Review Article

Decreasing ruminal methane production through enhancing the sulfate reduction pathway

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ABSTRACT

Methane (CH_4) production from ruminants accounts for 16% of the global greenhouse gas emissions and represents 2% to 12% of feed energy. Mitigating CH_4 production from ruminants is of great importance for sustainable development of the ruminant industry. H_2 is the primary substrate for CH_4 production in the processes of ruminal methanogenesis. Sulfate reducing bacteria are able to compete with methanogens for H_2 in the rumen, and consequently inhibit the methanogenesis. Enhancing the ruminal sulfate reducing pathway is an important approach to mitigate CH_4 emissions in ruminants. The review summarized the effects of sulfate and elemental S on ruminal methanogenesis, and clarified the related mechanisms through the impacts of sulfate and elemental S on major ruminal sulfate reducing bacteria. Enhancing the activities of the major ruminal sulfate reducing bacteria including *Desulfovibrio*, *Desulfohalobium* and *Sulfolobus* through dietary sulfate addition, elemental S and dried distillers grains with solubles can effectively decrease the ruminal CH_4 emissions. Suitable levels of dietary addition with different S sources for reducing the ruminal CH_4 production, as well as maintaining the performance and health of ruminants, need to be investigated in the future.

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1. Introduction

Methane (CH_4) is a greenhouse gas with a global warming potential 28 times that of CO_2 (Gerber et al., 2013). The global anthropogenic CH_4 emissions in 2020 were 380 Mt, of which 49 Mt/year were from livestock (United Nations Environment Programme and Climate and Clean Air Coalition, 2021). The CH_4 from ruminants accounts for more than 95% of the total CH_4 emissions from livestock, while that from non-ruminants is about 5% (Fig. 1). About 90% of the CH_4 from ruminants is produced in the process of rumen microbial methanogenesis (McAllister et al., 2015). Ruminal CH_4 production represents 2% to 12% of the total energy intake of ruminants (Johnson and Johnson, 1995), which could otherwise be used for animal growth or meat and milk production.

Ruminant production is developing rapidly to meet the needs of meat and milk by the increasing human population. It was predicted that milk and meat production must be increased by 63% to 76%, respectively, to meet the growing demands of the global population (Alexandratos and Bruinsma, 2012). Consequently, the CH_4 emissions from ruminants are expected to further increase with the development of ruminant production (Eshel et al., 2014). The contradiction between developing ruminant production and protecting the environment is becoming more divisional across the world. Decreasing the enteric CH_4 emissions is important for the sustainable development of ruminant production. The development of low-cost approaches to decrease the CH_4 emissions from ruminants is thus of great importance to complete CH_4 mitigation objectives.

The options for mitigating the ruminal CH_4 emissions mainly include inhibiting the activities of methanogens with tannins (Beauchemin et al., 2007; Jayanegara et al., 2015), defaunation of rumen protozoa (Morgavi et al., 2008), shifting H_2 from CH_4 production to other pathways with fumarate (Abrar et al., 2016), nitrates (Lund et al., 2014; Klop et al., 2016) and sulfate (van
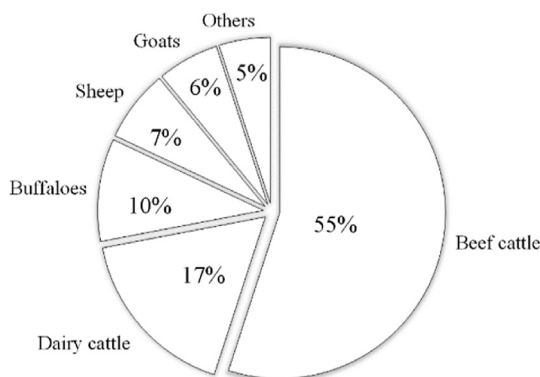
Fig. 1. Contributions of different animal species to the total CH_4 emissions from livestock. Calculated based on Food and Agriculture Organization of the United Nations (FAOSTAT, 1961–2019, http://www.fao.org/faostat/en/#data/GE).

