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Methane Production in Dairy Cows, Inhibition, Measurement, and Predicting Models

Mohammad Ramin, Juana C. Chagas, and Sophie J. Krizsan

Abstract

Methane is a potent greenhouse gas that is produced in many sectors. Agriculture and, more specifically, livestock contribute to this phenomenon. Methane is produced as a result of fermentation in the rumen of dairy cows with a significant amount of gas being released in the atmosphere via the mouth of ruminants. The total intake is the main factor influencing methane production followed by digestibility, fat, and the amount of fibre in the diet. Many strategies exist to reduce methane emissions such as chemicals, essential oils, and the red macroalgae in the diet of dairy cows. The majority of these strategies are either expensive or not feasible to use in a long-term period of time since the microbes in the rumen will adapt to this change. There is a wide range of methods and tools to measure methane emissions both in vitro and in vivo. The respiration chamber is the golden method to measure and quantify the fluxes (methane emissions) in dairy cows. In some cases where measurements of methane are impossible, vitro techniques together with modelling approaches could be used to predict methane emissions. Empirical and mechanistic modelling is a technique widely used to predict methane emissions. In this case by knowing some feed and animal characteristics methane could be reliably estimated.

Keywords

Methane · Livestock · Fermentation · Rumen · In vitro and In vivo · Respiration chamber · Empirical and mechanistic modelling

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M. Ahmed (ed.), *Systems Modeling*, https://doi.org/10.1007/978-981-15-4728-7_10

10.1 Methane Gas

Water vapour is the number one contributor to greenhouse gas (GHG) effect followed by carbon dioxide (CO_2) and methane (CH_4) (Kiehl and Trenberth 1997). Methane is a compound with relatively high energy combustion of 55.5 MJ/kg (Crutzen 1995) that contributes to about 20% of total anthropogenic GHG emissions as shown by Lassey (2007). Methane has a very short turn-over time of about 10 years in the atmosphere as compared to CO_2, but it can trap the heat 20 times greater than CO_2, playing a key part in global climate change. The concentration of CH_4 gas has been rising rapidly in the atmosphere over the past decade compared to three centuries ago; it has raised over 2.5-fold (Lassey 2007). Emissions of CH_4 lead to increased ground-level ozone, with significant damage to public health and agriculture (Howarth 2019), giving an estimated social cost of 2700 USD per ton of CH_4 (Shindell 2015).

10.2 Sources of Methane Emissions

There are many sources that CH_4 originates from; it can be from wetlands, rice paddies, termites, agriculture, and burning biomass (Immig 1996). The rice paddies have been shown to be an important contributor with annual emissions reported to be around 115 teragrams (Tg) per year (Thorpe 2009). The agriculture sector contributes to about 10–12% of the total global anthropogenic GHG emissions (McAllister et al. 2011) with livestock sector (enteric fermentation) contributing the most within the agricultural sector of around 37% of total anthropogenic CH_4 emissions. Other reports claim that CH_4 emissions from the livestock sector is about 51% of the total agricultural CH_4 emissions and that the contribution of rice paddies and livestock is rather similar, 100 and 110 Tg/year, respectively (Moss et al. 2000).

There is a high demand by the Intergovernmental Panel on Climate Change (IPCC) to evaluate the number of gases produced and to develop methods and strategies to reduce the emissions of GHG within a time frame (Ahmed 2017; Moss et al. 2000). Within the European countries, livestock, mainly the enteric fermentation, has been reported to be the leading CH_4 producer within the agriculture sector (Moss et al. 2000).

Within the European Union (EU-27) and based on data that was obtained in 2003, Lesschen et al. (2011) reported that dairy cow and beef cattle contributed to the most GHG emissions (Fig. 10.1).

As shown in Fig. 10.1, the enteric fermentation from dairy cow and beef cattle contributes the most to the GHG emissions followed by the N_2O soil emission and manure management.

