Methane Gas Emission from Ruminants

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Table of Contents

CHAPTER-1: ABSTRACT

The rearing of ruminants for domestic consumption and export invariably lead to the production of CH_4 as a product of digestion. CH_4 has more warming power than carbon dioxide. This study investigated the emission of CH_4 from Chittagong Veterinary and Animal Science University sheep farm. The study was conducted to estimate CH_4 from sheep. The study worked with 48 gas samples where 24 samples before taking breath of sheep and another 24 samples after taking breath of sheep. Here between two samples maintained 6 hours gap. All the samples collected from 500 liter water tank. After sampling, samples were calculated by gas chromatography for knowing the level of CH_4. In this study after gas chromatography analysis one sheep can produce 2.79 liter per day.

CHAPTER-2: INTRODUCTION

CH_4 is a green house gas (GHG) with a global warming potential 28-fold that of carbon dioxide (Geneva: IPCC; 2014. p. 15). Agriculture makes a significant contribution to total GHG production, with estimates varying according to country and calculation method (Hristov et al.,2013). It has been reported that (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 (Howden and Reyenga, 1999). 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). Increasing atmospheric concentrations of CH_4 have led scientists to examine its sources of origin. The level of CH_4 production results in estimates of the contribution by ruminants to global warming that may occur in the next 50 to 100 yr to be a little less than 2%(Johnson et al., 1995).Many factors influence CH_4 emissions from cattle and include the following: level of feed intake, type of carbohydrate in the diet, feed processing, addition of lipids or ionophores to the diet, and alterations in the ruminal microflora. Manipulation of these factors can reduce CH_4 emissions from cattle(Johnson et al., 1995)

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. CH_4 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. Many techniques exist to quantify CH_4 emissions from individual or groups of animals(Bhatta et al.,2007). Here we collect CH_4 gas cost effective face mask method (Oss et al.,2016;Silveira et al.,2019). Then measure CH_4 gas by gas chromatography. The primary advantages of this method are the simplicity and lower cost. It can also be used to collect the expired gas from the grazing animals periodically and estimate CH_4 production (Bhatta et al.,2007).

Objectives:

To estimate CH_4 gas emitted from ruminant animal.

CHAPTER-3: REVIEW LITERATURE

3.1.The SF_6 tracer technique:

This method is relatively new and was first described in 1993–1994 (Johnson et al., 1994; Lassey et al., 1997).. The main purpose of the method was to investigate energy efficacy in free ranging cattle (Zimmerman 1993), because it had been queried that results obtained in respiration chambers could not be applied to free ranging animals(Okelly et al.,1789–1793). The basic idea behind the method is that CH_4 emission can be measured if the emission rate of a tracer gas from the rumen is known. For this purpose a non-toxic(Nes et al.,2010) , physiologically inert(Johnson et al., 1994), stabile gas is needed. Furthermore, the gas should mix with rumen air in the same way as CH_4. SF_6 was chosen , because it fulfills the above criteria, is cheap, has an extremely low detection limit and is simple to analyze.The SF_6 tracer technique is based on inserting a calibrated source of SF_6 (sulfur hexafluoride) into the rumen of each participating animal. This inert tracer, which discharges from a 'permeation tube' (Lassey et al., 2001), has the virtue of being quantitatively detectable in gas samples at very low levels (parts per 1012). Time-integrated breath samples are collected, usually over 24 h, and the ratio of the CH_4 to SF_6 release rates is equated to the ratio of their background corrected concentrations as measured in the breath sample (Johnson et al., 1994; Lassey et al., 1997). Repeated 24-h samples collected over 5 successive days generally display good day-to-day consistency in inferred daily emission for each animal, such that the variance in per-animal daily emission averaged across the herd or flock is dominated by inter-animal variation (Lassey et al., 1997). Uncertainties inherent in the SF_6 tracer technique arise from: extrapolation of permeation tube performance (Lassey et al., 2001); variations in breath collection efficiency throughout the collection period (important only if the CH_4 production rate also varies); concerns that the imposition of sampling equipment may affect feeding behaviour; and a dearth of data on the proportion of CH_4 released from the anus (undetected by the SF_6 tracer technique). The SF_6 tracer technique is widely adopted in many countries, including the U.S.A. (Pavao-Zuckerman et al., 1999; Johnson et al., 2000b; Westberg et al., 2001; DeRamus et al., 2003), Canada (McCaughey et al., 1997, 1999; Boadi et al., 2002a, 2004), New Zealand (Lassey et al., 1997; Judd et al., 1999; Lassey and Ulyatt, 2000; Lassey et al., 2002; Ulyatt et al., 2002a,b, 2005; Pinares-Patin˜o et al., 2003d), Australia (Leuning et al., 1999), Ireland (F. O'Mara, University College Dublin, personal communication, 2001), France (Pinares-Patin˜o et al., 2003a), Brazil (Primavesi et al., 2004), India (A. K. Srivastava, National Dairy Development Board, Gujarat, India, personal communication, 2003), China (H. Dong, Agrometeorology Institute, Beijing, China, personal communication, 2003).

