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Diet effects on methane production by goats and a comparison between measurement methodologies

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SUMMARY

A series of studies were carried out to measure the methane (CH_4) production by Japanese goats fed 19 different diets (D_1–D_{19}) varying in nutritive composition in the open circuit respiration chamber (RC) and to compare them with CH_4 estimated by the *in vitro* gas production test (IVGPT). Adult Japanese goats (>2 years old) with a mean body weight of $26 \pm 5{\cdot}4$ kg were used in these experiments. Each diet was fed to four randomly selected goats and feeding was carried out at 1·1 maintenance (M) as per National Research Council (NRC) (1981) for goats. Average CH_4 emission by goats in the RC ranged from 0·23 to 0·39 (mean value 31 ml/g dry matter intake (DMI)); when it was expressed as a proportion of gross energy or, with methane conversion rate (MCR), it ranged from 5·0 to 8·2, with an average of 6·6. Incorporation of by-products like sweet potato vine silage (SPVS) ($P = 0{\cdot}016$), dried pumpkin ($P = 0{\cdot}052$) and brewers' grain in the diet suppressed ($P < 0{\cdot}01$) methanogenesis in goats, when compared with that of standard farm diet (D_1). The CH_4 output measured in the RC was very close to that estimated from the gas collected after 24 h and higher after 48 h of *in vitro* incubation. Although composition of the diets' acid detergent fibre (ADF) had a significant effect on methane emission, methane output estimated by IVGPT was very close to that measured in the RC demonstrating that this system could be used to estimate the CH_4 production potential from diets in preparing a database and also in the planning of mitigation strategies in small ruminants to improve their performance as well as to reduce greenhouse gas emissions.

INTRODUCTION

Methane (CH_4) is one of the main greenhouse gases contributing to global warming with a 100-year global warming potential (GWP), 23 times that of CO_2 (Intergovernmental Panel on Climate Change (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 0·20 of the greenhouse gas effect (IPCC 2001). However, there is uncertainty in the estimation of CH_4 production from small ruminants due to the scant availability of data to document the variability that exists and also due to the significant impact of diet on enteric CH_4 production. Methane production from goats fed different diets has not been studied extensively. Such data can contribute to more accurate estimates of emission factors used in greenhouse gas inventories as well as to developing nutritional and management strategies to reduce CH_4 production.

Many current inventories for enteric CH_4 production are based on measurements of emission rates from animals in open circuit respirometers in strictly controlled environments (Murray *et al.* 1999). There is a need to be able to measure the CH_4 production potential of a range of feedstuffs under different rumen environmental conditions in order to predict the amount of CH_4 produced by ruminants. *In vitro* methods have the advantage not only of being less expensive and less time-consuming, but also it is possible to maintain experimental conditions more precisely than *in vivo* trials (Getachew *et al.* 1998). *In vitro* techniques using rumen fluid as an inoculum could be considered as models of *in vivo* rumen digestion and have been applied to estimate digestibility of feedstuffs (Moss & Givens 2002). But there have been limited attempts to use the *in vitro* gas production technique (IVGPT; Menke *et al.* 1979) to estimate the CH_4 production potential of feedstuffs.

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Table 1. *Ingredient composition of the experimental diets* (*g*/*kg*)

Diets	Timothy hay	Alfalfa hay	Maize	Soybean meal	Concentrate	Others	
D_1	700	–	200	100	–	–	
D_2	350	–	100	50	–	500	SPVS*
D_3	350	–	100	50	–	500	SPVS treated with formic acid†
D_4	250	250	–	–	500	–	
D_5	250	250	–	–	500	–	
D_6	250	250	–	–	500	–	
D_7	–	850	–	–	150	–	
D_8	–	850	–	–	150	–	
D_9	–	1000	–	–	–	–	
D_{10}	180	–	–	–	820	–	
D_{11}	180	–	–	–	820	–	
D_{12}	180	–	–	–	820	–	
D_{13}	–	650	–	–	–	350	Pumpkin‡
D_{14}	600	–	250	–	–	150	Brewers' grain – freeze dried
D_{15}	600	–	250	–	–	150	Brewers' grain – dried at 60 °C
D_{16}	600	–	250	–	–	150	Brewers' grain – dried at 130 °C
D_{17}	160	160	–	–	680	–	
D_{18}	160	160	–	–	680	–	
D_{19}	160	160	–	–	680	–	

