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Enteric methane mitigation strategies in ruminants: a review¤

Estrategias de mitigación de metano enterico en rumiantes: revision de literatura

Estratégias de mitigação de metano entérico em ruminantes: revisão de literatura

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Summary

Livestock farming in Latin America has been criticized because of its large greenhouse gas (GHG) production resulting from the use of degraded forage and low-efficiency production performance. Agriculture contributes a significant amount of the three main greenhouse gases: methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O). Methane has a global warming potential 25 times greater than CO_2. Enteric methane is an important greenhouse gas responsible for approximately 15% of global warming. The trend and legal obligation of mitigating greenhouse gas emissions will likely directly influence improved efficiency of livestock systems, including animal nutrition and handling. The development of mitigation strategies and the viability of their practical applications have been researched around the world. Various nutritional strategies to mitigate enteric methane have been studied and developed. All of them differ in terms of viability, cost, and acceptance by the producers. Their adoption should be based on the capacity to reduce methane emissions in association with economic viability and animal performance. Animal performance improvement will be achieved in production systems (mainly those related to efficient forage use) associated with good management of nutrition, health and reproduction. These are important strategies to consolidate Brazil as a food producer to the world, respecting the demands regarding land, water, biodiversity conservation and emission of greenhouse gases.

Keywords: *climate change, global warming, greenhouse gas, livestock, sustainability.*

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Resumen

La industria pecuaria latinoamericana ha sido criticada por la emisión significativa de gases con efecto invernadero (GHG). Dicha crítica se fundamenta en los bajos indicadores zootécnicos observados en los sistemas de producción animal basados en pasturas degradadas o que se encuentran por debajo de su potencial de producción. La industria agropecuaria contribuye de manera significativa con la emisión de los tres principales GHG: metano (CH_4), dióxido de carbono (CO_2) y óxido nitroso (NO_2). El gas metano tiene un potencial de calentamiento global 25 veces mayor que el de CO_2. El metano entérico es un importante gas de efecto invernadero, que es responsable de aproximadamente el 15% del calentamiento global. La tendencia o la obligación legal de mitigar las emisiones de GHG tendrá una influencia directa sobre la necesidad del aumento de la eficiencia zootécnica en los sistemas pecuarios relacionado con el manejo nutricional de los animales que deberá ser adoptado. El desarrollo de estrategias de mitigación y la viabilidad de su aplicación práctica representan áreas de investigación alrededor del mundo. Existen diversas estrategias nutricionales que se han estudiado y desarrollado con el fin de mitigar el metano entérico. Dichas estrategias presentan diferentes viabilidades, costos y posibilidades para que sean aceptadas por los productores. La elección de la estrategia de mitigación a ser adoptada deberá estar centrada en la capacidad de reducción de las emisiones de metano asociada con la viabilidad económica y el mantenimiento del desempeño animal. El aumento de los indicadores zootécnicos que se obtendrán en los sistemas de producción (principalmente aquellos que utilicen de manera eficiente el forraje) asociado a una buena nutrición, salud y manejo reproductivo, son estrategias importantes para la consolidación de Brasil como un importante productor de alimentos para el mundo, teniendo en cuenta las demandas relacionadas con el uso del suelo, del agua, la conservación de la biodiversidad y de la emisión de gases con efecto invernadero.

Palabras clave: *calentamiento global, cambios climáticos, ganadería, gases de efecto invernadero, sostenibilidad.*

Resumo

A pecuária da América Latina tem sido criticada por emitir quantidades significativas de gases de efeito estufa (GHG). Tal crítica tem sido fundamentada nos baixos índices zootécnicos verificados em sistemas de exploração animal baseados em pastagens degradadas ou que se encontram abaixo do seu potencial de produção. A agropecuária contribui de forma significativa com a emissão dos três principais GHG: metano (CH_4), dióxido de carbono (CO_2) e óxido nitroso (NO_2). O gás metano apresenta potencial de aquecimento global 25 vezes maior que o CO_2. O metano entérico é um importante gás de efeito estufa, que é responsável por aproximadamente 15% do aquecimento global. A tendência ou obrigação legal de mitigar as emissões de GHG influenciará diretamente a necessidade de aumento da eficiência zootécnica nos sistemas pecuários, atrelado ao manejo nutricional dos animais a ser adotado. O desenvolvimento de estratégias de mitigação e a viabilidade da aplicação prática dessas estratégias são áreas atuais de pesquisa em todo o mundo. Existem várias estratégias de nutrição para mitigar metano entérico que têm sido estudados e desenvolvidos. Todos estes têm diferentes viabilidades, custos e possibilidades de serem adotadas pelos produtores. A escolha de qual vai ser utilizado deve basear-se na capacidade de reduzir as emissões de metano associadas com viabilidade econômica e a manutenção do desempenho do animal. O aumento nos índices zootécnicos que serão obtidos em sistemas de produção (principalmente os relacionados ao uso de forragem eficiente) associada a uma boa nutrição, saúde e manejo reprodutivo são estratégias importantes para consolidar o Brasil como um importante produtor de alimentos para o mundo, respeitando as demandas relacionadas ao uso da terra, da água, da conservação da biodiversidade e da emissão de gases de efeito estufa.

Palavras chave: *aquecimento global, gases de efeito estufa, mudanças climáticas, pecuária, sustentabilidade.*

Introduction

Growth of global population and increased purchase power has promoted a rapid increase in the demand for food from animal sources. The world population will have reached 9 billion by 2050, while the demand for meat and milk products is expected to increase to 465 million tons and 1.043 million tons, respectively (FAO, 2006). Latin America has a prominent position as an animal protein provider for the world (FAO, 2010).

Despite the importance of agriculture in food production and revenue, there is a lot of discussion about the environmental impact of livestock and agricultural activities in relation to climate change. Latin American livestock industries have been criticized for their large greenhouse gas production as a result of using degraded forage with performance below production potential. The inefficiencies of these low-production meat and milk systems cause large amounts of greenhouse gas emissions (IPCC, 2007).

Agriculture contributes a significant amount of the three main greenhouse gases: methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O). Methane has a global warming potential 25 times greater than $CO_{2,}$ persists 9 to 15 years in the atmosphere, and increases 7.0% each year (IPCC, 2006). Atmospheric methane results from anaerobic fermentation of organic matter in wetland environments, rice fields cropped by flood irrigation, enteric fermentation, anaerobic treatment of animal residues, and biomass burning.

Cattle produce methane from enteric fermentation (85 to 90%) and fecal excretion. A total of 95% of rumen methane is excreted via eructation and from the intestines, 89% of methane produced is exhaled and around 1% excreted *via* the anus (Murray *et al.*, 1976). Methane from enteric fermentation represents 25% of methane anthropogenic emissions (Wuebbles and Hayhoe, 2002).

