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Measurement of Methane Production from Ruminants*

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ABSTRACT: On a global scale agriculture and in particular enteric fermentation in ruminants is reported to produce about one fourth (21 to 25%) of the total anthropogenic emissions of methane (CH_4). Methane is produced during the anaerobic fermentation of hydrolyzed dietary carbohydrates in the rumen and represents an energy loss to the host besides contributing to emissions of greenhouse gases into the environment. However, there appears to be uncertainty in the CH_4 estimation from livestock due to the limited availability of data to document the variability at the farm level and also due to the significant impact of diet on the enteric CH_4 production. The methane mitigation strategies require robust prediction of emissions from rumen. There are many methods available which would be suitable for measuring CH_4 produced from the various stages of animal production. However, several factors need to be considered in order to select the most appropriate technique like the cost, level of accuracy required and the scale and design of the experiments to be undertaken. Selection of any technique depends on the accuracy as each one has its advantages and disadvantages. Screening of mitigation strategies may be evaluated using individual animal before large-scale trials on groups of animals are carried out. In this review various methods for the estimation of methane production from ruminants as well as for the determination of methane production potential of ruminant feeds are discussed. The advantages and disadvantages of the methods starting from respiration chamber, ventilated hood, facemask, sulphur hexafluoride (SF_6) tracer technique, prediction equations and meteorological methods to *in vitro* methods are detailed. (**Key Words:** Methane, Measurement Technique, Ruminant)

INTRODUCTION

It has been reported that methane (CH_4) promotes stratospheric ozone depletion (Blake and Rowland, 1988). The water vapour that is added to the stratosphere when CH_4 is oxidized may provide surfaces for heterogeneous reactions that destroy ozone. Worldwide initiatives such as the Kyoto Protocol demand that these emissions be reduced or at least prevented from further increase (Howden and Reyenga, 1999). Methane is the second major contributor to global warming with a 100-year global warming potential (GWP), 23 times that of CO_2 (IPCC, 2001). Thus despite being present in the atmosphere at far lower concentrations than CO_2, it was reported that CH_4 is responsible for approximately 20% of the greenhouse gas effect (IPCC, 1990; 1992). In ruminants, CH_4 is produced principally from microbial fermentation of hydrolyzed dietary carbohydrates such as cellulose, hemi-cellulose, pectin and starch in the rumen and emitted primarily by eructation. The primary substrates for ruminal methanogenesis are hydrogen and CO_2. Most of the hydrogen produced during the fermentation of hydrolyzed dietary carbohydrates, much of which is generated during the conversion of hexose to acetate or butyrate, ends up in CH_4. Significant quantities of CH_4 can also arise from microbial fermentation of amino acids, the end products of which are ammonia, volatile fatty acids, CO_2 and CH_4. Methane accounts for a significant energy loss to the ruminant animal, amounting to about 8% of gross energy at maintenance level of intake and falling to about 6% as the level of intake rises. Increased understanding and improved quantification of CH_4 production in the rumen has implications not only for global environmental protection but also for efficient animal production.

For the development of an accurate inventory, or to implement mitigation strategies, it is important that there be

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confidence in the accuracy of the CH_4 measurement technology. Methane emissions from livestock have been measured as part of the studies on ruminal fermentation, energy balance, evaluation of feed additives and most recently, to characterize and reduce the contribution of ruminants to the global CH_4 burden. Livestock CH_4 emissions have been measured using respiration calorimetry systems such as whole body chambers, head boxes, ventilated hoods and face masks (Johnson and Johnson, 1995). Data obtained from these techniques have been the foundation of the prediction equations used to generate mathematical models and countrywide and global inventories (Benchaar et al., 1998; Mills et al., 2001). To develop strategies to mitigate CH_4 emissions the precise quantification of CH_4 emissions from ruminants under a wide range of circumstances is very essential.

There are many methods available which would be suitable for measuring CH_4 produced from the various stages of animal production. However, several factors need to be considered in order to select the most appropriate technique like the cost, level of accuracy required and the scale and design of the experiments to be undertaken (Johnson et al., 2000).

Common abbreviations used in CH_4 measurement equipments: ECD-Electron capture detector; FID-Flame ionization detector; FTIR-Fourier transform infrared (spectroscopy); GC-Gas chromatography/Gas chromatograph; TCD-Thermal conductivity detector; TDL-Tuneable diode laser; TGA-Trace gas analyzer; SF_6- Sulphur hexafluoride.

Gas chromatography

The principle is based on the individual partitioning characteristics of different gases in the sample between a mobile phase (an inert gas such as Helium) and a stationery solid phase packed in a column. After separating the components in the gaseous mixture, each component was identified by its retention time on the column and quantified by a subsequent detector. The detector is the key part of the GC system. Three types of detectors are commonly employed for measurement of greenhouse gases: thermal conductivity detector (TCD)-generally for CO_2; flame ionization detector (FID)-sensitive to CH_4; electron capture detector (ECD)-commonly used for N_2O. The detectors may be individually connected to GC systems, or fitted in combination, thus allowing the simultaneous analysis of several gases (Sitaula et al., 1992). Crill et al. (1995) reported that detection limits below 200 ppb are possible with CH_4.