* Sweet potato vine silage (SPVS) contained (g/kg dry matter) 101 DM, 124 crude protein (CP), 851 organic matter (OM), 419 neutral detergent fibre (NDF) and 351 acid detergent fibre (ADF).
† SPVS was treated with 0·2 % formic acid (85 %).
‡ Composition (g/kg DM) 903 DM, 946 OM, 94 CP, 251 NDF, 97 ADF, 17 acid detergent lignin (ADL), 18·8 MJ/kg gross energy (GE).

The gas production system has been used primarily to describe the rate of degradation of feedstuffs and to study the formation of volatile fatty acids (VFAs) and stoichiometry of fermentation (Blummel & Ørskov 1993). The objectives behind using IVGPT in the current experiment are, simply, to demonstrate the large number of samples that can be analysed at a time and the ease with which the system can be established and managed compared with *in vivo* techniques. The present study was designed to examine two issues: first, to measure the CH_4 production by Japanese goats fed diets differing in composition in a respiration chamber (RC) and second, to compare that with CH_4 estimated by the *in vitro* gas production technique. It is thought that this is the first report in which CH_4 emissions from goats measured in the RC from a range of 19 different diet samples have been compared with that estimated using IVGPT.

MATERIALS AND METHODS

Animals and diets

Adult Japanese goats (2 years old) with a mean body weight of $26 \pm 5{\cdot}4$ kg were used for the current experiments. The details of the diets (D_1–D_{19}) fed to the goats are presented in Table 1. D_2 and D_3 each contained sweet potato vine silage (SPVS; 0·50); however, in D_3 SPVS was treated with formic acid (850 g/litre) at 0·002 (during ensiling) on fresh matter basis. The SPVS was produced after ensiling the stem and leaves of sweet potato for 28 days and it was fed to goats after mixing with other ingredients just before being offered. Diets 14, 15 and 16 contained 0·15 of brewers' grains. Brewers' grains added in D_{14} were freeze-dried, whereas in D_{15} and D_{16}, they were dried at 60 and 130 °C, respectively. Diets 4–6, 10–12 and 17–19 contained similar amounts of concentrates but the ingredient composition was different (Table 2) and that has resulted in the variation in their nutritional composition (Table 3). Feeding was at 1·1 M, as per NRC (1981) standard for goats.

Metabolism trial

Four goats were used in the trial; all four goats were fed the same diet at the same time and the 19 diets were fed to goats in succession. A metabolism trial for each diet was conducted for 21 days with a 7-day adjustment period and 7-day preliminary period followed by a 7-day collection period, which included 3 consecutive days for measurement of gas exchange in an open circuit RC. Each diet was fed randomly to avoid any risk of bias when comparing feeds that are close together in composition. Further, there was also a gap of 3 weeks between each trial for the goats to become acclimatized to the diet (preliminary period)

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Table 2. *Composition (g/100 g) of the concentrate mixture of different diets*

(a)

Ingredients	D_4	D_5	D_6
Brewers' grains	0	40	10
Maize	20	23	7
Soybean meal	40	5	5

(b)

Ingredients	D_{10}	D_{11}	D_{12}
Brewers' grains	3·4	12·3	12·3
Maize	35·8	28·1	28·1
Soybean meal	11·2	2·2	2·2
Cotton seed meal	10·1	0	0
Beet pulp	9·0	9·0	9·0
Wheat bran	4·4	11·2	11·2
Soybean hulls	3·4	12·3	12·3
Fish meal	0	1·1	1·1
Maize gluten meal	0	1·1	1·1
Molasses	2·2	2·2	2·2
Mineral premix	2·4	2·4	2·4

