a degree of host control over the rumen microbiome [40,41]. However, recent works using the Bayesian approach estimate that host genetics and microbiota explain 24% and 7% of the changes in CH_4 levels in the host, respectively [42].

Therefore, as mentioned, the two factors of the fermentation of rumen carbohydrates and the type of rumen microbiome are effective on the direct CH_4 release, and by controlling these two factors, the amount of rumen CH_4 release can be reduced. With the production of more soluble H, its consumption by the rumen microbiome increases and less dihydrogen is produced. As a result, CH_4 production from different pathways such as hydrogenotrophic and methylotrophic is reduced. In fact, increasing the efficiency of feed consumption and converting it into more energy can reduce CH_4 production. Also, the studies showed that the type of rumen bacteria, fungi, archaea, and protozoa are able to change the amount of rumen CH_4, but more studies are needed to confirm the results. In addition, studies showed that CH_4 production can be controlled by controlling the catalytic MCR enzyme.

3.2. CH_4 Measurement Methods in Dairy Cows

Respiratory calorimetry (closed or open circuit), polyethylene tunnel system, isotopic dilution, tracer gas (such as sulfur hexafluoride (SF6)), and micrometeorological mass balance are the most commonly used methods for evaluating CH_4 production in ruminants [43]. Additionally, new technologies are being created to measure the amount of CH_4 produced during feeding at robotic milking stations or pasture feeders, or by using rumen sensors [44] to monitor CH_4 concentrations hourly [45]. Also, researchers have used mathematical and experimental models to predict CH_4 production from dairy cows in some studies, and the correlation between digestibility and CH_4 production in their findings has led to a novel strategy for solving this issue [46]. However, the comparison of CH_4 production data obtained from in vitro and in vivo methods has not had a high correlation. For example, in the study of Hatew et al. [47], who examined the effect of feeding dairy cows with two sources of fast and slow fermentable carbohydrates, as well as the level of 270 and 530 g of concentrate per kg of dry matter of the diet, they reported that the unit of measurement of methane production affects the correlation between in vitro and in vivo data, so the correlation value (R^2) was 0.04 per unit of OM ingested and 0.54 per unit of estimated rumen-fermentable OM.

As mentioned, various methods have been used to measure CH_4 in ruminants. However, a suitable method should have low operating cost, good accuracy, high reproducibility, and no need for special equipment; it should also measure all the CH_4 produced in different ways (mouth, nose, and anus).

3.3. CH$_4$ Emission from Enteric Fermentation and Fertilizer

A process known as enteric fermentation is mainly carried out by ruminants and results from complex microbiological activities. This process, which takes place in the rumen and under anaerobic conditions, breaks down cellulose and other large molecules and releases hydrogen. CH$_4$ is created by methanogenic archaea from carbon dioxide and hydrogen, and it is released through the mouth and nose in the process of belching [48]. CH$_4$ is additionally created through the decomposition of organic compounds in feces and litter materials in anaerobic conditions as well as by manure. An anaerobic environment is a prerequisite for CH$_4$ production through the microbial metabolism of organic matter. In fact, manure is decomposed into substances such as volatile acids, and these substances are used by bacteria to produce CH$_4$.

After adding fertilizer to the soil, N$_2$Os is released from solid manure, surface layers of stored sewage, and livestock bedding. Ammonium is the main inorganic nitrogen (N) in fresh solid manure and wastewater. N$_2$O and nitrate are produced as a result of the nitrification of ammonium, which further increases the generation of N$_2$O through incomplete denitrification (the incomplete biological reduction of nitrate to N$_2$ gas) [49,50]. Environmental factors, including temperature, fertilizer composition, and management, impact how much CH$_4$ and N$_2$O are produced by fertilizer. Low temperatures and proper management of animal manure can be utilized as a strategy to effectively reduce gas production [12,51]. It should be emphasized that all domestic animal species contribute to the creation of CH$_4$ and N$_2$O in manure, but ruminants (cows, buffalo, sheep, and goats) are the main producers of CH$_4$ through enteric fermentation [10].

3.4. Herd Management Practices

In the context of investigating the effect of age on CH$_4$ emissions, it has been reported that Holstein heifers have an emission coefficient of 36.2 kg of CH$_4$ from birth to 12 months and 64.3 kg of CH$_4$ from 13 to 24 months of age [52]. This has a direct relationship with the increase in enteric CH$_4$ rate [53]. On the other hand, the increase in dry matter consumption is related to the increase in intestinal CH$_4$ production [54–56]. The increase in calving age in three-year-old heifers compared to heifers that gave birth at 2 years old, shows more CH$_4$ emission. In 3-year-old heifers, 50% of intestinal CH$_4$ emission occurs from birth to calving [53], which is caused by the increase in DMI and the long growth period in non-productive heifers [52,57].

