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Comparison of methods to measure enteric methane emissions from ruminants: an integrative review

Comparativo de metodologias para mensuração das emissões de metano entérico em ruminantes: uma revisão integrativa

Comparación de metodologías para medición de las emisiones de metano entérico en rumiantes: una revisión integrativa

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Abstract

The agricultural industry is the main emitter of methane (CH_4) and nitrous oxide (N_2O), gases that each have a global warming potential that is greater than that of CO_2 by 23 and 298 times, respectively. On a global scale, enteric fermentation from ruminant animal production (especially of cattle and sheep) is responsible for between 21% and 25% of total anthropogenic CH_4 emissions. The search for effective, simple, and fast methods to measure the production of CH_4 and other products from ruminal fermentation has been the objective of several studies on ruminant nutrition. Thus, techniques have been developed under experimental conditions of Brazil and other international countries to quantify CH_4 emissions of ruminants. The objective of this literature review is to discuss and compare the existing techniques of measuring enteric CH_4 from ruminants. Each technique has a shortcoming or disadvantage in its characterization of ruminal fermentation. The ex-situ technique of measuring CH_4 from ruminal fermentation has been quite promising, because it facilitates the measurement of CH_4 and other fermentation products, such as short chain fatty acids (SOFA). CH_4 detection by a portable laser is effective in monitoring fluctuations in emission and is recommended especially for short-term measurements of respiring and eructating animals reared in a feedlot system. Other methods are being improved and better adapted for practical use in the ongoing quest for more efficient uses of ruminal fermentation products.

Keywords: Climate change; Livestock; Ruminal fermentation; Greenhouse gases.

Resumo

O setor pecuário é o principal emissor de metano (CH_4) e óxido nitroso (N_2O) para a atmosfera, gases com potencial de aquecimento global, respectivamente, 23 e 298 vezes maior que o CO_2. Particularmente a fermentação entérica em animais ruminantes (predominantemente bovinos e ovinos) produz, em escala global, entre 21 e 25% do total das emissões antropogênicas de metano. A procura por métodos acurados, simples e rápidos para mensurar a produção de metano e outros produtos da fermentação ruminal tem sido objetivo de pesquisas na nutrição de ruminantes. Desta forma, técnicas foram desenvolvidas com o objetivo de quantificar-se a emissão de metano por ruminantes, sob diferentes condições experimentais brasileiras e estrangeiras. Esta revisão de literatura teve como objetivo discutir, fazendo um comparativo entre as metodologias existentes para mensuração do metano entérico em ruminantes. Desta forma, entende-se que cada método desenvolvido possui alguma inadequação ou inconveniente quando se objetiva a caracterização da fermentação ruminal. A técnica de fermentação ruminal *ex-situ* de mensuração de metano tem se mostrado bastante promissora, pois permite mensurar a produção de CH_4 e outros produtos da fermentação como os ácidos graxos de cadeia curta. O detector de metano a laser portátil é eficiente ao cruzar as informações dos picos de metano na eructação e respiração e recomendado para mensurações curtas, especialmente para animais em sistema de fedlot. Entretanto algumas metodologias estão sendo aprimoradas e adequando-se em busca de melhores resultados sobre os produtos da fermentação ruminal.

Palavras chave: Aquecimento global; Fermentação ruminal, Gases de efeito estufa, Pecuária.

Resumen

La industria pecuaria es el principal emisor de metano (CH_4) y óxido nitroso (N_2O) para la atmósfera, eses gases tienen un potencial de calentamiento global, respectivamente, 23 y 298 veces mayor que el CO_2. La fermentación entérica y particularmente en animales rumiantes (principalmente ganado vacuno y ovino) producen, en una escala global, entre el 21 y el 25% de las emisiones antropogénicas de CH_4. La demanda de métodos precisos, sencillos y rápidos para medir la producción de metano y otros productos de la fermentación ruminal ha sido objeto de investigación en nutrición de rumiantes. Por lo tanto, se desarrollaron técnicas para cuantificar la emisión de metano por los rumiantes, brasileños y extranjeros bajo diferentes

condiciones experimentales. Esta revisión de literatura destinada a discutir haciendo una comparación entre las metodologías existentes para la medición de metano entérico en rumiantes. Por lo tanto, se entiende que cada método ha desarrollado algunos insuficiencia o molestias cuando se pretende caracterizar la fermentación ruminal. La técnica de medición de la fermentación ruminal ex-situ de metano ha sido bastante prometedora, ya que permite medir la producción de CH_4 y otros productos de fermentación tales como ácidos grasos de cadena corta. Láser portátil detector de metano es eficaz cuando se cruza la información de los picos de metano en eructos y la respiración y se recomienda para mediciones cortas, sobre todo para los animales en el sistema feedlot. Sin embargo, algunas metodologías se están mejorando y satisfaciendo en busca de mejores resultados en productos de la fermentación ruminal.
Palabras clave: Calentamiento global; Fermentación ruminal; Gases de efecto invernadero; Ganado.

1. Introduction

Cattle rearing is one of the main sectors of Brazilian agribusiness, which is a major contributor to the worldwide market. Over the last decade, Brazil has stood out among major players in the global market, being the largest exporter of beef, and having the second largest volume of cattle slaughter and the largest commercial herd. According to projections from USDA (2014), such rates are expected to be similar in 2020.