(c)

Ingredients	D_{17}	D_{18}	D_{19}
Brewers' grains	0	3	6
Maize	30	22	14
Soybean meal	10	6	2
Cotton seed meal	8	8	8
Beet pulp	9	9	9
Wheat bran	5	4·5	4
Soybean hulls	0	5	10
Rice bran	0	4·5	9
Linseed meal	2	2	2
Molasses	2	2	2
Mineral premix	2	2	2

and also to avoid any residual effect. The daily allowances were weighed and fed once a day. Refusals, if any, were collected and measured before feeding on the next day. Representative samples of feeds and refusals were taken, oven dried (60 °C for 24 h) and ground to pass through a 1 mm sieve for chemical analysis. The total faecal and urinary outputs were preserved daily. Urine was collected in the urine buckets where 25 ml (0·01 of urine volume) of 20 g/litre sulphuric acid (H_2SO_4) was placed before collection. After total collection, faeces and urine were mixed thoroughly and sub-sampled for N determination. For further analysis, faecal samples were oven dried (60 °C for 24 h) and then ground similarly. Urine (20 ml) was measured onto a polyethylene film

Table 3. *Composition (g/kg DM) of the experimental diets fed to goats*

Diets	DM	OM	CP	EE	aNDF	ADF	GE (MJ/kg DM)
D_1	886	939	128	21	554	314	18·3
D_2	876	886	137	30	478	357	18·5
D_3	888	890	136	30	484	349	18·1
D_4	874	933	175	40	429	219	19·1
D_5	874	934	164	45	540	244	19·1
D_6	874	932	170	43	552	211	19·3
D_7	887	889	180	44	461	297	18·1
D_8	888	929	180	34	459	294	18·0
D_9	889	883	186	23	522	345	17·7
D_{10}	872	926	158	39	375	211	18·7
D_{11}	852	926	172	45	435	211	19·0
D_{12}	873	922	165	43	371	203	18·9
D_{13}	881	914	145	39	382	279	18·3
D_{14}	895	956	111	34	559	284	18·9
D_{15}	893	957	113	34	564	283	18·8
D_{16}	898	957	111	21	580	293	18·8
D_{17}	886	937	163	51	339	227	18·9
D_{18}	884	930	171	61	346	226	18·8
D_{19}	875	934	164	75	356	253	19·7
S.E.M. (D.F. = 38)	9·6	12·7	9·0	2·8	15·4	10·0	1·03

Values represent mean of the triplicate analysis.
D_1–D_{19}, diets 1–19 fed to goats during the RC studies.
DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; aNDF, neutral detergent fibre; ADF, acid detergent fibre; GE, gross energy.

and freeze-dried for determination of gross energy (GE) using the method described by Itoh & Tano (1977).

RC

Total air flow, consumption of oxygen (O_2), the concentration of CH_4 and carbon dioxide (CO_2) produced were measured for 3 consecutive days by direct measurement of gaseous output using an open circuit RC, details of which are as outlined by Iwasaki *et al.* (1982). The chamber was maintained under controlled temperature conditions at 20 °C and relative humidity at 60% throughout the measurement periods.

IVGPT

In vitro gas production was determined as per the procedure described by Menke & Steingass (1988). In brief, samples (200 mg) were weighed into 100 ml calibrated glass syringes (Haberle Labortechnik, Germany) with pistons lubricated with Vaseline. Buffered mineral solution (Menke & Steingass 1988) was prepared and placed in a water bath at 39 °C

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under continuous flushing with CO_2. Rumen fluid was collected before the morning feed from three rumen-cannulated, non-lactating and non-pregnant Holstein cows (weighing 466 kg body weight) fed 5·8 kg timothy hay, 1·6 kg crushed maize and 0·80 kg soybean meal (SBM). Rumen fluid and contents were collected into a prewarmed insulated flask, transported to the laboratory, homogenized and filtered through four layers of nylon cloth. The reducing fluid was prepared and mixed with buffer. 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 and immediately placed in thermostatically controlled shaking water bath at 39 °C. Three syringes containing 30 ml inoculum served as blanks. The incubations were carried out in triplicate for each feed sample and these incubations were repeated three times, with intervals of 1 week. Incubations without feed sample served as blanks with every set. The difference in the composition and activity of rumen inoculum among incubations was controlled by parallel incubation of reference standard feedstuffs obtained from the Hohenheim University, Germany as suggested by Menke *et al.* (1979).