Intestinal CH$_4$ per daily weight gain in two- and three-year-old heifers (at the time of first calving) was 208 and 304 g, respectively. Morrison et al. [57] showed that heifers emit 252 g of CH$_4$ per kg of body weight until the age of 23 months. Nutritional strategies can have a clear effect on the amount of annual intestinal CH$_4$ emission. In different studies, CH$_4$ emission per unit of DMI (CH$_4$ per DMI) has varied from 20.6 to 22.7 and an average of 21.5 [53,58,59]. Adding corn silage and concentrate to the diet can reduce intestinal CH$_4$ per DMI, which is caused by changes in the fermented substrate [54]. The substitution of non-structural carbohydrates with structural carbohydrates in forages, greatly changes the chemical and physical conditions in the rumen and microbial populations and thus changes CH$_4$ production [56,60].

In addition, grazing grass has higher organic matter digestibility compared to pasture, which in turn can reduce CH$_4$ emissions per kg of DMI [61,62]. Holstein dairy cows, with an annual milk production of 8400 kg, produce 25% less intestinal CH$_4$ than Norman breed cows with an annual milk production of 6215 kg, which shows the importance of the breed and genetics of dairy cows in the emission of greenhouse gases [53]. Strategies aimed at improving the quality of productivity in dairy cows reduce CH$_4$ production by 20 to 30% [55,63]. Genetic selection and nutrition strategies have a 19 and 15% effect in CH$_4$ reduction [55]. Management factors in dairy cattle breeding units, feeding strategy, breed, age of first calving, and replacement rate can directly affect productivity in dairy cows, pregnancy, and feed yield, and among the mentioned factors, breed has the highest effect on the reduction of intestinal CH$_4$ production with a rate of 17.1%, followed by nutritional

strategy with a rate of 14.8% [53]. Finally, it can be concluded that dry matter consumption, feeding strategies, replacement rate, genetics and breed can affect the amount of CH_4 produced, especially when it is expressed per kg of milk produced from livestock.

3.5. Existing Solutions to Reduce CH_4 Emissions

3.5.1. Ruminal Microbial Population

Complex plant carbohydrates are transformed into energy because of the evolution of ruminant animals and microbes, which is advantageous for the host animal and the symbiosis of microbes. Bacteria, protozoa, fungi, and archaea are ruminal microbes (Figure 3 and Table 2). In contrast to other anaerobic ecosystems (such as marshes and estuaries), the rumen ecosystem decomposes plant materials quickly as an anaerobic environment, and various fermentation products are produced. Amino acids and soluble proteins are quickly fermented by ruminal microbes, resulting in the production of gases like hydrogen, ammonia, and carbon dioxide.
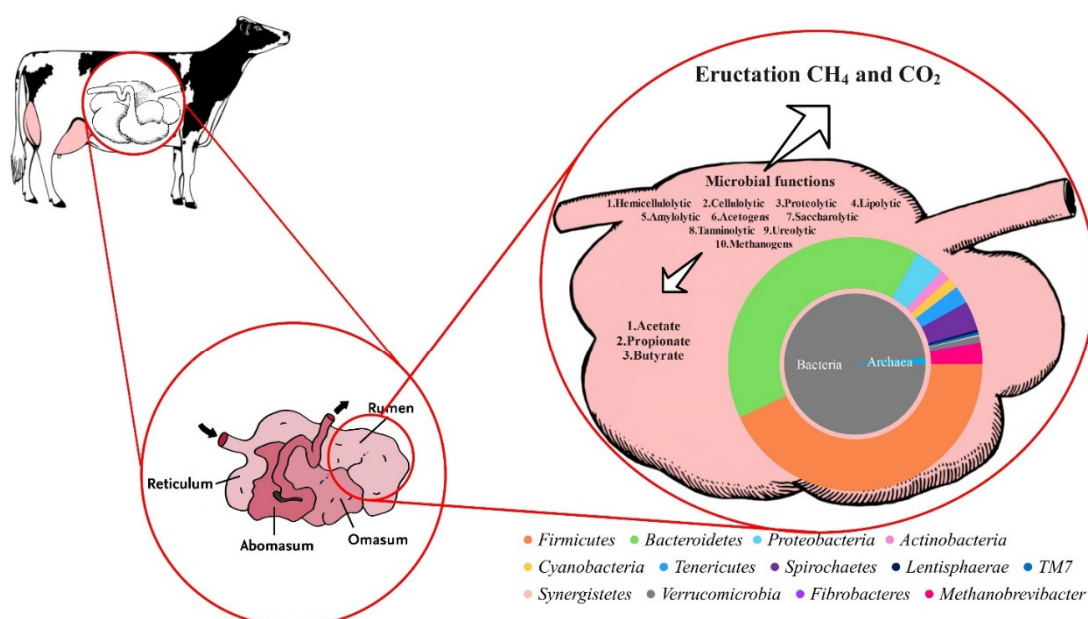