In 2016, the cattle and buffalo herds accounted for 218,23 and 1,37 million heads respectively (IBGE, 2016), a little over 20% of all cattle worldwide (USDA, 2014), with breeds adapted to the very different regions and ecosystems of Brazil. Forage based animal production has a crucial impact on supplies of nutrient-rich food for human beings. According to Berchielli et al. (2012), the main environmental challenges of extensive cattle rearing include the emission of methane (CH_4) from the enteric fermentation of ruminants, the emission of nitrous oxide (N_2O) from animal manure, and the exchange of carbon dioxide (CO_2) between soil and plants.

In this context, the Brazilian livestock industry has been criticized for the emission of significant amounts of greenhouse gases (GHG) from enteric fermentation (Pereira, 2013), the deforestation and expansion of pastures, and the relative lack of zootechnical advancement in the use of *Brachiaria* species of grasses in extensive systems, accompanied by degradation and/or low productive potential (MCTI, 2009).

Cows are important animals that provide high quality food. Their role in sustaining forage based production systems is crucial because they do not require grains or cereals, which are major sources of energy for human beings (Chaudhry, 2008). Because the gastrointestinal tracts of ruminant animals differ from those of monogastrics, they are able to transform different fiber rich plant materials, which have little to no nutritional value, into nutritious foods of high biological value, such as milk and meat.

Ruminants are mammals that have fore-stomachs with anatomical structures that are adapted to the process of fermentation, which thereby allows the animal to utilize fibrous plants in the diet (Furlan et al., 2011). Microbial fermentation of ingested feed in the rumen is an anaerobic process that produces volatile fatty acids, such as acetic, propionic, and butyric acids, which are energy sources for the animal. In addition, the process emits gases such as CO_2 and CH_4 that the animal eliminates by eructation. The group of obligate anaerobic organisms responsible for methane production is referred to as methanogenic *archaea* (Arcuri et al., 2011).

Increased GHG emissions currently present a major environmental challenge. Changes in GHG concentrations may lead to an increase in the average temperature of the planet by up to 5.8°C over the next 100 years (IPCC, 2007). Among the GHG, CO_2, CH_4, and N_2O are the most significant in the agricultural industry. Even though the concentrations of CH_4, and N_2O in the environment are lower than that of CO_2, these gases each have a global warming potential that is greater than that of CO_2 by 23 and 296 times, respectively (Snyder et al., 2008).

In environmental terms, CH_4 is wasted energy, because that dissipated by eructation accounts for 2–12% of the energy consumed and ultimately contributes to global warming and climate changes. On average, 250–500 L of CH_4 is produced and emitted per animal per day (Johnson and Johnson, 1995).

CH_4 is a potent and prevalent GHG. Anthropogenic activities have been responsible for 70% of global emissions of CH_4 and livestock production accounts for 29% of that amount. CH_4 is the second most abundant GHG, responsible for 14% of global emissions, which persists in the environment for 12 years (GMI, 2014). According to the MCTI (2009), Brazil emitted 11.659 Gg of CH_4 from cattle rearing in 2005, comprising a portion of 62.5% of total emissions. Several industrial sectors (energy, manufacturing, agriculture and livestock production, waste treatment, and land-use change and forestry) also contributed to that figure; however, the primary source of gaseous emissions was enteric fermentation from beef cattle, accounting for 55% (10.258 Gg), and to a lesser extent, from dairy cattle, accounting for 7.5% (1.401 Gg) of emissions. Furthermore, according to the MCTI (2014), CH_4 emissions from enteric fermentation increased by 22.2% between 1995 and 2005. In the same document, a reduction in enteric CH_4 emissions between 2005 and 2012 was reported, representing a decrease of just 3.9%.

Studies have shown that ruminants possess unique qualities that facilitate the development of animal production management strategies that have the potential to reduce GHG emissions per unit of product. Thus, more efficient, sustainable production technologies and systems can be realized with potentially increased income for the farmer. This review aims to discuss and compare the available methods of measuring enteric CH_4 from ruminants.

2. Metodology

Systematic reviews built through a critical analysis of given topic, considering a theoretical view, contribute to debates aimed at the development of the subject by the scientific population, collaborating to update knowledge for a short period of time (Torrelio et al, 2009; Pereira et al., 2018).

To carry out this study, followed the norms for the elaboration of scientific research obtained by Pereira et al. (2018), scientific articles were selected, available in the electronic databases: Base de Dados da Pesquisa Agropecuária Embrapa: BDPA, Scopus (Elsevier), Scielo.ORG, Wiley Online Library and Google Scholar without restriction of the year of their publications from the object of study now proposed.

3. Sistematic Review

3.1 Expansion of farming and livestock raising in Brazil and the environmental implications

Cattle rearing in Brazil is almost exclusively based on the use of cultivated pasture as feed. Between 1970 and 2010, the area of available pasture grew by 39%, to occupy 170 million hectares. During this period, the national herd grew by 251%, to 204 million animals (MAPA, 2014). Thus, production grew from 0.47 to 1.2 animals per hectare. These data reveal a distinction of Brazilian cattle that can potentially benefit from the 259 million hectares of land, which feature native vegetation in various ecosystems of Brazil, such as the region of the Amazon rainforest and the Cerrado. Thus, Strassburg et al. (2014) stated that Brazil could become more efficient in animal production. According to those authors, just 34% of the potential capacity of cultivated pastures in Brazil is currently utilized. If this figure could be increased to 52% or about 1 AU/ha, it would be sufficient to meet the demand for meat, crops, wood products, and biofuels until 2040, without further conversion of natural ecosystems. As a result, emissions of CO_2 could be reduced by about 14 Gt CO_2 Eq.