Beef cattle and sheep produce 107 to 300 g and 17.8 to 39.3 g CH_4/day, respectively (Czerkawski, 1969; Holter and Young, 1992; McAllister *et al.*, 1996), resulting in 39.1 to 109.5 kg and 6.5 to 14.4 kg annual emissions, respectively. India and Brazil are the highest emitters of enteric methane, with 14.5 and 10.3 (x 10^{12}) g/year, respectively. Brazil is the greatest emitter of beef cattle methane followed by India and the U.S. (9.6, 8.6, and 5.1 x10^{12} g/year, respectively; Thorpe, 2009). By 2005, agriculture was responsible for 22% of methane emissions in Brazil (MCT, 2009).

Enteric methane, responsible for 15% of global warming, is directly related with rumen fermentation efficiency because of the loss of carbon and consequent loss of energy, which affects animal performance (Cotton and Pielke, 1995; Bell *et al.*, 2011). It is important to understand methane synthesis mechanisms. The challenge is to develop diets and handling strategies to minimize methane production (CH_4/kg of milk, meat, or wool), increase production efficiency and decrease livestock contribution to global warming.

Beef cattle have been labelled as the greatest culprit of climate change, yet most of the criticism is not scientifically based. We need to develop and validate accurate methodologies to measure methane emissions and create specific databases for the production systems in each region (Lima *et al.*, 2006; Grainger *et al.*, 2007). Misguided media information regarding this issue could be used as an excuse to create non-tariff obstacles to exporting Brazilian livestock products.

Discussions on how to reduce greenhouse gas emissions have focused on production and supply chain modifications of food through significant changes in consumption patterns. Significant reductions in the consumption of food from animal sources have been proposed to decrease greenhouse emissions; however, the nutritional value of different foods needs to be considered to evaluate the impact of their production on the climate (Machado *et al.,* 2011).

NDCI unit (nutrient density/greenhouse emission) was proposed by Smedman *et al.* (2010) and is comprised of the ratio between nutritional density and climate impact by combining the nutrient density of food with the gas emissions from its production. The authors compared greenhouse gas emissions from the production of milk, soft drinks, orange juice, beer, wine, sparkling water, soy drinks and oat drinks. A total of 99 g of CO_2 were produced per 100 g of milk—much greater compared to the other drinks. However, when emissions were compared using NDCI values, milk had an advantage because of its high nutritional value (Table 1). This result represents a good argument to be presented in media discussions, which sometimes encourage reducing consumption of animal products to decrease the environmental impact associated with animal production.

Livestock production is likely to be increasingly affected by carbon emissions limits and environmental laws. The trend and legal obligation to mitigate greenhouse gas emissions is likely to have a

Table1. Nutritional density according with climate impact.

Food	Percentage of NNR in 100 g of product	Number of nutrients ≥ 5% of NNR	Nutritional density	Greenhouse gas emission	Ratio NDCI
Milk	126	9	53.8	99	0.54
Soft drink	7	0	0	109	0
Orange juice	90	4	17.2	61	0.28
Beer	18	0	0	101	0
Red wine	24	1	1.2	204	0.01
Sparkling water	2	0	0	10	0
Soy drink	53	3	7.6	30	0.25
Oat drink	32	1	1.5	21	0.07

NNR = Nordic Nutritional Recommendations; Nutritional density = Percentage of NNR per 100 g of product x number of nutrients ≥ 5% of NNR/21; Greenhouse Gas Emissions (g of CO_2 produced per 100 g of product); Ratio DNIC = Ratio of Nutritional Density to Climatic Impact (nutritional density/ greenhouse gas emissions). Source: Smedman *et al.* (2010).

direct influence on the efficiency of livestock systems, including animal nutrition and handling. Improvement of food practices can reduce methane emissions per kg of food intake or per kg of product (McAllister, 2011). Some alternatives to reduce methane production include specific agents and diet additives. Development of mitigation strategies and their viability have been researched around the world (Thornton, 2010).

Enteric methane production and its function in rumen ecosystem

Fermentation of diet components by rumen microbiota results in the production of short chain fatty acids (SCFAs)—an energy source for ruminants—and gases (CO_2 and CH_4) excreted via eructation (Martin *et al.*, 2009a). Rumen fermentation involves an oxidation process, generating reduced co-factors (NADH, NADPH, and FADH), which are then re-oxidized (NAD+, NADP and FAD+) by dehydrogenation reactions, releasing hydrogen in the rumen. As an electron acceptor process, methanogenesis removes hydrogen gas (H_2) from the rumen. Methane production is therefore essential for obtaining a high-performing rumen ecosystem because H_2 accumulation, which could inhibit dehydrogenase activity in re-oxidation co-factors, is avoided. An efficient H_2 capture in the rumen contributes to increase the rate of fermentation by the lack of its inhibitory effect on the microbial degradation of vegetative material (Wolin, 1979; McAllister and Newbold, 2008).

Enteric methane is derived from the activity of the methanogen *Archaea*, a microbial group distinct from eukaryotes (protozoa and fungi), bacteria with its own co-factors (coenzymes M, F420, and F430), and fat (isoprene-glycerol esters). Despite the central function of H_2 in the metabolism, methanogenesis is important to rumen function and animal nutrition although methanogens comprise only a small part of the rumen's microbial biomass (Janssen and Kirs, 2008). *Archaea* methanogens are responsible for methane production in ruminants. Therefore, considerable research efforts have been made to gather more information about them (Attwood *et al.*, 2008; Attwood *et al.*, 2011). Identification of their metabolic activities and diversity is required for developing strategies to mitigate enteric methane emissions. Sequencing of their genomes will provide important information to develop such strategies (Buddle *et al.*, 2010). Other microorganisms provide an appropriate environment to facilitate methanogen survival or produce substrates that would be available for methanogens. Metabolic pathway for H_2 production and interspecies relationships between methanogens and other microorganisms of the ruminal ecosystem should be considered in the strategies to control methane emission by ruminants. The H_2 produced by microbial fermentation is an energy source to *Archaea* methanogens for methane production. Formate can be used to produce methane by methanogens; however, it is a less important methane precursor than H_2 and is responsible for approximately 18% of the methane produced (Hungate *et al.*, 1970). Ruminal fermentation products are not equivalent in terms of H_2 production; their amount depends on short chain fatty acid (SCFA) concentration and the relative ratio between acetate, propionate and butyrate (Owens and Goetsch, 1988; Eun *et al.*, 2004; Martin *et al.*, 2009a). Quantitative mathematic models consider fermentation stoichiometric calculations to balance formation of H_2, SCFAs and other products for predicting methane production (Bannink *et al.*, 2006; Ellis *et al.*, 2008a).