Figure 3. Microbial and archaea populations associated with CH_4 emissions in dairy cows have been identified (adapted from [64]). At the phylum level of the bacterial community, CH_4 production is dominated by Firmicutes and Bacteroidetes, the relative average of which is more than 83%. Also, *Methanobrevibacter* and *Euryarchaeota* have a significant role in CH_4 release at the level of archaea. At the level of the bacterial family, the most abundant family is *Provetella* with an average relative abundance of 54%. Other genera such as *Succiniclasticum* and *Ruminococcus* are involved in the next stages of CH_4 emission.

Among the bacterial phyla, *Bacteroidetes* and *Firmicutes* are the most abundant rumen microbiota in dairy cows [68]. It has been found that fibrinolytic bacteria, especially *Cellulolytic Ruminococcus* and several species of *Eubacterium*, are the main hydrogen producers, while *Fibrobacter* spp. do not produce hydrogen. Also, *Bacteroidetes* have been identified as the main hydrogen consumers [69]. In addition, ciliate protozoa and ruminal fibrinolytic species are hydrogen producers that counter CH_4 reduction strategies [70]. However, continuous removal of hydrogen is critical to maintaining ruminal biological fermentation function, because excessive hydrogen accumulation limits carbohydrate fermentation by inhibiting NAD^+ regeneration [71]. By increasing of concentrate-to-forage ratio in dairy cows' diet (with the same amount of consumable dry matter), it is possible to decrease the number of archaea and, as a result, CH_4 production. A decrease in the number of arches and subsequently a decrease in CH_4 production with an increase in dietary concentration to forage ratio of dairy cows (with the same amount of dry matter [DM] consumed) has

been reported, which is caused by a higher speed of feed passing through the rumen and a decrease in the binding of methanogens to feed particles [72]. Feeding with carbohydrates that ferment quickly reduces the pH of the rumen and the number of cellulolytic bacteria and protozoa, the result of which is a decrease in the digestion of fiber, a decrease in the ratio of acetate to propionate, and finally, a reduction in the production of CH_4 [73]. A possible explanation for this phenomenon is related to the competition for the substrate, because *Methanobrevibacter* species are hydrogenotrophic and use hydrogen and formate as substrates for CH_4 production. These findings indicate that archaea populations, ciliate protozoa, and dominating rumen microbes may be involved in the biological adaptation of the host to decrease CH_4 [74].

Table 2. Some of the most important ruminal methanogens in dairy cows (over 95% similarity was selected).

Taxon	GenBank Accession No	Reference
Methanosphaera stadtmanae	AY196684	
Methanosphaera sp. *R6*	AB022186	
Methanobrevibacter smithii ATCC 35061	CP000678	
Methanobrevibacter gottschalkii	U55238	[65]
Methanogenic archaeon CIRG-GM02	FJ951431	
Methanobrevibacter ruminantium	CP001719	
Methanobrevibacter millerae	AJ009958	
Methanobrevibacter olleyae	AY65201	[66]
Methanomassiliicoccaceae	-	[34]
Methanobacterium aarhusense	AY386124	[67]
Methanobrevibacter thaueri	GCA_003111625.1	

Firmicutes richness and the ratio of *Firmicutes* to *Bacteroidetes* are associated with daily weight gain due to a lower acetate to propionate ratio [68], and they are positively correlated with increased CH_4 emissions [2]. Furthermore, it has been claimed that CH_4 generation is correlated with higher *Firmicutes* populations and lower volatile fatty acid concentrations. Additionally, the ratio of *Firmicutes* to *Bacteroidetes* can be an important indicator for the investigation of the rumen microbiome and GHG emissions [75]. Furthermore, it has been noted that there is a positive relationship between fecal methanogen archaea concentration and CH_4 emission [73]. In fact, the reduction of methanogenesis or methanogens in the rumen should be accompanied by the reduction of methanogenic archaea. Additionally, because 20 percent of rumen methanogens are attached to ciliate protozoa and because of their function in CH_4 generation and the significant interaction effect between the two, these species may be an excellent choice for reducing CH_4 emissions [76,77].

3.5.2. Feeding and Composition of Diet

The ration is considered to be a determining factor for the microbial population in the host animal, because the various physical and chemical properties of the feed can cause significant variations in the dominant population of archaea [78]. Methanogenic archaea can synthesize CH_4 through methanogenesis from low carbon-number substrates such as fumarate, pyruvate, methylamine, acetate, and carbon dioxide. This process depends on the availability of ATP from the anaerobic rumen bacteria's intestinal fermentation [79].