Infrared photo acoustic spectrometer-trace gas analyzer

Beck-Friis (2000) described the operating principle behind the TGA. A gas sample is contained in a sealed cell and irradiated with chopped infrared (IR) light of selected wavelength. The wavelength is specifically absorbed by the gas to be studied and is selected using filters. The energy absorbed by the gas leads to an increase in its temperature and pressure. Since the IR light is chopped, this causes a series of pressure pulses in the cell, which were detected by microphones. The voltage generated by the microphones is proportional to the gas concentration in the cell. TGA is very sensitive to CH_4 (detection limit 100 ppb) and the instrument has the advantage of being portable and is also able to give on-line measurements, however it is very expensive.

Fourier transform infrared (FTIR) absorption spectroscopy

The principle involves infrared light being split into two paths by a two-beam interferometer. When the beams are combined at an infrared detector, constructive and destructive interference produces a modulated signal that is a function of the optical path difference between the two beams. This so-called interferogram is converted into a spectrum by a complex Fourier transform. In FTIR spectroscopy the unique infrared absorption of different molecules are used to quantify their concentration. A number of gases of interest in climate change research could be uniquely and simultaneously determined (Greatorex, 2000).

Tuneable diode laser absorption spectroscopy

This is based on the absorption of an infrared laser beam as it travels along a path through the gas sample. The sensitivity of the TDL based instruments depends on the path according length and the strength of the absorption line, with highest detection sensitivities for gas having strong absorption lines in the spectral region emitted by the laser (Freibauer, 2000). Typical laser emission line widths are small relative to typical absorption line widths, and a high spectral resolution could be achieved in resolving individual absorption lines at atmospheric and low pressures, without interference from other gases (Edwards et al., 1994). In TDL based instruments, detection limits are possible in ppt; its main limitation is the number of gases that could be measured simultaneously with the same diode (Freibauer, 2000) and the cost.

Semiconductor chip sensor

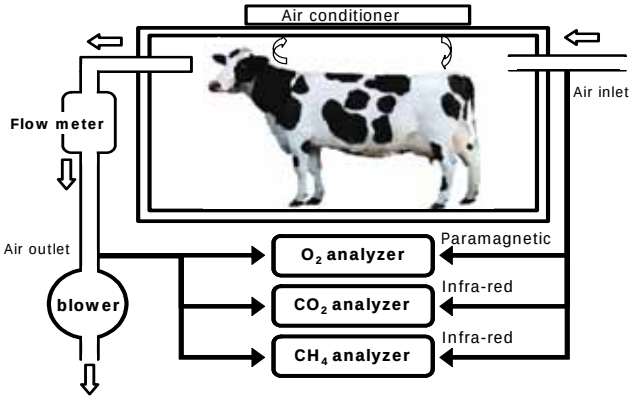
Takenaka et al. (2004) used a novel gas analyzing system using a semiconductor chip sensor (SCGA) designed for the analysis of human expired gases to analyze the environmental gas samples. This sensor can detect hydrogen, CH_4 and carbon monoxide gas within 5 min and with a sensitivity of 0.1 ppm for each gas. The correlation between CH_4 concentrations detected by SCGA and GC was 0.86.

METHANE SAMPLING

There are many options available by which CH_4 emissions from ruminants could be measured. Selection of a technique depends on the accuracy as each one has its advantages and disadvantages. Screening of mitigation strategies may be best evaluated using individual animal before large scale tests on herds of animals are conducted (Johnson et al., 2000).

Respiration calorimeter

The classical standard for ruminant CH_4 measurement by nutritionists is the respiration chamber, or calorimeter. Respiration calorimetry techniques such as whole animal chambers, head boxes, or ventilated hoods and face masks have been used effectively to collect most of the available information concerning CH_4 emissions in livestock. The predominant use of calorimeters has been to measure gaseous exchange as part of energy balance measurements, CH_4 loss being a necessary part of this procedure. There are various designs of calorimeters (Blaxter, 1962), but the most common one being the open circuit calorimeter. The principle behind open-circuit indirect-respiration techniques is that outside air is circulated around the animal's head, mouth and nose and well mixed inside air is collected



Open circuit - indirect calorimeter

(Mclean and Tobin, 1987). The animal is placed in open-circuit respiration chamber for a period of several days, the inputs (feed, oxygen, CO_2) and outputs (excretion, oxygen, CO_2 and CH_4) were measured from the chamber. The chamber should be well sealed and capable of a slight negative pressure. This ensures that all leaks will be inward and not result in a net loss of CH_4. Air conditioning, dehumidification, feeders, waterers and a method by which faeces and urine could be removed are necessary in order to create a comfortable environment within the chamber. Animal movement and normal behaviour should be provided for as much as possible however, some degree of restraint is necessary within the chamber. Experimental factors that should be considered are a) restrictions to the animal's intake to ensure that the experiment can be reproduced, b) stresses on the animal from being in confinement c) lack of environmental stresses on the animal (e.g. lack of heat stress) d) lack of exercise e) experimental duration.

Advantage

The ability to make accurate measurements of emissions including CH_4 from ruminal and hindgut fermentations

Disadvantages

While this technique is satisfactory for measuring CH_4 emission from dried diets, there are difficulties in deriving values that are applicable to the grazing ruminant.

i) Grazing ruminant select their diet, maximum intakes in a chamber are considerably lower than in grazing animals, fresh pasture continue to respire in the chamber.
ii) The restriction of the animal movement
iii) The expenses associated with the construction and maintenance of the chambers.

Ventilated hood

A ventilated hood could also be used to quantify CH_4 emissions using the same principles. This technique involves the use of an airtight box (as shown in the picture)

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that surrounds the animal's head. A sleeve or drape could be placed around the neck of the animal to minimize air leakage. The box must be big enough to allow the animal to move its head in an unrestricted manner and allows access to feed and water.