Recently published data based on radioactive carbon (C^{14}) content in CH_4 indicates that anthropogenic emissions of CH_4 in recent decades have been higher than previously estimated (Petrenko et al. 2017). Satellite data (Howarth 2019) suggest that the increased global CH_4 emissions in the period 2005–2015 were

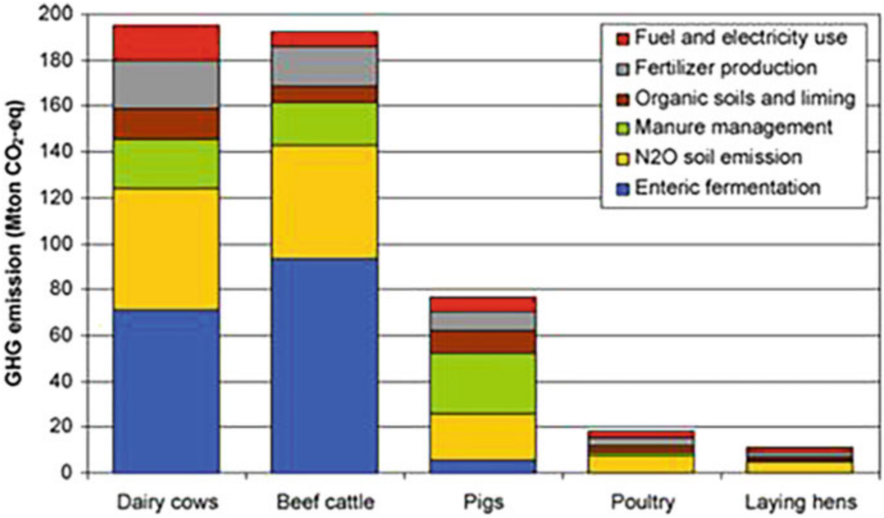

Fig. 10.1 Greenhouse gas emissions from the livestock production in the EU-27. (Source: Lesschen et al. 2011)

mostly due to increased extraction of shale gas and that the natural gas and oil industry contributes twice as much CH_4 emissions as animal agriculture.

10.3 Methane in Ruminants

Methane is produced in the rumen of ruminants with a minor contribution from the hindgut as a result of food digestion and fermentation. The majority (95%) of CH_4 gas is produced during the enteric fermentation and is lost to the atmosphere via belching, whereas the remaining 5% is emitted through the rectal (Fig. 10.2).

The food eaten by dairy cows (mainly silage and concentrates) is then fermented in the rumen by the help of microorganisms. A result of this fermentation is hydrogen (H_2) gas which then needs to be absorbed in order to make this fermentation pathway happening all time. There are specific microorganisms in the rumen belonging to the domain of Archaea (*Methanobrevibacter* spp.) which uses the H_2 to produce CH_4 gas. One of the dominant species of methanogenic bacteria living in the rumen is *Methanobacterium ruminantium* (Miller et al. 1986). The phenomenon of CH_4 emission starts around 4 weeks after birth in dairy cows when the rumen is almost shaped, and solid particles are kept in the rumen (Johnson and Johnson 1995). Methanogenic bacteria are mainly in both the liquid and solid phases in the rumen (Morgavi et al. 2010). The food entering the rumen (stomach) of a cow is first digested by microorganisms that contain mainly bacteria, protozoa, and fungi. The simple monomers produced by primary microorganisms are then used by both primary and secondary fermenters to produce end products such as volatile fatty acids (VFA), CO_2, and H_2 (McAllister et al. 1996). In the final step of fermentation,

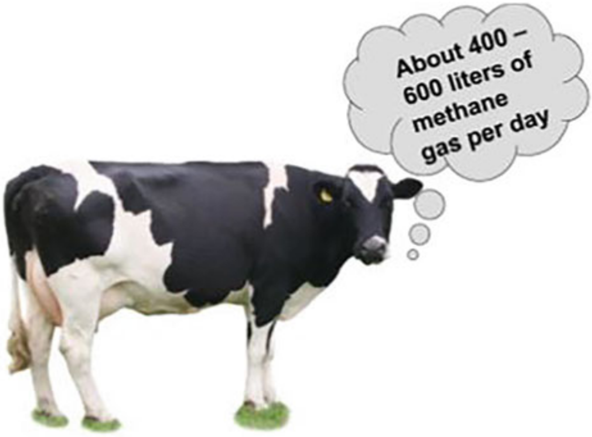

Fig. 10.2 Picture showing that the majority of CH_4 is eructated from the mouth of dairy cows

the H_2 that is produced in previous steps is then used together with CO_2 to produce CH_4 gas by methanogens in the rumen (Eq. 10.1).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{10.1}$$

Methane emission from dairy cows depends on many factors, such as type of feed, the amount of feed intake, quality of the feed, and digestibility. Grass contains energy; this energy is called gross energy (GE) and once eaten by dairy cows a part of this energy is lost as CH_4 gas. Depending on the factors mentioned above, CH_4 emission as a proportion of GE varies between 2% and 12% of GE intake (Johnson and Johnson 1995).