Zijderveld et al., 2010), manipulation of dietary concentrates to forage ratio (Lovett et al., 2003), and improving animal performance to decrease the CH_4 production per unit of product (González-Recio et al., 2020). Considering that ideal feed additives should reduce CH_4 production while not adversely affecting feed digestion and animal performance, one promising approach to decrease the ruminal CH_4 emission is to manipulate the critical biochemical pathways for synthesizing CH_4 in the rumen. Of all the options, mitigating ruminal CH_4 emissions through shifting the ruminal H_2 from CH_4 production via the administration of sulfate or elemental sulfur (S), is garnering more attention, not only because the approach is effective to decrease the ruminal CH_4 production, but it is also related to dietary S supply for ruminants. The objectives of this review were to summarize the advances in sulfate reducing pathway to mitigate the ruminal CH_4 emissions, clarify the inherent mechanisms through the functions of the major sulfate reducing bacteria, and propose perspectives for further research.

2. Methanogenesis in the rumen

A complex microbial community exists in the rumen of ruminants. The rumen microorganisms are mainly classified into bacteria, protozoa and anaerobic fungi. *Methanobrevi bacterruminantium* and *Methanomicrobium mobile* were considered to be the dominant methanogens in the rumen (Min et al., 2014). Rumen microorganisms enable the hosts to efficiently digest fibrous feeds through microbial-mediated fermentation, and dietary carbohydrates can be extensively fermented by rumen microorganisms to produce many end products; mainly including volatile fatty acids (VFA), CO_2 and H_2 (Janssen and Kirs, 2008). The H_2 produced in the rumen fermentation process is released from glycolysis and pyruvate oxidative decarboxylation to acetyl-CoA (Lan and Yang, 2019). Methanogenic archaea are able to use H_2 and CO_2 as substrates for synthesizing CH_4. This pathway is considered to be the main H_2 sink within the rumen (Kumar et al., 2014).

There are three pathways for CH_4 production in the rumen (Fig. 2). Firstly, CO_2 is reduced successively to CH_4 by H_2 as the primary electron donor through formyl, methenyl, methylene and methyl intermediates and 1 mol of CH_4 and 2 mol of H_2O can be produced from 1 mole of CO_2 and 4 mol of H_2. The reduction of the carbon moiety involves 7 steps catalyzed by a number of unique cofactors and enzymes (Haque, 2018). The CO_2–H_2 reduction is the predominant pathway of CH_4 production in the rumen and accounts for about 82% the CH_4 production (Ellis et al., 2008).

Through genome sequencing, the prominent methanogens, such as *M. bacterruminantium*, were found to be responsible for CO_2 reduction through the CO_2–H_2 reduction pathway in the rumen (Qiao et al., 2014), which can partially decrease the H_2 pressure and therefore favor the continuous rumen fermentation. Without the removal of H_2, the accumulated H_2 inhibits the re-oxidation of NADH, NADPH and FADH, and consequently decreases VFA production (Ellis et al., 2008). Secondly, synthesis of CH_4 may be completed using acetate and formate as substrates. *M. bacterruminantium* and *Methanobrevi bactergottschalkii* are the major hydrogenotrophic archaea which account for more than 74% of the methanogenic archaeal community in the rumen (Henderson et al., 2015). Although acetate is highly available in the rumen, acetoclastic methanogenesis has minor importance for CH_4 production (Ungerfeld et al., 2015) because of the low growth rate of the acetate-utilizing methanogen *Methanosarcinales* and the high outflow rate from the rumen (Thauer et al., 2008), and low affinity of the acetogens to H_2 (Morgavi et al., 2010). Formate is used by many of hydrogenotrophic rumen methanogens as an alternative substrate for CH_4 production (Carroll and Hungate, 1970). However, it accounts for only 16% to 18% of the total CH_4 production in the rumen (Ungerfeld et al., 2015). Thirdly, methyl groups present in the methanol and methylamines can be used as another category of substrates for methanogenesis in the rumen (Sun et al., 2021). However, only 3% to 5% of CH_4 is produced through this pathway (Ungerfeld et al., 2015). Of the three pathways for the CH_4 production in the rumen, it could be found that inhibiting the CO_2–H_2 reduction processes is most important because most of the ruminal CH_4 is produced through the pathway.

