

Recent Nutritional Advances to Mitigate Methane Emission in Cattle: A Review

Review Article

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ABSTRACT

Climate change and preventative regulations on greenhouse gas (GHG) emissions have forced countries to focus on reducing the emission of GHG by the causative factors. The rapid increase in the world population, the culture of urbanization and enhanced income of human societies over the past few decades have raised concerns about more effective and sustainable ways of the food supply with minimum adverse effects on the environment. The livestock sector is very important in terms of meat, milk, and eggs, that all of them are important and high-quality constituents of human nutrition. Despite the value of these products, livestock and poultry have not ever been without a detrimental effect on the environment, and the challenge for researchers and scientists in this field has tried to minimize these adverse effects. GHGs such as CH₄, CO₂ and N₂O, and nitrogen and phosphorus disposal are some of them, which affect both the livestock and poultry sector. About 14.5% of total global anthropogenic GHG per year has been attributed to the domestic animal production sector, which is equal to 7.1 gigatonnes of the annual CO₂ equivalent (CO₂_{equ}) of GHG. Approximately, 44%, 29% and 27% of the sector's emissions are CH₄, N₂O, and CO₂, respectively. Methane production and N₂O emission in ruminants are not only effective on the environment but also on animal performance, so the use of multipurpose strategies to reduce the emission of these compounds can improve livestock performance in addition to positive environmental impacts. Since more than 54% of the annual production of CO₂_{equ} has been accounted for beef cattle, using different mitigation strategies in this section is more essential. The present review aimed to summarize the current knowledge and findings of the influencing factors on GHG emissions from beef cattle.

KEY WORDS beef cattle, emission, greenhouse gas, methane.

INTRODUCTION

Climate change, besides environmental degradation, population growth, widespread poverty and increasing food insecurity, are considered as the most important challenges of the 21st century. Unfortunately, the effects of climate change such as rising sea levels, increasing flood risks and changing climate patterns will be irreparable (FAO and GDP, 2018). Forecasts suggest that the world population will reach from 7.6 to 9.8 billion in 2050 but the food de-

mand will double, mainly due to increasing urbanization and income level (FAOSTAT, 2020). Therefore, agriculture, and especially the livestock sector, will play a very critical and challenging role in meeting the rising demand for this growing population. Iran, as one of the largest countries in the Middle East, plays a key role in the region's economy, especially agriculture and livestock production. According to the FAOSTAT (2020) and the Statistical Center of Iran (2017), there were more than 4.8 million cattle in Iran producing more than 6.8 million tons of milk and

477000 tons of meat annually. In addition to being economically important, the profession has also employed over 3.1 million people (about 3.8% of the population) in the country. Because of significant differences in the number of livestock between Iran compared to United States, Brazil, China, Turkey, and European Union (EU) (Table 1), the contribution of Iran to greenhouse gas (GHG) emission from livestock production appears to be less. However, since Iran is located in a hot and dry region will affect more than other regions and will not be safe from climate change. Therefore, to the goal of “sustainability” of global food system, any effort to minimize the adverse impact of ruminant husbandry on the environment will be valuable. “Sustainability” is more than environmental impacts and balances environmental, social concerns and economic conditions (Flachowsky *et al.* 2018; Gleason and White, 2019; Lan and Yang, 2019).

A significant source of greenhouse gas (GHG) emissions is the agricultural sector (Burney *et al.* 2010). According to FAO (2018), the three main GHGs emitted from agriculture activities are CH₄, CO₂, and N₂O. The GHGs emission sources remarked including Enteric fermentation and manure management; Application of fertilizers and associated products; Energy consumption (directly or indirectly like livestock production, farm facilities and feed manufacturing and processing practice); and Land use changes. Generally, it is estimated that ruminants contribute around 80% of the total global livestock emissions and recognized as major contributors through the production of methane (Gerber *et al.* 2013). According to EPA (2018), beef cattle were predominant contributors to CH₄ emissions and were responsible for 71% of total enteric CH₄ emissions from livestock in 2016. In addition, as described by Mitloehner (2018) and White and Hall (2017) United States beef cattle enterprises account for 52% and 25% of emissions from animal agriculture and of all agricultural emissions, respectively. The magnitude of the impact of each GHGs on global warming is calculated using a conversion factor as CO₂ equivalent, which is 1, 34 and 298 for CO₂, CH₄ and N₂O, respectively (FAO, 2018).

The environmental impact of animal-derived foods are currently quantified by so-called CO₂_{equ} footprints (CFs) (Flachowsky and Hachenberg, 2009). The CFs for animal originated food depends on numerous of affecting factors like animal species, type of production, feeding of animals, level of animal performance, system boundaries, and output/endpoints of production (Flachowsky and Kamphues, 2012). Edible protein from ruminants is mainly defined by a higher CFs because of the high GHGs potential of CH₄ produced in the rumen. In addition, the energy and protein conversion efficiency from feed into food of animal origin is low and may vary between 3% (energy-beef) and up to

40% (energy-dairy; protein-chicken for fattening); (Cassidy *et al.* 2013; Flachowsky *et al.* 2018). However, CFs for beef cattle husbandry usually extend from the inputs to the harvesting system through the feedlot or slaughterhouse gates. GHG emissions from beef cattle rearing are including CO₂ emissions from commercial fertilizer synthesis, herbicides, seeds and other inputs to the farm system; CO₂ emissions from field management and transportation; CH₄ emissions from enteric fermentation and manure storage. Direct and indirect N₂O emissions from manure management; CO₂ emissions from infrastructure upkeep; and other sources (Gleason and White, 2019). For most beef cattle producers in the United State, the cow-calf operation contributes the greatest to the whole-system emission primarily because of enteric fermentation from the herd level of cow (Beauchemin *et al.* 2007; Asem-Hiablie *et al.* 2019).