There is a negative correlation between rumen pH and gas production. As the pH decreases, the ratio of acetate to propionate decreases. Inhibition of CH_4 production may be due to direct or indirect (or both) inhibition of methanogens through reduced H_2 production due to reduced acetate and greater propionate production. At the same time, the value of propionate production increases as the electrons in the rumen are used for the fermentation of this material, and this itself leads to a decrease in CH_4. On the other hand, the high content of lactic acid also leads to the regulation of methane because it uses H_2 to produce propionic acid, which is the source of CH_4 production. In general, it seems that the decrease

in the amount of forage is due to the reduction of CH_4 production caused by the conversion of lactic acid to propionic acid and the reduction of protozoa and methanogens in the rumen [80].

The addition of animal fat or vegetable oils to ruminants' diets can affect the microbial community with a toxic effect or limit the microbial colonization of feed particles affecting the access of microbial enzymes to substrates. Consequently, feed digestibility may be adversely affected by the addition of lipids, hence vegetable oils can be a good alternative. However, the amount and type of lipid added, and the composition of the diet determine the effect of dietary lipid supplements on nutrient digestibility. The remarkable thing in the studies is that other microbial groups involved in fiber degradation were not affected by vegetable oils. Also, the amount of propionate increases with the addition of vegetable oil, which leads to hydrogen consumption. On the other hand, vegetable oils reduce the number of protozoa and methanogenic archaea [81].

Previous studies show that the amount of CH_4 produced in the rumen can be affected by changing the amount of carbohydrates in the ration or the fat content of the feed [21,23,82]. Compared to rations based on fodder, changing from fiber carbohydrates to starch or grain reduces intestinal CH_4 production and release [82]. Starch fermentation increases the production of propionate in the rumen, and by creating an alternative hydrogen reservoir for methanogenesis, it reduces the pH of the rumen and thus prevents the growth of methanogens.

Another nutritional strategy is the use of fat (lipid) supplements and additives such as calcium salts of fatty acids and hydrogenated fats, unaltered extracts of animal sources (such as tallow) or vegetable sources (such as soybean, rapeseed, cottonseed, and sunflower seeds) [82,83]. Dietary fat as a source of dense and non-fermentable energy provides the possibility of replacing part of the feed with fat. This causes a decrease in fermentable energy, which supports microbial growth in the rumen by changing the amount of carbohydrates in the ration [84]. However, using this method may have unexpected consequences, such as decreasing fiber digestibility [85].

Briefly, highly productive forages with high nutritional value, such as grasses and legumes, were considered as a complete diet for ruminants [86], which, by changing the nature of carbohydrates from fibrous to non-fibrous, lead to a reduction in CH_4 emissions [87]. On the other hand, with an increase in the ratio of fiber to starch, the amount of energy decreases in all required levels, including maintenance, growth [88], and lactation [89]. In addition, replacing corn silage with alfalfa silage has caused a decrease in CH_4 (g/kg of DM and gross energy intake) by Holstein dairy cows [60]. Also, the reduction of CH4 emissions by replacing corn silage with grass silage in the diet has been reported [90]. Moreover, van Gastelen et al. [91] reported in a study a reduction in CH_4 emissions in corn silage diets when replacing grass silage or legume silage. The inclusion of forage legumes in the diet of ruminants also can reduce the emission of CH_4 and greenhouse gases due to the reduction in the consumption of nitrogen fertilizer and fertilizer production, as well as the increase in biodiversity and the reduction of parasitism in these animals [92].

As mentioned in Table 3, the effects of supplementing vegetable oils and animal fat are similar. Both sources lead to a decrease in feed consumption, an increase in the ratio of propionate to acetate, and a decrease in the population of methanogens, which subsequently leads to a decrease in CH_4 emission through increasing the energy content, reducing dietary fiber, and reducing the population of methanogens. In addition, vegetable oils have a significant content of medium- and long-chain fatty acids [79]. In various studies, the use of vegetable oil sources changes the methanogens population or reduces feed consumption, leading to decreased CH_4 production. For example, the use of coconut oil has caused a reduction in CH_4 from 13 to 73% [93]. Also, in beef bulls, supplementing diets with 6% refined soybean oil (DM of diet) caused a 39% decrease in CH_4 production [94]. The addition of linseed oil (5% DM of diet) has resulted in 55% reduction of CH_4 in dairy cows [95]. Also, sunflower oil caused a decrease of methanogens by 11.5 to 22% [96]. Other sources of fat, such as tallow, have also had a reducing effect on methane emissions. For

example, Beauchemin et al. [42] reported that adding 34 g of tallow to heifer diets reduced CH_4 by 11%. The effect of dietary lipids on reducing CH_4 production is largely dependent on the ration components [79].