Enteric methane and energy losses

At an energetic content of 55.22 MJ/kg (Brouwer, 1965), methane represents a significant amount of energy in a production system (Table 2). Approximately 5.5 to 6.5% of raw energy ingested is converted to methane (Johnson and Ward, 1996). However, measurements in respiratory chambers (indirect calorimetry) show greater methane emissions: from 2 to 12% of raw energy ingested (Johnson and Johnson, 1995). Generally, as digestibility increases variation in methane production also increases. According to Johnson and Johnson (1995), there are two causes of methane production variation: the amount of carbohydrates fermented in the rumen, and the ratio of propionate to acetate produced.

Table 2. Typical variations of methane emissions between three kinds of ruminants, energy losses as methane, and the estimation of how many annual grazing days are lost.

Animal class	Live weight average (kg)	CH_4 (kg/head/day)	CH_4 energy losses (MJ/head/day)[a]	Daily energy requirements (MJ/head/day)[b]	Grazing days lost per year[c]
Adult sheep	48	10-13	1.5-2.0	13	43-55
Steer	470	50-90	7.6-13.6	83	33-60
Dairy cow	550	91-146	13.6-22.1	203	25-40

[a] Assuming energy density of 55.22 MJ/kg of CH_4 (Brouwer, 1965).

[b] Standing Committee of Agriculture (1990).

[c] Days lost grazing annually = (loss of energy/ daily requirements) x 365.25.

Source: Eckard *et al.* (2010).

While evaluating methane production of steers fed forage-based diets or diets with 80% concentrate, Harper *et al.* (1999) observed that 8.1% and 2.1% of raw energy was lost as methane, respectively. According to Kaharabata *et al.* (2000), a dairy cow weighing 600 kg can produce 268 to 450 g CH_4 per day. This energy loss (13,344 kcal/g) would be enough to produce between 4.55 and 7.65 kg of milk containing 4% fat. Johnson *et al.* (1994) reported 256 L/day methane produced by steers (9.1% of raw energy ingested), 193.9 L/day by heifers (5.6% of raw energy), and 548.2 L/day (5.7% of raw energy) by lactating cows.

It is important to consider the enteric methane production per unit of animal product produced (kg of milk, meat, or wool). A balance can be established between the necessity of food produced for the growing population and the emission of greenhouse gases. A reduction of enteric methane production without compromising animal productivity is thus desired to mitigate greenhouse gas emissions and improve ruminant feed conversion efficiency.

Improving product quality through reduction of greenhouse gas emission levels can enhance efficiency of systems in Latin America. According to Barioni *et al.* (2007), increasing birth rate of cows from 55 to 68%, reducing slaughter age from 36 to 28 months, and reducing mortality from 7 to 4.5% in animals younger than one year of age could reduce methane emissions by 18% in relation to the equivalent level of carcass production in Brazil by 2025. This could be possible even with a 25.4% increase in meat production. This means that actions oriented to improve production efficiency would proportionally reduce methane emissions because more products (meat, milk, or wool) will be obtained using the same resources (Guimaraes Jr. *et al.*, 2010).

Yan *et al.* (2010) evaluated data from 20 energy metabolism studies in open flow respiratory chambers involving 579 lactating cows with varied genetic merit, lactation number, lactation phase, and live weight. The authors studied enteric methane emissions using energy, efficiency, and productivity. Results indicate that methane losses in relation to raw energy ingested or milk energy are negatively related to milk yield, metabolizable energy (q), and efficiency to utilize the metabolizable energy for lactation (K_l). Therefore, selection of highly producing lactating cows and a more efficient use of energy represent an effective methane-mitigation strategy.

Nutritional strategies to mitigate enteric methane

The H_2 produced in the rumen is critically important to the rumen ecosystem, mainly during the fermentation process. H_2 should remain reduced, allowing for the reoxidation of NADH, in order to degrade nutrients for SCFA production. In this methanogenesis process, H_2 manipulation in the rumen is the key to controlling methane emissions (Joblin, 1999).

According to Martin *et al.* (2009a), the metabolic pathways involved in H_2 production and use and methanogen populations should be considered in strategies to control methane emissions. Strategies need to focus on reducing H_2 production without spoiling digestion, stimulating H_2 use through alternative production pathways for ruminants, and/or *Archeaea* methanogenic inhibition (number and/or activity), associated with stimulation of pathways that consume H_2 to avoid the negative effects of increasing partial H_2 pressure in the rumen.

Diet composition and quality

Concentrate. Increasing the amount of concentrate in the diet reduces the proportion of dietary energy that is converted to methane (Blaxter and Clapperton, 1965). In other words, the addition of concentrate promotes the methane emission reduction as a proportion of ingested energy or expressed as per unit of animal product (milk and/or meat).

Fibrous carbohydrate substitution (cellulose and hemicellulose) for non-fibrous carbohydrates (starch and sugar) results in significant modifications in both the physiochemical conditions in the rumen and microbial populations. Increase of amylolytic bacteria results in a change in SCFA production, promoting a proportional increase of propionate and a reduction of acetate. Consequently, methane production is reduced because of low H_2 availability in the rumen.

However, according to Martin *et al.* (2009a), the low acetate to propionate ratio does not always occur when animals are fed concentrate-rich diets. In this situation, the reduction of methane emissions can be explained by the reduction in both pH and ciliate protozoa. The low rumen pH can inhibit growth and/or activity of methanogens and cellulolytic bacteria.

In high concentrate diets, the factors that induce methane reduction are: increasing propionate, which reduces H_2 in the rumen, methanogenic (Hegarty, 1999), cellulolytic bacteria (Brossard *et al.*, 2004), and ciliate protozoa inhibition via pH reduction. According to Clarke (1977) rumen ciliated protozoa are sensitive to pH changes and they cannot survive if pH increases above 7.8 or decreases below 5.0. Dehority (2005) reported death of *in vitro* protozoa at pH values below 5.4 and bacteriocin production by lactic bacteria, which inhibits methanogenic activity (Rodriguez and Campos, 2007).

Methane losses are relatively constant in diets with 30 to 40% concentrate (6 to 7% of raw energy ingested) while methane losses decrease rapidly to low values in diets containing 80 to 90% concentrate (2 to 3% of raw energy ingested; Lovett *et al.*, 2003; Beauchemin and McGinn, 2005; Martin *et al.*, 2007).

Berchielli *et al.* (2003) reported a quadratic relationship to methane production in beef cattle fed different dietary forage to concentrate ratios. According to the authors, the results suggest that concentrate addition in low amounts offers favorable conditions for microorganisms by providing energy to degrade fiber fractions in the rumen. However, when 60% concentrate is added to the diet the rumen environment becomes spoiled by microbial methanogenesis, evidenced by a lower rumen pH. Primavesi *et al.* (2004) also reported that substituting forage with concentrate results in the maximum methane emission when concentrate was added as 40% of DM.

Concentrate addition to reduce methane emission has economic and environmental limits. Possible metabolic consequences of diets rich in non-fibrous carbohydrates include ruminal acidosis, reduced milk fat and shorter productive life of animals. The economic viability of production systems with high levels of concentrate is questionable in climates more conducive to forage-based production, as in Brazil and other tropical countries.