According to Opio *et al.* (2013), cattle annually emitted 4.6 gigatonnes CO₂_{equ}, of which 46% derived from dairy and 54% from beef cattle. However, buffalos and small ruminants released only 0.62 and 0.47 gigatonnes CO₂_{equ}, respectively. In addition, enteric CH₄ contributed almost 45% of the combined CO₂_{equ} emissions from dairy and beef cattle. It is reported the meat production by beef cattle systems is about 35 million tonnes/year, while by dairy cattle systems is only 27 million tonnes/year. GHG emission intensity of meat protein from beef cattle, and integrated milk and meat protein intensity from dairy cattle differ from about 200-1100, and 50-350 kg CO₂_{equ}/kg edible protein, respectively, related to the region of the world (Opio *et al.* 2013).

Feed, as the major variable inputs cost, plays a critical role in cattle production, and a cattle operation can be profitable when the feed used efficiently to meet nutrient requirement (Johnson *et al.* 2019). Determining efficient beef cattle breeds and their adoptability to suitable production systems is a major challenge of meat production around the world, with the raising concern about the environmental effects of beef productions (Rowntree *et al.* 2016). Recently, the EPA (2018) and Rotz *et al.* (2019) reported that beef cattle have emitted about between 132 to 142 Tg CO₂_{equ}/year through enteric fermentation and manure management.

Anaerobic digestion and microbiology of methanogenesis

Anaerobic digestion is a very complicated process of dissociation of organic compounds including a sequence of biochemical processes, consisting of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Due to the complexity of this digestion process, a specific group of microorganisms performs each step with different rates; for instance, the most critical of them are hydrolytic-fermentative, aceto-

clastic and hydrogenotrophic methanogenic archaea, proton-reducing acetogenic and their metabolic intermediates (Zabranska and Pokorna, 2018). Since methanogenesis, the process of CH₄ formation, has the slowest rate, the balance among different steps of anaerobic digestion is required to achieve the optimum process efficiency (Demirel and Scherer, 2008) (Table 2). Briefly, the complex organic compounds are enzymatically metabolized by all mentioned groups of microorganisms through series of metabolic intermediates like CO₂, H₂, alcohols, and low fatty acids, especially volatile fatty acids (VFA) such as propionic and butyric acids.

Then, propionic and butyric acids are hydrolysed by syntrophic acetogens into direct and simpler precursors of methanogenesis such as CH₃-COOH (acetate), CO₂, and H₂. Finally, CH₄ be generated by methanogenic archaea from a limited number of substrates, CO₂ and H₂, acetic acid, C1-compounds, and methyl group donors (e.g. methanol, methylamines, and methylsulfides) (Costa and Leigh, 2014; Zabranska and Pokorna, 2018).

Methanogens are a specific community of microorganisms, which are exclusively producing CH₄ through the methanogenesis and belongs to domain archaea. Despite their division and taxonomy are included to four classes (*Methanobacteria*, *Methanococci*, *Methanomicrobia*, and *Methanopyri*).

These microorganisms have living conditions requirements and greatly specific substrates and become frequently the restrictive community of the completely anaerobic digestion. The principal specifications of given methanogenic archaea, particular and limited precursors, as shown in Figure 1, (H₂, CO₂, formate, methanol, acetate, and methylamines), and requirements for the cultivation conditions like optimal temperature (30-83 °C) and optimal pH ranges (5-8.5) (Zabranska and Pokorna, 2018; De la Fuente *et al.* 2019). Hydrogenotrophic methanogens use H₂ and CO₂ or formic acid to generate CH₄. In addition, acetotrophic methanogens produce methane from acetic acid, but methylotrophic methanogens only use C1- and methylated as precursor compounds (Demirel and Scherer, 2008). Interestingly, *Methanosarcina* spp. are the only methanogens, which are capable to utilize all the substrates mentioned above and metabolize up to nine several different substrates (Galagan *et al.* 2002). Furthermore, it should be considered that until now only around 10% of rumen microbes are known and there are undetected rumen microbial genera and species especially involving on methanogenesis (Pers-Kamezyc *et al.* 2011). Fortunately, findings have been detected new rumen microbial species through molecular biology techniques like Real-time PCR, polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-

DGGE), and fluorescence *in situ* hybridization (FISH) (Mohammed *et al.* 2011; Szumacher-Strabel *et al.* 2011).