3. Potentials of inhibiting ruminal methanogenesis through enhancing the sulfate reduction pathway

Except for the pathway of CO_2–H_2 reduction, other pathways for H_2 disposal also exist in the rumen, such as propionate production, reductive acetogenesis, sulfate reduction, nitrate/nitrite reduction and fumarate reduction (Fig. 3). Although nitrates can be used as effective H_2 sink (Haque, 2018) and have an inhibitive effect on ruminal CH_4 production (van Zijderveld et al., 2010; Lee, 2014; Feng et al., 2020), the toxicity of the intermediate product, nitrite, limits the utilization of nitrates as CH_4 inhibitors (Lund et al., 2014). Fumarate can also be used as a ruminal CH_4 inhibitor. However, some experiments showed dietary addition with fumarate had minor effects on CH_4 production (Lin et al., 2013; Mbiriri et al., 2017). Therefore, sulfate may be more important than nitrate and fumarate as the H_2 sink to decrease ruminal CH_4 production.

3.1. Sulfur metabolism and sulfate reducing bacteria in the rumen

Coleman (1960) first isolated the sulfate reducing bacteria (SRB) *Desulfotomaculum ruminis* from the rumen of sheep fed hay. Later, Huisingh et al. (1974) isolated the SRB *Desulfovibrio* from the rumen of sheep fed a diet containing sulfate. The concentration of SRB in the rumen, mainly including *Desulfovibrio* and *Desulfotomaculum*, is approximately 10^5 to 10^6 cells/mL (Huisingh et al., 1974). More recently, Drewnoski et al. (2014) found that the top three genera of SRB were *Desulfovibrio*, *Desulfohalobium* and *Sulfolobus*, and the reads of *Desulfovibrio* were 1.65 times greater in the rumen of steers fed 0.6% S diet than 0.3% S diet, whereas *Desulfohalobium* and *Sulfolobus* were relatively similar between the two diets. By using 16S rRNA gene sequencing, Zhao et al. (2020) found that the first three genera of SRB were *Desulfovibrio*, *Desulfobulbus* and *Desulfuromonas*, and dietary addition with sulfate increased the relative abundances of *Desulfovibrio* and *Desulfobulbus*. Wu et al. (2015) reported that adding sulfate increased the population of
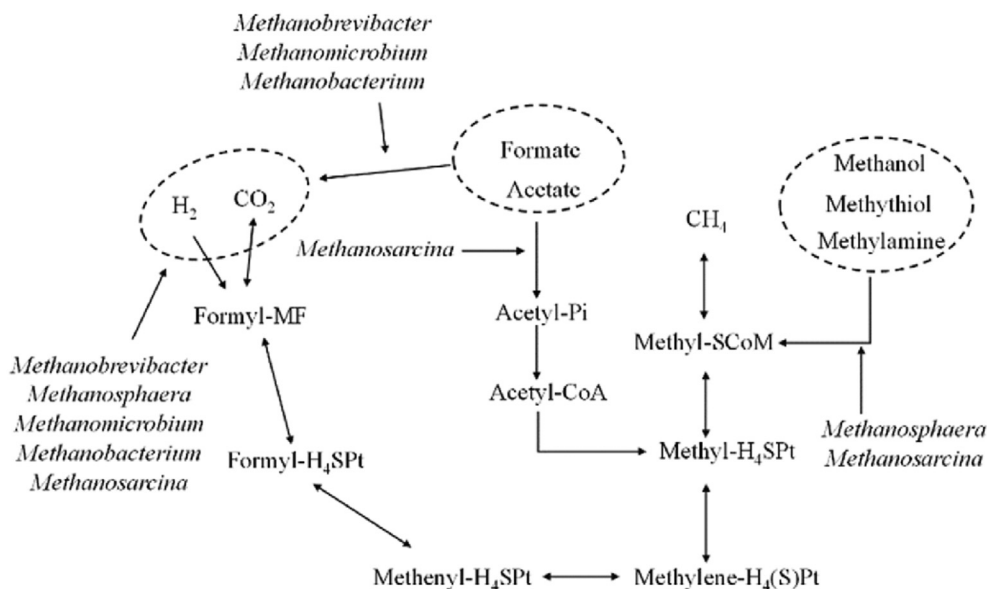
Fig. 2. Simplified methanogenesis pathways and related methanogens genera in the rumen. Modified based on Honan et al. (2021).
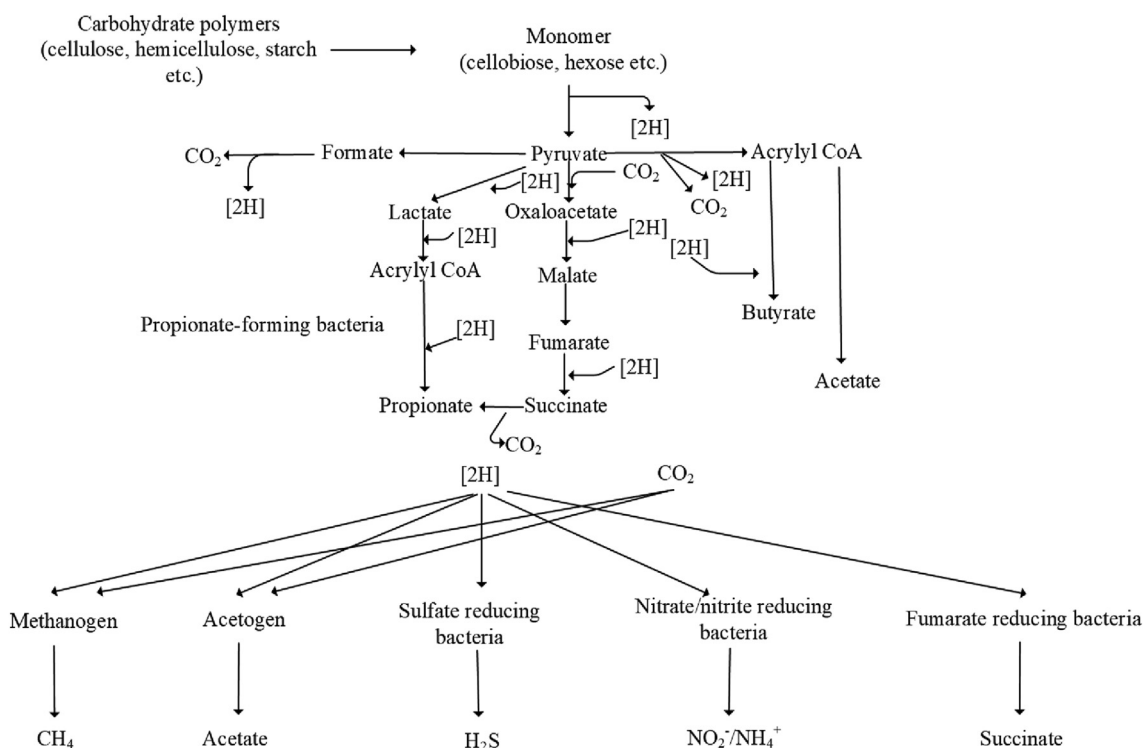