In addition, the consequences of the increased energy density of diets should be analyzed. Greenhouse gas emissions, such as as CO_2 and nitrous oxide (N_2O), originating from grain production, harvesting and transportation can overcome the reduction of enteric methane emissions caused by its inclusion in ruminant diets. Johnson *et al.* (2002b) and Lovett *et al.* (2006) have reprted the flow of greenhouse gases in production systems.

Concentrate composition also influences methane production. Lovett *et al.* (2006) evaluated the effect of pasture supplementation with concentrate composed of fiber by-products (32.8% insoluble neutral detergent fiber–NDF) on enteric methane emissions. An increase of daily methane production (from 346 to 399 g/cow/day) was observed when concentrate was added (due to its high fiber and low starch levels). However, the authors observed a tendency for methane emission/kg of milk produced to reduce because concentrate promotes milk yield.

Forage. Methane emission (g/kg dry matter ingested) is influenced by the type of forage the animal has been ingesting. Usually, animals fed legumes have less methane emissions than animals fed grasses. According to Benchaar *et al.* (2001), the substitution of Timothy-grass hay *(Phleum pratense*) for lucerne *(Medicago sativa*) reduced methane emission by 21% (expressed as percentage of digestible energy). McCaughey *et al.* (1999) observed 10% reduction in methane production per unit of product in beef cattle at pasture, when a grass-exclusive diet was substituted for alfalfa and grass (ratio 70:30). The effect of legume use on methane emission can be explained by the presence of condensed tannins (Waghorn, 2007), different levels of fiber, increased ingestion of dry matter (DM) and consequent increased rate of passage in the rumen (O´Mara *et al.*, 2004).

There are many differences among the composition of carbohydrates in forage, which influence their methanogenic potential. C_4 grasses can produce more methane per kg of DM ingested than grasses with C_3 photosynthesis (Ulyatt *et al.*, 2002; Archimède *et al.*, 2011). Corroborating this, Primavesi *et al.* (2004) observed 121 to 147 kg CH_4/animal/year emissions in lactating cows under tropical conditions. These values were higher in comparison to those in North America (118 kg of CH_4/animal/year for animals weighing 600 kg, lactation of 6,700 kg of milk/year, and ingestion of 2.7% live weight of DM), and Eastern Europe (100kg of CH_4/animal/year for cows weighing 550 kg, lactation of 4200 kg of milk/year and ingestion of 2.5% live weight of DM; IPCC, 1995; Johnson and Ward, 1996). The authors attribute this difference to the lower quality of tropical forage compared to temperate forage, especially because of higher fiber content and lower digestibility. Archimède *et al.* (2001) reported that methane emissions (L/kg of DM ingested) were 17% higher when ruminants were fed C_4 grasses as compared to C_3 grasses.

Another factor that lowers methane production by lactating cows grazing on temperate pastures is grain inclusion higher than 50%. The percentage of CH_4 produced in relation to raw energy ingested ranges from 5.5 to 6.5% in North America and Eastern Europe (United States, 1990). Primavesi *et al.* (2004) obtained 8.3%, and 10.6% in crossbred Dutch lactating cows kept in fertilized tobiatã grass pastures *(Panicum maximum* cv. Tobiatã*)* and brachiaria *(Brachiaria decumbenses* Stapf.*),* respectively.

Conservation methods and forage processing should also be considered. According to Beauchemin *et al.* (2008), methanogenesis tends to be lower in silage compared to hay and lower in finely-ground feed or pellets compared to roughly picked feed. Forage milling and pelleting reduce methanogenesis (methane production decreases 20 to 40% per unit of diet; Blaxter, 1989) due to increased passage rate. However, these effects are not apparent when feed consumption is restricted. Ammoniation or protein supplementation of low quality forage increases methane losses in proportion to digestibility, although methane production per unit of product is reduced (Johnson and Johnson, 1995).

Handling practices that improve forage quality increase animal performance and productivity per unit of area. Associated with performance increments, an increase in methane emission is expected as a result of greater ruminal fermentation. However, the amount of methane per unit of product (milk or meat) is reduced when animal production or growth increases.

Wins *et al.* (2010) evaluated the effects of two DM levels in pre-pasture forage (low: 1000 kg/ha and high: 2200 kg/ha) on methane emissions, voluntary DM intake (VDMI), and milk yield of cows. Methane emissions were measured in two experiments through a SF_6-tracer technique. The authors concluded that low mass of pre-pasture forage improved the pasture nutritional quality and consequently reduced methane emissions (g/day; g/kg of milk; g/kg of milk solids and g/kg of DM ingested). These results are in agreement with Blaxter and Clapperton (1965) who observed that CH_4 decreases while digestibility increases with higher intakes (two or three times the maintenance level). Despite intake being the most important factor in methane production, Wins *et al.* (2010) showed that other factors are involved in methane emissions.

Robertson and Waghorn (2002) observed that methane production per lactating cow increases as forage matures (5 and 6.5% of raw energy ingested for spring and summer, respectively). The relatively low methane emissions observed for young forage can be explained by higher levels of soluble carbohydrate and linoleic acid. Hegarty (2001) analyzed the effect of nutritional improvement of pasture on methane production in Merino, sheep, finding that the proportion of ingested energy losses in the form of methane decreased from 6.6 to 6.0% with increasing forage digestibility.

Therefore, implementation of adequate pasture handling increases feed quantity and quality and is a suitable strategy to mitigate enteric methane by increasing energy efficiency, reducing livestock's impact on the environment, and improving feed efficiency and profit (Chaves *et al.*, 2006).

Lipid addition. Dietary supplementation with non-protected lipids reduces methane emissions through multiple mechanisms: reduction of fermentable organic matter (lipids are not a source of energy for rumen bacteria); reduction of methanogenic activity due to the presence of medium-chain fatty acids; toxic effects on cellulolytic bacteria (Nagajara *et al.*, 1997) and protozoa (Doreau and Ferlay, 1995) due to the effect of polyunsaturated fatty acids (PUFAs) and biohydrogenation of PUFAs.

Toxic effects of long chain fatty acids occur through their action on cell membranes, particularly gram-positive bacteria. Linoleic acid is toxic to cellulolytic bacteria *(F. succinogenes, R. albus,* and *R. flavelasciens)* because it affects cell integrity and fungus *Neocallimastix frontalis* growth (Maia *et al.*, 2007). Changes in rumen microbial populations favor propionate production, increasing H_2 captured in the process.

Despite PUFAs biohydrogenation resulting in H_2 capture, they have little influence on methanogenesis because a complete hydrogenation of 1 mol of linolenic acid prevents the production of 0.75 mol of CH_4 (Martin *et al.*, 2009). The use of metabolic hydrogen during unsaturated fatty acids biohydrogenation is lower (1%) compared to the reduction of CO_2 (48%), SCFAs synthesis (33%), and bacterial cell synthesis (12%; Czerkawski, 1986).