Advantages

The primary advantage of this technique is the relatively lower cost of the ventilated hood system as compared to a

whole animal chamber.

Disadvantages

i) As with the chamber, use of a hood also requires a restrained and trained animal
ii) The inability to measure all the hindgut CH_4

Facemask

Facemasks may also be used to quantify CH_4 production (Liang et al., 1989). The principle behind the use of the facemask is the same as that of the chamber and hood.

Advantages

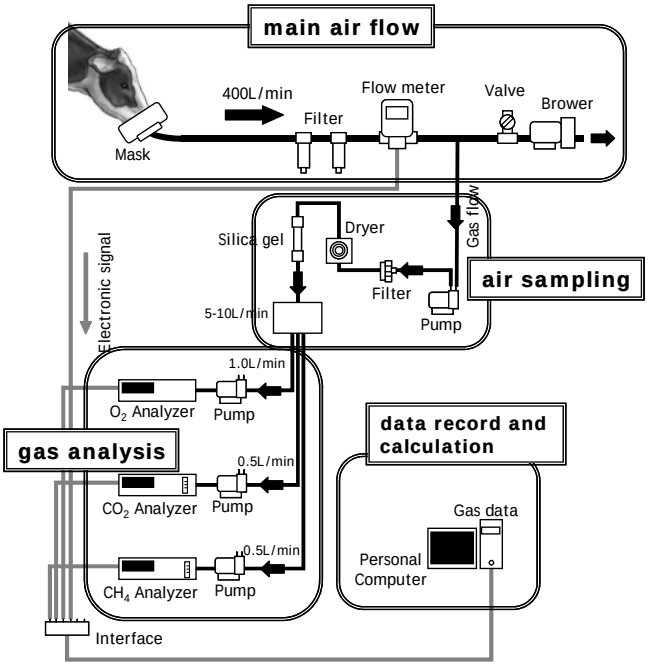
The primary advantages of this method are the simplicity and lower cost. They can also be used to collect the expired gas from the grazing animals periodically and estimate CH_4 production.

Disadvantages

The facemask, compared with chamber methods, underestimates heat production and likely CH_4 as well by an average 9% (Liang et al., 1989). It requires animal cooperation and eliminates its ability to eat and drink. Because of the normal daily variation in emissions meaningful CH_4 emission measurements is difficult and hence short term measurements might lead to erroneous results (Johnson and Johnson, 1995).

Ventilated flow-through method with a face mask

In the facemask technique, the mask for the collection of expired air has to be tightly sealed around the face of the animal, which may be stressful for the animal and also the Douglas-bag for the collection of gas is not easy to handle. Kawashima et al. (2002) developed a ventilated flow-through method with a facemask. The system consists of four major components 1) main airflow system component 2) air-sampling component 3) gas analysis component 4) data record and calculation system.



(Courtesy-Dr. T. Nishida, JIRCAS)

Terada (1999) examined 3 factors viz. individual animal, daily difference and diurnal changes to influence the accuracy of the CH_4 measurement. The variance related to diurnal change was the largest among the three factors. It was suggested, based on these analyses, that a respiration trial should be conducted for 2-3 days, 4-6 times a day with 4 experimental animals.

Calculation

The principle is based on the Brouwer's equation for measurement of heat production.

$$HP = 16.18 \times V\,O_2 + 5.16 \times V\,CO_2 - 5.90 \times N - 2.42 \times V\,CH_4$$

Where, HP: heat production (kJ); V O_2: oxygen consumption (liter at STP); V CO_2: carbon dioxide production (liter at STP); N: urinary nitrogen excretion (g); V CH_4: methane production (liter at STP).

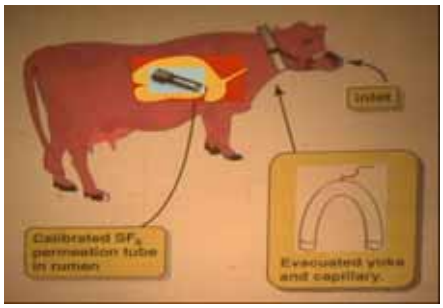
TRACER GAS TECHNIQUES

Methane emission from ruminants can also be estimated by using the ERUCT (Emissions from Ruminants Using a Calibrated Tracer) technique. The tracer can either be isotopic or non-isotopic. Isotopic tracer techniques generally require simple experimental designs and relatively straightforward calculations, at least for the lower number pools (Johnson and Johnson 1995). Isotopic methods involve the use of (^{3}H-) methane or (^{14}C-) methane and ruminally cannulated animals (Murray et al., 1975). Using the continuous infusion technique, infusion lines deliver the labeled gas to the ventral part of rumen and sampling of gas takes place in the dorsal rumen. After determining the specific activity of the radio-labeled methane gas, total methane production can be calculated. It is also possible to measure CH_4 production from a single dose of injection of tracer (France et al., 1993). France et al. (1993) described models for up to three and higher CH_4 pools.

Disadvantages

Difficulty in the preparation of the infusion solution is the major limitation when isotopic tracers are used because of low solubility of CH_4 gas.

Non-isotopic tracer techniques are also available for measurement of CH_4 emissions. Johnson et al. (2000) described a technique using sulphur hexafluoride (SF_6), an inert gas tracer. Emissions from groups of animals in a



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room or groups on pasture are possible through the release of a tracer into the room or pasture area.