It should be noted that enteric CH₄ emission in ruminants along with being an environmental negative impacts leading to a loss of 10–11% of the total gross energy (GE) intake of the animal (Flachowsky and Brade, 2007; Tamminga *et al.* 2007; Valli, 2020). Therefore, suppress CH₄ emission from ruminant is crucially required (Lan and Yang, 2019). In recent decades, various strategies and intensive research have been developed to mitigate enteric CH₄ emissions without negatively effect on animal productivity. For example, nutritional strategies, rumen manipulations as well as management or breeding techniques can be mentioned. Feeding and nutritional strategies are more practical and conventional approaches to reduce enteric CH₄ emissions and can be more easily practiced under field conditions by farmers. Furthermore, to the direct relationship between enteric CH₄ production and dry matter intake, total methane emission of high-producing cattle will be higher than low-producing animals. However, the amount of CH₄ intensity (g/kg of meat or milk) from higher dry matter intake of the high-yielding animals will be reduced. In other words, despite higher GHGs emissions, the main advantages of raising high producing, more health and fertile, and longer life expectancy animals ultimately reduce the GHGs intensity per unit of products (milk/meat) (Özkan *et al.* 2015; Özkan *et al.* 2018; Von Soosten *et al.* 2020).

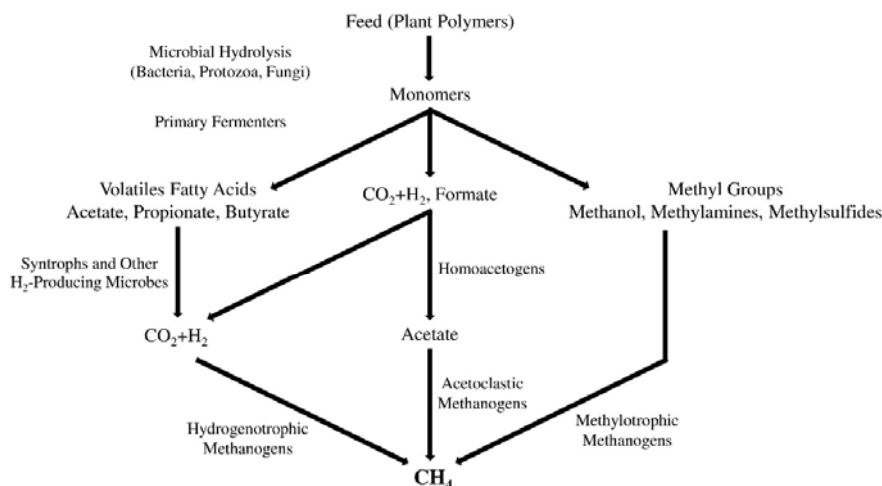
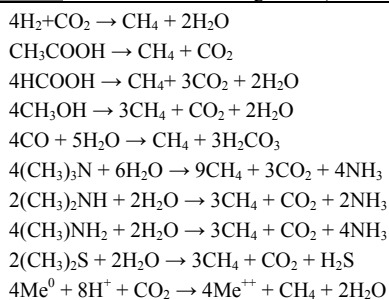
Various strategies, which manipulate rumen conditions and subsequently reduce enteric CH₄ emissions are increasing of concentrate to forage ratio, increasing levels of fatty acids and lipid supplementation, plant secondary metabolites, bacteriocins, ionophores, probiotics, halogenated CH₄ analogues, nitroxy compounds, fungal metabolite, and microalgae.

Dietary strategies to mitigate CH₄ emissions

Various methods and strategies have been proposed to reduce enteric CH₄ production in ruminants, such as dietary modification, manipulation of ruminal fermentation, and preventing methanogenic archaea using specific inhibitors. Methanogenesis inhibitors might be potentially efficient reducing agents if they apply the evolutionary determined of methanogenic archaea (Moate *et al.* 2016). In addition, archaea are evolutionarily distinct from other rumen microorganisms (including bacteria, protozoa, fungi, and viruses), and all methanogenic archaea contribute a similar biochemical pathway of methanogenesis (Hedderich and Whitman, 2013). Hence, the preventers of the methanogenesis pathway may exclusively prevent only methanogens without directly affecting other useful rumen microorganisms (Moate *et al.* 2016; Patra, 2016).

Table 1 Statics data of cattle production in Iran comparing to other countries in 2017 (FAOSTAT, 2017)

Country	Cattle Qty. (×10 ⁶)	Meat production (ton)	Milk production (ton)
Iran	4.879	477887	6811482
China	83.355	6911741	30772422
United States	93.705	11907239	97734736
Brazil	214.890	9550000	33490810
Turkey	14.080	987482	18762319
European Union	121.396	10504465	221362061

Table 2 Reactions of methanogenesis (Demirel and Scherer, 2008; Zabranska and Pokorna, 2018)**Figure 1** Schematic anaerobic fermentation of organic matter to methane

The main substrates and microbial groups catalyzing the reactions are indicated (De la Fuente *et al.* 2019)

Several reviews on CH₄ reduction approaches and options have been published previously (Patra, 2016; Knapp *et al.* 2014; Cottle *et al.* 2011). In this section, further recent advances in nutritional CH₄ mitigation strategies are mentioned.

Increasing concentrate:forage ratio

One of the most reliable strategies to reduce CH₄ emissions in dairy and beef cattle is using higher level of concentrate (Knapp *et al.* 2014).

Providing higher amounts of concentrate is mitigated gross energy (GE) loss dramatically (Johnson and Johnson, 1995) and is decreased CH₄ emissions by 3-6.5% (Beauchemin *et al.* 2007). Increased percentage of concentrate in diets consequently decrease fiber levels (cellulose and hemicellulose) and increase starch levels caused widespread physiological changes in the rumen environment. These changes are due to changes in microbial populations such as amylolytic bacteria, increasing in the production of VFAs, enhancing the ratio of propionate to acetate, which

reduces CH₄ production by reducing the availability of H₂ in the rumen (Ribeiro Pereira *et al.* 2015).