The effectiveness of lipid addition on reducing methane emissions depends on supplementation level, lipid source, method of supply (e.g. refined oil, oil seeds) and diet type (Beauchemin *et al.*, 2008).

Despite the possibility of a methane reduction greater than 40% when high levels of lipids are added (Machmuller and Kreuzer, 1999; Jordan *et al.,* 2006b), a reduction from 10 to 25% is more likely to be obtained (Beauchemin *et al.,* 2008). It is recommended that lipid supplementation does not exceed 6 to 7% DM to avoid the decrease of VDMI. Multiple action of lipid supplementation can affect the number and activity of rumen microbes, which can compromise digestion when toxic effects promote H_2 accumulation.

Beauchemin *et al.* (2008) revised 17 studies on beef cattle and sheep and established a connection between levels of lipid addition (% of VDMI) and methane emission (g/kg of DM intake) in different oil and fat sources. Methane emissions would be reduced by 5.6% per 1% lipid addition. The authors found considerable variation among lipid sources on methanogenesis. A sharp methane decline was observed (g/kg of DM intake) in studies with coconut oil (63.8% reduction with 7% addition; Machmuller and Kreuzer, 1999) and myristic acid (58.3% reduction with 5% addition; Machmuller *et al.*, 2003).

Martin *et al.* (2009a) summarized *in vivo* studies (67 diets supplemented with lipids, from 28 publications) evaluating the effect of lipid sources on methane emissions from beef cattle and sheep. The results were 3.8% methane reduction (g/kg of DM ingested) per 1% fat added to the diet (% of VDMI).

It is evident that fatty acid effects on methanogenesis depend on their chemical nature. Lipid supplements rich in medium chain fatty acids (12 to 14 carbons) such as coconut, palm or canola oils (rich in lauric acid), or purified myristic acid, are more effective in depressing methane emissions in diets rich in concentrate and low in Ca (Machmuller *et al.*, 2003). According to Dohme *et al.* (2001), lauric acid (C 12:0), and myristic acid (C 14:0) showed similar effects when provided separately, but when combined they promoted a synergistic sharp-reducing effect on methane emissions (Soliva *et al.*, 2004).

Few studies have evaluated the effects of monounsaturated fatty acids (such as oleic acid in canola), and saturated fatty acids (SFA, such as palmitic and stearic in tallow) on rumen methanogenesis. A 30% decline in methane production was observed when 12% tallow was added to the diet of lactating dairy cows (Van der Honing *et al.*, 1983). However, this effect was not observed in other cow (Johnson *et al.*, 2002a; Woodward *et al.*, 2006) and sheep studies (Cosgrove *et al.*, 2008).

The mechanism of action of saturated fatty acids has been related to their ability to damage cell membranes, leading to K+ leakage—an indicator of damaged membrane—followed by cell death. Among SFA, the most toxic to bacteria membrane is lauric acid (C 12:0), followed by myristic (C 14:0) acid, and both are used as antibacterial agents (Zhou *et al,* 2013). The aforementioned authors tested the effects of SFA on methanogenesis and *Methanobrevibacter ruminantium* viability and observed higher toxicity for lauric and myristic acids, which caused a greater decrease on methane production.

Grainger *et al.* (2010b) evaluated methanogenesis when cottonseed was added to dairy cow diets over 12 weeks. They observed a lasting methane emissions reduction (mean 3.5 g CH_4/kg of DM ingested) over 12 weeks as a result of cottonseed addition (2.61 kg of DM/cow/day). This effect increased from 5.1% in the first week to 14.5% in the twelfth week.

A compulsory inclusion of 5% biofuel to diesel fuel since 2010 is driving Brazilian agriculture to adapt oil seed production for non-food purposes. Some options of raw materials have been studied (soybean, castor bean, cotton, jatropha, palm kernel, licuri palm, babassu palm, macauba palm, radish, peanut, sunflower, canola, and coconut). Consequently, many byproducts have been produced (milled meals, pressed meals, and glycerin) and there is increased availability of a variety of oils used in biodiesel production, which have potential for ruminant diets, possibly contributing to mitigating enteric methane.

Quantifying methane-mitigation potential by using biodiesel byproducts is important because the benefits of byproduct and oil inclusions in ruminant diets can be combined with the benefits of biodiesel as energy source (reduction of CO_2 emission) and thereby contribute to consolidate Brazil as a global reference in biofuels.

Additives. Another strategy to mitigate enteric methane is the use of additives. Ruminal ecosystem manipulation is an important tool used by nutritionists to increase feed conversion efficiency and animal performance. In the past, research was focused on antimicrobial use (e.g. monensin). However, the growing societal pressure against the use of this additive in animal feed has encouraged the search for other alternative methods to manipulate the rumen environment.

The ionophores are anti-methanogenic effects of ionophores are more related to the inhibition of methane precursors (formate and H_2) than a direct effect on methanogen populations because methanogens are more resistant to ionophores than H_2-producing bacteria. Reduction of methane precursors would be responsible for 45% of ionophore's effect on methane production while the remainder would be a consequence of decreased feed intake (Nagajara *et al.*, 1997). The decline of methane as an effect of ionophores can be associated with growth inhibition of ciliate protozoa that produce H_2 and are colonized by methanogens (McAllister *et al.*, 1996).

Johnson and Johnson (1995) revised ionophore additions to grain-based and forage-based diets finding a great variation in rumen methanogenesis reduction (4 to 31%). They concluded that any effect is short-lasting and methane returns to normal levels after two weeks. Methane reduction was most likely associated with decreased DM rather than a direct effect on methanogenesis. Monensin effect on methane reduction is dose-dependent. Studies revised by Beauchemin *et al.* (2008) showed that doses lower than 15 ppm have no effect on methanogenesis (g CH_4/day or g CH_4/kg DM ingested) in dairy cows. Higher doses (24 to 35 ppm) reduced methane production (between 4 and 10% g/day; and 3 to 8% g/kg DM ingested) by beef and dairy cattle (Sauer *et al.,* 1998; McGinn *et al.,* 2004; Van Vugt *et al.,* 2005; Odongo *et al.,* 2007). A 30% methane reduction was reported when 33 ppm monensin was included in low or high forage diets (Guan *et al.,* 2006).

Grainger *et al.* (2010a) evaluated the use of a higher monensin dose (471 mg/day) in cows fed on ryegrass pasture supplemented with 4 kg/day of barley grain. Methane emissions were estimated in pasture animals using both SF_6-tracer gas technique and respiration chambers. In both conditions, the monensin addition did not increase milk production and did not promote any effects on enteric methane emissions (g/day, g/kg of milk and g/kg of DM ingested). The authors concluded that monensin does not represent a viable strategy to mitigate methane emissions from dairy cows when they are fed concentrate-supplemented pasture.