Fig. 3. Ruminal fermentation of carbohydrates and H_2 disposal ways. Modified based on Lan and Yang (2019).

Desulfovibrio in in vitro rumen fermentation. The results of the studies indicated that *Desulfovibrio* were closely related to the sulfate metabolism in the rumen.

The metabolism of S-containing compounds in rumen is a reversible process. Fig. 4 shows that sulfate can be transformed to an active form as adenosine-5-phosphosulfate (APS). Then, APS is reduced to sulfite and consequently converted to sulfide. Ruminal microorganisms play the key role in S metabolism and the rate of S transfer between sulfate, sulfide and hydrogen sulfide (H_2S) (Drewnoski et al., 2014). Two classes of bacteria, assimilatory and

dissimilatory, are responsible for S metabolism in the rumen (Suttle, 2010). Assimilatory bacteria are able to incorporate sulfide into S-containing amino acids or cofactors such as biotin and pantothenic acid (Bradley et al., 2011). Dissimilatory bacteria which are SRB are able to transform sulfide to H_2S as the end product (Bradley et al., 2011). Ruminal SRB can produce sulfide mainly using lactate and sulfate as the substrates, even though other carbon sources can be utilized. Then, sulfide can combine with H_2 to produce H_2S (Suttle, 2010). About 70% to 85% of H_2S eructated from the rumen can be inhaled (Pogge, 2016) and may be absorbed into
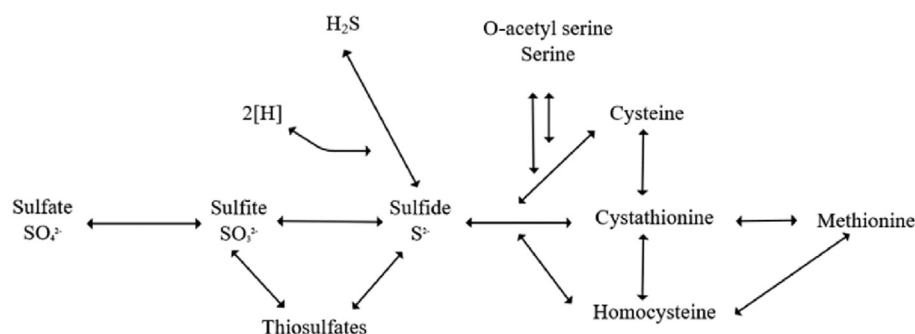
Fig. 4. Metabolism of sulfur in the rumen (Pogge, 2016).

blood to cause polioencephalomalacia (PEM), which is the syndrome of S toxicity at high S intake.

3.2. Mechanism of decreasing CH_4 production through the sulfate reduction pathway

Ruminal SRB utilize different forms of S, e.g. sulfate, sulfite, thiosulfate and elemental S, as the optional H_2 sinks (Hinsley and Berks, 2002). As the end product of the sulfate reduction pathway, H_2S can inhibit methanogenic activity and consequently reduce ruminal CH_4 production (van Zijderveld et al., 2010). Factors affecting the effect of the dissimilatory SRB on sulfate reduction mainly include the dissimilatory sulfite reductase enzyme activity (Bradley et al., 2011), fermentable carbohydrates, nitrogen, pH, and other minerals (Underwood and Smitasiri, 1999). Because SRB require H_2 to reduce sulfate to sulfide and methanogens require H_2 to reduce CO_2 to produce CH_4, the most important relationship between SRB and methanogenesis is the competition for H_2.

The ability of SRB in the competition for H_2 with methanogens in the rumen mainly depends on sulfate levels (Jiang et al., 2010; Singh and Lin, 2015). Increasing sulfate level in the rumen can improve the capacity of SRB to be a H_2 sink (Bryant et al., 1977). Table 1 shows that the dissimilatory sulfate reduction by SRB is thermodynamically more favorable ($\Delta G = -21.1$ kJ/mol H_2) than hydrogenotrophic methanogenesis ($\Delta G = -16.9$ kJ/mol H_2) (Ungerfeld et al., 2015).

Some SRB are versatile organisms that possess the ability to oxidize H_2S to sulfate and prevent the accumulation of toxic levels of H_2S (Dröge et al., 2005). A newly isolated group of SRB, known as *Fusobacterium*, decreased the 72 h CH_4 production by 62% in in vitro rumen fermentation without H_2S accumulation (Paul et al., 2011). Thus, selecting specific SRB that have the ability to oxidize H_2S is a promising strategy to decrease the ruminal CH_4 production. Nitrate-reducing-sulfide-oxidizing bacteria (NR-SOB) are a subset bacterial group that can use sulfide as the electron donors for nitrite ammonification (Hubert and Voordouw, 2007). However, the presence of NR-SOB is limited in the rumen due to the low concentration of ruminal sulfide. It was reported that ruminal SRB

Wolinella succinogenes have physiological similarities with NR-SOB *Sulfurospirillium deleyianum*, which are able to use sulfide as an electron donor for their growth (Simon, 2002).

4. Impacts of different S sources on ruminal CH_4 production and microorganisms

4.1. Sulfate

The actual sulfate reduction in the rumen is limited by the amount of S-containing compounds (Zinder, 1993). The level of S in normal/healthy animal diets is recommended to be about 0.15% dry matter (DM) (National Academies of Sciences, Engineering and Medicine, 2016), usually not high enough to permit sulfate-reducers to outcompete methanogens as the major H_2 sink in the rumen. It was reported that the concentration of sulfate from 10 to 22 mmol/L could effectively decrease the rate of methanogenesis in in vitro rumen fermentation (Siegert et al., 2011).

van Zijderveld et al. (2010) reported that adding sulfate (0.85% total S in DM) to the diet containing nitrate reduced CH_4 production by 21%, compared with the diet without sulfate in growing sheep. Silivong et al. (2011) showed that adding S at 0.8% DM from sulfate to the diet of goats also reduced ruminal CH_4 production by 14.2%. Similarly, Arif et al. (2016) reported that when sheep were fed a diet containing nitrate at 2.5% DM, adding sulfate at 0.4% DM reduced ruminal CH_4 yield and the CH_4/CO_2 ratio by 7% and 8%, respectively. These results demonstrated that dietary addition with sulfate effectively decreased the ruminal CH_4 production in different species of ruminants, and did not show any adverse effects on the animals.