However, the expansion of the cattle industry has been strongly associated with changes in the entire agricultural industry. According to Millen et al. (2011) and Meyer et al. (2013), over the last few decades (1991–2010), cattle rearing has undergone expansion in the Central-western states of Brazil and presently accounts for about 34.3% of the national herd (IBGE, 2014). Recently, an increase in soybean production has led to the displacement of cattle farmers to the Northern regions of Brazil, where the price of land is 10% lower than in other regions. The sugar industry places an additional pressure on land prices, particularly in areas of high agricultural potential that have qualities such as fertile soils, well drainage, and flat topography, as well as other factors, such as proximity to large urban centers with good infrastructure and proximity to ports. The Amazon rainforest is located in the Northern region of Brazil and is one of the richest biomes worldwide. The Brazilian government established the macro-region referred to as the “Legal Amazon” for administrative purposes and economic planning. This region encompasses the states of the Northern region of Brazil (Acre, Amapá, Amazonas, Pará, Rondônia, and Roraima) and parts of the states of Mato Grosso, Tocantins, and Maranhão (Sudam, 2015). The area of this macro-region is 5.2 million km^2, or 61% of Brazilian territory. With the migration of livestock rearing to this area, about 15% of the Amazon rainforest was depleted and approximately 80% of the deforested areas have now been replaced by cultivated pastures (Veiga et al., 2002).

According to Rivero et al. (2009), cattle rearing occurs on both small and large properties, is strongly correlated with deforestation, and has grown almost continuously in recent history, with various technological advances in the industry. This movement of livestock production into new geographical areas is associated with increased demands for meat and the progressive integration of livestock production generally within global markets (Rivero et al., 2009).

However, Barcellos et al. (2008) demonstrated that the rearing of beef cattle in Brazil was developed as an early activity that was based on a production model that entailed the intensive use of land and natural resources near the borders. For this reason, cattle rearing was established as a major industry because of its liquid funds, low risk, and the adoption of extensive pastures. A growing number of farmers incorporate the use of technology into livestock production. However, other farmers continue to use management techniques of the past, some of which lead to low yields and foster deforestation (Dias-Filho, 2011). International organizations have pressured the Brazilian government to curb deforestation.

A significant proportion of Brazilian cattle is reared on cultivated pastures, which supports the country’s competitiveness in livestock production. Therefore, there has been an international pressure on the Brazilian cattle industry, which has been described as a villain of sustainable development. Brazil has been referred to as a major producer of CH_4, a reputation that can ultimately lead to an embargo on Brazilian livestock products (Pedreira et al., 2009). Environmental pressures currently guide the development of research studies on factors associated with the feed required for livestock production and existing ruminant production systems that aid in reducing GHG emissions.

In a study by (Assis et al., 2019) they concluded that supplementation with virginiamycin can provide additional gains in the performance of lactating calves and reduce the production of enteric methane per kilogram of body weight gain, in addition to reducing the environmental impact.

3.2 Description of techniques for measuring CH_4 emitted by ruminants

The global climate changes extensively discussed by experts at the Intergovernmental Panel on Climate Change (IPCC) in 1995, 1996, 2006, and 2007 ushered in the measurement of GHG emissions in agricultural ecosystems. Benchmark reports generated by the working group coordinated by the Ministry of Science, Technology, and Innovation (MCTI) in Brazil emphasize that livestock rearing is the main source of CH_4 emissions from agricultural activities. The second Brazilian Inventory of Anthropogenic GHG Emissions released by the MCTI (2010) states that 96.9% of CH_4 emissions originated from enteric fermentation of beef and dairy cattle and 91.1% of total CH_4 emissions in 2005 were from livestock. In the same document, between 1990 and 2005, estimates of enteric CH_4 emissions of Brazilian livestock grew by 35.8%, totaling 11.129 Gg of CH_4 in 2005.

However, the methods by which enteric CH_4 emissions from Brazilian livestock are measured are based on equations recommended by the IPCC (1996). Thus, the technical coefficient of CH_4 production considers the aspects of feed and the production potential of the animals only, unlike the more holistic approach that is necessary in Brazil. Therefore, the estimated value of enteric CH_4 emissions may be different from the actual value. Based on the Tier 2 approach (IPCC, 1996), the MCTI (2009) estimated that enteric CH_4 production from male, young, and female beef cattle amounted to 42, 48, and 67 kg per animal per year, respectively; and from dairy cattle, 65 kg per animal per year. Caliman et al. (2012) used the technique of tracer gas sulfur hexafluoride (SF_6) in Nellore cattle and concluded that the average CH_4 emissions from males and females with a mean age of 290 days and a mean weight of 216 kg was equivalent to 52.6 kg per animal per year.

