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Comparison of *In vivo* and *In vitro* Techniques for Methane Production from Ruminant Diets*

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ABSTRACT: This study was conducted to compare the methane (CH_4) production estimated by *in vivo* (sulfur hexafluoride tracer technique (SF_6)) with that of two *in vitro* rumen simulation (RUSITEC) and gas production (IVGPT)) techniques. Four adult dry Holstein cows, aged 7.4±3.0 years and weighing 697±70 kg, were used for measuring methane production from five diets by the SF_6 technique. The experimental diets were alfalfa hay (D_1), corn silage + soybean meal (SBM) (910: 90, D_2), Italian rye grass hay +SBM (920: 80, D_3), rice straw +SBM (910: 90, D_4) and Sudan grass hay +SBM (920: 80, D_5). Each diet was individually fed to all 4 cows and 5 feeding studies of 17 d each were conducted to measure the methane production. In the RUSITEC, methane production was measured from triplicate vessels for each diet. *In vitro* gas production was measured for each of the diets in triplicate syringes. The gas produced after 24 and 48 h was recorded and gas samples were collected in vacuum vials and the methane production was calculated after correction for standard temperature and pressure (STP). Compared to the SF_6 technique, estimates of methane production using the RUSITEC were lower for all diets. Methane production estimated from 24 h *in vitro* gas production was higher ($p<0.001$) on D_1 as compared to that measured by SF_6, whereas on D_2 to D_5 it was lower. Compared to SF_6, methane production estimated from 48 h *in vitro* gas production was higher on all diets. However, methane estimated from the mean of the two measurement intervals (24+48 h/2) in IVGPT was very close to that of SF_6 (correlation 0.98), except on D_1. The results of our study confirmed that IVGPT is reflective of *in vivo* conditions, so that it could be used to generate a database on methane production potential of various ruminant diets and to examine strategies to modify methane emissions by ruminants. (**Key Words :** Sulfur hexafluoride, RUSITEC, *In vitro* Gas Production, Methane)

INTRODUCTION

Due to its low abundance in the atmosphere, the importance of methane on climatic impacts has often been undervalued in the past. Methane accounts for a significant energy loss to the ruminants, amounting to about 8% of gross energy at maintenance level of intake and falling to about 6% as the level of intake increases (France et al., 1993). Methane is one of the main greenhouse gases contributing to global warming with a 100-year global warming potential (GWP) 23 times that of carbon dioxide (CO_2) (IPCC, 2001). Thus, despite being present in the atmosphere at far lower concentrations than CO_2, it is estimated that CH_4 is responsible for approximately 20% of the greenhouse gas effect (IPCC, 2001).

Many current inventories for enteric CH_4 production are based on measurements of emission rates from ruminants in open circuit calorimeters under strictly controlled environments, with specific diets linked to energy balance (Murray et al., 1999). Accurate, yet simple, predictions of methane production of ruminants on any feeding regime are important in the nutrition of ruminants, and in modelling their contribution to methane emissions (Blummel et al., 2005). The correlation between methane outputs estimated in ruminants by respiration chamber and SF_6 tracer technique is very high (Johnson et al., 1994). The SF_6 technique involves the direct measurement of methane emissions from livestock. A small permeation tube containing SF_6 is placed in the cow's rumen, and SF_6 and CH_4 concentrations are measured near the mouth and nostrils of the cow. The methane emission rate can be calculated from the known SF_6 emission rate and the measured SF_6 and methane concentrations. Although SF_6 is less expensive and time consuming than respiration chamber studies, still it needs large quantities of feed and is unsuitable for screening a wide range of feeds. According to

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Freibauer (cited by Greatorex JM. 2000) the use of SF_6 for measurement of methane production in individual ruminants have been restricted in the USA due to concern over SF_6 residues in meat and milk. Further SF_6 itself is a greenhouse gas with a global warming potential (GWP) 23,900 times that of CO_2 and an atmospheric lifetime of 3,200 years (Machmuller and Hegarty, 2005).