The possible transitory effects of ionophores associated with the growing pressure to decrease antimicrobial use in animal production suggest that this strategy of methane mitigation does not represent a lasting solution for the problem.

Organic acids (malate and fumarate) represent an alternative to antimicrobial use in ruminant nutrition. These substances can stimulate lactate capture by *Selenomonas ruminantium* bacteria (Martin and Park, 1996) and act as a buffer to prevent rumen acidosis when the diet is high in energy-rich concentrate. In addition, organic acid supplements, which are direct precursors of propionate, demonstrate a positive dose-dependent effect on methanogenesis reduction (Asanuma *et al.*, 1999; O´Mara, 2004).

Commercial use of organic acids is limited for ruminants because of their cost. Considering this, forage can be provided as a source of dicarboxylic acid. Intermediate components of the tricarboxylic acid cycle accumulate in plant tissue. However, according to O'Mara (2004), there is a great variation in accumulation (0.6 to 7.5% of DM). Callaway *et al.* (1997) conducted a study to determine malate concentrations present in hay of five alfalfa varieties at different maturity stages. At more mature stages, malate concentration was reduced from 6.5 to 7.0% in young-harvested alfalfa and 2.9 to 4.5% when it was harvested later. Martin (1998) suggested that high levels of malate in fresh forage at initial stages of growth, especially in alfalfa, can promote significant changes in rumen microbial fermentation.

There is growing interest in the use of plant secondary compounds (plant extracts) to mitigate methane since this natural alternative avoids the use of chemical additives. Some plants produce secondary metabolites to protect them from fungi, bacteria, insects and herbivores. The effects of these molecules on rumen methanogenesis are highly variable. Most studies have focused on tannins, saponins, and essential oils. When high levels of these substances are ingested, adverse effects on animal performance and health can occur, but in low concentrations they can improve rumen fermentation (Morais *et al.*, 2006; Beauchemin *et al.*, 2008).

Tannins are polyphenolic substances with varied molecular weight and complexity, and are classified as either hydrolysable or condensed. Antimethanogenic activity of tannins found in plants has been associated with condensed tannins.

Dairy cows presented lower methane emissions when fed *Lotus corniculatus* (26.9 g CH_4/kg of DM ingested and 378 g CH_4/kg of milk solids) compared to ryegrass silage-fed cows (35.23 g CH_4/kg of DM ingested and 434 g CH_4/kg of milk solids) (Woodward *et al.*, 2001). Oliveira *et al.* (2006) did not observe any effect on methanogenesis when low and high tannin levels in sorghum silage diets were fed to beef cattle.

Saponins in *Brachiaria decumbes* and alfalfa *(Medicago sativa)* are glucosides with a direct effect on rumen microorganisms. Saponins reduce protein degradation and simultaneously favor protein synthesis and microbial biomass synthesis; both processes result in reduced H_2 availability to methanogenesis (Martin *et al.*, 2009a). The main antimethanogenic mechanism of saponins is related to its toxic effects on ciliate protozoa. This compound emulsifies the lipid cell membranes of protozoa, altering permeability and consequently causes cell death (Wallace *et al.,* 2002).

Hess *et al.* (2004) observed a 54% decrease in protozoa numbers and 20% reduction in *in vitro* methane production when saponins were used in high levels (12 mg/g of DM). Guo *et al.* (2008) observed methanogenesis reductions of 8% and protozoa reductions of 50% when saponins were used *in vitro*. The authors reported a decline in methanogenic activity (76%), measured through mcrA (methyl coenzyme-M reductase) gene expression with no effect on methanogen numbers.

Essential oils are secondary metabolites responsible for the smell and color of some plants. Some molecules present in essential oils have antimicrobial activities that act on gram-positive and gram-negative bacteria. Among the essential oils studied, garlic oil *(Allium sativa)* extracted through vaporization and distillation showed some effect on *in vitro* methanogenesis. Busquet *et al.* (2005) evaluated the effect of garlic oil and four of its components (diallyl sulphide, diallyl disulphide, allyl mercaptan, and allicin) on *in vitro* ruminal fermentation. Methane production after 17 hours of fermentation was reduced significantly by garlic oil, allyl mercaptam, and diallyl disulfide. McAllister *et al.* (2008) studied a commercially available allicin product, finding no effect on daily SCFAs or ammonia (N-NH3) production at levels of 0, 2 and 20 µg/mL. However, at 20 µg/mL, methane production was reduced significantly; this can be related to the reduction in methanogen populations in relation to total bacteria.

Watabane *et al.* (2010) evaluated cashew nut shell liquid (CNSL). CNSL contains phenolic compounds (e.g. anarcadic acid) that selectively inhibit Gram-positive bacteria. The authors carried out *in vitro* experiments using a concentrate-rich diet (30:70 forage to concentrate) to evaluate different doses of raw and thermal processed CNSL. Results indicated that raw CNSL could be used for rumen manipulation, increasing propionate production and reducing methane emissions.

Mitigation strategies via alternative pathways to use H_2

Redirection of H_2 towards processes that produce beneficial products to ruminants is another strategy to mitigate methane. Some examples of these processes are the addition of substrate, which can stimulate propionate production and the attempts to insert bacteria, which express reductive acetogenesis in the rumen. These processes increase propionate and acetate production, respectively, as well as reduce H_2 availability for methanogenesis (Van Zijderveld *et al.*, 2010). Nitrate and/or sulphate salts have also been evaluated because they provide an alternative pathway for H_2 use.

Acetogenic probiotics. In hindgut fermentation species (such as humans, hamsters, rabbits and rats), reductive acetogenesis is a natural mechanism to use H_2 in the gastrointestinal tract. It is known that acetogenesis occurs in the rumen, but the hydrogenotrophic capacity and environmental significance are not well understood.

Eubacterium limosum was the first acetogenic microorganism discovered in the rumen. It was isolated in sheep fed a molasses-based diet (Gethner *et al.*, 1981). It demonstrated an ability to grow in a medium with CO_2 and H_2 and produce acetate. Due to the difficulty of isolating acetogenic bacteria, it was concluded that these microorganisms were foreign to the rumen and acetogenesis was not considered a relevant ruminal process. However, with the increasing discussion about the influence of methane on global warming, the acetogenesis process is starting to be considered as a potential methane mitigation strategy.

In addition, acetate (final product of the reaction) has an advantageous characteristic because it is an additional source of energy to the host animal. However, when comparing acetogenesis and methanogenesis as competitors for reduction equivalents in the rumen, acetogenesis is less efficient than methanogenesis because it requires a higher concentration of H_2 to reduce CO_2 to acetate than methanogens need to reduce CO_2 to CH_4. The latter reaction is thermodynamically favorable (Weimer, 1998).