Altogether, the effects of increasing the amount of concentrate on CH₄ production depend on several factors. The most important factors are the type and quality of forage and the level of supplementation of concentrate or forage. In general, these effects are exacerbated when the amount of concentrate in low quality forage diets increases from zero to around 50% or from 70-75% to more than 90%. Conversely, there were at least changes in CH₄ emission when a moderate amount of concentrate in higher quality forages (such as grass silage) diets included (increase from 25-30% to 70-75%) (Huhtanen and Huuskonen, 2020). The type of grains used in the concentrate has shown that can change the CH₄ production too. For example, when the main grain source of concentrate was corn, 30% greater decrease in CH₄ production was shown compared to barley. Also, the reduction of CH₄ emission has been dramatically increased when optimum dietary balance and high digestible and nutritive ingredients were used in grazing cows fed with the high amount of concentrate (Beauchemin and McGinn, 2005).

It should be noted, providing a higher ratio of concentrate in cattle diet to reduce CH₄ emission has special considerations and limitations. High levels of concentrate could decrease the ruminal pH, increase the production of lactic acid and subsequently promote ruminal acidosis and shorten the productive life span of animals. Furthermore, the economic explainability of concentrate supplementation should be considered. Increasing the concentrate to forage ratio will negatively affect the digestibility of crude fiber which could lead to loss of productivity potential and will also result in increased concentration of fermentable organic matter in manure and is presumably to increase CH₄ emissions from manure management (Lee *et al.* 2012).

The increased price of forage in Iran as a result of consecutive droughts has been makes beef cattle operators to include more concentrate in the ration (Statistical Center of Iran, 2017).

Supplementation of lipid and fatty acid

Supplementation of lipid, oils and fatty acids is considered as reliable solution to mitigate enteric methane emission of dairy and beef cattle (Beauchemin *et al.* 2007; Patra and Yu, 2013a; Bayat *et al.* 2018). More recent studies have also proven that supplementation of plant oils, fats or fatty acid supplementation in beef cattle diets can effectively decrease enteric CH₄ emissions (Aviles-Nieto *et al.* 2019; Winders *et al.* 2019). According to Patra and Yu, (2013b), each 1% increase in dietary fat supplementation decreases CH₄ emission by 4.30%. In addition, in a meta-analysis using 33 treatments (Beauchemin *et al.* 2007), each 1 per-

centage of dietary fat addition resulted in a 5.6% mitigation in CH₄ (g/kg of dry matter intake (DMI)) maximum to 36%. In general, there are three ways that dietary lipids reduce methane: 1) biohydrogenation of fatty acids, 2) increased propionate production from lipolysis converting triglycerides to glycerol, which is then converted to propionate by *Anaerovibrio lipolytica* bacteria, and 3) reduction in available fermentable substrate in the rumen as fatty acids are not fermentable (Winders *et al.* 2019). Dietary supplementation of different type of lipids might decrease dry matter intake in many kinds of diets, eventually can indirectly influence on enteric CH₄ emission (Eugène *et al.* 2008; Rabiee *et al.* 2012; Hristov *et al.* 2013). It should be considered that the physical form of lipid (free oils comparing oilseeds) could affect its potential to reduce enteric CH₄ emissions. For example, supplementation of whole sunflower seeds has been mitigated CH₄ more than it's free oil (Beauchemin *et al.* 2007). In contrast, in further studies by Brask *et al.* (2013) and Fiorentini *et al.* (2014) were not found any positive impact of the physical form of lipids on CH₄ emissions when cattle fed total mixed rations. A recent *in vitro* research (Beck *et al.* 2018) has shown that supplementation of whole cottonseed to grazing beef cattle is an efficient solution to reduce enteric CH₄ emission intensity.

In addition, Beck *et al.* (2019) reported fat supplements varying in physical form (whole cotton seed meal, bypass fat and soybean oil) can improve beef cattle performance and reduce methane emission divergently. In summary, using unsaturated fatty acid sources (soybean oil and whole cottonseed) has reduced approximately 12% of methane production (g/d) comparing control and bypass fat powder. Although, dietary fat supplementation has emitted CH₄ emission (g/kg average daily gain (ADG)) nearly 50%. However, it seems differences in oil and fat source can shift the rumen microbial communities (Wang *et al.* 2017). Alternatively, supplementation of oilseeds may be gradually released or only be partially available to the rumen (Beck *et al.* 2019).

Plant secondary metabolites

Antibiotics are widely supplemented to beef cattle rations in order to their ability as rumen modulators, optimizing animal productivity (D'Aurea *et al.* 2019; Vieco-Saiz *et al.* 2019) and decreasing enteric CH₄ production (Bodas *et al.* 2012). However, present regulations by health organizations have been banned or limited antibiotic usage in animal husbandry. This issue has forced different workers looking for antibiotics alternatives such as natural feed additives or plant secondary metabolites (Ornaghi *et al.* 2019). Several plant secondary metabolites, such as saponins, tannins, and essential oils (EO), in different forages and plant extracts have been proven to be efficient for enteric CH₄ reduction

(Hristov *et al.* 2013; Knapp *et al.* 2014; Patra, 2016). Plants contain a high amount of tannins and saponins have reported being potential to mitigate CH₄ emission in cattle (Suybeng *et al.* 2019; Wu *et al.* 2019). As recently reviewed by Aboagye and Beauchemin (2019), tannins play as rumen modifiers and able to influence methanogenesis although their mechanism is still unclear.