For individual animal measurement, a calibrated source of SF_6 is placed in the rumen *per os* prior to an experiment. The source of SF_6 is a permeation tube, and the rate of release of SF_6 is controlled by a permeable Teflon™ membrane held in place by a porous stainless steel frit and a locking nut. The release rate of the gas permeation tube is calibrated at 39°C by regular weighing for a period, prior to its insertion into the rumen. The tubes for sheep, typically 35 mm length by 11 mm in external diameter, are made from brass rods and weigh about 32 g.

Each test animal is fitted with a halter, which supports an inlet tube that is placed so that its opening is close to the nose. As the vacuum in the sampling canister/yoke slowly dissipates a steady sample of the air around the mouth and nose of the animal is taken. By varying the length and diameter of the capillary tube the duration of sampling may be regulated. The yoke is easily isolated for daily changing by means of a shut-off valve and quick connect fittings. Yoke volumes are typically 1.7 and 2.5 litres for sheep and cattle respectively, and the capillary system is designed to deliver half this volume during the collection period of 24 h. An identical apparatus needs to be placed each day to collect an integrated background air sample. After collection of a sample the yoke is pressurized with nitrogen, and CH_4 and SF_6 concentrations are determined by gas chromatography.

Methane emission rate is calculated as follows;

$$QCH_4 = QSF_6 \times [CH_4]/ [SF_6]$$

Where QCH_4 is the emission rate of methane in g/day; QSF_6 is the known release rate (g/day) of SF_6 from the permeation tube; $[CH_4]$ and $[SF_6]$ are the measured concentrations in the canister.

Johnson et al. (1994) compared 55 measurements using the SF_6 technique with 25 chamber measurements of cattle, and showed that while the SF_6 estimates were 0.93 of those in the chamber, the difference was not significant. Kurihara (personal communication) compared a set of 27 SF_6 measurement data where, each data was the average of 3-4 SF_6 measurements, with another set of 27 chamber data where, each data was the average of 2-3 chamber measurements from Holstein heifers or dry cattle and observed that the SF_6 estimates were 0.84-1.02 (mean = 0.99; $p>0.1$) of those in the chamber. Pinares-Patino (2000) in New Zealand found in an experiment with 10 sheep fed chaffed Lucerne hay that CH_4 production estimated from SF_6 was 0.95 chamber emission. Boadi et al. (2002) compared estimates of CH_4 production using the SF_6 tracer technique (137.4 L/d) with an open circuit hood calorimeter (130.0 L/d) using yearling beef heifers and found no significant difference ($p = 0.24$). However, Pinares-Patino (2000) in two experiments with sheep obtained SF_6 results that were extremely variable compared to chamber. McCrabb and Baker (cited by Ulyatt et al., 1999) measured CH_4 production from 5 Friesian calves fed Rhodes grass (*Chloris gayana*) hay, using both SF_6 and a confinement-type respiration chamber. The estimate of CH_4 production made with the respiration chamber (7.7±0.67 L/h) was twice that ($p<0.005$) estimated using SF_6, either in pens (4.1 ±0.35 L/h) or in the chamber (4.0±0.46 L/h). SF_6 has also been used successfully as tracer to estimate the total CH_4 emission from all the cattle in a barn (Kaharabata and Schuepp, 2000).

Advantages

i) This technique eliminates the necessity to restrain or enclose the animal, thus allowing the animal to move about and graze.
ii) It is also not necessary to sample directly from the animal's rumen or throat because the use of the tracer accounts changes in dilution associated with head or air movement.

Disadvantages

i) SF_6 is a greenhouse gas itself, with a GWP 23,900 times that of CO_2 and an atmospheric lifetime of 3,200 years (Machmuller and Hegarty, 2005).
ii) The residue of SF_6 in meat and milk from farm animals is another issue.
iii) It is necessary to train the animal to wear a halter and collection yoke/canister.

Class	Tracer	Advantages	Disadvantages
Isotopes	$^{14}CH_4$, CH^3_4 radioisotopes	Very low detection limits	Radioactive- not useful in food chain
	$^{13}CH_4$ stable isotope	Little C transfer to other molecules OK for food chain	Very expensive
	CH^2_4 stable isotope	Cheap, safe, OK for food chain	Hydrogen transfer to other molecules
Noble gases	Argon	Low detection limit and moderate cost	High background concentration
	Xenon	Low background	High cost
	Krypton	Low background	Very high cost
Other Gases	Ethane	Availability	High detection limit
	Propane	Availability	High detection limit

Source: Hegarty et al. (2004)

iv) This tracer technique does not measure all of the hindgut CH_4. Any CH_4 from the hindgut that is absorbed into the blood stream will be expired and collected but any CH_4 that escapes absorption and is released from the rectum is not collected.

Ethane (C_2H_6) has also been used as a marker to estimate CH_4 emission (Moate et al., 1997; Mlbanzamihigo et al., 2002) using essentially the same principle as SF_6. The major difference in the use of the two tracers is that ethane has to be bubbled from an external source into the rumen, and so is not suitable for use in grazing experiments. Hegarty et al. (2004) listed possibility of other markers for measuring enteric CH_4 emissions and approaches being considered. Machmuller and Hegarty (2005) identified ethane and stable isotopes of methane as promising alternative tracer gases for the ERUCT technique.