Recent studies indicate that ruminants have at least a small population of acetogenic bacteria, the density of which is influenced by the diet. Acetogenic presence in the rumen is a defense mechanism to avoid H_2 accumulation when methanogenesis is inhibited; therefore, these microorganisms do not compete with methanogens (Hegarty, 2001). Acetogenic bacteria are present in high numbers when methanogenesis is not established in newborn calves (Morvan *et al.*, 1994) and when cattle are fed on low forage : concentrate diets (Leedle and Greening, 1988). Recent isolation of new gastrointestinal bacteria species using H_2 (Klieve e Joblin, 2007) represents a new perspective for this mitigation strategy.

Nitrate and sulphate salts. The use of nitrate as an alternative to H_2 is not recommended because of the toxic effects of nitrite, an intermediate compound from the reduction of nitrate to ammonia. Nitrate to nitrite reduction (ΔG_T = -130 kJ/mol H_2) and subsequent reduction from nitrite to ammonia (ΔG_T = -124 kJ/mol H_2) releases more energy than reduction from CO_2 to CH_4 (ΔG_T = -16.9 kJ/mol H_2; Ungerfeld and Kohn, 2006). This process could be the main pathway to eliminate H_2 if sufficient nitrate was available in the rumen. Reduction of nitrate to ammonia consumes eight electrons and each mol of reduced nitrate can reduce 1 mol of methane. The ammonia produced could be available to other anabolic processes and would be an important source of fermentable N in diets deficient in crude protein, where lower concentrations of ammonia in the rumen limit microbial protein synthesis (van Zijderveld *et al.*, 2010).

For animals not adapted to the use of nitrate in the diet, the ability of rumen microorganisms to reduce nitrate to nitrite is greater than the ability to reduce nitrite to ammonia. This nitrate compound is absorbed in the ruminal epithelium and promotes the conversion of blood hemoglobin from ferrous form (Fe^{2+}) to ferric form (Fe^{3+}), which inhibits hemoglobin's ability to carry O_2 to tissues (methemoglobin), resulting in general anoxia, decreasing animal performance, and in severe cases, leading to fatality (Ozmen *et al.*, 2005). Supplementation with sulphur or cysteine can decrease nitrite accumulation in the rumen because sulphate is a reducer (ΔG_T = - 21.1 kJ/mol of H_2) that competes for electrons, which decrease methane production (Ungerfiel and Kohn, 2006).

Van Zijderveld *et al.* (2010) evaluated the effects of nitrate and sulphate addition on methane emissions with sheep diets (2.6% DM) in respiratory chambers. Methane production was reduced while the supplements were used (nitrate: 32% decrease; sulphate: 16% decrease; nitrate and sulphate: 47% decrease). The reduction in methane emissions due to nitrate use was more pronounced when it was used after feeding, while the sulphate effect was observed throughout the day. The authors concluded that when these compounds were provided in a safe way, nitrate and sulphate salts are potential agents to mitigate enteric methane.

Vaccination against rumen methanogens. The efficiency of vaccination depends on the connection between saliva antibodies and the methanogen surface resulting in their inactivation or removal. Therefore the vaccine's primary targets in the methanogens are surface proteins or proteins associated with membranes (Buddle *et al.*, 2010). This strategy involves vaccination of animals to induce production of saliva antibodies that are released in the rumen to neutralize methanogen effects or reduce methane emissions.

Cook *et al.* (2008) utilized a passive immunization technique using chicken egg yolk as a quick, economical and non-invasive source of antibody production (IgY) from a bird vaccine prepared from integral cells of three ruminal methanogen strains. The authors observed that the addition of high levels of bird antibody (IgY) reduced methane production in cultured rumen liquid *in vitro*. However, these results were not permanent; this was attributed to either the possible instability of antibodies in rumen liquid or to the presence of methanogens not grown in a prepared bird vaccine and therefore unaffected by IgY antibodies.

A large amount of rumen methanogens cannot be cultivated in a laboratory (Wright *et al.*, 2006); therefore, it is possible that these non-cultivable bacteria, of which there are no antibodies developed for, may grow to replace the methanogens that have developed antibodies (McAllister *et al.*, 2008). Methanogen diversity in the rumen can be influenced by diet and geographic location (Wright *et al.*, 2007). There is a challenge to develop a vaccine with a vast action spectrum against methanogens that can be effective in different conditions and regions.

Wright *et al.* (2004) evaluated sheep immunization using prepared integral cells of three methanogens and observed 7.7% reduction in methane emissions. However, when the study was repeated using five methanogens the vaccine did not promote immunization, although a change occurred in rumen microbial fauna (Williams *et al.*, 2009). These results emphasize the difficulty in producing an effective vaccine that can reduce enteric methane emissions using prepared methanogen cells (Buddle *et al.*, 2010).

The development of a recombinant vaccine against cell surface proteins existing in several species of methanogens can improve the effectiveness of vaccination as a method to mitigate enteric methane (McAllister *et al.*, 2008). Buddle *et al.* (2010) proposed the development of vaccines against proteins essential to the growth of methanogens and/or methanogenesis, with cross-reactions to other species through the genetic sequence information of *M. ruminantium*.

Bacteriophages and bacteriocins. Biological control strategies, such as the use of bacteriophages and bacteriocins, can be effective to directly inhibit *Archaea* methanogens and redirect H_2 to reductive rumen bacteria that may be propiogenic or acetogenic (McAllister *et al.*, 2008).

Bacteriophages are present in all biological ecosystems and have the ability to penetrate and consequently cause lysis in the host cell. This effect of bacteriophages and their genes can be a potential strategy to mitigate methane (Buddle *et al.*, 2010). Only six *Archaea* bacteriophages have currently been genetically sequenced and described and only two are methanogenic bacteriophages: *Methanobacterium* phages psi M1 and M2, and *Methanothermobacter* phage psi M100 (Pfister *et al.*, 1998; Luo *et al.*, 2001). The quick adaptation of microorganisms to bacteriophages challenges the use of this strategy and as a result, bacteriophages have to be identified, sequenced and characterized (Buddle *et al.*, 2010). Bacteriophages are host-specific, which is another limiting factor for using this strategy to reduce methane due to the high number of methanogen species in the rumen (Janssen and Kirs, 2008; McAllister *et al.*, 2008).

Bacteriocins, bactericidal peptides produced by bacteria, could also be used (McAllister *et al.*, 2008). However, there is scarce information on their effects on methanogenesis. Nisine, a bactoriocin produced by *Lactococcus lactis,* has been studied as a tool for mitigating methane. Sar *et al.* (2005) evaluated the effects of different concentrations of nisine on methane production *in vitro* in a continuous culture system. As its concentration increased from 5 to 30 µmol/L, methane production was reduced from 14 to 40%. Cattle HC5 bacteriocin, produced by *Streptococcus bovis,* inhibited *in vitro* methanogenesis up to 50% (Lee *et al.*, 2002).

Identification of stable bacteriocins in the rumen environment and specific to methanogenic bacteria is an area for future research. *In vivo* studies are necessary to establish the lasting adaptability and effectiveness to use bacteriocins as a feed additive (Boadi *et al.*, 2004; McAliister *et al.*, 2008).