3.2.Respiration Chamber:

Respiration chamber is a well-established, well-documented and reliable CH_4 measurement system, as it is the "gold standard" that accurately measures total CH_4 production from rumen and hindgut fermentation(Hammond et al., 2011; Zhao et al., 2015; Hynes et al.,2016). In this technique, an animal is held in a sealed chamber which is large enough to comfortably accommodate them and which is maintained under slightly negative atmospheric pressure.

This ensures that any undetected or unavoidable gaseous leaks flow inwards rather than outwards, thereby avoiding any loss of gaseous product (Johnson et al., 1995; Storm et al., 2012). CH_4 emissions are calculated by the measured airflow multiplied by the difference in concentrations between the inlet and outlet air. This is facilitated by automated sampling and analysis using an infrared gas analyzer, which repeatedly determines the concentration of CH_4 in both the inlet and exhaust air. Often, a multi-gas analyzer which integrates the measurements of CH_4 CO_2, O_2 and NH_3, etc., is used to investigate the GHG emissions and heat production of the animals simultaneously(Zhao et al.,2016; Yang et al.,2019).

3.3.Supplementary feed intake determinations:

Because the CH_4 is derived from ingested feed, measuring CH_4 emitted without also measuring feed ingested limits both data utility and opportunities to investigate emission determinants. A more universal measure of emission is the dimensionless 'CH_4 conversion factor', also known as the 'CH_4 yield', Ym, which is the CH_4 emitted per unit of feed intake with both CH_4 and intake expressed as energies of combustion. Most feeds contain about 18.4 MJ of gross energy (GE) per kg of dry matter (DM) and CH_4 has energy content 55.65 MJ/kg, so that a typical Ym value of 6% corresponds to 19.8 g CH_4/kg DM intake. However, determining feed intake by grazing animals is particularly difficult (discussed below), and intake estimates will usually be the biggest source of uncertainty in SF_6-based estimates of Ym for individual animals. When averaged across a herd or flock, a confounding uncertainty will be inter-animal variation 122 K.R. Lassey / Agricultural and Forest Meteorology 142 (2007) 120–132 in Ym (Lassey et al., 1997). While confining the animals under controlled feeding conditions will markedly reduce intake uncertainty, it may also alter the feeding behaviour and feed selection relative to freely grazing animals. Determining feed intake by a grazing animal is perforce indirect and fraught with uncertainty. It is usually determined by estimating the fraction of the feed that is not digested and therefore voided, together with the daily faecal output of each animal. The former is usually taken as a property of the feed alone (the complement of feed digestibility) and determined for example by near-infrared reflectance spectroscopy (Norris et al., 1976). Collecting daily faecal output is feasible only for small male animals such as sheep (i.e., not to cattle because of the quantity voided), but the burden of a collection bag plus the need for regular mustering can affect grazing behaviour. A biologically inactive marker such as a compound of chromium or ytterbium can be used in place of total faecal collection (Prigge et al., 1981): from the marker concentration in intermittent faecal samples, together with the dose rate or intra-ruminal release rate of the marker, the faecal production can be inferred. However, the concentration of such markers can show marked diurnal variation and lead to unreliable or biassed feed intake estimates, difficulties which can be overcome by using slow intraruminal release capsules of n-alkanes, typically C32 (Dove and Mayes, 1991). However, there remain concerns that with some n-alkane formulations the pre-calibrated release rate may not be matched intraruminally (G. Waghorn, Dexcel, N.Z., personal communication, 2003). With such concerns in mind, some investigators have preferred to compute the feed intake for individual cattle by applying an energy requirements model (Section 4) in conjunction with easily measured characteristics such as liveweight and milk production (Lassey et al., 1997; Ulyatt et al., 2002a,b), arguing that this provided the more dependable feed-intake estimate (Ulyatt et al., 2002).Such