METEOROLOGICAL TECHNIQUES

There is a range of meteorological techniques, classified as 'bottom up' or direct measurement of emissions from a known number of animals at the ground level, or 'top down' techniques that infer land-based emissions from their atmospheric signature that have been employed to try and validate predictions of greenhouse gas inventories (Beswick et al., 1998; Denmead et al., 2000).

Tunnel technique

Lockyer and Jarvis (1995) and Lockyer (1997) described a system in which air was drawn across animals enclosed in a 4.3×9.9 m polythene-clad tunnel placed over pasture. Various numbers of sheep and calves were enclosed for up to 10 days; and CH_4 emission was estimated to be on an average 13-14 g/d for sheep and 74.5 g/d for calves. In both the studies CH_4 emission declined with time, probably in response to declining feed availability given the very high stocking rates (470 sheep or calves per ha with two animals in the tunnel and 2,818 sheep/ha with 12 sheep in the tunnel).

Murray et al. (1999) conducted two experiments to compare CH_4 emissions from sheep housed either in a poly-tunnel system or in open-circuit respiration chambers. In each system, the sheep received maintenance levels of either cut grass or high temperature dried grass pellets. The results indicated that CH_4 production from chamber was greater (31.7 L/kg DMI) than the tunnel system (26.9 L/kg DMI).

Disadvantage

The method is not suited for evaluating differences between imposed experimental treatments (Denmead et al., 1998).

Denmead et al. (1998), Harper et al. (1999) and Leuning et al. (1999) described a variant of the mass balance approach in which animals were fenced in a 22×22 m enclosure, and gas was sampled from many ports on a framework up to 3.5 m high surrounding the enclosure. Wind speeds were measured from anemometers at the same levels as the sample ports. The advantage of this technique is that it can accommodate changes in wind direction. This technique was used by Leuning et al. (1999) to measure CH_4 emission for five days from 14 sheep grazing a grass and legume pasture. Methane concentration from the sample ports was measured on-line by high precision FTIR spectroscopy. Seven of these animals were used concurrently to measure CH_4 emission using the SF_6 tracer technique. The daily mean values for the two techniques were similar: 11.7 g/d for the SF_6 technique and 11.9 g/d for the mass balance measurements.

Disadvantages

i) With this technique, a very high stocking rate (289 sheep/ha) is required to achieve a differential in gas concentration that can be measured.
ii) It is also not suitable for experiments where treatments need to be evaluated (Denmead et al., 1998).

Top down techniques

Denmead et al. (2000) reported a range of meteorological techniques that have been developed to determine areal emissions from their atmospheric signature.

References	Technique	Animals	BW (kg)	Milk (kg/d)	CH_4 (L/h/d)
Sechen et al. (1989)	Respiration calorimetry	6 lactating cows	603	37.1	557
Sechen et al. (1989)	Respiration calorimetry	6 lactating cows	603	41.3	470
Kirchgessner et al. (1991)	Respiration calorimetry	67 lactating cows	583	17	420
Kinsman et al. (1995)	Mass balance	118 lactating cows	602	28.5	552
Sauer et al. (1998)	Mass balance	88-109 lactating cows	600	29	622
Kaharabata et al. (2000)	Atmospheric-SF_6 technique	90 Holstein cows	600	28.5	542
Kaharabata et al. (2000)	Atmospheric-SF_6 technique	147 dry heifers	ND	-	631
Westberg et al. (2001)	Internal SF_6 tracer	4 lactating cows	673	22	623
Westberg et al. (2001)	Internal SF_6 tracer	4 lactating cows	673	22	566
Johnson et al. (2002)	Room SF_6 tracer	36 lactating cows	600	32.3	543
Johnson et al. (2002)	Room SF_6 tracer	36 lactating cows	600	39.3	550
Johnson et al. (2002)	Room SF_6 tracer	36 lactating cows	600	39.1	637

Source: Boadi et al. (2004)

These vary in scale from flux gradient analysis designed to measure at the paddock scale, to boundary layer techniques that integrate fluxes over larger areas of the landscape (Beswick et al., 1998; Denmead et al., 2000; Wratt et al., 2001). Judd et al. (1999) used a micrometeorological flux gradient technique to estimate CH_4 fluxes for 5 days across a paddock grazed by sheep. Samples of air were drawn from two heights (3.8 and 1.2 m) on a tower sited on the downwind boundary of the experimental area; wind speed and direction were also measured from the tower. Four 3 ha paddocks upwind of the tower were stocked at 20 sheep/ha, and 11 of these sheep were used to estimate CH_4 emission for the 5 days using the SF_6 tracer technique (Lassey et al., 1997). The CH_4 emission estimated by this technique was 19.5±4.8 g/d, which compared well with the SF_6 tracer measurements of 19.4±4.2 g/d. A similar measurement system was described by Denmead et al. (2000) who found good agreement with inventory predictions, but the error was too high for detection of small changes that might be important for inventory, regulatory or animal science experimental purposes.

Disadvantages

i) The method of Judd et al. (1999) is inflexible in that it requires a large fetch of undisturbed air on the upwind side of the sampling tower, it can only be used on rainless days when the wind is in one direction, and it can be affected by the movement of animals within the measurement footprint.

ii) It measures from groups of animals and thus is not suited to evaluating differences between treatments.