Pasture handling and crop livestock systems

The majority of enteric methane emissions in Brazil come from extensive cattle-farming systems

(Lima, 2002) and grazing on degraded pastures. This scenario generates inefficient production processes that cause more methane production per unit of animal product (Guimares Jr. *et al.,* 2010). Among the alternatives to mitigate greenhouse gas emissions from livestock enterprises is to use forage with higher nutritional value, associated with adequate pasture handling (DeRamus *et al.*, 2003; Lassey, 2007).

Investing in recuperating degraded pasture is another potential strategy. According to a report from FAO (2006), pasture (native and cultivated) represents the second largest source of global potential carbon (C) capture, draining 1.7 billion tons per year from the atmosphere. This is second only to forest capture, which can drain 2 billion tons of C per year. Adequate pasture handling for improving soil fertility can help accumulate soil C by a ratio of 0.3 T of C/ha/year (IPCC, 2000) and mitigate 1.1 T of equivalent CO_2/ha/year. This would be enough to offset approximately 80% of the annual methane emission from one beef cattle unit estimated at 57 kg (IPCC, 1996), which is equivalent to 1.42 T of CO_2 (57 kg x 25 CH_4/year global warming potential of the gas = 1.42 T of equivalent CO_2). Productive and well-handled forage can therefore provide favourable conditions to significantly increase animal performance and absorb large amounts of carbon emitted from livestock, becoming an important component in the balance of greenhouse gases (Guimaraes Jr. *et al.*, 2010).

Well-managed foraging areas can be important sites for carbon accumulation and support stocking rates of 1 to 3 animal units per ha. Recuperation of degraded areas is an option for improving animal production and to retain chemical and physical traits of the land, while it simultaneously increases carbon stock (Boddey *et al.*, 2001).

Crop-livestock integration has been recognized as an alternative to reduce greenhouse gas emissions from agriculture. The Brazilian Government added the crop-livestock integration technology to the proposal presented at the 15th Conference of the Parties (COP 15) by the Intergovernmental Panel on Climate Change as a mitigation activity that can be applied nationally to reduce greenhouse gas emissions. The Government committed to implement this technology on 4 million hectares, expecting to reduce between 18 and 22 million T of equivalent CO_2. It is therefore expected that the incentive to use this technology in Brazil in the coming years will grow through public development policies (Guimaraes Jr. *et al.*, 2010).

Methodologies to evaluate enteric methane emissions

Before using mitigation strategies, it is necessary to have enteric methane emissions measured accurately to determine emissions from each management technique and to prepare national inventories.

Different techniques have been developed to quantify methane emissions. Validation and application in different production systems gives credibility to activities related to national inventories of greenhouse gas emissions from livestock and to develop public policies towards tending to global demands of reducing the environmental impacts of agriculture.

Methane emissions can be measured with *in vivo* and *in vitro* methodologies (McAllister, 2011). The use of experimental animals represents high costs. Consequently, *in vitro* methodologies are the primary option to evaluate methane reduction or inhibition. *In vitro* techniques are less costly and allow for rapid screening of diets and their combinations to evaluate the effects of a wide range of additives and feed ingredients on methanogenesis (Makkar and Vercoe, 2007). Diet additives and inhibitors able to reduce methane *in vitro* can later be evaluated *in vivo* with increased costs and details, addressing more practical feeding situations.

The *in vivo* reference method (gold standard) to quantify enteric methane production involves the use of respiratory chambers and gas collection (Rodriguez *et al.*, 2007). McAllister (2011) indicated respiratory chambers are the reference method to compare methane-mitigation agents.

Respiratory chambers require costly investments and labor, restrict animal movement, and can only evaluate a limited number of animals. Descriptions about the conventional system of open flow respirometry can be found in studies by Yong *et* *al.* (1975), Bryant *et al.* (1977), McLean and Tobin (1987), and Miller and Koes (1988); modern systems are described by Grainger *et al.* (2007), Odongo *et al.* (2007), and Rodríguez *et al.* (2007).

Methane emissions can be measured by inserting indicators in the rumen, such as the sulphur hexafluoride (SF_6) tracer gas methodology (Johnson *et al.*, 1994), which has been adopted as a standard method for grazing animals.

Tracer-SF_6 gas technique has been used to measure methane emissions in grazing animals (Johnson *et al.*, 1994; Lassey *et al.*, 1997; Woodward *et al.*, 2006). A small permeation tube with SF_6 of a known release rate is inserted in the rumen. Expired air is sampled through a stainless steel capillary tube (adapted to halter) connected to a vacuum yoke (built of high-resistance PVC pipe), which is connected to a metal valve with a sampling septum and a quick coupling. The CH_4 and SF_6 concentrations are determined by gas chromatography. Methane flow emissions can be calculated from the release ratio of SF_6 in the rumen and the concentration of CH_4 and SF_6 in the sample (Johnson and Johnson, 1995; USEPA, 2000). This technique does not require animals to be caged, allowing them to move and graze (Johnson *et al.*, 2007).

Pinares-Patiño *et al.* (2011) reported that the tracer gas methodology presents larger variability when compared with respiratory chambers; therefore more animals will be necessary to detect differences among treatments.

At IPCC (2006), specific information from each country was reported; the models used to predict enteric methane emissions included data such as diet composition, enteric fermentation product composition, seasonality, characterization of animal population, feed quality and availability, and methane mitigation strategies. Enteric methane emission measurements are necessary to complete these documents. National and international inventories of greenhouse gases are based on mathematical models. Mechanistic models and regression models allow for the analysis of causes and variations in methane production (Ellis *et al.*, 2008a). Multiple regression equations have been reported in the literature (Kriss, 1930; Axelsson, 1949; Blaxter and Clapperton, 1965; Moe and Tyrrel, 1979; Mills *et al.*, 2003; Ellis *et al.*, 2007; Ellis *et al.*, 2008a). The optimal equation to predict methane production will depend upon which diet will be used and whether the equation considers the variates for each specific situation (Ellis *et al.*, 2008a). Modeling has been applied to methane emission studies and is an important tool in developing greenhouse gas inventories and mitigation strategies.

Final considerations

Ruminant methane emissions are a consequence of gastrointestinal fermentation processes, which allow animals to transform cellulose-rich roughage into milk and meat. A survey of methane emission potential of agriculture systems and evaluation of mitigation strategies should be holistic, considering carbon dynamics and balance in the entire production system.

Several nutritional strategies have been studied and developed to mitigate enteric methane. They have different viability and cost. The choice of which one to adopt should be based on its capacity to reduce methane emissions associated with economical viability and animal performance.

Improving production parameters related to efficient forage-use and associated with good nutritional, health and reproductive management is an important strategy to consolidate tropical countries as food producers for the world, attending the demands related to land, water, biodiversity conservation, and greenhouse gases emissions.

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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