10.4 Factors Affecting Methane Emission

There are many factors influencing CH_4 emission in dairy cows. The main element is dry matter intake. In addition to intake, diet digestibility, amount of fat and fibre in the diet has effects on CH_4 emission in dairy cows (Ramin and Huhtanen 2013).

There are some feed characteristics influencing CH_4 emission in dairy cows as there is a close relationship between rumen-fermented organic matter and CH_4 emission (Ramin and Huhtanen 2013). Diets that contain high amounts of digestible fibre will increase the digestibility in the diet resulting in higher emissions of CH_4. The forage to concentrate ratio in the diet also affects CH_4 emission, for example, feeding high concentrate proportions (above 90%) in the diet of feedlot beef cattle can reduce CH_4 significantly (Johnson and Johnson 1995). Moss et al. (1995) showed that CH_4 as a proportion of GE increased more when the concentrate was increased in the diet of sheep fed on a low level of intake. The effect of fat in the diet is another factor influencing CH_4 emission (Grainger and Beauchemin 2011). There are some theories behind the effect of fat on CH_4 emissions: (1) unsaturated fatty acids are bio-hydrogenated in the rumen, a process that utilizes H_2, (2) inclusion of fat in the diet simply reduces the supply of carbohydrates resulting in less fermentable substrates, and (3) inclusion of fat in the diet favours the pathway of propionic acid production (H_2 sink) in the rumen (Ramin and Huhtanen 2013).

10.5 Factors Inhibiting CH_4 Emissions

To date, there are many strategies to reduce CH_4 emission in dairy cows, ranging from chemicals to algae. Some show direct effects on methanogenic bacteria and some act by interrupting the last step in the biochemical process of producing CH_4 in the rumen. For the chemicals, the efficient methane inhibitor identified is 3-nitrooxypropanol (3NOP). The 3NOP has proven to be the most effective inhibitor without showing any adverse effect on milk production (Hristov et al. 2015). The amount of 3NOP needed to reduce enteric methane from cows is very small, 80 mg per kg of DM intake showed reductions up to 30% of methane production from high producing dairy cows (Hristov et al. 2015). In addition, other chemicals have been reported in the literature decreasing CH_4 emissions, such as 2-nitroethanol and bromoform (Chagas et al. 2019; Zhang and Yang 2011).

Regarding dietary strategies with the potential to mitigate CH_4 emission, the rapeseed oil added to a grass silage-based diet reduced ruminal CH_4 emissions from lactating cows as reported by Bayat et al. (2018), where the decrease in CH_4 was explained by reductions in DM intake and the dilution effect on fermentable organic matter. Franco et al. (2017) in an in vitro study replaced soybean meal by dried distiller's grain in grass silage-based diet, and the authors reported a decrease in predicted in vivo CH_4 production, which was related to a shift in the ruminal fermentation pattern to decreased acetate and butyrate production and increased propionate production. Further, the use of oats in the diet has also been shown as a potential strategy to reduce CH_4 emission, and a recent study conducted by Fant et al. (2020) showed that predicted in vivo CH_4 emission was 8.5% lower for a diet that used oats compared to barley.

Several studies have recently reported the potential of essential oils to reduce enteric CH_4 production, primarily in vitro and short-term trials. The most common essential oils reported in the literature as methane mitigate strategies are derived from thyme, oregano, horseradish, rhubarb, frangula, and highlighting garlic, cinnamon, and its derivatives (Benchaar and Greathead 2011). However, the authors draw attention to the need for in vivo investigation to propose whether these ingredients/ additives can be used successfully to inhibit rumen methanogenesis, without depressing feed intake, digestibility, and animal productivity.

Recently, the red macroalgae *Asparagopsis taxiformis* (AT) has shown promising effects on reducing CH_4 emission from dairy cows. In vivo (Stefenoni et al. 2019) and in vitro (Chagas et al. 2019) studies showed a decrease of 80% on CH_4 emission by adding 0.5% of AT on a dry matter basis and inhibition of CH_4 by adding 0.5% of AT on organic matter basis, respectively. Previous in vitro studies also had reported the potential to mitigate methane emission to adding AT in ruminants diets (Machado et al. 2014, 2016).