The total gas volume after 2, 4, 6, 8, 10, 24 and 48 h were recorded. The gas samples were collected after 24 and 48 h in evacuated vials and subjected for CH_4 analysis in a gas chromatograph. Methane production was calculated from the cumulative gas produced after 24 and 48 h incubation. Methane production estimated by IVGPT was corrected for standard temperature and pressure (STP) for comparison with that of RC as follows:

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

Chemical analysis

Methane estimation

From the *in vitro* gas samples, concentration of CH_4 was analysed by gas chromatograph GC-8A (Shimadzu Corp., Kyoto, Japan) on Polapack Q (Waters Corp., MA, USA) column.

Diet samples were analysed for total ash and nitrogen by the Kjeldahl method (Association of Official Analytical Chemists (AOAC) 1995). Neutral detergent fibre (aNDF) was analysed as per Van Soest *et al.* (1991) and acid detergent fibre (ADF) as per Robertson & Van Soest (1981). The NDF was analysed without sodium sulphite, but with the addition of a heat stable-amylase. Both aNDF and ADF were expressed with residual ash. Gross energy content of the samples was analysed in an adiabatic bomb calorimeter (CA-4 PJ, Shimadzu, Japan).

Statistical analysis

The independence and homogeneity of variance of the 19 diets over time was tested by a time series plot of residuals (Box–jung satistical analysis) (Ljung & Box 1978). The autocorrelation with lags 1 and 2 was also performed for all the diets.

Data on digestibility and methane production from different diets measured in RC were analysed with the experiment being included in the model (mixed model) and animal as random effect. The data were analysed using SPSS 11.0. Stata 8.0 and Systat 8.0 and Microsoft Word and Excel were used to generate graphs and tables.

RESULTS

Composition of diets

Maximum crude protein (CP) was recorded in diets 7, 8 and 9 (180 g/kg) and the lowest in 14, 15 and 16 (112 g/kg). Ether extract (EE) content ranged from 21 to 75 g/kg. Maximum aNDF was in D_{16} followed by D_{15} and D_{14} and the lowest in D_{17}, followed by D_{18} and D_{19}. The ADF was more than 200 g/kg in all the diets, maximum being in D_2. Gross energy content (MJ/kg) varied from 17·7 to 19·7 with an average of 18 (Table 3). The digestibility of nutrients and the nutritive value of the diets are presented in Table 4. Maximum dry matter (DM), GE and ADF digestibility coefficients were recorded in D_{10}, D_{11} and D_{12} containing maximum concentrate (0·82). Lowest dry matter digestibility (DMD) of 0·62 was in sole alfalfa (*Medicago sativa*) diet (D_9). Maximum crude protein digestibility (CPD) was in D_{11} and the lowest in D_{16}. The aNDF digestibility coefficients of D_{10}, D_{11} and D_{12} could not be estimated. Among the diets, D_{11} contained maximum total digestible nutrients (TDN) (804 g/kg); it was very similar to D_{10}, D_{14} and D_{15} (795 g/kg) and lowest was in D_3. Metabolizable energy (MJ/kg DM) was more than 10 in all the diets, with the maximum being in D_{17}. The difference in the digestibility and the nutritive value of different diets are not discussed further. Methane productions from the diets are discussed in relation to their nutritional composition.