In vitro methods have the advantage of being less expensive and time-consuming and allow maintenance of more precise experimental conditions than *in vivo* studies. However, an efficient laboratory method should be reproducible and correlate well with actually measured *in vivo* parameters (Getachew et al., 1998). Devised by Czerkawski and Breckenridge (1977), in RUSITEC solid feeds are confined in bags that are normally replaced by new bags once a day and it is possible to study the digestive, fermentative and microbial parameters at the same time. The close association between rumen fermentation and gas production has long been recognized and the history of rumen fermentative gas measuring techniques started in the early 1940s (Quin, 1943). The *in vitro* gas production test (IVGPT) became a routine method of feed evaluation after Menke et al. (1979), reported a high correlation between gas production *in vitro* and *in vivo* apparent digestibility. The objective of the present study was to assess the potential of *in vitro* methods to estimate the methane production potential of ruminant diets by comparing SF_6 with two *in vitro* techniques.

MATERIALS AND METHODS

Sulphur hexafluoride tracer technique

Four adult non-lactating Holstein cows of 7.4±3.0 years of age and weighing 697±70 kg were used for measuring methane emissions by the SF_6 technique (Johnson et al., 1994). Cows were housed in individual stalls in a well ventilated shed with an automatic drinking water facility. A calibrated source of SF_6 (4 permeation tubes) was placed in the reticulo-rumen of each animal *per os* few weeks before the start of the measurement period. These permeation tubes remained the source of SF_6 throughout the 5 experimental periods of 10 to 12 d each. In each measurement period of 5 consecutive d, samples of expired gas were collected continuously into evacuated polyvinyl chloride (PVC) collection canister which was placed just beside the cow. The ratio CH_4/SF_6 was determined by gas chromatography using flame ionization (CH_4) and electron-capture (SF_6) detectors. Methane emission rate (g/d) was calculated as the product of this ratio and the SF_6 permeation rate and was expressed as ml/g DMI.

Diets and feeding

A total of five experimental diets were examined, alfalfa hay (D_1); corn silage +SBM (910: 90, D_2); Italian rye grass hay +SBM (920: 80, D_3); rice straw +SBM (910: 90, D_4) and sudan grass hay +SBM (920: 80, D_5). Each diet was individually fed to all 4 cows and the 5 feeding studies consisted of 10 to 12 d preliminary feeding periods followed by 5 consecutive days of measurement period, during which the methane production from each diet was measured. The daily feed allowance was calculated according to Japanese feeding standards (1999) for energy maintenance level and was offered in a mash form twice daily.

SF_6 estimation

The SF_6 concentration was determined using a gas chromatograph (Shimadzu GC 14 A, Kyoto, Japan) equipped with a ^{63}Ni electron capture detector (ECD). The pre-column and the main column (i.e. molecular sieve 5.0 m, 60 to 80 mesh and 4.0 m) were run with 15 ml/min N_2 of carrier flow at 60°C. Calibration was performed using SF_6 standard gases (150 to 1,500 ppt SF_6).

Rumen simulation technique

The fermenter used was the semi-continuous system similar to the one developed by Czerkawski and Breckenridge (1977). The system (Kajikawa et al., 2003) was equipped with 16×1 L vessels and in this study 15 were used to incubate the diets in triplicate. Treatments were allocated at random to 3 vessels each. All fermenters were filled with 400 ml of strained rumen fluid and 400 ml of artificial saliva (Mc Dougall, 1948). Rumen inoculum for the fermentation vessels were obtained from a pooled sample of strained rumen contents and rumen solids removed before the morning feeding from a cannulated cow receiving timothy hay: soybean meal (920:80) fed for energy maintenance. A precision pump guaranteed a continuous buffer infusion rate set at 0.035/h. The composition of the diets used in RUSITEC were the same as in SF_6, however they were ground to pass through 1 mm sieve and the total DM in each bag was 9.6 g. The feed for the fermentation vessel was provided in nylon bags (10×20 cm, mean pore size 50 μm, ANKOM Technology, New York., USA). At the beginning of the experimental period, one of the two nylon bags was filled with 70 g solid rumen content for easier establishment of favorable fermentation conditions and the other with the respective diet, which were gently agitated in the liquid phase. Subsequently, each day, one bag was replaced starting with the bag containing solid rumen content thus achieving a general feed incubation period of 48 h. While the bag was being changed, the vessels were flushed with CO_2 to help maintain anaerobic conditions. The experiment lasted for 17 d with all the samples collected during the last 5 d.