Nevertheless, various theories have been reported that how tannins reduce CH₄ emission in ruminants: (a) tannins can directly impact on methanogens; (b) they influence protozoa that are related to methanogens; (c) tannins effect on fibrolytic bacteria and decrease rumen fiber digestibility, and (d) they act as an H₂ sink.

Probably, the tannin type (molecular weight, source or subunit), concentration, dietary substrate, and animal type are the most significant factors can affect CH₄ production and might be divers in an extensive range (*in vivo*=6.0% to 68% and *in vitro*=4.3% to 70%). In beef cattle, supplementation of hydrolysable tannin subunit (i.e. gallic acid) has the potential to reduce the environment impact of cattle husbandry (lower CH₄, N₂O and ammonia emissions), without affecting animal productivity (Aboagye *et al.* 2019).

In a recent *in vitro* study, using different levels of eucalyptus oil (2, 4, 6, 8, and 10 mL.kg⁻¹ DM) and a high-protein diet has decreased the CH₄ emission even with minimum oil amounts (Abdelrahman *et al.* 2019). Eucalyptus oil acts a definitive role in CH₄ reduction in order to it's highly desaturation point, which led to toxicity for methanogenic archaea (Prins *et al.* 1972).

Recent *in vitro* study demonstrated that using a basal dietary plant-like alfalfa silage (rich in secondary metabolites, especially saponins) can reduce enteric CH₄ emission and methanogens counts (Kozłowska *et al.* 2020). This kind of investigation can more feasible and acceptable for farmers to use inexpensive and more available compound instead of saponins rich sources.

The good potential of garlic and citrus extracts (15 g.d⁻¹.animal⁻¹) has been showed to mitigate CH₄ production and yield in Angus × Hereford feedlot cattle (Roque *et al.* 2019). Allicin, a biologically active compound in garlic extracts, can affect CH₄ emission through reductions in on methanogenic archaea and protozoa populations (Ma *et al.* 2016) with it's highly permeable potential through cell membranes (Miron *et al.* 2000). According to Eger *et al.* (2018) a blend of citrus and garlic extracts may decreased CH₄ production by changing the population of methanogenic archaea such that the proportion of *Methanobacteriaceae* was emitted without affecting negative impacts on rumen fermentation.

Dietary supplementation of a mixture of natural additives (1.5, 3.0, 4.5, or 6.0 g.d⁻¹.animal⁻¹, containing 37.5% each

of clove essential oil (vanillin, eugenol and thymol) plus 12.5 % of castor and cashew oils) linearly reduced CH₄ production (76%) in cross-bred Angus × Nellore beef cattle. Moreover, measurement of abundance of Archaeal community demonstrated a reduction (79%) in the main CH₄ producing genera including: *Ferroplasma*, *Halorhabdus*, *Methanoplanus*, and *Picrophilus*. The greatest generators of acetate in the rumen, *Fibrobacter* and *Lactobacillus*, have been declined by 71% leading to inhibition of H₂ production and reduction of CH₄ formation (Ornaghi *et al.* 2019).

Berry fruits and their by-products contain several biologically active compounds like tannins, saponins, flavones, phenolic acids, ellagic acid, vitamins C and E, folic acid, and β-sitosterol that can be applied in animal nutrition (Roj *et al.* 2009). Supplementation of hemp and blueberry oils (as unconventional oils high in polyunsaturated fatty acids, (PUFA)) has been showed which can reduce enteric CH₄ emission by 10-16% without compromising effect on rumen fermentation and degradability (Embaby *et al.* 2019). Adding of berry seed residues showed profitable economically and nutritionally for dairy cattle production and but reduced CH₄ emission numerically (Bryszak *et al.* 2019).

The effect of bioactive compounds and secondary plant metabolites on CH₄ mitigation may also depend on the basic nutrient components (like crude protein and crude fiber) (Patra and Saxena, 2009; Cieslak *et al.* 2013; Cieslak *et al.* 2014). There are some evidences that basic nutrient components can interact with bioactive compounds and consequently the bioactive compounds become physically less available for microbiota. For instance, increasing the amount of NDF and ADF inhibits microbial activity through a reduction in the availability of slowly fermented carbohydrates (Wilson and Hatfield, 1997). In addition, variations in the chemical composition of the herbs (such as Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) can affect the concentration of short chain fatty acids (Njidda and Nasiru, 2010) and the ruminal pH and can suppress methanogen growth, hence mitigating CH₄ production per unit of fermented organic matter (Van Kessel and Russell, 1996). Furthermore, the results confirmed that fumarate supplementation with herbal mixture in high concentrate diet can reduce *in vitro* CH₄ emission by 10-11% and increase propionate ranging from 5 to 13%; however, it's effect depends on many parameters, such as the type or nature of diet, fumarate concentration, ruminal pH, and different microbial community in batch culture (Pisarčíková *et al.* 2016).