experiments would commonly impose feeding conditions that enable feed intakes to be directly and accurately measured. Despite the above caveats that could account for some. It is noteworthy that Ym values reported in those tables broadly support recommendations in the range 6–7% by the IPCC Good Practice Guidance (IPCC, 2000)

3.4. Facemask:

A facemask is another technique using a similar mechanism of gas concentration analysis to that of a ventilated hood and RC but in a manner of spot sampling (Oss et al.,2016;Silveira et al.,2019)The mask fully covers the muzzle by a strap attached around the neck of the animal. Gas sampling was performed by a tube that connected the mask to a mass flow controller and then gas analyzers . The animal is usually confined within a squeeze chute to assist the measurement and the measurement typically lasts for 30 min and is done every 2–3 h, for a maximum of seven times a day . The measurement frequency could further reduce to only once a day at 6h after morning feeding for 2–3 days (Oss et al.,2016;Silveira et al.,2019)since there is evidence that the sampling conducted at that time is strongly correlated with total daily CH_4 emissions.

3.5.Measuring CH_4 by Means of Chambers

Different chamber systems or respiration chambers have been used for the last 100 years with the main purpose of studying the energy metabolism of animals (Johnson et al., 2007; Mclean et al.,1987). CH_4 loss is an inherent part of the energy metabolism in ruminants, and various types of chambers are valuable tools in the investigation of mitigation strategies for CH_4 emissions. The principle of the chambers is to collect all exhaled breath from the animal and measure e.g., the CH_4 concentration. Animal calorimetric systems, where air composition is measured, are divided into two main types: The closed-circuit and the open-circuit(Wainman et al.,1998) . The CH_4 emission is calculated from flow and gas concentration in inlet and outlet air from the chamber, but more complex calculations have been developed that also take into account the small differences in inflow and outflow and changes in chamber concentration of gases (Brown et al.,1984). Chamber systems can be used to examine nearly all aspects of nutrition, and this technique gives results with a day-to-day CV, which can be below 10%, but the variation is dependent on e.g., feeding level. Considerations about design and placement of the chambers can eliminate the risk of reduced feed intake.

3.6. In Vitro Gas Production Technique for CH_4 Measurements:

The in vitro gas production technique (IVGPT) has been used to simulate ruminal fermentation of feed and feedstuffs (Rrymer et al.,2005) for decades. With the increasing interest in green house gas (GHG) emissions from agriculture in recent years, the traditional IVGPTs have been modified to include measurement of CH_4 production e.g.(Pellikaan et al.,2011; Navarro-Villa et al.,2011) The basic principle of IVGPTs is to ferment feed under controlled laboratory conditions employing natural rumen microbes. Feedstuffs, e.g., subjected to different treatments, are incubated at 39 °C with a mixture of rumen fluid, buffer and minerals for a certain time period, typically 24, 48, 72, 96 or 144 h . The amount of total gas produced during incubation is measured and its composition analyzed, to obtain data on the in vitro production of CH_4. At the same time it is possible to determine in vitro degradation of the feedstuffs, making it possible to determine whether a reduction in CH_4

production is at the cost of total feed degradation. The output of IVGPT experiments is usually reported as amount of CH_4per gram dry matter (DM), per gram degraded DM (dDM) or per gram degraded NDF (dNDF).

Various IVGPT systems have been employed for CH_4 determination as for example syringes Bhatta,2006; Blümme et al., 1993) , rusitec (Blümme et al., 1993) , closed vessel batch fermentations;(Navarro-Villa et al.,2011) and lately fully automated systems(Pellikaan et al.,2011) .