Fig. 10.3 This cartoon shows the side effects of dietary CH_4 inhibitors. (Reprinted from: An Introduction to Rumen Studies by J.W. Czerkawski, page 172. Copyright © (1986))

One major problem with additives used in the diet is the excess of H_2 gas in the rumen if there is no other sink to uptake the H_2 production (Fig. 10.3).

10.6 Methods and Models for Measuring or Predicting CH_4 Emission

There are many tools and models in the literature to predict CH_4 emission. Respiration chamber is the most accurate method of measuring CH_4 emission in dairy cows (Johnson and Johnson 1995). The animal is basically allocated in a chamber for 2–3 days in which all exhaled breath is measured including CH_4. The technique is laborious with high construction costs. The alternative to in vivo techniques measuring CH_4 emissions, in vitro methods, is also used. In the in vitro method, a small sample size (1 g) is incubated in fermentation units in which buffered rumen fluid is added. The fermentation takes place in an anaerobic condition at the same temperature of the rumen (39 °C). The unit is then gently shacked for about 48 h.

The in vitro gas production system's main advantage is that it provides a large number of data points, which allow accurate estimates of CH_4 emissions. In another hand, this system has some limitations compared with in vivo studies (e.g. no absorption of VFA over time and the intake dynamics).

Recently, Ramin and Huhtanen (2012) developed the application of an in vitro method so CH_4 emission could be predicted in vivo by applying the data obtained from the in vitro in a rumen model. The method allows estimation of CH_4 emissions every 20 min of incubating a sample up to 48 h. Figure 10.4 shows the curve of CH_4

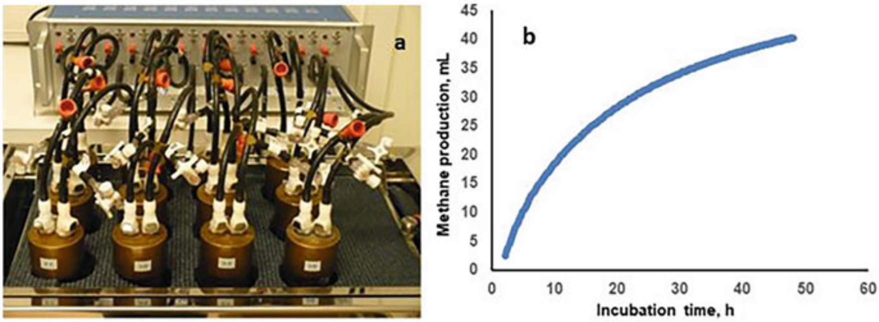

Fig. 10.4 In vitro method (**a**) and methane emission (**b**) over a 48 h incubation period from a silage-based diet using the model as described by Ramin and Huhtanen (2012)

emission over a 48 h incubation time for a diet consisting of silage. One main advantage of in vitro systems is that it allows digestion kinetics to be evaluated from feeds and that the method could be used as a screening tool for assessing different CH_4 inhibitors.

Danielsson et al. (2017) evaluated the in vitro system developed by Ramin and Huhtanen (2012) by formulating 49 diets used in 13 in vivo studies in which CH_4 emission was measured by the respiration chamber. The results indicated that the in vitro system predicted in vivo CH_4 emissions very well with a high $R^2 = 0.96$. However, the values obtained (mean 399 L/d) also showed a slight underestimation compared to the observed (mean 418 L/d) in vivo CH_4 emissions (Fig. 10.5).

Models are developed from data sets that consist of animal and dietary characteristics. The most widely used models to predict CH_4 emissions are the empirical models. However, models can be categorized into two main groups: empirical models (e.g. Ellis et al. 2007; Ramin and Huhtanen 2013; Niu et al. 2018) or dynamic mechanistic models (Huhtanen et al. 2015).

Empirical models relate CH_4 emissions to the total amount of intake and dietary composition (Ramin and Huhtanen 2013). The empirical models developed by Ramin and Huhtanen (2013) use a data set in which no additive study is used. It is also advisable to use a mixed model regression analysis so that random study effect will be taken into account (St-Pierre 2001) when developing models predicting CH_4 emission. The model predicting CH_4 as a proportion of GE developed by Ramin and Huhtanen (2013) takes into account total dry matter intake per kg of body weight (DMIBW), organic matter digestibility estimated at the maintenance level of feeding (OMDm), and dietary concentrations of neutral detergent fibre (NDF), non-fibre carbohydrates (NFC), and ether extract (EE).