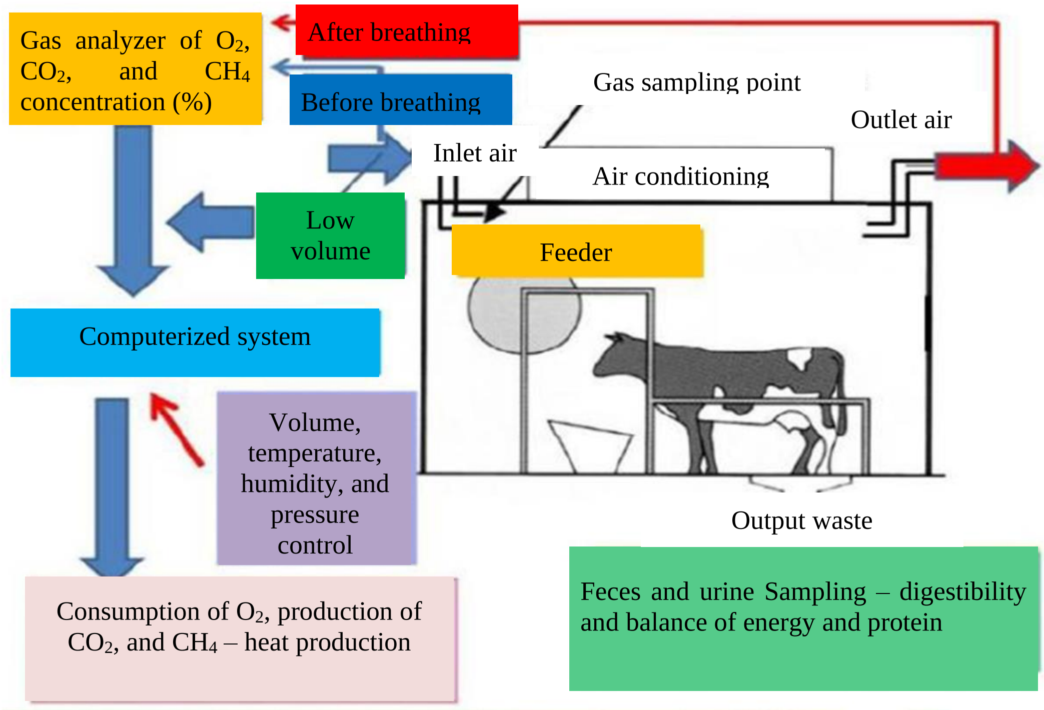
To select the most appropriate of the available scientific techniques, one must consider factors such as accuracy, dimensions, cost, and the experimental model used. The available techniques measure enteric CH_4 with consideration of a particular individual or herd (direct measurement), or model (indirect measurement). It is therefore necessary to have an awareness of the feasibility and restrictions of each technique.

The quest for quick, accurate, and simple methods to measure CH_4 and other products of ruminal fermentation has been the focus of several studies on ruminal nutrition. Thus, techniques have been developed in Brazil and other countries to quantify CH_4 emission by ruminants under various experimental conditions. Such techniques have been developed from an animal nutrition perspective, with the purpose of quantifying energy losses from ruminants fed different diets and are quite useful for monitoring the environmental efficiency of the production system. Each method has its advantages and disadvantages in terms of representing a holistic definition of ruminal fermentation.

3.3 Calorimetric chamber

The classic standard of measuring CH_4 from ruminants under various conditions in Brazil and other countries is the breathing chamber or calorimetric chamber (Bhatta et al., 2007). The main objective of this technique is to determine the energy produced from normal metabolism of the animals. These chambers are valuable tools in investigating mitigation strategies for CH_4 emissions. The principle of the chamber is based on recording measurements (within a controlled environment) of the concentrations of CH_4, O_2, and CO_2 in air exhaled by the animal. In ruminant metabolism, CH_4 represents an inherent energy loss mainly through enteric fermentation and emissions through the mouth, nose, and rectum (Broucek, 2014). Use of the calorimetric chamber is generally restricted to analysis of an individual animal, because of the costs of construction and the requirement of technical skills to operate (Storm et al., 2012). However, the technique can provide continuous and accurate data about the atmospheric composition over extended periods. There are two categories of chamber systems, in which atmospheric composition (particularly the concentration of CH_4) can be measured - closed and open (Storm et al., 2012). The open system is more widely used in research studies, and is illustrated in Figure 1.

Figure 1. A schematic diagram of an open breathing chamber (adapted by Rodriguez et al., 2007).



Source: Authors.

In its simplified form, the open breathing chamber represents an environmental system that collects air within the chamber in which the animal is housed. A pump adapted as a flow meter is used to control a specific proportion of input and output gases. During the process of air outflow, a gas analyzer quantifies and identifies CH_4 and other gases. The chamber is also equipped with sensors that measure and control the relative humidity of air, temperature, and internal barometric pressure. This system provides adequate accommodation and permits the supply of feed and water ad libitum. In addition, excreta can be collected with minimal disturbance to the internal environment of the chamber. Enteric CH_4 emission is derived from the difference between input and output air.

Calorimetric chambers are considered a reference tool in the estimation of CH_4 emissions by ruminants, because of the reliability of the measurements, which can facilitate the calibration of instruments (Storm et al., 2012). There is the possibility of experimental error however, because an artificial environment is created that may affect the animals' behavior and other variables such as dry matter intake (DMI), which is directly related to the emission of enteric CH_4. Therefore, use of this technique is not recommended in extensive ruminant production systems because it restricts movement and displaces the animals (Bhatta et al., 2007).