4.2. Elemental S

Li et al. (2013) reported that increasing dietary S from 0.15% to 0.56% DM via inclusion of elemental S to a nitrate-containing diet (1.88% DM) reduced the ruminal CH_4 emissions from lambs by 37.8%. Uniyal et al. (2020) showed that supplementation with elemental S in goat diets up to 0.16% DM tended to reduce the ruminal CH_4 emissions. Similarly, Rebelo et al. (2019) observed that addition with elemental S to nitrate-containing diets (5.1% DM vs. 2.7% DM) did not alter the ruminal CH_4 emissions in steers. The results suggested that elemental S was not as effective as sulfate to decrease ruminal CH_4 production. The reason may be due to the fact that the utilization efficiency of elemental S was lower than sulfate by rumen microorganisms (van Zijderveld et al., 2010).

4.3. Dried distillers grains with solubles

Wu et al. (2015) reported that dietary inclusion of corn dried distillers grains with solubles (DDGS) at 60% DM decreased CH_4

Table 1
Reductive process and reaction thermodynamics of hydrogen-utilizing microorganisms in the rumen.

Substrates	Products	ΔG (KJ/H_2)	Microorganisms
$CO_2 + 4H_2$	$CH_4 + 2H_2O$	−16.9	Methanogens
$CO_2 + 4H_2$	$C_2H_4O_2 + 2H_2O$	−2.2	Reductive acetogens
$SO_4^{2-} + 4H_2 + H^+$	$HS^- + 4H_2O$	−21.1	Sulfate reducing bacteria
$NO_3^- + 4H_2 + H^+$	$NH_4 + 3H_2O$	−124	Nitrite reducing bacteria

ΔG = free energy change, which indicates how energetically favorable it is; i.e. the higher ΔG, the more energy utilization. Negative ΔG indicates the energy release. Adopted from Haque (2018).

production by 59%, whereas inclusion of sulfate or sulfuric acid at 0.32% DM only slightly reduced CH_4 production in in vitro rumen fermentation. The results suggested that different S sources varied in the inhibitive effects on CH_4 production.

The S content of most DDGS ranges from 0.6% to 0.8% DM (Kim et al., 2012). The S-containing compounds in DDGS mainly include sulfuric acid and S-containing amino acids (Drewnoski et al., 2014). Hünerberg et al. (2013) reported that feeding finishing beef cattle a diet containing corn DDGS at 40% DM decreased the enteric CH_4 by 19.5%. Benchaar et al. (2013) reported that dietary inclusion of corn DDGS at 30% DM decreased the CH_4 emissions of lactating dairy cows by 14%. The results suggested that including suitable levels of DDGS in the diet would be beneficial to decrease the ruminal CH_4 production in cattle.

4.4. Combination of sulfate and nitrate

In contrast to these positive results, Pesta (2015) reported that dietary addition with sulfate (0.54% DM) alone did not affect the ruminal CH_4 emissions, whereas adding the combination of nitrate (2% DM) and sulfate (2% DM) decreased the CH_4/CO_2 ratio and tended to decrease CH_4 production/kg DM intake (DMI). Patra and Yu (2014) reported that inclusion of sulfate at 0.84% DM decreased the in vitro ruminal CH_4 production by 16%, whereas inclusion of the same level of nitrate decreased the in vitro ruminal CH_4 production by 30%. The results were consistent with van Zijderveld et al. (2010), who reported that supplementing sulfate or nitrate (both at 2.6%DM) decreased the ruminal CH_4 production in sheep by 16% or 32%, respectively. Stoichiometrically, one mole of sulfate can decrease CH_4 production by one mole if sulfate is reduced to H_2S solely by H_2 (Muyzer and Stams, 2008). The reason for the less effective impact of sulfate on decreasing ruminal CH_4 production than nitrate, could possibly be due to incomplete reduction, less favorable thermodynamics and lower competitiveness for H_2 by sulfate than nitrate.