Boundary layer techniques

Variations of the boundary layer technique have been used to estimate CH_4 emissions over larger land areas. Basically, vertical profiles of gas concentration are determined through the depth of the atmospheric boundary layer, and these data have been used in various modeling techniques to infer emissions over a specified land area. In one series of experiments in Australia, a 22 m tower and an aircraft were used to collect samples of gas at different heights within the convective boundary layer, and gas budgeting techniques were used to estimate CH_4 emissions (Denmead et al., 2000; Griffith et al., 2002). In another application in New Zealand (Lassey et al., 2000b; Wratt et al., 2001; Gimson et al., 2002) air samples were collected by light aircraft from two columns of air, one upwind and another towards the downwind boundary of the target site. Night time measurements utilizing the gas concentrating effect of the nocturnal boundary layer have also been made, usually taking gas samples at various heights up a profile using a balloon (Denmead et al., 2000; Harvey et al., 2002) or sampling from a tower (Griffith et al., 2002). Harvey et al. (2002) have also proposed an isotope dilution/ mass balance technique for use in conjunction with the nocturnal boundary layer method. Denmead et al. (2000) reviewed the strengths and weaknesses of a range of meteorological flux measurement techniques and concluded that these methods provide estimates of CH_4 emission that have reasonable agreement with inventory estimates. However, the error was generally too high for detection of small changes that might be important for inventory, regulatory or animal science research.

Advantages

i) The technique gives an integrated net emission of all sources in their footprint

ii) In terms of farm management the technique is non-invasive

Disadvantage

i) Inflexible in terms of limited climatic conditions in which they can operate.

Boadi et al. (2004) compared the methane production measured by respiration calorimetry, mass balance and SF_6 tracer technique in dairy cows and heifers.

PREDICTION EQUATIONS

Feed characteristics can also be used to calculate CH_4 production. Number of empirical regression equations, based on the results of calorimetric experiments, has been developed. The Blaxter and Clapperton (1965) equation is the basis from which most all estimates of CH_4 production from ruminants have been derived.

$$CH_4 (\% GE) = 1.30+0.112 D+L (2.37-0.50 D)$$

Where, D = energy digestibility at maintenance level of feeding; L = feeding level

The relationship was derived from a series of CH_4 production measurements from mature sheep fed a range of diets. Moe and Tyrell (1979) proposed another equation;

$$CH_4 = 3.406+0.510 (\text{soluble residue}) +1.736 (\text{hemi-cellulose}) +2.648 (\text{cellulose})$$

Where, CH_4 in MJ/day and soluble residue, hemi-cellulose and cellulose in kg fed/day (R^2 = 0.67). The relationship was derived from measurements made from cattle fed high-quality dairy rations and relates soluble residue, hemi-cellulose, and cellulose to CH_4 production.

Wilkerson et al. (1995) evaluated number of equations for accuracy of prediction of CH_4 production for Holstein cows against a set of data compiled at the USDA, Beltsville and concluded that the equation of Moe and Tyrell (1979) was the most accurate for the prediction of CH_4 production from dry and lactating cows. However, the mean absolute error of prediction (11.0% of the mean) was still high. Further the equation is probably limited in application to dairy cows confined indoors and fed the typical high concentrate type of diet used in the US (Denmead et al., 2000). Yan et al. (2000) published a specialized equation for dairy and beef cattle offered grass silage-based diets where the independent variates are digestible energy intake, the proportion of ADF in the diet, and the level of feeding above maintenance. The R^2 was 0.89, indicating a reasonably good fit.

$$CH_4\text{-E (MJ/day)} = \text{DEI (MJ/d)} (0.094+0.028_{ADFI}/T_{ADFI}) -2.453 (FL-1)$$

$$CH_4\text{-E (MJ/day)} = \text{DEI (MJ/d)} (0.096+0.035_{DMI}/T_{DMI}) -2.298 (FL-1)$$

McCourt et al. (2005) developed several prediction equations for beef cattle and concluded that factors such as forage proportion (FT), feeding level (FL) and live weight improved the accuracy (R^2= 0.77)

$$CH_4 = 9.98 DEI-0.95 LW-432.3 FL+2.54 F/T+470.7$$

Shibata et al. (1992) also developed set of equations after trials with Holstein heifers, Corriedale wethers and Japanese goats and concluded that CH_4 production from ruminants fed below 1.5 times maintenance can be predicted from DMI alone (r = 0.992)

$$CH_4 (L/day) = 0.0305 DMI (g/day)-4. 441$$

From the results obtained from 190 energy balance trials with dairy cattle, beef cattle, sheep and goats Shibata et al. (1993) developed a quadratic equation for CH_4 production (Y, L/day)

$$Y = -17.766+42.793 X-0.849 X^2 \qquad (r = 0.966)$$

Where, X = DMI (kg/day)

Pelchen and Peters (1998) developed equations for sheep from 1,317 sets of data from the literature. There was a wide range of diet types, including a few from animals fed fresh herbage. By grouping the sets of data according to criteria such as digestibility (<65, 65-70, 70-75, >75) and crude fibre (<15, 15-20, 20-25, 25-30, >30) they derived regressions for predicting CH_4 emission (g/d) with R^2 = 0.7-0.85.