Resende et al. (2006) used the calorimetric chamber to determine daily production of CH_4 in dairy heifers of average weight 240 kg that were fed hay (*Cynodon dactylon*), which they found to be 162 L CH_4 per animal per day. Ferreira (2014) used the breathing chamber technique with purebred bulls with an average weight of 302 kg. The animals were fed diets based on a forage (corn silage; F) to concentrate (C) ratio of 58F:42C (on a dry matter basis). The animals gained an average of 900 g of body weight per day and the maximum production of CH_4 per animal was 126.61 g per day.

3.4 Head chamber

This technique involves the use of an airtight box that encases the ruminant's head and a curtain or sleeve around the neck to minimize air exchange between the internal and external environments of the chamber (Bhatta et al., 2007). The box should be sized to permit unrestricted movement of the animal's head and access to feed and water.

The main advantage of this technique compared to the calorimetric chamber is the lower cost; however, it does not provide shelter for the entire animal (Bhatta et al., 2007). In addition, the head chamber can only measure CH_4 emissions from the airways (nose and mouth) of the animal, and as with the calorimeter chamber, the measurements must be done individually on trained animals.

3.5 Face mask

The face mask is another method to measure CH_4 from ruminants that is based on a similar principle to that of the calorimetric chamber or the head chamber (Johnson; Johnson, 1995). The technique consists of mounting and adjusting the mask on the animal's head to collect exhaled air from the airways. The animal must go through a period of adaptation to the equipment, which typically occurs over a limited time of 7 daily occurrences of 6 minutes each (Odai et al., 2010). The animal is unable to feed or drink and analyses are conducted in a similar manner to that of the open calorimetric chamber (Bhatta et al., 2007).

The main advantage of this technique is the lower cost of construction, as compared to the calorimetric chamber or the head chamber. The face mask can also be used in animals on pasture (Bhatta et al., 2007). The limitation of this technique however, is its prevention of the intake of feed and fluids. The production of CH_4 is also underestimated because of the lack of measurement of gas emissions from the rectum. In addition, short-term measurements of CH_4 emissions can result in errors, owing to daily fluctuations of humidity, temperature, and other environmental factors (Bhatta et al., 2007).

Wang et al. (2009) used the facial mask technique to measure CH_4 production from Nellore bulls with an average weight of 750 kg, and reported 111.51 g of CH_4 per day per animal. For the duration of that study, the animals were fed a diet with a forage (corn silage):concentrate (corn, soybean, and urea based) ratio of 70F:30C.

3.6 Polyethylene tunnel

This technique employs a tunnel similar to that used as an agricultural greenhouse that is constructed on pasture and equipped with two layers of inflatable polyethylene, walls, and a large access door (Bhatta et al., 2007). This is an alternative to the calorimetric chamber, with simpler principles of operation and data collection. Within the tunnel, air is sucked at a constant speed and air samples can be continuously collected from an exhaust port and subjected to gas analysis or gas phase chromatography (Kebreab et al., 2006). Alternatively, air samples can be collected manually and thereafter submitted for analysis (Bhatta et al., 2007). The system is equipped with sensors for the control of temperature, air humidity, and the flow of gas produced by the animals. This technique is typically used to collect CH_4 emissions on areas of green forage, allowing the animals to express normal behavior. It also permits the control of selected forage within the confined space of the tunnel (Bhatta et al., 2007). After consumption of all the forage, CH_4 emissions are quantified and the tunnel can be moved to another location where there is greater availability of forage to the individual or group of confined animals.

The advantages of this technique include the free movement of the animals within the tunnel and the low cost associated with acquisition and installation (Kebreab et al., 2006). However, temperature control within the tunnel during times of high ambient temperatures is not practical. Most studies employing this technique have been conducted on sheep because of the limitation of pasture space (Bhatta et al., 2007). In addition, the technique is not suitable for experiments that evaluate various treatments.

3.7 Sulfur hexafluoride (SF_6) tracer gas

The SF_6 technique developed by Washington University in the US and adapted by Primavesi and collaborators (2004a) in Brazil, is another method to measure CH_4 that uses a small permeation capsule (a metal tube with a porous plate at one end) containing sulfur hexafluoride (SF_6). The capsule is first placed in a thermostatic water bath for 30 days and thereafter inserted into the rumen of the animal. A halter equipped with a capillary tube is placed over the animal's head and connected to a yoke (made of PVC tubes). The sample of the gases collected over a given period is subjected to a vacuum, after which it is taken to the laboratory for measurement by gas chromatography.

A valve in the PVC yoke regulates collection of the air exhaled through the nares of the animal at a constant rate. The sampler system (halter and yoke) is calibrated to cease collection within a pre-determined period (normally 24 hours), when the sample occupies half of the storage capacity of the system (approximately 51 kPa or 0.5 atm). The time taken to collect a single sample can be modified by varying the length or diameter of the capillary tube. After sample collection, the pressure on the yoke is measured with a digital meter, and adjusted by subjection to high purity nitrogen to achieve a pressure of approximately 122 kPa (1.2 atm). This pressurization is required for dilution and injection of the samples into the equipment for further analysis. The concentrations of CH_4 and SF_6 are determined by gas chromatography. The emission rate of CH_4 is derived from the emission rate of the permeation capsule in the rumen, and these two variables are indicative of the concentrations of CH_4 and SF_6, respectively in the sample.