Table 3. General strategies of public nutrition management to reduce CH_4 emission.

Strategy	Effects	Reference
Decreasing forage to concentrate ratio	• Reducing ration energy by changing the carbohydrate source from fiber to starch • Decline rumen pH and inhibiting methanogens • Hydrogen consumption with propionate production	[54,97]
Supplementation of fats (animal fat and vegetable oil)	• Replacing the energy density source and reducing the consumption of dry matter • Decrease the source of fibrous carbohydrates in the diet • Reducing the fermentation of organic matters in the rumen and subsequently decrease the activity of methanogens and protozoa • Increasing propionate to acetate (P/A) ratio, with reduced fiber digestion and increased energy density	[98]
Feeding corn and grain silage instead of grass silage	• Increase DMI efficiency • Increasing P/A ratio • More fluid passage through rumen and amplification of digestion in abomasum	[54,97]
Improving pasture management	• Shifting from fiber to rapidly fermentable carbohydrates	[99,100]
Secondary plant compounds (phenolic compounds, tannins, saponins and essential oils)	• Reduction of methanogenesis pathways • Tannin and saponin: reduction of microbial activity (cellulolytic and protozoa), optimizing ruminal fermentation and bio-hydrogenation • Coating with dietary fiber by hydrogen bonding • Essential oils preventing the deamination of amino acids	[101–104]
Ionophores (such as monensin, antibiotics)	• Feed intake adjustment • Inhibition of bacteria and protozoa • Increasing P/A ratio • Bloating control and modulation of rumen microbial diversity	[105,106]
Feed additives Yeast/Bacterial	• Improve microbial activity • Stabilizing rumen pH and preventing acidosis	[54,107,108]
Enzyme supplementation	• Increasing fiber digestion • Enhancing volatile fatty acids and ammonia nitrogen	[109]
Addition hemicellulose/starch	• Increasing digestibility and rate of passage of fluid through rumen • Increasing P/A ratio • Decline in rumen pH and subsequent reduction of fibrolytic bacteria	[79,110,111]
Rumen manipulation with chemical additives	• Elimination of methanogens and protozoa • Removal of hydrogen and formate producers as CH_4 precursors	[112]
Methanogen vaccine	• Inhibition of methanogens activity and induction immune responses	[79,113]
Organic acids: aspartate, fumarate, malate	• Stimulation of lactate utilization by ruminal bacteria such as *Selenomonas ruminantium* by adding aspartate, fumarate, and malate • Use of malate as an electron sink for hydrogen in the succinate-propionate pathway in *Selenomonas ruminantium*	[79,114,115]

3.5.3. Adding Inhibitors

In contrast to ration management strategies, introducing inhibitors is a direct way to prevent methanogens' growth and activity in the rumen. They can also act as electron acceptors, diverting the flow of electrons away from the reduction of carbon dioxide and CH_4 by consuming the reducing equivalents produced during fermentation [116,117]. "Specific" or "non-specific" inhibitors are terms used to describe chemical substances that prevent the formation of methanogen. While non-specific inhibitors impact on the activity of both methanogens and non-methanogens, specific inhibitors target the enzymes present in methanogens [77]. Table 4 shows a selected list of compounds and their approximate effective dose that caused the reduction of CH_4 production in the ration.

Over the past 50 years, several studies have been conducted on the effects of some of these compounds [46,99]. For instance, among the most well-known and active compounds that have demonstrated CH_4-reducing properties in very low concentrations are bromoform and chloroform (cows produce 40% less CH_4 when fed 1.5 mL per day, and the decreases lasts until the 42nd day) [125]. However, these compounds may have long-term harmful effects and may cause serious reactions when taken directly in large doses by humans and animals (at concentrations of 200–1000 mg/kg/day in rats) [126]. As a result, the direct treatment of ruminants with these compounds may cause problems. However, dietary substitutes that naturally contain these compounds in very low doses and are still effective in the prevention of CH_4 production may provide an alternative [127,128]. Up to 70% of bromoform, a short-lived volatile molecule that depletes ozone layer [129], is thought to be produced by algae. However, this molecule is produced and stored by the red alga species *Asparagopsis* as a natural chemical defense against bacteria [77]. Due to its volatility, its accumulation inside algae cells is unusual, which makes red algae unique for use in ruminant diets [130]. Red algae have the potential to reduce CH_4 emissions, but further research is required before these products may be recommended for long-term use [131]. The main effects of algae on CH_4 reduction are caused by bromoform as a CH_4 inhibitor.