Dynamic and mechanistic models to predict CH_4 from ruminants have also been established to simulate ruminal fermentation under a variety of nutritional conditions (Mills et al., 2001). Benchaar et al. (1998) showed that mechanistic models allow the prediction of CH_4 production more accurately than simple regression equations, under a large variation of diet composition. Regression analysis showed good agreement between observed and predicted results by modeling experimental data taken from the literature (r^2 = 0.76, root means square prediction error = 15.4%; Mills et al., 2001). Predictions with Blaxter and Clapperton (1965) and Moe and Tyrell (1979) regressions were poor, with R^2 = 0.57 and 0.42 respectively. Johnson et al. (1993) compared the actual CH_4 measurements and those predicted by Blaxter and Clapperton equation. The equation predicted CH_4 production to range from 6 to 10% with most points in the 6 to 8% range. Actual CH_4 measurements ranged from 2 to 11%. Although these models have usefulness in the prediction of CH_4 production from animals under the conditions from which the equations or models are developed, they are of limited use in the prediction of CH_4 production when intake is unknown or when the rumen is disturbed (Johnson et al., 2001). Recently Blummel et al. (2005) after comparing CH_4 production in sheep measured in open circuit respiration chamber with that calculated from VFA profile in IVGPT concluded that the large variation in CH_4 production from a

range of diets renders these equations less useful which assume constant relationships of CH_4 production. Equations for predicting CH_4 emissions were developed mostly from data using the respiration calorimetry chamber to define the relationship between energy intake and CH_4 production are based mainly on the diet characteristics. The environment inside the respiration chamber is controlled and the animals are under feed restriction during measurement. Therefore the data from the chamber cannot be applied under every farm situation, especially where animals are grazing and pasture quality is changing. Also they are not accurate enough for use in determining small differences between animals in experiments.

IN VITRO TECHNIQUES

Although *in vivo* studies using actual animals are ultimately needed, however an *in vitro* simulation of the rumen can often be effective and efficient because of its relative readiness and cheapness of operation. It can also be used to define the effects of a specific factor which might be concealed in an *in vivo* study behind the complexity of a lot of related factors in the rumen environment.

Rumen simulation technique (RUSITEC)

In the RUSITEC, solid feeds are confined in nylon bags that are normally replaced by new bags once a day. The amount of ration is small (10-25 g DM/day/L of vessel), and the set points of the liquid dilution rate are also small (2-5%/h) as compared with the actual *in vivo* values.

Calculation of methane production

The gas produced from each fermenter is collected in polythene/rubber bags and the volume of gas is recorded using a dry gas meter. From the gas samples, the concentration of CH_4 is measured in gas chromatograph (x). The volume of CH_4 gas produced is calculated from the total volume of gas produced after 24 h in the fermenter (y). The CH_4 production should be converted to STP value (1 atm, 0°C) for comparison with CH_4 measured by other techniques.

$$\text{Methane in ml (at STP)} = (\text{Methane ml}) \times [273/(273+39)] \times \frac{(\text{atmospheric pressure at the experiment})}{(\text{standard atmospheric pressure})}$$

Methane is expressed as ml/g DM incubated in the fermenter or ml/g DDM (if DM digestibility is estimated) or as % of GE.

Bhatta et al. (2006_b) observed that RUSITEC ($p<0.001$) underestimated CH_4 production as compared to that estimated by SF_6 technique. Eun et al. (2004) reported that CH_4 estimated from head space gas in dual-flow fermenters was significantly lower than CH_4 estimated from the stoichiometric calculation at a dilution rate of 3.2 %/h from diets ranging in concentrate and roughage ratio (Table 1).

Advantages

i) Constructional simplicity and operational easiness.
ii) The easy operation of the device can increase the number of fermenters that can be used at a time.

Disadvantages

i) A higher dilution rate of more than 4.0 %/h would make the concentration of the fermentation products lower than the values normally observed in the actual rumen (Czerkawski and Breckenridge, 1977).
ii) The difficulty in obtaining a uniform sample because of the stratification of the fermenter contents into compartments that are typically depicted in three parts: free liquid, a solid associated part which can be washed out and a solid associated part which cannot be washed out. The effluent sample from the RUSITEC, however only represents the liquid part of the contents, and this liquid has a lower microbial density than the solid associated compartments.
iii) Protozoa numbers in the effluent gradually decreases as the incubation proceeds and settles at around 3,000/ml after the 8th day for 3.0 %/h dilution rate (Kajikawa et al., 2003). Hence, CH_4 output will be significantly low, if the gas sampling is commenced after the 8th day (Bhatta et al., 2006b).

In vitro gas production technique (IVGPT)

Various aspects of *in vitro* gas production test have been reviewed by Getachew et al. (1998). Initial works on gas measurement (McBee, 1953; El-Shazly and Hungate, 1965; Czerkawski and Breckenridge, 1969, 1970) were centered on investigations of rumen microbial activities using manometric measurements. A manometric method of gas measurement for the evaluation of rumen microbial activity with respect to cellulose and hemi-cellulose fermentation

Table 1. Comparison of CH_4 production from stoichiometric equation and head space gas (Eun et al., 2004)

	HF	MF	LF
CH_4 estimated* (mmol/d)	13.1	13.1	13.4
CH_4 measured** (mmol/d)	9.6	6.1	4.2

HF = High forage (70% roughage: 30% concentrate); MF = Medium forage (50% roughage: 50% concentrate).
LF = High forage (30% roughage: 70% concentrate); Dilution rate: 3.2%/h.
* (Acetate, mmol/d) + (2×butyrate, mmol/d)-(CO_2, mmol/d); ** Methane measured from headspace gas.