Nitrate supplementation

Researchers suggested that nitrate (NO₃⁻) acts as a CH₄ inhibitor by changing the population of rumen microbiome in

the following two methods: a) toxicity by nitrite (NO_2^-), an intermediate of nitrate reduction; b) competition for H_2 (Zhao *et al.* 2015). In the other word, nitrate prevents methanogenesis playing as H_2 alternative sinks and directly preventing the methanogenic archaea. As described by Patra (2016) two benefits are introduced for nitrate supplementation: (a) reducing of CH_4 production, as mentioned above, and (2) providing ammonia to growth of rumen microbial community resulting in reduced dietary protein inclusion. Therefore, nitrate can influence as an efficient CH_4 suppressor and a possible non-protein nitrogen (NPN) resource for beef cattle, playing as an electron sink and adding NH_4 -based N to the rumen (Nolan *et al.* 2010; Zhao *et al.* 2015).

Encapsulation of nitrate (NO_3) has been investigated to make sure nitrate slowly release inside the rumen environment and enhance the efficiency of microbial community to reduce NO_3 to NH_4 completely, hence keeping down the risk of NO_3/NO_2 toxicity (Alemu *et al.* 2019). Feeding slow release nitrate (encapsulation nitrate (EN), 2.5% encapsulated calcium ammonium nitrate (NO_3^-)) in feedlot cattle fed high-grain finishing diets reduced CH_4 yield (10.06%), dry matter intake and slaughter weight without affecting ADG; however, more days on-feed may be required to reach slaughter weight which may compensate some of the benefits of improved G:F (9.7%) and reduced CH_4 emissions (Romero-Pérez *et al.* 2018). Supplementation of NE in substitution of urea mitigated enteric CH_4 emissions (13%) although has not been shown positive impact on beef cattle performance (Alemu *et al.* 2019).

In grazing steers, NO_3 encapsulation can positively influence enteric CH_4 emission, thereby reducing *Methanobrevibacter* abundance in the rumen. Moreover, EN supplementation can stimulate the growth of fumarate-reducer and lactate generator bacteria, thereby increasing propionate:acetate ratio through rumen fermentation (Granja-Salcedo *et al.* 2019). Finally, information about the factors affecting the efficiency of nitrate reduction in the rumen is scarce. Encapsulation nitrate, amount of nitrate consumed and the rate of nitrate intake as well as the type of diet (e.g., concentrate:forage ratio, nitrogen and sulfur concentrations) and the type of animal affect the ruminal nitrate consumption, and subsequently, the reduction of CH_4 emissions. In addition, the period time of a dietary nitrate added may influence its efficacy in decreasing CH_4 emission over time (Alemu *et al.* 2019).

Lactic acid bacteria (LAB) supplementation

Sustainable food production can be achieved when innovative and creative strategies are used to reduce CH_4 emissions from livestock. One of these recent strategies is the application of LAB (Vieco-Saiz *et al.* 2019).

This kind of microorganisms are suitable probiotics and gram-positive bacteria producing lactic acid, as a main end product of carbohydrates fermentation. In addition, LAB-probiotics are intrinsic inhabitants of the mammalian gut microbiome and are among the most relevant microorganisms used in food fermentation. Lactic acid bacteria are simply isolated from the digestive tract of ruminants and used in various forms of direct-fed microbials or silage inoculants (Doyle *et al.* 2019). In addition, it has been suggested that LAB can be used to decrease CH_4 production in ruminant livestock (Haque, 2018).

The researchers examined 45 bacterial strains, including strains of LAB, *Propionibacteria*, and *Bifidobacteria*, for their potential to reduce methanogenic archaea (Jeyanathan *et al.* 2016). They suggested that LAB could stimulate the growth of lactic acid-consuming bacteria, which would increase propionic acid production and subsequently reduce hydrogen availability for methane production. On the contrary, it should be noted that the subsequent work of these researchers (Jeyanathan *et al.* 2019) using similar strains had no effect on reducing methane emissions. However, LAB supplementation can be an effective, viable and intrinsic solution for reducing enteric CH_4 production (Doyle *et al.* 2019), although reliable research and data in this area are still scarce to promote these strategies.

Hydrogen-utilizing bacteria

Hydrogen is recognized as the major substrate for ruminal methanogenesis. There is a closely relevant between H_2 metabolism, its related microbiome and methane producing archaea (Figure 2) (Russell and Wallace, 1997; Lourenço *et al.* 2010). Specific microbes can compete with methanogenic archaea and could convey H_2 apart from methanogenesis consequently reduce enteric CH_4 emission. This strategy may inhibit detrimental effects of chemical additives like microbial resistance or toxicity and increase the availability of feed gross energy of the animal (Lan and Yang, 2019). To explore this method of CH_4 mitigation different types of bacteria have been introduced; e.g., propionate producing bacteria (PPB), sulphate (SO_4^{2-})-reducing bacteria (SRB), nitrate/nitrite-reducing bacteria (NRB) or the homoacetogens. Thermodynamically, PPB, SRB and NRB groups have some special benefits compared to methanogenic archaea when using H_2 as an electron sink. However, their metabolism would be limited in normal ruminal environment due to their low abundance or lack of essential substrates (Lan and Yang, 2019).

Recently, two reliable strategies have been developed to improve the propionate production pathway in the rumen, including the use of propionate precursors such as malate or fumarate or supplementation of propionate-producing bacteria.