Many artificial and natural inhibitors have been investigated in earlier studies [77,112,119]. The findings show the necessity for more research on inhibitor concentration, characteristics, side effects, and application due to their inconsistent efficacy. This variation has been attributed to ration components, rumen microbiome changes, digestion kinetics, and the animal's adaptive responses to such compounds [14]. For example, numerous compensatory mechanisms cause the inhibitory effects of halogenated compounds on methanogens to wear off over time [54,132]. Due to this, the long-term storage of these compounds has received increased focus. For instance, the delivery of these anti-methanogenic compounds via slow-release capsules is comparable to that of the monensin antibiotic [133,134]. To be acceptable to the farmers (breeders), these items must be produced at a reasonable cost, which is not the case now.

Previous research demonstrates that ruminants' microbial methanogenesis is decreased by adding microalgae to the ration, which also enhances growth performance [135–137]. For example, in laboratory conditions, CH_4 generation was reduced by 98% when rumen fluid contained 0.5 to 2% of biomass from the red algae *Asparagopsis* sp. [138]. Additionally, a different study found that using *Asparagopsis armata* algae at a rate of 0.5 to 1% of diet decreased CH_4 production by 43 to 60% [139]. However, the addition of macroalgae *Ulva rigida*, *Gracilaria vermiculophylla*, and *Saccharina latissima* to the amount of 25% of the rumen liquid has no effect on CH_4 reduction [140]. In a recent study, the amount of iodine in the diet with the addition of 2% and 5% of the *Asparagopsis taxiformis* was 0.08 and 0.15 PPM, respectively [120]. Also, there is a linear relationship between dietary iodine concentration and milk iodine levels. Therefore, iodized supplements such as algae should be used with caution in dairy cows' diets. Considering that the amount of iodine in red algae is equal to 38 mg per 100 g of algae DM, it should be noted that the amount of adding algae should not be higher than the dietary iodine recommendations (0.5 mg of iodine per kg of DM) [141].

Table 4. Effective compounds on CH$_4$ mitigation.

Additives	Effects	Dosage	Mitigation Enteric CH$_4$	Reference
3-nitrooxypropanol (NOP)	• Decrease in the rumen acetate to propionate ratio • Limited effects on the growth characteristics of rumen protozoa and bacteria	1.6 g/cow per day	28%	[118]
		80 mg/kg of DM	28.4%	[112]
		100 mg/kg of DM	54%	[119]
Bromoform/ bromochloromethane	• These compounds and halogenated analogues react with B12 cofactors and inhibit cobamide-dependent CH$_4$ production	*Asparagopsis taxiformis* contained bromoform at a concentration of 7.8 mg/g dry weight	86%	[120]
Hydroxy methyl glutaryl-SCoA (HMG-CoA) reductase inhibitors	• Inhibition of the synthesis of methanogenic archaebacteria (such as *Methanobrevibacter*) by lovastatin	150 mg/kg of DM	38%	[121]
Halogenated aliphatic hydrocarbons	• Inhibition of methyl coenzyme M reductase or aconitase in the Krebs cycle • Inhibition of corrinoid enzymes • A shift in the microbiota toward more bacteroidetes and a decrease in Archaea	15.6 g/cow per day	58%	[122]
Polyunsaturated fatty acids (PUFA) and medium-chain fatty acids (MCFA), lauric, myristic, capric and caprylic acids	• Hydrogens consumption through bio-hydrogenation • Modulating ruminal bio-hydrogenation	60 g/kg DM of camelina oil	29.5%	[123]
		24 to 37g/kg DM of cottonseed oil	42%	[124]
		25 and 8 g/kg DM of soybean oil and grape seed tannin extract, respectively	28%	[101]

Before its commercial use, there are several advantages and disadvantages to using algae (macroalgae and microalgae) that should be considered. In general, algae must be produced in open or closed systems, harvested, and then dried to be used commercially as supplements for ruminant feed. Microalgae are less than 100 μm in size, which makes them more difficult to harvest and dry. In contrast, macroalgae can be more efficient [142]. Currently, there is a lot of research in the field of reducing production costs and the carbon footprint of algae. Additionally, the type of algae, the location in which it is found, and the way it is transported to the end-use site all have the potential to affect the carbon footprint, which must be carefully balanced against any potential advantages of using it as ruminant feed [78].