This technique allows free movement and normal grazing activity and eliminates the need to confine animals in cages or barometric chambers (Primavesi *et al.*, 2004b). Training is necessary to condition the animal to the fitted equipment and daily handling to replace the PVC tubing. With this technique, the production of rectal methane is not measured; therefore, the production of gases is underestimated.

According to the same authors (2004b) states that the SF_6 tracer technique can be used generally to measure ruminal CH_4 under field conditions in Brazil. This technique was used in milk producing cows on pastures of *Brachiaria decumbens.* The researchers reported emissions of 188 g of enteric CH_4 per day from crossbred heifers on pastures without fertilizer, and 295 g of enteric CH_4 per day from dry cows on fertilized pastures in September and November, respectively.

3.8 In vitro semi-automatic gas production technique

The in vitro technique of measuring CH_4 production measures the total gas produced by fermentation of feed incubated in ruminal fluid with a buffer solution (Broucek, 2014). The technique involves the simulation of metabolic processes that normally occur in the rumen, particularly the microbial fermentation of feed in an environment conducive to the survival of microorganisms. The method has been in use since the 1950s and uses gas chromatography to quantify and identify the gases produced during the incubation of the feed (Jayanegara et al., 2009). Measurements of CH_4 emission can be obtained with the modified technique of Broucek (2014), which features three steps: collection of rumen contents and preparation of the solution to be fermented; production and storage of the gases generated by the fermentation process; and qualitative analysis of the gases produced.

The in vitro gas production technique is a promising, low cost, practical, and reliable way to quantify and identify the gases produced during the process of fermentation in ruminants (Jayanegara et al., 2009). The technique has been used extensively because in comparison to other techniques it requires fewer samples and produces faster results (Oliveira et al., 2014). With this technique however, it is impossible to simulate the complexity of the rumen within a container.

Bueno et al. (2015) evaluated CH_4 production from the fermentation of ruminal fluid of cattle fed diets of various forage:concentrate ratios (70%, 50%, or 30% forage, on a dry matter basis) and reported an average production of 398 mL of CH_4. Increased levels of concentrates in the feed resulted in higher CH_4 production.

3.9 Ex-situ technique (micro-rumen)

The ex-situ technique was recently developed at the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo. According to Rodrigues et al. (2012), the technique entails the incubation of both solid and liquid ruminal contents in glass bottles (micro-rumen) placed in a thermostatic bath. Ruminal conditions are simulated for 30 minutes, after which the process of fermentation is stopped in an autoclave. After the bottles achieve ambient temperature, the collected sample of gas is extracted for identification and quantification by gas chromatography. The advantage of this technique is its ability to measure the final products of ruminal fermentation (CH_4 and short chain fatty acids [SCFA]), and determine the energy lost by CH_4 production relative to the total energy produced (Rodrigues et al., 2013). The technique also permits evaluation of the fermentation profile of the ruminant, by sampling ruminal contents at various intervals throughout the day. However, the ex-situ technique can also underestimate CH_4 production, because it does not simulate the stages of digestion that occur during the passage of ingesta to the intestine. In addition, the technique does not measure rectal CH_4 production.

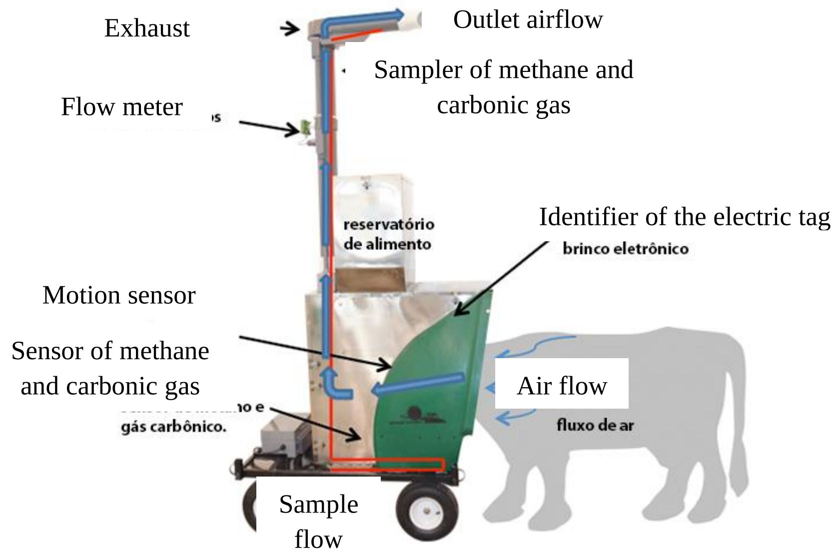
3.10 Automatic feeder technique (Greenfeed)

The Greenfeed is an automatic feeder used to measure GHG emissions of cattle. It is one of the most recently developed technologies with enhanced speed and reliability. The Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária; Embrapa) was the first institution in Latin America to use the technique. The automatic feeder was used by researchers at Embrapa Livestock Southeast (SP) to measure emissions of CH_4 and CO_2 from animals. The Greenfeed technique recognizes an electric tag on the animal as it places its head in the area of the feeder. From that moment, the gases emitted as the animal eats are measured every second. Thus, it is possible to monitor individual emission rates over time.