During the last 5 d of the experiment, culture pH was

Table 1. Chemical composition* (g/kg DM) of the ingredients used in the experiments

Ingredients	DM	OM	CP	EE	aNDF	ADF	Lignin (sa)	Ash	GE (MJ/kg DM)
Alfalfa hay	934	882	189	25	398	317	70	118	18.3
Corn silage	934	951	75	35	408	252	32	49	18.8
Italian ryegrass hay	897	950	45	11	663	397	70	50	18.3
Rice straw	937	827	28	15	665	404	47	173	15.8
Sudan grass hay	939	909	79	13	704	394	53	91	18.0
Soybean meal	904	932	516	18	128	92.1	7.2	69	19.6

* Values represent triplicate analysis of single sample.

measured using a pH electrode in samples of fermentation fluid withdrawn at the time of feeding. Total gas produced in each fermenter was collected in gas-proof bags. Gas production was quantified in a dry gas meter (set at 25°C) and CH_4 was analyzed by gas chromatograph and expressed as ml/g DM incubated.

In vitro gas production technique

Gas production was determined by the procedure of Menke and Steingass (1988). The ground samples (200 mg) were weighed into 100 ml calibrated glass syringes (Haberle Labortechnik, Lonsee-Ettlenschieβ, Germany) with pistons lubricated with Vaseline. Buffered mineral solution (Menke and Steingass, 1988) was prepared and placed in a water bath at 39°C under continuous flushing with CO_2. Rumen fluid was collected before the morning feeding from a rumen cannulated, non-lactating and non-pregnant Holstein cow (weighing 466 kg) fed 7.5 kg timothy hay and 0.65 kg of soybean meal. Rumen fluid and contents were collected into a pre-warmed insulated flask, transported to the laboratory, homogenized and filtered through 6 layers of nylon cloth. All handling was with continuous flushing of CO_2. The reducing fluid was prepared and mixed with rumen fluid. The well-mixed and CO_2 flushed rumen fluid was added to the buffered mineral solution. The mixture was kept stirred under CO_2 in a water bath at 39°C, using a magnetic stirrer. Buffered rumen fluid (30 ml) was dispensed into each syringe containing the weighed diet samples. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and clips were opened to remove gas by pushing the piston upwards to achieve complete gas removal. The clip was closed, initial volume recorded, and the syringes were immediately placed in a thermostatically controlled shaking water bath at 39°C. Three syringes containing 30 ml inoculum served as blanks. Incubation was completed in triplicate within each run and the runs were replicated thrice.

Gas produced after 24 and 48 h was recorded and gas samples were collected in evacuated vials and subjected to methane analysis in a GC as described in the next section. Methane production was calculated from the gas produced after 24 h and 48 h of incubation and expressed as ml/g DM incubated.

Methane production estimated by RUSITEC and IVGPT were corrected for standard temperature and pressure (STP) for comparison with that of SF_6 as:

Methane in ml (at STP) = (Methane ml)
×(273/ (273+25 for RUSITEC or 39 for IVGPT))
×((atmospheric pressure at the experiment)
/(standard atmospheric pressure))

Chemical analysis

In the gas samples, concentration of CH_4 was analyzed by gas chromatograph GC-8A (Shimadzu Corp., Kyoto, Japan) on Polapack Q (Waters Corp., Massachusetts, USA) column.