The molecular structure of 3-nitrooxypropanol (NOP) makes this compound of very high solubility in the cell and it is quickly metabolized into nitrate and nitrite [143]. It has also been reported that it is metabolized to 1, 3-Propanediol, which is a compound with very low toxicity, and turns into 3-hydroxypropionic acid (3-HP). In addition 3-HP causes the synthesis of propionyl coenzyme-A as a substrate for gluconeogenesis [144]. In addition, NOP residues are absent or trace in milk and meat, and its metabolites do not have mutagenic and genotoxic potential [144]. However, for the absence of NOP residues in manure, milk, or meat, more safety studies are needed. So far, no specific government regulations have been reported for these compounds. Also, the consumption of bromoform in dairy cows in the amount of 41 mg/kg of dry matter of the consumed feed did not cause tissue accumulation, but it was reported to be excreted through urine and feces [145]. No official institution has reported a specific standard for its consumption or addition to the ration of dairy cows. Also, other compounds such as halogenated aliphatic hydrocarbons do not have official government approval, so more studies are needed. Since the formation of metabolites, toxicity, and exact elimination kinetics of these compounds are not known, there is a need for short-term and long-term studies in ruminants, especially in dairy cows. As many of these compounds may create additional costs for livestock farmers, to encourage their use, the government should provide financial assistance to livestock farmers in order to reduce environmental costs.

3.5.4. Vaccination

Another potential strategy that has so far shown contradictory results is vaccination against rumen methanogens. To neutralize methanogens, this strategy is based on the formation of an antibody response that is transported to the rumen by salivary secretions. Many producers would find it simple to implement this vaccination as they already follow annual vaccination protocols. Consequently, compared to alternative techniques, vaccination might be more costly [146]. It has been found that the concentration of avian anti-methanogen antibodies (IgY) can reduce CH_4 production in vitro [147].

In a study on 30 sheep, a 7-methanogens mixture and a 3-methanogens mixture were provided to the sheep. The time between the primary and secondary subcutaneous vaccinations was 153 days. Next, the amount of CH_4 produced was calculated using an SF6 and a breathing room technique. Four weeks following secondary immunization in sheep, the outcomes showed a significant 7.7% decrease in intestinal CH_4 generation in a mixture of 3-methanogens [148]. Strong antibody responses including both IgG and IgA responses with antisera selected from methanogen fractions have been found in saliva, even though intestinal CH_4 release was not measured in this study [149]. In another study, the use of a vaccine that tried to capture 52% of the methanogens in the rumen, contrary to the expected effect, CH_4 production increased by 18% in sheep after three vaccination cycles, which indicates the ineffectiveness of the vaccination [150]. In another research, vaccination against a methanogen derived from *Methanobrevibacter ruminantium* was administered to 5-month-old male Holstein-Friesian calves. A strong IgG response and a moderate IgA response were found in the serum and saliva of animals inoculated. Additionally, rumen fluid sample analysis showed the presence of antibodies [151].

Trials of in vivo vaccination, however promising, may be limited by our current knowledge of the rumen methanogen population. Additionally, in some studies, the use of vaccination has not only reduced CH_4, but also slightly has increased it [150]. Furthermore, it has been said that because each vaccine formulation is unique to a diet and environment, existing research is unable to cover its full range [79,150]. On the other hand, most rumen methanogens are difficult to produce, and the majority of the species have not yet been isolated [152]. As methanogen populations can vary widely by region and diet, it is crucial to successfully cultivate high-yielding methanogen species and to do so in production systems for the vaccination approach to be effective in the future [79].

As stated, rumen microbial population control strategies, feeding, addition of inhibitors and vaccination are proposed as strategies to control CH_4 production, each of which needs further investigation. For example, by controlling the physical and chemical characteristics of the diet, the population of methanogenic arcs in the rumen can be controlled; or by changing the diet of animal towards a diet containing starch and grains and increasing the amount of lipid in the ration, CH_4 production can be reduced. On the other hand, feed additives such as algae can suppress methanogens and act as an electron acceptor. Vaccination has also received much attention today, but its results are contradictory and need further investigation. All these strategies can be effective, but the side effects of each, cost, public access, and the amount of CH_4 reduction in each method should be evaluated. To increase the effectiveness of these methods, the possibility of their simultaneous use also needs to be assessed.

3.6. Pros and Cons of CH_4 Mitigation Methods

Fat supplementation is considered a strategy to increase the energetic level of the ruminant diet [153]. Also, improved reproduction has been reported with supplemental fat sources, possibly due to improved energy balance as well as the effects of fatty acids on hormones that play a vital role in reproduction [154]. Perhaps the most important disadvantage of this method is the limitation of its amount in the rations of dairy cows because of its negative effects on fiber digestion in the rumen [155].

One of the most important advantages of implementing vaccination is its easy use for farmers, because they use a kind of annual vaccination protocol. Also, vaccination

can be cost effective [146] and cause strong antibodies in ruminants [149]. On the other hand, it is difficult to cultivate most species of arc methanogens in vitro, which is one of the disadvantages of this method [152].

Improving pasture quality can improve feed digestibility and lead to reduced enteric CH4 emissions [54,90,156]. Flavonoids, tannins, pectin, glycosides, terpenoids, and sesquiterpene lactones are known as the most important plant secondary metabolites. Some of these compounds are toxic to the animal or act as an inhibitory agent through the sense of taste [157]. However, some of these compounds show promise for improving animal productivity and health and reducing enteric CH_4 production. The most important compounds are saponins, concentrated tannins, and essential oils [90].