Another advantage of the automatic feeder is its ability to register the emissions per animal in real time and log these readings in a computer connected to the feeder. More than 90% of the gases produced from ruminants are released by eructation through the mouth and nostrils. Thus, the device generates a very reliable database for research studies aimed at reducing levels of GHG.

According to Berndt (2015), the main advantage of the automatic feeder over traditional technologies is the number of data generated. That author points out that emissions can be measured using various diets in confinement. The traditional method provides a single reading per animal per day, for one week. In contrast, with the Greenfeed technique, emissions are registered by the system each time the animal visits the feeder (about 10 times a day). According to Berndt (2015), more data can be obtained from the same cow and various animals can be monitored simultaneously. The same author states that the Greenfeed technique provides a small quantity of feed to attract the animal to the feeder. The animals are motivated to eat by the system, which is pre-programed to release feed automatically. After placing its head in the feeder, the animal is identified by a reading off the electronic tag that is equipped with radio frequency technology (RFID). A fan is immediately triggered to suck the air exhaled through the animal's nostrils and mouth. The sensors within the equipment measure gas concentrations, the volume of gas emitted, and other environmental parameters (Figure 2).

Figure 2. Automatic feeder, Greenfeed used to measure enteric CH_4 in real time (Adapted from Embrapa, 2015).



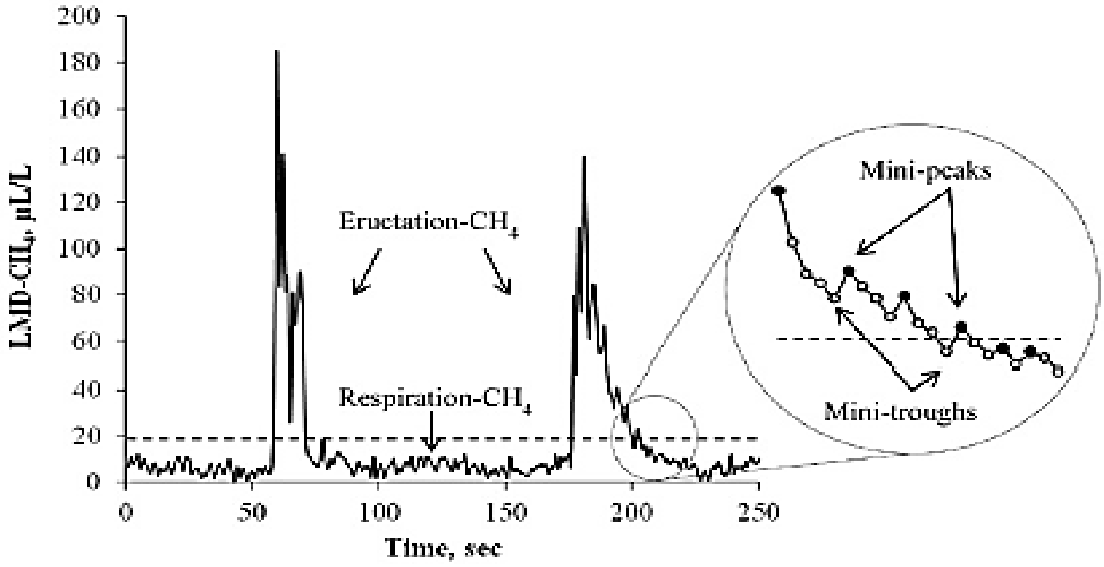
Source: Authors.

The limiting factor of the Greenfeed, particularly in studies conducted on larger numbers of animals, is its prohibitive cost, which has made its implementation virtually impossible in various research centers. The time required to condition the animals (particularly Zebu and other native commercial breeds) to the use of the equipment also needs to be considered when planning studies that employ this technique.

3.11 Laser CH_4 detector technique

The laser methane detector (LMD) is a non-invasive technique that facilitates the individual measurement of CH_4 emission per animal (Ricci et al., 2014). It has been proposed as an alternative method of determining enteric CH_4 emissions from animals in their natural habitat, with the convenience of a portable gas meter that permits field measurements (Chagunda; Yan, 2011; Ricci et al., 2014). The technique provides detailed information on CH_4 emission and facilitates the identification of the size and interval of output peaks after eating, for short periods of time (Figure 3). The correlation between eructation and respiration peaks facilitates a more comprehensive understanding of the dynamics of CH_4 production during the fermentation process.

Figure 3. Example of output data (solid line) from sheep during observation period obtained with the laser CH_4 detector (corrected for background CH_4, LMD-CH_4; µL/L). Data consist of mini-peak and mini-trough values (amplified section). Source: Ricci *et al*. (2014)



Source: Authors.

Fast and efficient monitoring of the dynamics of CH_4 production is also possible with this technique, which thereby facilitates the selection of breeding animals and the evaluation of strategies to classify and distinguish the effects of various diets (Hegarty, 2013).