Diet samples were analyzed for DM, total N, ether extract, ash (AOAC, 1990), neutral detergent fiber (Van Soest et al., 1991), acid detergent fiber (ADF) and sulfuric acid lignin (Lignin (sa) (Robertson and Van Soest, 1981). Neutral detergent fiber (aNDF) was analyzed without sodium sulfite and with the use of a heat stable-amylase. Both aNDF and ADF are expressed with residual ash. Gross energy content of the samples was analyzed in an adiabatic bomb calorimeter (CA- 4 PJ, Shimadzu, Kyoto, Japan).

Statistical analysis

The methane production data was analyzed in two stages. In the first stage diets and measurement techniques were treated as main effects and the interaction of diets and techniques was also tested. In the second stage, methane production from individual diets was compared among the three techniques by analysis of variance using SAS/STAT Version 9.1. Tukey's Studentized Range Test was used to test for difference among the means.

RESULTS

Composition of the diets

The nutrient compositions of the individual ingredients as well as the diets are presented in Table 1 and 2. Among the roughages alfalfa hay contained (g/kg DM) maximum CP (189), corn silage and sudan grass hay contained almost similar CP (mean 77) while rice straw possessed the lowest protein concentration (28). Lignin (sa) content was similar in alfalfa hay and Italian ryegrass hay (70). Lignin (sa) was lowest in corn silage (32) followed by rice straw (48) and

Table 2. Chemical composition* (g/kg DM) of the different diets used in the experiments

Diet		Forage	SBM	DM	OM	CP	EE	aNDF	ADF	Lignin (sa)	Ash	GE MJ/kg
D_1	Alfalfa hay	1,000	-	934	882	189	25	398	317	70	118	18.3
D_2	Corn silage	910	90	931	949	114	33	383	238	30	51	18.8
D_3	Italian ryegrass hay	920	80	898	949	83	12	620	372	65	51	18.4
D_4	Rice straw	910	90	933	836	70	15	619	377	44	164	16.2
D_5	Sudan grass hay	920	80	936	911	113	13	659	370	49	89	18.2

* Values represent triplicate analysis of individual samples.

Table 3. Comparison of methane production measured by SF_6 technique with that of RUSITEC and IVGPT

Diets	SF_6	RUSITEC	IVGPT		SEM	p value	
			24-h	48-h		Method	Method×diet
CH_4 (ml/g DM)							
All	29.5^b	9.4^d	27.0^c	34.3^a	0.026	0.0001	0.0001
D_1	27.4^c	9.80^d	31.6^b	34.8^a	0.058	0.0001	-
D_2	37.1^b	9.70^d	34.6^c	41.5^a	0.058	0.0001	-
D_3	30.3^b	8.04^d	24.6^c	33.3^a	0.058	0.0001	-
D_4	24.5^b	8.03^d	20.6^c	28.7^a	0.058	0.0001	-
D_5	28.1^b	11.5^d	23.8^c	33.3^a	0.058	0.0001	-
CH_4 (% GE)							
All	6.47^b	1.97^d	5.91^c	7.49^a	0.006	0.0001	0.0001
D_1	5.95^c	2.00^d	6.83^b	7.54^a	0.029	0.0001	-
D_2	7.77^b	1.94^d	7.25^c	8.62^a	0.006	0.0001	-
D_3	6.52^b	1.66^d	5.27^c	7.03^a	0.006	0.0001	-
D_4	5.98^b	1.88^d	5.01^c	6.99^a	0.006	0.0001	-
D_5	6.12^b	2.39^d	5.19^c	7.26^a	0.006	0.0001	-

Means in a row with different superscripts differ significantly ($p<0.0001$).
D_1: Alfalfa hay. D_2: 910 Corn silage+90 SBM. D_3: 920 Italian ryegrass hay−80 SBM.
D_4: 910 Rice straw−90 SBM. D_5: 920 Sudan grass hay+80 SBM.

sudan grass hay (53), respectively. GE content was more than 18 MJ in all the roughages, except rice straw (15 MJ/kg DM), due to higher ash content (173). In the mixed diets, addition of soybean contributed to an increase in the CP and GE and proportionate reduction in the fibre components, except alfalfa.