3.7. The CO_2 Technique:

A newly developed method for estimating CH_4 emissions from livestock is based on the use of CO_2 as a tracer gas(Madsen et al.,2010) . Instead of using externally added SF6, the naturally emitted CO_2 is used to quantify CH_4 emission. The CH_4/CO_2-ratio in the production of air of the animal(s) in question is measured at regular intervals and combined with the calculated total daily CO_2 production of the animal(s). The calculations are the same as for the SF6 tracer technique, only with CO_2 as the tracer gas instead of SF_6. The use of CO_2 as a quantifier gas is based on knowledge compiled over more than 100 years from experiments measuring feed requirements and feed composition.

3.8.Methods Based on Whole Buildings or Areas:

The methods described to far are focused on single animal measurements that fit well within a traditional experimental agricultural setup and are well suited for comparing different treatments. Unfortunately, all these methods will affect animal behavior to some extent, and they are not suitable for measuring e.g., interactions between CH_4 emission and barn design, exchange of CH_4 between grazing animals and their surroundings or whole farm emissions. During the last decades methods suitable for estimating CH_4 emission both from barns, whole farms, feedlots and paddocks have been develop. The methods can roughly be divided into non-micrometeorological techniques and micrometeorological techniques. Micrometeorological methods are defined as measuring fluxes of gas in the free atmosphere and relating these fluxes to animal emissions (Harper et al., 2011)Two non-micrometeorological methods, which focus on systems rather than individual animals are described by (Harper et al., 2011).

3.9.Intra-Ruminal Gas Sensor

An intra-ruminal device, which measures the concentrations of CH_4 and CO_2 dissolved in rumen fluid, but does not measure flux (emission), has recently been fabricated (CSRO, 2014). The rumen environmental conditions may be specifically unfavorable for an electronic device, which may cause corrosion of electrical circuits. In addition, the dissolved gases in rumen fluid must permeate quickly through the membrane of the intra-ruminal device in order to dynamically analyze the concentrations of gases (Motate et al.,2016). Information on internal rumen pressure, rumen size, and eructation pattern can be integrated to estimate the gas production rates (Hill et al., 2016)). Thus, further research would be required to develop an approach to measure CH_4 production from individual animals from the in situ measurements of gas concentrations in the rumen. The measurement of CO2 and CH_4 concentrations in rumen and breath (respiratory and eructated) at the same time would be

advantageous to assess the feasibility of using CO_2 as a tracer gas and this could guide to the use of low-cost handheld systems to estimate CH_4 production.

3.10. Combined Feeder and CH_4 Analyzer :

A newly patented system called GreenFeedTM (C-lock Inc., USA) combines an automatic feeding system with measurements of CH_4 and CO_2. The animals entering an automatic feeding system are recognized and concentrations of CH_4 and CO_2 are measured. Air is continuously pumped through the automatic feeding system to quantify flow and thereby CH_4 and CO_2 emitted during eating. To ascertain how much of the expiration air is collected the system can perform recovery experiments automatically by releasing small amounts of a tracer gas inside the feeders head cabin. Possible applications are inside AMSs, in conventional tie-stalls, and for grazing animals fed supplements. A disadvantage is that it only measures CH_4 emissions when the animals have their head in the feeder and are eating. Correlations with whole-day emissions must therefore be examined thoroughly.

3.11. Proxy Methods:

Another type of method is being developed with the aim of examining many animals at a time without invasive intervention and large experimental set-ups. These so-called proxy-methods correlate CH_4 emissions with parameters that can be measured in easily obtainable biological samples like milk or feces. Several studies have examined the fatty acid profiles of milk and correlated these with CH_4 production of the animals. The theory is that certain fatty acids or fats in the milk or feces are correlated with either the feed composition(Chilliard et al.,2009)or the amount of methanogenic archae in the rumen(Vlaeminck et all, 2006) , which both have an effect on the production of CH_4. Two recent studies(Dijkstra et al.,2011;Montoya et all,2011)indicate some correlations between milk fatty acid profiles and CH_4 emissions, but further studies are required.