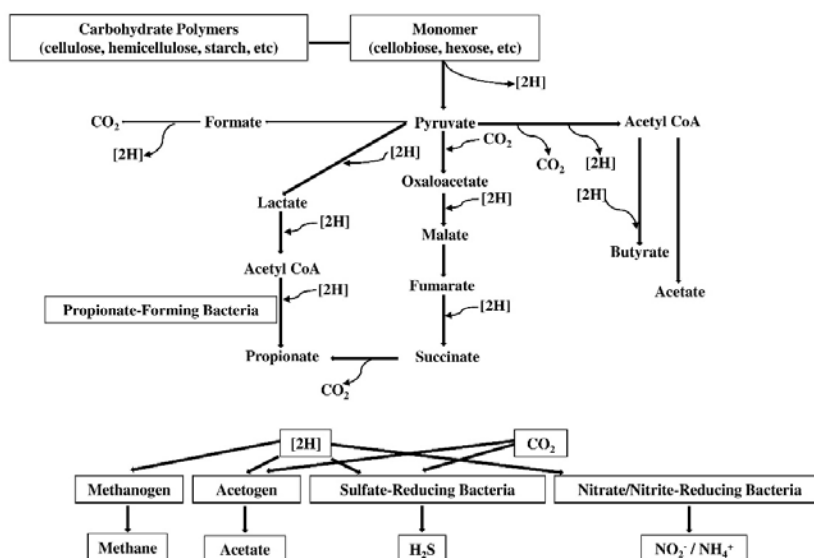


Figure 2 Feed fermentation and H₂ disposal pathways in the rumen (Lan and Yang, 2019)

Because of the small concentration of NO₃⁻ and SO₄²⁻ in the rumen medium, the use of SO₄²⁻ or nitrate as additives is a preferred approach to stimulate SO₄²⁻ and nitrate reducing bacteria. In order to prevent the toxic effects of these compounds in the rumen, the use of SO₄²⁻ or nitrate/nitrite reducing bacteria is recommended. Despite the dynamic nature of rumen microorganisms, the development and generalization of these methods will require more extensive research on methane emission reduction, both *in vivo* and in larger scale studies (Lan and Yang, 2019).

Nitroxy compounds

3-nitroxypropanol (3-NOP) is a recently developed compound that have particular anti-methanogenic effects and can mitigate enteric CH₄ production by 25 to 45% in several studies while maintaining animal performance (Romero-Perez *et al.* 2014; Hristov *et al.* 2015; Vyas *et al.* 2016; Vyas *et al.* 2018). In addition, McGinn *et al.* (2019) indicated that there was a large CH₄ emission reduction of about 70% (±18%) because of 3 nitroxypropanol dietary adding. This additive has been demonstrated to exclusively target the nickel enzyme methyl-coenzyme M reductase (*mcr*) in methanogenic archaea, thereby preventing the final phase of CH₄ production by reversibly oxidizing the nickel enzyme cofactor from Ni(I) to Ni(II) (Duin *et al.* 2016). Furthermore, dietary adding 3-NOP at 100 mg.kg⁻¹ DM decreased CH₄ yield by 18% when beef steers were fed a low concentrate diet but no reduction was reported when a high concentrate diet was fed (Kim *et al.* 2019).

There are some inconsistencies between methane mitigation studies when 3-NOP was fed, although the reasons are still unclear. However, animal type and variation, experimental design and duration, dietary composition, and methane measurement technique may have attributed to the variability (Huhtanen *et al.* 2019). As described by Vyas *et al.* (2016) the rumen concentration of *mcr* may be decreased for a high grain comparing to low grain diet, resulting in greater efficacy of 3-NOP in CH₄ reduction. In addition, Kim *et al.* (2019) reported that by preventing of rumen CH₄ production, fermentation process shifts from acetate to propionate production for 2H⁺ removal. Valerate, as an alternative sink for 2H⁺ in the rumen, has increased when 3-NOP was fed too.

Fungal metabolites

Lovastatin is known as a secondary fungal metabolite that inhibits the activity of a critical enzyme in cholesterol synthesis, 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase (Jahromi *et al.* 2013; Candyrine *et al.* 2018). Researches have shown that the use of fungal strain *Aspergillus terreus* containing lovastatin as well as fungal strain *Mortierella wolfii* reduced the ruminal population of methanogenic archaea and methane production (Cosgrove *et al.* 2012).

In addition, other fungal metabolites such as “mevastatin” and “pravastatin” also increased the proportion of propionate to acetate and thereby reducing the production of enteric CH₄ production (Morgavi *et al.* 2013).

Microalgae

Studies using microalgae, as methane reducing agents, have shown that CH₄ production is reduced by 99% even with 2% *Asparagopsis* supplementation *in vitro* condition (Machado *et al.* 2014). Use of algae *Chlorella vulgaris* improved rumen bacterial growth as well as increased total VFAs and enhanced milk production in dairy cows (Anele *et al.* 2016; Kholif *et al.* 2017; Tsiplakou *et al.* 2017). This strain of algae has also been identified as a reliable candidate for reducing methane emissions (Bohutskyi *et al.* 2014; Tsiplakou *et al.* 2017; Wild *et al.* 2019). Furthermore, *Oedogonium*, a member of *Filamentous* microalgae, was reported to reduce enteric methane production (Machado *et al.* 2014). *Cystoseira trinodis* and *Dictyota bartayresii* members of brown algae can inhibit methane production *in vitro* conditions. In addition, Sucu (2019) reported that careful selection and combination of substrate and algae (*Chlorella vulgaris* and *C. variabilis*) may positively manipulate rumen fermentation and may inhibit CH₄ production.