The pH of the rumen fluid from D_1 to D_5 RUSITEC fermenters was 7.04, 6.96, 7.05, 7.17, and 7.02 and their corresponding IVDMD was 0.665, 0.646, 0.520, 0.457, and 0.554, respectively.

Methane production estimated by different techniques

Methane production estimated by different techniques is presented in Table 3. Methane out put estimated by RUSITEC was lower ($p<0.0001$) as compared to that by either SF_6 or IVGPT, in all the diets. Except D_1, methane production (ml/g DM) estimated after 24 h IVGPT was lower ($p<0.0001$) as compared to that of SF_6; however, that estimated after 48 h incubation (from 24 and 48 h *in vitro* gas samples) was higher ($p<0.0001$) in all the diets. The correlation coefficients between methane production measured by SF_6 and IVGPT were 0.749 after 24 h and 0.939 after 48 h. The hypothetical mean of the methane production measured at two intervals ((24+48)/2) was 33.2, 38.1, 29.0, 24.7 and 28.6 (ml/g DM) and 7.19, 7.94, 6.15, 6.00 and 6.23 (% GE) from G_1 to G_5, respectively.

DISCUSSION

Methane is the most abundant organic gas in the earth's atmosphere, and annual CH_4 concentration is increasing globally at a rate of between 0.7% and 1.0% (Crutzen, 1995). The correlation between methane output estimated from ruminants by respiration chamber and SF_6 is very high (Johnson et al., 1994). To our knowledge, this is the first report wherein SF_6 (*in vivo*) technique was compared with two *in vitro* techniques (RUSITEC and IVGPT) for their potential to estimate methane production from ruminant diets.

Methane output (ml/g DM) from the all the diet samples as well as the interaction effect of method×diet showed significant ($p<0.0001$) difference. Similarly, CH_4 output from individual diets also differed ($p<0.0001$) among the techniques. The trend was also similar when methane out put was expressed as % of GE. Estimates of methane production in the RUSITEC were lower ($p<0.0001$) from all the diets as compared to that of either SF_6 or IVGPT. The pH of the rumen fluid from D_1 to D_5 RUSITEC fermenters was 7.04, 6.96, 7.05, 7.17, and 7.02 and their corresponding IVDMD was 0.665, 0.646, 0.520, 0.457, and 0.554,

Table 4. Correlation of methane production (ml/g DM) measured by SF_6 with that of RUSITEC and IVGPT at different incubation intervals

	SF_6	RUSITEC	IVGPT		
			24-h	48-h	(24 h+48 h)/2
Diets D_1 to D_5					
SF_6	1				
RUSITEC	0.1654	1			
24h-IVGPT	0.7485	0.2715	1		
48h-IVGPT	0.9394*	0.3498	0.9137	1	
(24 h+48 h)/2	0.8512	0.3128	0.9830*	0.9728*	1
Diets D_2 to D_5					
SF_6	1				
RUSITEC	0.2031	1			
24h-IVGPT	0.9817*	0.2444	1		
48h-IVGPT	0.9854*	0.3459	0.9895*	1	
(24 h+48 h)/2	0.9860*	0.2926	0.9977*	0.9970*	1

* $p<0.05$.