3.12. Laser CH_4 detector:

Laser CH_4 detector (Tokyo Gas Engineering Solutions Inc., Tokyo, Japan) is a hand-held device that can remotely measure CH_4 concentrations in the air between the LMD and the muzzle of the animal using the infrared absorption spectroscopy technique Chagunda et al.,2013; Ricci et al.,2014)The distance between the LMD and the animal is in a range of 1 to 3 m and the measurement period is typically between 2 to 4 min each time (Garnsworthy et al.,2019; Hammondet al.,2016)The unit of the CH_4 concentration is then displayed as parts per million-meter (ppm-m). The LMD can normally be operated in an environment of −17 ◦C to 50 ◦C with 30% to 90% relative humidity .

3.13.Mitigation of Enteric CH_4 Emissions in Ruminant:

For reducing enteric CH_4 we can give Lipid Supplementation, Plant Secondary Compounds, Nitrate Supplementation,Halogenated Compounds, Nitrooxy Compounds, Fungal Metabolites, Microalgae(Patra et al., 2016)

CHAPTER-4: METHODOLOGY

4.1.Study area:

The study was conducted in CVASU animal farm and postgraduate laboratory under the Department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University (CVASU) Khulshi, Chattogram, Bangladesh.

4.2.Animal preparation:

We needed to habituate the animal to wear the face mask. So we offer the mask to the animal periodically before 1 week of gas collection. Thus the animal habituated with the mask. Then we apply the mask with proper restraining. We applied the mask for five minutes. In this time it take oxygen from the empty plastic tank.

4.3.Face mask preparation:

We make a cost effective facemask with the help of some available things. We used water bottle, rubber gloves, wash basin pipe for making the mask. The mask was connected with the 500 litre plastic tank. We also used stainless still lock nut, plastic tank adapter for the connection. The plastic tank was fully vacuumed by sandy clay.

4.4.Breath collection:

The face mask fitted with plastic tank placed on nose and mouth area of the animal. All exhale and inhale breath collected into the plastic tank for five minutes. we maintained a time protocol for collecting gas sample.

Table 1: 1st day sampling (after 6 hours)

Time	Before breath	After breath
11 am	3 sample	3 sample
5 pm	3 sample	3 sample
11 pm	3 sample	3 sample
5 am	3 sample	3 sample

After 36 hours later we took another 24 samples. We took samples same way, before breathing and after breathing from the tank.

Table 2: 2nd day sampling (after 6 hours)

Time	Before breath	After breath
6 pm	3 sample	3 sample
12 am	3 sample	3 sample
6 am	3 sample	3 sample
12 pm	3 sample	3 sample

4.5.Air sample collection:

Air sample collected from the plastic tank 2 times

1. Before fitting the mask to the animal: 3 samples
2. After fitting the mask to the animal: 3 samples (animal fitted with the mask for 5 minutes)

We used syringe and vacutainer tube for air collection from the tank. after collection we maintained the cool chain of the sample. we preserved the samples in refrigerator at 4 degree celsius.

Before collecting the sample we marked the samples in a particular name. They are:

Table 3: 1st day sample name

Time	Protocol	Name of sample
11 AM	Before breath	0-B1
		0-B2
		0-B3
	After breath	0-A1
		0-A2
		0-A3
5 PM	Before breath	6-B1
		6-B2
		6-B3
	After breath	6-A1
		6-A2
		6-A3
11 PM	Before breath	12-B1
		12-B2
		12-B3
	After breath	12-A1
		12-A2
		12-A3
5 AM	Before breath	18-B1
		18-B2
		18-B3
	After breath	18-A1
		18-A2
		18-A3

Table 4: 2nd day sample name

Time	Protocol	Name of sample
6 PM	Before breath	2-0-B1
		2-0-B2
		2-0-B3
	After breath	2-0-A1