CH₄ inhibitors

Monensin has been widely investigated and accounted to enhance the productivity of beef cattle (Pancini *et al.* 2020). This ionophoric antibiotic isolated from *Streptomyces cinnamonensis* and has antifungal and antiprotozoal (anticoccidial) characteristics. Monensin is commonly utilized in different commercial livestock production, as a growth promoter or improving the ruminal fermentation, body weight gain (BWG) and FCR or as a coccidiostat (Ipharraguerre and Clark 2003; Mimouni *et al.* 2014). Monensin can reduce acetate to propionate proportion, enteric CH₄ and NH₄⁺ production, thereby improving efficiency of energy metabolism, feed efficiency and BWG (Hemphill *et al.* 2018; Gupta *et al.* 2019).

In a meta-analysis study by Appuhamy *et al.* (2013), monensin remarkably reduced CH₄ emissions in beef steers and dairy cows (-19 and -6 g.day⁻¹, respectively). The reducing impact of monensin on methanogenesis is because of preventive effect on protozoa and gram-positive bacteria, which promote propionate formation and reduce acetate, butyrate and formate production, leading to lower substrate availability for methanogenic archaea and subsequent CH₄ production.

Combination use of CH₄ inhibitors

In recent years, a large number of CH₄ inhibitors have been investigated, mainly individually. However, these compounds usually have special effects on nutrient digestibility and ruminal fermentation, especially if supplemented at high concentration levels for greater inhibition effect on methane emission (Patra, 2016). Some of these compounds

also lead to animal toxicity when used at high doses (Patra, 2012). Supplementation of lower doses of CH₄ inhibitors can compensate for the toxicity problems but the methanogenesis inhibition effect is not highlighted at low doses. However, combinations of inhibitors with a supplementary mode of actions may mitigate CH₄ emission synergistically and improve their efficiency without using any harmful impact on rumen fermentation or nutrient digestion at low levels (Patra and Yu, 2013a; Narvaez *et al.* 2013). Recently, it has been demonstrated that combinations of two relevant CH₄ inhibitors (saponin with nitrate) can be more effective and practical than individual inhibitors (reduced 32.92% and 25.04% with nitrate and nitrate+saponin, respectively). Different mechanisms have been reported for these inhibitors such as antimethanogenic actions or inhibit different microbial communities involved in CH₄ production or SO₄-reduction (Wu *et al.* 2019).

Genetic control of GHGs

Nowadays, the mitigation of enteric CH₄ of cattle has critical importance. In general, there are four main methane-controlling parameters: 1) rumen microbial community, 2) dry matter intake and feed composition, 3) host physiological conditions, and 4) host genetics (De Haas *et al.* 2016). Recent studies have shown that genetic factors in which controlling enteric CH₄ is a heritable trait with a high correlation with dry matter intake (De Haas *et al.* 2016; Garnsworthy *et al.* 2019). Different studies have illustrated that intrinsic variation between cattle exists in enteric methane emission and there is a possibility to decrease CH₄ production ranging from 10 to 20% by breeding (Waghorn and Woodward, 2006; Grainger *et al.* 2007). However, it should be considered that nutritional and management strategies to mitigate enteric CH₄ emission leading to short-term reduction, but breeding and genetic strategies can provide long-term and persistence reduction in order to their improvement are cumulative and permanent (Garnsworthy *et al.* 2019). It should be stressed that the genetic control of GHGs are mainly focused on dairy cattle and information from beef cattle are scarce (Barwick *et al.* 2019; Fennessy *et al.* 2019).

Regardless of the reduction approaches, measurement methods of enteric CH₄ emission are critically essential to achieve a highly accurate and precise date. In addition, measuring CH₄ on a large quantity of cattle is a strict challenge. However, different scientists around the world have tried to focus on efficient measurement methods to achieve a highly accurate date with a large number of animals (Jonker *et al.* 2020). However, recent findings confirmed that there is a sufficient correlation among different direct and indirect methods measuring enteric methane emission (Garnsworthy *et al.* 2019).

CONCLUSION

Practical strategies to reduce enteric CH₄ emission in ruminants can be effective both in achieving international commitments due to climate change and in improving gross energy efficiency and livestock performance. Increasing livestock productivity through production systems improves the livelihoods of livestock farmers and ensures food security. Although innovative and novel strategies to reduce CH₄ emissions have been explored, only a few of them have been developed due to efficiency, feasibility, and cost-effectiveness, which will subsequently be developed on farms. It seems that combining several strategies to reduce CH₄ production at the farm level would significantly reduce the rate of CH₄ emit from cows to a considerable extent compared to using a single or an individual strategy. Therefore, CH₄ reduction strategies that show both nutritional and environmental benefits are likely to be better accepted by farmers. For example, increasing the level of concentrate and fat and oil supplements can reduce the production of CH₄ as well as improve animal productivity. Likewise, dietary nitrate supplementation can reduce crude protein levels in the diet and ultimately reduce methane emissions and enhance productivity. Future research, however, on reducing greenhouse gas emissions, particularly methane and N₂O, should focus on achieving both environmental and nutritional approaches to sustainable development.

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