respectively. Bhatta et al. (2006a) in a recent RUSITEC experiment also recorded similar values. The pH and IVDMD values indicated normal fermentation of all the diets in RUSITEC fermenters. After 24 h incubation, the net gas produced (mean) from 5 diets was about 1664 ml in RUSITEC and that of IVGPT was 1732 ml (based on mean gas volume as 36.1 ml/200 mg DM in IVGPT; calculated for equivalent of 9.6 g substrate in RUSITEC). This volume was also almost similar between the two techniques, reflecting normal fermentation of all the diets in RUSITEC fermenters. However, the major difference observed was in the composition of the gas samples. The gas samples from RUSITEC contained 0.334 CO_2 and 0.059 CH_4, whereas it was 0.701 and 0.165, respectively in the gas collected after 24 h incubation from IVGPT. This difference in the composition of the gas could be the main reason for lower estimate of methane production in RUSITEC (Bhatta et al., 2005), which could be attributed to the gradual reduction in the protozoa. It was established in earlier studies (Kajikawa et al., 2003) that the protozoa numbers in the effluent gradually decreased as the incubation proceeded and settled at around 3,000/ml after the 8th day for 0.030/h dilution rate. It has also been reported that owing to their relatively long generation time and their association with the liquid phase of Ruminal contents holotrich protozoa cannot survive in RUSITEC (Martin et al., 1999). Since part of the methanogens in the rumen cohabit with ciliate protozoa and have been shown to be responsible for 9-25% of methanogenesis in the rumen fluid (Newbold et al., 1995), decrease in the protozoal count results in reduced methane out put. Further, protozoa are also known to produce butyric acid (one mol of butyric acid production results in production of 4 hydrogen molecules); a reduction in protozoa population would also results in lower butyric acid and in turn lower hydrogen as well as CH_4 production. Sampling from the RUSITEC should be undertaken only after the system has reached a steady state conditions (after 8 to 10 days). Since protozoa numbers gradually decreases as the incubation proceeds, this point needs to be considered while using RUSITEC for methane measurement studies.

Average CH_4 production (ml/g DM) recorded in RUSITEC was 9.4 at a dilution rate of about 840 ml/d as compared to 29.5 in SF_6 and 27.0 after 24 h (or 34.3 after 48 h) in IVGPT. Dohme et al. (1999) reported 16.1 ml/g DM in RUSITEC at a dilution rate of 520 ml/d, and 14.7 at a dilution rate of 530 ml/d in another experiment (Dohme et al., 2001). Sliwinski et al. (2002) observed 17.8 and 17.5 whereas Hess et al. (2003) recorded 2.47 and recently Soliva et al. (2004) reported 13.9 ml/g DM in RUSITEC experiment. The overall CH_4 production (ml/g DM) in all these RUSITEC experiments ranged from 2.47 to 17.8 with a mean value of 16.0. This reflects that generally the CH_4 produced in RUSITEC fermenters was lower as compared to that of either SF_6 or IVGPT. The CH_4 production recorded in our RUSITEC study (9.4 ml/g) was within the range reported in other studies, but slightly on the lower side, which might be attributed to higher dilution rate compared to other studies. With dual flow fermenters Eun et al. (2004) observed that CH_4 production measured from headspace gas was lower ($p<0.05$) at a dilution rate of 0.032 (0.035 in our study) as compared to that calculated from VFA stoichiometry.

Compared to methane (ml/g DM) measured by SF_6, that estimated from the gas samples collected after 24 h incubation (IVGPT) was higher from D_1, whereas it was lower ($p>0.0001$) from D_2 to D_5 (Table 3), however, after 48 h of incubation, it was higher from all the diets. Similar trend was also observed when methane out put was expressed as % of GE. D_1 was alfalfa hay (Table 2) and contained maximum CP (190 g/kg DM) with high digestibility (0.66) that contributed for the early onset of fermentation and subsequent gas production. Whereas all other diets contained SBM and CP was less than 120 g/kg

DM; aNDF levels of D_3 to D_5 were also higher (>620) as compared to that of D_1 (398). The recorded difference in the methane production among diets by IVGPT was attributed to their nutrient composition since it was reported (Getachew et al., 2005) that slowly digestible fraction of feed was associated with higher methane production. The hypothetical average of the methane production measured at two intervals ((24 h+48 h)/2) were 33.2, 38.1, 29.0, 24.7 and 28.6 (ml/g DM) and 7.19, 7.94, 6.15, 6.00 and 6.23 (% GE) from G_1 to G_5, respectively. These values are very close to those measured by SF_6 both in terms of ml/g DM as well as % GE, except D_1; and the correlation coefficient was 0.986 (Table 4). It appeared that for diets containing low protein, high fibre with low digestibility values, 24 h IVGPT estimated lower methane out put, whereas 48 h incubation overestimated it. It may be inferred that for those feeds, 36 h incubation in IVGPT would be optimum to estimate the methane production potential.