The use of ionophores in ration improves nitrogen and energy efficiency [54,90]. Also, ionophores inhibit gram-positive bacteria by disrupting the osmotic chemical gradient. [158]. In addition, these compounds help to produce propionate with increased energy efficiency, which can act as a sink of H and reduce CH_4 production [159]. However, the effect of ionophores on CH_4 production has been inconsistent and sometimes not detected in studies in grazing systems [159,160].

4. Discussion and Conclusions

Regarding population growth and their urgent need for animal products (milk, dairy products, and meat) and malnutrition in many countries of the world, it is not possible to reduce the number of ruminants, especially dairy cows. However, ruminants have a significant contribution to the production of greenhouse gases, especially CH_4. To achieve the reduction of global warming effects caused by greenhouse gases, especially CH_4, changes in animal breeding systems and the adoption of new strategic approaches is required. In this paper, several methods for mitigating CH_4 emissions were studied and discussed. To directly reduce ruminant's CH_4 emissions, these strategies include changing the composition of the feed (Table 3), using CH_4 inhibitors (Table 4), and vaccination, and may potentially reduce greenhouse gas emissions. At present, it is difficult to decide which method is best for CH_4 mitigation considering the studies and the contradictory results in some of them.

Nutritional strategies such as changing the ratio of forage to concentrate are one of the efficient approaches to reduce methane production. However, there are some limitations to using high amounts of non-fibrous carbohydrates in the ration of ruminants, such as increasing the price of the diet, sub-acute acidosis, etc.

Currently, most of the commercial CH_4 inhibitors have a very high price, and this problem has caused a decrease in the interest in using them on dairy farms. In addition, the toxicity of these chemical compounds is being investigated in the latest studies and the decision to use them widely has in an aura of ambiguity. Also, our current knowledge of the ruminal microbiome is very limited, and this microbial flora is highly dependent on feeding conditions, which has made it difficult for experts manipulate rumen or limit the species of methanogens. Identifying methanogens and investigating their coexistence with other bacteria, fungi, and protozoa can also be very useful as a practical approach to reducing CH_4. However, algae represent a potential research field in this context, with the goal of reducing ruminant CH_4 emissions and carbon footprints without compromising product quality. Before the large-scale feeding and commercialization of algae are considered in an attempt to reduce CH_4 emissions without affecting animal production, there are some crucial issues that must be resolved. The practical feeding dose and range that can be used to produce optimal effects on rumen fermentation without impacting animal intake and productivity is a crucial component of the diet management strategy. Palatability is important when choosing any new feed (or food), as it is important to carefully evaluate the trade-off between the amount that can be supplemented and the amount that can be tolerated. So far, 40 species of macroalgae and less than 10 species of microalgae have been studied for their CH_4 reduction potential, and most of these studies have been conducted in vitro (Table 4). Algae can impact the quality of milk or meat, as evidenced by earlier

studies, when taken as a feed additive. These effects can be positive or negative and are again highly dependent on the dose, among other variables. More research is essential to ensure that the decrease in CH_4 production does not reduce animal productivity.

Therefore, the adoption of any of the methods by farmers (breeders) is a fundamental challenge that requires advertising and government incentives to encourage the use of these strategies, such as carbon credit policies.

Research capacity to quantify greenhouse gas emissions in developing countries is limited. The global dairy industry is under great pressure to reduce greenhouse gas emissions. There is great potential for developing countries to contribute to this reduction, as a large proportion of dairy cows are part of agricultural systems in these countries with a higher emission intensity compared to the dairy industry in developed countries.

Dairy cows have a great multi-functional value in animal husbandry and agriculture, which should be considered in terms of sustainable dairy cattle breeding industry research in developing countries and production development. CH_4 emissions can be reduced by improving forage quality by adding more cold-season forages and legumes and rotationally grazing animals, including forage with beneficial secondary compounds such as dense tannins and saponins, which also have the potential to reduce CH_4. Providing nutritional supplements that improve the nutritional status of livestock and feed energy efficiency has the potential to reduce CH_4 emissions from grazing cattle. CH_4 reduction strategies in grazing environments are limited, but producer decisions that improve animal nutritional status, forage base quality, and supplementation with compounds known to reduce CH_4 can reduce CH_4 production. Now that slightly more expensive and easier-to-use tools are available, researchers should conduct more long-term monitoring experiments and focus on reducing CH_4 production in animals, because the reduction potential is greatest. However, local expertise and facilities can be supported by appropriate research technologies from developed countries to help understand the source and scale of the greenhouse gas emissions from ruminants in different agricultural and livestock systems.

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