was developed by McBee (1953). El-Shazly and Hungate (1965) measured rumen fermentation rates with rumen contents and mineral salt solutions where, different amounts of hay were incubated using the constant volume manometric method. Czerkawski and Breckenridge (1969) developed a gas measuring manometric apparatus to investigate the effect of fatty acids on the fermentation of sugar-beet pulp and sucrose by mixed rumen micro-organisms. Although the manometric methods permits a quantitative determination of acids and gases evolved during the fermentation, and allow incubation of a large amount of sample (Hungate et al., 1955), the number of samples that could be handled is less. Getachew et al. (1998) concluded that manometric methods of gas measurement do not have wide acceptability in routine feed evaluation since there was no provision for the mechanical stirring of the sample during incubation. Wilkins (1974) developed an automated pressure transducer method for measuring gas production by microorganisms and it created a basis for the development of the pressure transducer method for feed evaluation. The advantages of the gas measuring techniques over the other *in vitro* techniques (Tilley and Terry, 1963) for feed evaluation have been outlined by Blummel and Orskov (1993) and Makkar et al. (1995). There are basically two approaches for measuring gas volumes: i) measuring gas collected at atmospheric pressure and its volume determined directly or ii) measuring gas accumulated in a fixed volume container, and the volume is calculated from pressure changes. Within these broad groups, there are different gas measuring techniques developed for the evaluation of feed quality. The available gas measuring techniques are i) Hohenheim gas method or Menke's method (Menke et al., 1979) ii) Liquid displacement system (Beuvink et al., 1992) iii) Manometric method (Waghorn and Stafford, 1993) iv) Pressure transducer systems: Manual (Theodorou et al., 1994); Computerized (Pell and Schofield, 1993); Combination of pressure transducer and gas release system (Devies et al., 1995; Cone et al., 1996).

Menke et al. (1979) developed a feed evaluation system using an *in vitro* gas measuring technique. Fermentations were conducted in 100 ml calibrated syringes containing the feedstuff and a buffered rumen fluid. In this system, gas production in 24 h observed on incubation of 200 mg feed dry matter correlated well with digestibility of organic matter determined *in vivo* with sheep. The method of Menke et al. (1979) was modified by Blummel and Ørskov (1993) in that feed samples were incubated in thermostatically controlled water bath instead of a rotor in an incubator. Blummel et al. (1993) and Makkar et al. (1995) further modified this method. The main advantage

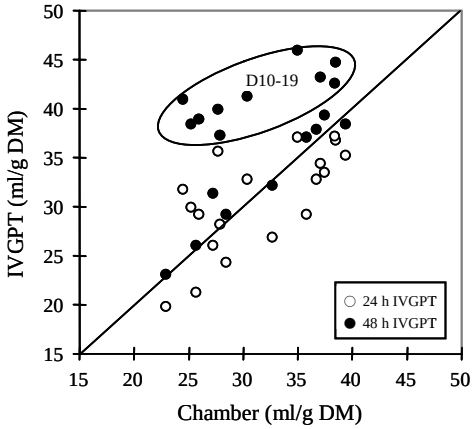
IVGPT

(National Institute of Livestock and Grassland Science - Japan)

of the modified method over the original method of Menke et al. (1979) is there is only a minimum drop in the temperature of the medium during the period of recording gas readings on incubation of syringes in a water bath. At the National Institute of Livestock and Grassland Science, Japan studies are being conducted to compare CH_4 production measured in respiration chamber and by SF_6 technique with that of IVGPT. The feed samples (200 mg DM) were incubated in 100 ml syringes kept in shaking water bath as shown above. Gas samples were collected in small-evacuated vials after 24 and 48 h of incubation. The concentration of CH_4 (%) in the gas samples was determined by the gas chromatograph. Methane production potential of the feeds was calculated based on the net gas produced (sample gas-blank). Methane production potential was expressed as ml (STP)/g DM incubated. The results indicated that the CH_4 output estimated by IVGPT was very close to that of SF_6.

Getachew et al. (2005) in a study with total mixed rations of dairy has reported similarity between measured and calculated CH_4 values with IVGPT and suggested that CH_4 production can be calculated if only gas and VFA production is measured. Blummel et al. (2005) suggested that CH_4 production in forage fed ruminants could be predicted by a simple *in vitro* technique that measured gas production and true substrate degradability, if feed intake is known.

In a series of experiments methane production from goats measured in open circuit respiration chamber from 19 diets were compared with that estimated by IVGPT. Methane production estimated from the cumulative gas collected after 48 h *in vitro* incubation was similar to that measured in respiration chamber in samples D_1 to D_9 and significantly ($p<0.05$) higher in D_{10} to D_{19} (Figure). Diets D_1 to D_9 contained higher fibre components (NDF and ADF) and the TDN values were lower compared to D_{10} to D_{19}. It was concluded that for diets containing low fibre and with higher digestibility, 36 h incubation produced good estimate to CH_4 compared to that measured in respiration



Figrue 1. Comparison of CH_4 production (ml/g DM) measured in respiration chamber with that of CH_4 estimated after 24 h and 48 h IVGPT (Bhatta et al., 2007).

chamber. These results indicate that IVGPT could be used to estimate the CH_4 production from a range of diets to have a database and to plan mitigation strategies in ruminants to improve the performance as well as to reduce the greenhouse gas.

Various methods are available for measurement of CH_4 production from ruminants as well as from ruminant feeds. The choice of a method depends on the accuracy; however the critical factor is the budgetary provisions. *In vitro* gas production technique appears to have the capacity to determine the CH_4 production potential of ruminant diets. However, further studies are needed to evaluate this technique to reflect the treatment difference among the feeds.

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