The proportion of CH_4 in the total gas produced ranged from 163 to 184 ml/L and didn't differ among diets after 24 and 48 h and it increased as the incubation time progressed. This confirms the recent finding of Getachew et al. (2005), that slowly digestible fraction of feed is associated with higher methane production. Although the proportion of CH_4 in total gas increased with time of incubation, the actual amount of CH_4 produced was much higher during the first 24 h of incubation. Methane produced after 24 h of incubation from all the diets was 27 ml/g DM (mean), and it was 29.5 by SF_6 technique. Shibata et al. (1992) measured 28.4 and 25.9 (ml/g DMI) in Holstein heifers and Corriedale wethers fed at 1.5 maintenance, respectively. In another study, Shibata et al. (1992) recorded 27.2, 33.8 and 33.9 ml/g DMI in Holstein lactating cows, Holstein pregnant cows and fattening steers, respectively. Kurihara et al. (1999) reported 113, 257 and 160 L/d of CH_4 in Brahman heifers consuming (kg/d DM) 3.58 (low quality hay), 7.07 (medium quality hay) and 7.31 (high grain diets), which was equivalent to 31.6, 36.4 and 21.9 ml/g DMI, respectively. Sauer et al. (1998) measured about 622 L/d of CH_4 from lactating cows, equivalent to 38.9 ml/g DMI. Holter and Young (1992) reported 420 L/d in lactating cows consuming only 14.4 kg/d DM, which was equivalent to 29.2 ml/g DMI. While Wilkerson et al. (1995) reported an average methane production by lactating Holstein cows of 497 L/cow/d. Our results (i.e. 27 ml/g DM by IVGPT and 29.5 ml/g DM by SF_6) are very close to those reported by Moss (2001) in sheep (31.0 ml/g DMI). The CH_4 produced after 24 h of incubation averaged 0.79 of total CH_4 produced at 48 h of incubation. In our SF_6 technique, animals were fed diets at maintenance level. Reports suggest that there was a strong negative relationship between intake and methane production, as the intake increases the percentage of dietary energy lost as methane decreases (Ulyatt et al., 2002). Future experiments are recommended on the comparative studies of methane production from feed samples in animals fed at maintenance and above maintenance level with that of IVGPT, since large part of the cattle population would be fed at above maintenance levels.

The results of our study established that this *in vitro* technique is reflective of *in vivo* conditions so that it could be used to have a database on the methane production potential of various ruminant diets and also to examine the strategies to modify CH_4 emissions by ruminants. Bhatta et al. (2006b) in another study with Japanese goats, in which methane production was measured in respiration chambers was compared with that of IVGPT, also reported similar findings. Recently, Getachew et al. (2005) reported similarity between measured and calculated methane values and suggested that methane production could be calculated if only *in vitro* gas volume and VFA production is measured. Blummel et al. (2005) suggested that methane production in forage fed ruminants could be predicted by a simple *in vitro* technique that measures gas production and true substrate degradability. This is also important since majority of the laboratories will not have expensive instruments to measure the methane production. The present study focused on forage-based diets. Results may differ with concentrate diets where VFA profiles change substantially and pH changes in buffer may be larger.

CONCLUSIONS

This is the first report wherein *in vivo* (SF_6) method was compared with two *in vitro* techniques (RUSITEC and IVGPT) for their potential to estimate methane production. It was established that the methane out put estimated by RUSITEC was lower as compared to that of SF_6. Methane production estimated by IVGPT was very close to that measured by SF_6 method. The results of the present study suggest the potential of IVGPT for estimating the methane production in ruminant diets. The high cost and technical difficulties involved in animal experimentation to measure methane production makes IVGPT suitable for developing data base that could be used for planning mitigation strategies through detailed *in vivo* environmental studies. However, further studies are recommended with more number of samples and with varying nutritional profiles.

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