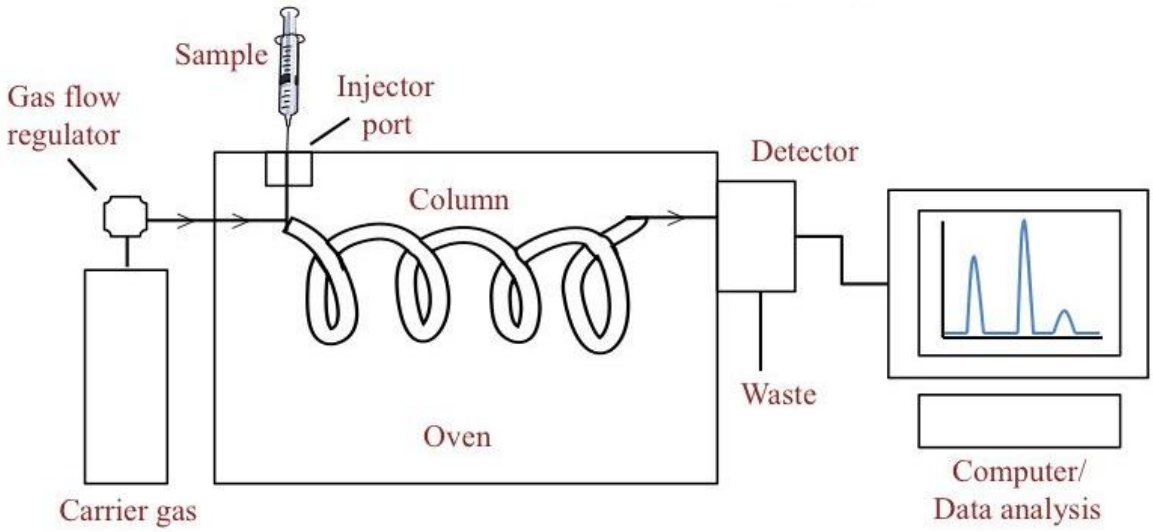
		2-0-A2
		2-0-A3
12 AM	Before breath	2-6-B1
		2-6-B2
		2-6-B3
	After breath	2-6-A1
		2-6-A2
		2-6-A3
6 AM	Before breath	2-12-B1
		2-12-B2
		2-12-B3
	After breath	2-12-A1
		2-12-A2
		2-12-A3
12 PM	Before breath	2-18-B1
		2-18-B2
		2-18-B3
	After breath	2-18-A1
		2-18-A2
		2-18-A3

4.6. Gas chromatography analysis:

Chromatography is a separation method in which the components of a sample partition between two phases: one of these phases is a stationary bed with a large surface area, and the other is a gas which percolates through the stationary bed. The sample is vaporized and carried by the mobile gas phase (the carrier gas) through the column. Samples partition (equilibrate) into the stationary liquid phase, based on their solubilities at the given temperature. The components of the sample (called solutes or analytes) separate from one another based on their relative vapor pressures and affinities for the stationary bed. (McNair et al., 2019).

The "official" definitions of the International Union of Pure and Applied Chemistry (IUPAC) are: "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. Elution chromatography is a procedure in which the mobile phase is continuously passed through or along the chromatographic bed and the sample is fed into the system as a finite slug".(Ettre etr al., 1993; Ettre 1993)

The basic parts of a simple gas chromatograph-carrier gas, gas flow regulator, injector, column, detector, and data system. The heart of the chromatograph is the column; the first ones were metal tubes packed with inert supports on which stationary liquids were coated. Today, the most popular columns are made of fused silica and are open tubes (OT) with capillary dimensions. The stationary liquid phase is coated on the inside surface of the capillary wall (McNair et al., 2019.)


Sample
Injector
port
Gas flow
regulator
Detector
Column
Waste
Oven
Carrier gas
Computer/
Data analysis

CHAPTER-5: RESULT AND DISCUSSION

After gas chromatography machine analysis we found the amount of CH_4 gas in our samples.