Both in vitro and in vivo studies showed that co-addition of sulfate and nitrate effectively decreased ruminal CH_4 production. van Zijderveld et al. (2010) reported that combination of nitrate and sulfate (2.6% + 2.6% DM) decreased the ruminal CH_4 production by 47% in sheep. Patra and Yu (2014) showed that combination of nitrate and sulfate (both at 0.84% DM) reduced the CH_4 production by 36% in in vitro rumen fermentation. The results indicate that the combination of nitrate and sulfate has greater effect on decreasing CH_4 production than sulfate or nitrate alone, suggesting a synergistic effect exists between sulfate and nitrate. Suitable combination ratios between nitrate and sulfate and adequate dosages of the mixture for effectively decreasing ruminal CH_4 production need to be investigated in the future.

4.5. Ruminal microorganisms

Drewnoski et al. (2012) reported that including DDGS containing high S (0.6% total dietary S) in the diet of steers increased the relative abundances of the ruminal SRB compared with low S DDGS (0.3%), whereasruminal methanogens were unaffected. Wu et al. (2015) reported that supplementing sulfate increased the relative abundances of the total bacteria and the SRB in vitro, but did not affect the total archaea and protozoa. Similarly, Patra and Yu (2014) reported that neither sulfate nor nitrate, alone or in combination, altered the abundance of the total bacteria or archaea in in vitro rumen fermentation. However, van Zijderveld et al. (2010) found that inclusion with sulfate in the diet of sheep tended to decrease the relative abundance of ruminal methanogens and increased that of ruminal bacteria and SRB, whereas it did not affect protozoa. The decrease in methanogens could be resulted from the negative effect of the end product H_2S produced from sulfate reduction (van Zijderveld et al., 2010). The results suggest increasing dietary S intake would increase the abundances of the ruminal SRB and consequently decrease the CH_4 production through the pathway of sulfate reduction.

5. Perspectives

Present results clearly indicate that ruminal SRB are highly competitive to methanogens for H_2. Therefore, enhancing the ruminal SRB activities can inhibit ruminal methanogenesis and decrease CH_4 production. Many S sources including sulfates, elemental S and S-containing compounds in DDGS are able to improve the ruminal SRB activities, whereas sulfates have better effect than other compounds.

The S requirements of growing beef cattle, dairy cattle and growing sheep are 0.15% DM (National Academies of Sciences Engineering and Medicine, 2016), 0.20% DM (National Research Council, 2001), and 0.18% to 0.26% DM (National Research Council, 1985), respectively. Although elevated dietary S can inhibit ruminal methanogenesis, excess S intake may have detrimental effects on DMI, average daily gain and health in cattle (Loneragan et al., 2001; Spears et al., 2011). It was reported that H_2S eructated from the rumen could be respired into the lungs and induce PEM at high S intake in cattle (Castro et al., 2021). Increasing the total dietary S from 0.13% to 0.46% by using $(NH_4)_2SO_4$ as the S source decreased the DMI and average daily gain of steers by 17.5% and 20%, respectively (Spears et al., 2011). The decreased DMI associated with high dietary S intake could be resulted from the increased H_2S production in the rumen (Uwituze et al., 2011). The maximum tolerable dietary S level is recommended to be 0.4% DM (National Academies of Sciences, Engineering, and Medicine, 2016). A recent study showed that adding Na_2SO_4 at 0.86%DM to achieve 0.39% DM of dietary S did not affect the DMI, nutrient digestion and health in feedlot cattle (Castro et al., 2021). It should be noted that high dietary S intake may increase the S excretion in feces and urine in cattle, and have negative effects on the environment (Hansen et al., 2016) by odorous S compounds emitted from excreta (Andriamanohiarisoamanana et al., 2015). Further studies are required to investigate the effects of different S sources and the associative effects between sulfate and nitrate on decreasing ruminal CH_4 production, and other approaches that can enhance the ruminal SRB activities in ruminants.

Author contributions

Yuchao Zhao: Writing and original draft preparation. **Guangyong Zhao**: Writing, reviewing, editing, and supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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