Ricci et al. (2014) compared the LMD to the gas chamber, with stimulated CH_4 emissions from sheep and cows. They recorded greater levels of CH_4 production with the LMD between 3 and 5 hours after eating, whereas the gas chamber detected greater volumes of CH_4 over longer periods. Although this technique is practical and provides a good indication of CH_4 production, it is sensitive to the type of ruminal fermentation and the animal's respiration rate. The type of rumen fermentation is influenced by the ratio of forage:concentrate in the diet and affects the respiratory rate and timing of peaks in CH_4 emission (Ricci et al., 2014).

Pickering et al. (2015) used the LMD to measure the CH_4 emissions of 1,726 dairy cows, in consideration of genetic and zootechnical aspects of production, such as feed and energy intake and requirements based on milk production, live weight, feed consumption, and body condition. Those researchers reported a high genetic correlation (0.92) between DMI and PME (predicted methane emission). In addition, they demonstrated that levels of CH_4 emission are hereditary and can be predicted with moderate accuracy by genomic selection. The researchers also identified the genomic regions that may be associated with CH_4 emissions, which they associated with the incidence of some diseases during lactation.

3.12 Methods of estimating enteric CH4 emission

There are various methods available to determine the amount of CH_4 generated by ruminants. Some methods provide approximate values and consider the animal population, and other methods provide information on important parameters, such as climatic factors, nutrition, age, and DMI, among others.

National CH_4 emission inventories for enteric fermentation have been projected by the IPCC (1996, 2006). Their estimates inform the use of methods that estimate CH_4 emissions from enteric fermentation, and facilitate the classification of these methods into three levels: Tier 1, Tier 2, and Tier 3. The basis for Tier 1 is the animal population and only subgroups of that population are distinguished to quantify CH_4 emissions. In Tier 2, the emission factors are estimated in a more complex manner that requires detailed data from individual countries about energy consumption and CH_4 conversion factors for specific livestock categories. The Tier 2 approach entails deeper analysis and is therefore recommended for key animals that are responsible for a significant portion of the country's total emissions. A proper description of the herd and identification of the energy coefficients of the feed on a national level are therefore required. As with Tier 1, the estimate of emissions must be presented in Gg (the equivalent of 1, 000 tons). The IPCC encourages further improvement of proposed Tier 2 methods or the development of a Tier 3 method to estimate CH_4 emissions and recommend the consideration and development of enteric CH_4 mitigation devices. Some factors that could potentially affect feed consumption are not considered in Tier 2, including breed or genotype, temperature, digestibility, and intake. Other factors may affect estimations of energy losses by CH_4, but are not included in Tier 2; they include digestibility, consumption, and composition of dry matter in the diet, digestion kinetics, particle passage rates, and variations in the microbial population of the digestive tract. However, the equations used in these approaches are static and regardless of the extent to which the definitions may be elaborate, environmental variables are not considered to obtain actual values of emission. Ominski et al. (2007) estimated enteric CH_4 emissions from Canadian cattle and found significant differences in values between the Tier 2 and Tier 1 methods. Those authors reported greater values for beef and dairy cattle with the Tier 2 method than with the Tier 1 method (15.27% and 14.7%, respectively). They also pointed out that although the Tier 2 method prioritizes information about production and management strategies, the accuracy of the derived estimates can be improved with greater consideration of population data for all classes and production cycles of the herd.

One of the pioneering research studies in Brazil that applied the prediction equation with pre-determined variables that had been obtained in experiments of ruminal nutrition and microbiology was performed by Malafaia et al. (1997). Those researchers concluded that the use of CH_4 production estimates yield realistic values of ruminant energy losses. In addition, they reported that less productive animals (determined by kg of meat or L of milk produced) emit greater levels of CH_4. They also identified a reduction in CH_4 emissions with the inclusion of lipid supplements in the feed. In comparison to the data generated by the IPCC, the findings of Malafaia et al. (1997) feature a deeper analysis of the zootechnical and nutritional aspects of the systems.

The equations to estimate gas production were derived from results obtained in the field, with beef cattle in production systems of the Southeastern region of Brazil, using the SF_6 tracer gas technique. CH_4 emissions in the ICL system were estimated to be 34, 36, and 43 kg per animal per year, for the years 2006, 2007, and 2008, respectively, with an average of 39 kg per animal per year, over three years. Based on data obtained from the estimated equations, it can be inferred that the ICL system effectively contributes to reducing CH_4 production per kg of meat produced. In addition, with this system, larger quantities of quality feed can be provided, and animal productivity can be enhanced. In this model, CH_4 production from the individual animal is significantly lower than in the IPCC (2006) model, which uses the standard values of 63 kg CH_4 for beef cattle and 56 kg CH_4 for dairy cattle, per animal per year. It should be noted that the SF_6 tracer gas technique is also considered an accurate method of estimating emissions, despite the fact that it is less accurate.

4. Final Considerations

The quest for accurate, simple, and fast methods of measuring CH_4 and other products of ruminal fermentation has been the objective of several studies on ruminant nutrition. However, each method that has been developed has some shortcoming in its holistic definition of ruminal fermentation.

The ex-situ technique of measuring CH_4 from ruminal fermentation has been quite promising, because it facilitates the measurement of CH_4 as well as other fermentation products such as SCFA, and quantifies the relative energy loss of CH_4 in comparison to other fermentation products.

The Green Feed technique is recommended for its approximation to Brazilian cattle production systems, for which the main source of feed is pasture. In contrast, the laser technique is recommended for the evaluation of emissions from animals in confined production systems.

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