Name of sample	Amount of CH_4 gas (ppm)
0-B1	4.78
0-B2	4.49
0-B3	4.423
0-A1	25.997
0-A2	23.125
0-A3	24.746
6-B1	4.401
6-B2	5.327
6-B3	4.386
6-A1	17.149
6-A2	18.143
6-A3	18.879
12-B1	4.998
12-B2	4.325
12-B3	4.316
12-A1	32.567
12-A2	30.673
12-A3	35.452
18-B1	4.7027
18-B2	4.4696
18-B3	5.2067
18-A1	24.248
18-A2	23.378
18-A3	23.451
2-0-B1	3.855
2-0-B2	4.491
2-0-B3	4.347
2-0-A1	21.229
2-0-A2	21.405
2-0-A3	20.365
2-6-B1	4.117
2-6-B2	4.303
2-6-B3	3.064
2-6-A1	21.678
2-6-A2	19.304
2-6-A3	22.517
2-12-B1	4.837
2-12-B2	4.447
2-12-B3	5.094
2-12-A1	23.835
2-12-A2	25.109
2-12-A3	24.222
2-18-B1	4.214
2-18-B2	4.296

2-18-B3	3.488
2-18-A1	24.388
2-18-A2	24.065
2-18-A3	24.933

Now we calculate mean of every 3 samples of before and after. Then we calculate difference between after and before.

1st day:

1)11am

Mean of before (0B) = (0B1+0B2+0B3)÷3 =4.564333

Mean of after (0A) = (0A1+0A2+0A3)÷3 =24.62267

Difference= 0A-0B = 20.058

2) 5pm

Mean of before (6B) = (6B1+6B2+6B3)÷3 =4.705

Mean of after (6A) = (6A1+6A2+6A3)÷3 = 18.057

Difference= 6A-6B =13.352

3) 11pm

Mean of before (12B) = (12B1+12B2+12B3)÷3 =4.546

Mean of after (12A) = (12A1+12A2+12A3)÷3 =32.897

Difference= 12A-12B = 28.351

4)5am

Mean of before (18B) = (18B1+18B2+18B3)÷3 = 4.793

Mean of after (18A) = (18A1+18A2+18A3)÷3 = 23.692

Difference= 18A-18B = 18.899

2nd day :

5)6pm:

Mean of before (20B) = (20B1+20B2+20B3)÷3 = 4.231

Mean of after (20A) = (20A1+20A2+20A3)÷3 = 21

Difference= 20A-20B = 16.769

6) 12am:

Mean of before (26B) = (26B1+26B2+26B3)÷3 = 3.828

Mean of after (26A) = (26A1+26A2+26A3)÷3 = 21.166

Difference= 26A-26B = 17.338

7)6am:

Mean of before (212B) = (212B1+212B2+212B3)÷3 =4.793

Mean of after (212A) = (212A1+212A2+212A3)÷3 =24.389

Difference= 212A-212B = 19.596

8)12pm:

Mean of before (218B) = (218B1+218B2+218B3)÷3 =3.999

Mean of after (218A) = (218A1+218A2+218A3)÷3 = 24.462

Difference= 218A-218B = 20.463

Now we do mean of all differences=(20.058+13.352+28.351+18.899+16.769+ 17.338+19.596+20.463) ÷ 8 =19.353 ppm

Tank size= 500 liter = 500×1000 = 500000 ml

PPM of CH_4 is = 19.353 ppm = 19.353/1000000

1000000 ml contains ml CH_4

1 ml contains =19.353 /1000000 ml CH_4

500000ml contains= 19.353 ×500000/1000000ml CH_4

= 9.6765 ml

9.6765 ml CH_4 produces within 5 minutes

5 minutes produce= 9.6765ml

1 minutes produce= 9.6765 /5 ml

=1.9353 ml

60 minutes produce= 1.9353×60

=116.118 ml

24 hours produce = 116.118 × 24

=2786.832 ml per day

= 2.79 liter per day

The composition of the animal feed is a crucial factor in controlling the amounts of CH_4 produced, but a sheep can produce about 30 litres of CH_4 each day and a dairy cow up to about 200(GreenHouse Gas Online.org © 2002, 2003, 2004, 2005 and 2006)

In our study we found one sheep can produce 2.79 liter CH_4 per day. The reasons of the variation of our result are:

In other country sheep are large in size. But in our country sheep are small in size. Sheep ingest lower quality and lower amount of feed, so sheep can not produce much CH_4.

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