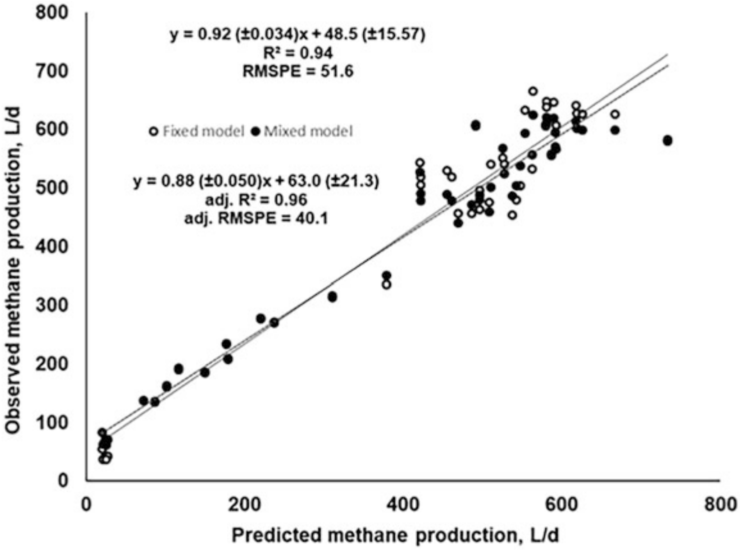

Fig. 10.5 Relationship between predicted in vivo CH_4 emission by the in vitro technique and observed CH_4 emission in vivo (L/d; $n = 49$), with fixed and mixed model regression analysis. (Source: Danielsson et al. 2017)

$$
\begin{aligned}
CH_4 - E/GE\ (kJ/MJ) = {} & -0.6\ (\pm 12.76) - 0.70\ (\pm 0.072) \\
& \times \mathrm{DMIBW}\ (g/kg) + 0.076\ (\pm 0.0118) \\
& \times \mathrm{OMDm}\ (g/kg) - 0.13\ (\pm 0.020) \\
& \times \mathrm{EE}\ (g/kg\ DM) + 0.046\ (\pm 0.0097) \\
& \times \mathrm{NDF}\ (g/kg\ DM) + 0.044\ (\pm 0.0094) \\
& \times \mathrm{NFC}\ (g/kg\ DM)
\end{aligned}
\tag{10.2}
$$

And the equation predicting total CH_4 emission (litres per day) developed by Ramin and Huhtanen (2013) was closely related to total DMI, and further adding other variables improved the model:

$$
\begin{aligned}
CH_4(L/d) = {} & -64.0\ (\pm 35.0) + 26.0\ (\pm 1.02) \times \mathrm{DMI}\ (kg/d) \\
& - 0.61\ (\pm 0.132) \times \mathrm{DMI2}\ (\mathrm{centered}) + 0.25\ (\pm 0.051) \\
& \times \mathrm{OMDm}\ (g/kg) - 66.4\ (\pm 8.22) \times \mathrm{EE\ intake}(kg\ DM/d) \\
& - 45.0\ (\pm 23.50) \times \mathrm{NFC}/(\mathrm{NDF} + \mathrm{NFC})
\end{aligned}
\tag{10.3}
$$

Mechanistic models are a little bit more complicated as compared to empirical models. Mechanistic models simulate CH_4 emissions using mathematical formulas and descriptions of ruminal fermentation biochemistry, making it a great tool for understanding the mechanisms and factors influencing CH_4 emissions in the rumen. Karoline is a dynamic, deterministic, and mechanistic simulation model of a lactating dairy cow developed by Danfær et al. (2006). The sub-model predicting CH_4 emission was further developed by Huhtanen et al. (2015). A recent evaluation of the