Methane production from different diets

Methane production by goats measured in the RC is presented in Table 5. Autocorrelation at lag 1 and lag 2 levels for all the 19 diets were statistically non-significant. The Box–Ljung statistical method (Ljung & Box 1978) to test the independence of serial

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Table 4. *Component digestibility* (*as a coefficient*) *in goats and nutritive value of experimental diets*

Diets	DMD	OMD	CPD	EED	aNDFD*	ADFD	GED	TDN	DE MJ/kg	ME MJ/kg
D_1	0·685	0·691	0·753	0·599	0·628	0·606	0·663	0·664	12·1	9·93
D_2	0·674	0·682	0·676	0·687	0·616	0·624	0·666	0·631	12·3	10·1
D_3	0·667	0·673	0·648	0·693	0·614	0·605	0·644	0·624	11·6	9·37
D_4	0·766	0·785	0·787	0·838	0·627	0·612	0·774	0·774	14·8	12·2
D_5	0·749	0·767	0·750	0·834	0·656	0·645	0·754	0·763	14·4	12·0
D_6	0·763	0·784	0·765	0·832	0·681	0·654	0·770	0·775	14·8	12·3
D_7	0·638	0·677	0·786	0·726	0·484	0·483	0·660	0·638	14·1	11·9
D_8	0·650	0·696	0·792	0·683	0·471	0·480	0·668	0·649	14·3	12·0
D_9	0·620	0·653	0·785	0·487	0·507	0·507	0·632	0·591	14·6	11·2
D_{10}	0·784	0·816	0·780	0·840	†	0·713	0·791	0·796	12·9	10·8
D_{11}	0·790	0·816	0·803	0·863	†	0·722	0·796	0·804	13·3	11·1
D_{12}	0·779	0·803	0·787	0·854	†	0·680	0·784	0·786	13·0	10·9
D_{13}	0·713	0·735	0·740	0·715	0·560	0·519	0·703	0·705	12·9	11·1
D_{14}	0·644	0·801	0·683	0·796	0·541	0·487	0·643	0·796	12·2	10·6
D_{15}	0·650	0·801	0·685	0·792	0·551	0·500	0·642	0·792	12·1	10·5
D_{16}	0·646	0·806	0·636	0·752	0·572	0·516	0·635	0·752	12·0	10·3
D_{17}	0·756	0·778	0·788	0·860	0·573	0·561	0·757	0·784	14·3	12·3
D_{18}	0·735	0·753	0·770	0·872	0·539	0·529	0·729	0·770	13·7	12·2
D_{19}	0·695	0·713	0·746	0·864	0·491	0·490	0·694	0·753	13·63	11·8
S.E.M. (D.F. = 54)	0·0099	0·0086	0·0092	0·0082	0·0165	0·0155	0·0107	0·0092	0·0018	0·0016

Values represent mean of the triplicate analysis.
* For aNDFD (D.F. = 45).
† aNDF digestibility was not estimated.
D_1–D_{19}, diets 1–19 fed to goats during the RC studies; DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility; EED, ether extract digestibility; aNDFD, neutral detergent fibre digestibility; ADFD, acid detergent fibre digestibility; GED, gross energy digestibility; TDN, total digestible nutrients (in proportions); DE, digestible energy; ME, metabolizable energy.

measurement based on 19 diets indicated that they were not statistically significant, thereby confirming independence and homogeneity of variance between times or, in other words, methane output measured from successive diets from each goat were independent. To have a relative estimate of methane emission by Japanese goats fed different diets, it was assumed D_1 containing 0·70 timothy hay + 0·20 maize + 0·10 SBM with 130 g CP/kg and 18 MJ of GE, as the standard (control) diet. This is the diet usually fed to goats in commercial farms. The methane output recorded in goats fed D_1 was 35·8 ml/g DM or 0·008 of GE. Addition of SPVS resulted in a 7·5% reduction ($P < 0·016$) in methane output, when compared with D_1. However, when SPVS was treated with formic acid there was no significant ($P = 0·145$) effect on methane output.

Methane emission in goats fed diets D_4–D_6 containing 0·50 of concentrate mixture + 0·25 each of timothy hay and alfalfa hay was similar ($P > 0·05$) to that of D_1. This trend was also seen in diets D_{10}–D_{12} containing 0·82 concentrate and 0·18 of timothy hay (Table 5).