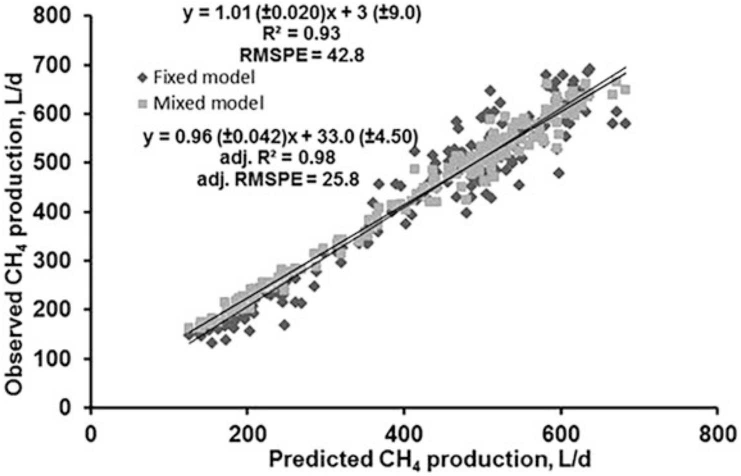

Fig. 10.6 Relationship between predicted (using the Karoline model) and observed CH_4 emissions (L/d) ($n = 184$) with fixed and mixed model regression analysis. (Source: Ramin and Huhtanen 2015)

Table 10.1 The comparison of empirical and mechanistic models in predicting CH_4 emission

Reference	Observation	R^2	RMSE
Empirical models			
Axelsson (1949)	175	0.75	0.131
Ellis et al. (2007)	172	0.71	0.296
Ramin and Huhtanen (2013)	184	0.93	0.104
Mechanistic models			
Mills et al. (2001)	32	0.76	0.154
Ramin and Huhtanen (2015)	184	0.93	0.101

Karoline model using a data set developed from studies that respiration chamber was used to measure CH_4 emission suggested that the model has a potential to predict CH_4 emissions accurately and precisely as shown in Fig. 10.6 (Ramin and Huhtanen 2015). Furthermore, evalution of CH_4 at whole farm scale is need of time (Ahmed et al. 2020).

Table 10.1 summarizes some empirical and mechanistic models developed in the literature. The empirical model developed by Ramin and Huhtanen (2013) predicted CH_4 emission better than other models as observed by a smaller root mean square error (RMSE). The mechanistic model Karoline also showed better predictions of CH_4 emission (Table 10.1) compared to the mechanistic model evaluated by Mills et al. (2001).

There are many equations developed in the literature predicting CH_4 production. Equations are basically developed from larger data sets in which intake and dietary factors are gathered. Since dry matter intake is the driving force in predicting CH_4

Table 10.2 Equations predicting CH_4 production

Source	Equation
Ellis et al. (2007)	CH_4 [MJ/d] = 3.41 + 0.520 × DMI^a [kg/d]−0.996 × ADF^b [kg/d] + 1.15 × NDF^c [kg/d]
Jentsch et al. (2007)	CH_4 [kJ] = 1802–21.1 × DMI [g/kg BW]
Bell et al. (2016)	CH_4 (g/kg DM intake) = 0.046 (±0.001) × $DOMD^d$–0.113 (±0.023) × EE^e–2.47 (±0.29) × (feeding level – 1)
Ramin and Huhtanen (2013)	CH_4 (L/d) = −64.0 (±35.0) + 26.0 (±1.02) × DMI (kg/d)−0.61 (±0.132) × $DMI^2{}_{\text{(centred)}}$ + 0.25 (±0.051) × $OMD^f{}_m$ (g/kg)−66.4 (±8.22) × EE (kg DM/d)−45.0 (±23.50) × NFC^g/(NDF + NFC)

[a]*DMI* dry matter intake, [b]*ADF* acid detergent fibre, [c]*NDF* neutral detergent fibre, [d]*DOMD* digestible organic matter, [e]*EE* ether extract, [f]OMD_m organic matter digestibility at maintenance level of feeding, [g]*NFC* non-fibrous carbohydrate

production, often all equations require this parameter for predicting CH_4 production. Table 10.2 summarizes some equations predicting CH_4 production in dairy cattle.

10.7 Conclusion

Methane is emitted from ruminants as a result of fermentation in rumen. There are many strategies to inhibit CH_4 emissions from ruminants. Most strategies reducing CH_4 emission require adaptation of the inhibitor used in the rumen and that the rechannelling of H_2 remains unclear in the rumen upon using any inhibitor. There are both in vitro and in vivo methods to measure CH_4 emission from dairy cows. Empirical and mechanistic models such as the Karoline model usually predicts CH_4 emission reliably in which they could be used by national inventories and advisory services for predicting CH_4 emission.

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