When alfalfa hay constituted the major part (850 g/kg) of the diet (D_7–D_9), there was a significant ($P < 0·05$) reduction in the methane output. Goats produced minimum amount (23·03 ml/g dry matter intake (DMI)) of methane, when they were fed with sole alfalfa diet (D_9). The reduction was approximately 13%, when compared with D_1. When brewers' grains were added to timothy hay (0·60)- and maize (0·25)-based diet, then methane emission was also reduced. Maximum CH_4 reduction (12%; $P = 0·025$) was recorded in freeze-dried brewers' grains (D_{14}) when compared with those dried at 60 °C D_{15} (10%; $P = 0·044$) or 130 °C D_{16} (8%; $P = 0·079$). Addition of pumpkin (0·35 D_{13}) also reduced the methane output (8%; $P = 0·052$), in goats, when compared with that of D_1 (Table 5).

Methane output recorded in goats fed D_{17} was similar, whereas it was significantly lower ($P < 0·087$) in D_{18} and D_{19} ($P < 0·029$); the reduction was 5·6 and 10·7%, respectively, when compared with D_1. These three diets contained 0·68 of concentrate and 0·16 each of timothy hay and alfalfa hay.

The correlation between CH_4 production (ml/g) with organic matter (OM) and ADF content of the diet consumed were −0·13 and −0·63, and when they were expressed as a proportion of GE, it was −0·13 and −0·55.

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Table 5. *Methane production in Japanese goats measured inRC fed different diets*

Diets	CH_4 production ml/g DMI	ml/g DDMI	ml/g DOMI	% Change from D_1
D_1	36	52	55	–
D_2	28	42	47	7·5
D_3	32	49	50	3·3
D_4	39	51	38	−3·5
D_5	37	49	34	−0·8
D_6	38	49	33	−1·7
D_7	26	40	42	11·9
D_8	27	42	45	8·7
D_9	23	37	38	13·0
D_{10}	39	49	41	−2·5
D_{11}	38	49	41	−2·3
D_{12}	35	45	37	1·2
D_{13}	28	39	39	8·2
D_{14}	25	38	32	12·0
D_{15}	26	40	34	10·1
D_{16}	28	43	38	8·2
D_{17}	37	49	35	−1·2
D_{18}	30	41	29	5·6
D_{19}	25	36	24	10·7
S.E.M. (D.F. = 54)	1·0	1·6	0·5	

$P < 0·05$.
D_1–D_{19}, diets 1–19 fed to goats during the RC studies.
DMI, dry matter intake; DDMI, digestible dry matter intake; DOMI, digestible organic matter intake; GE, gross energy.

Comparison of measurement techniques

Methane production (ml/g DM and proportion of GE) from all the diets measured in the RC and that estimated by IVGPT are presented in Table 6. When IVGPT was taken as a single treatment the values were similar to the chamber in D_1–D_2, D_5–D_9 and D_{11}. However, when CH_4 production from individual diets measured in the RC was compared with that estimated from the gas collected after 24 h IVGPT incubation, it was lower from D_1–D_6 and D_{17}; similar from D_7–D_{12} and D_{15}, D_{16} and D_{18} and higher ($P < 0·0001$) from D_{13}, D_{14} and D_{19}. The trend was similar when expressed as a proportion of GE or methane conversion rate (MCR). However, after 48 h of incubation it was similar to that measured in RC from D_1–D_9 and ($P < 0·0001$) higher from D_{10}–D_{19}. The correlation coefficient between the CH_4 produced (ml/g DM) after 24 h *in vitro* incubation and the nutritive composition of the diets were: −0·55 DM; −0·52 OM; −0·42 aNDF and −0·84 ADF and with the digestibility of nutrients were 0·87 DMD; 0·79 OMD; 0·57 ADF digestibility (ADFD) and 0·83 TDN.

It was observed that a simple comparison between the two methods shown in Fig. 1 ($R^2 = 0·37$) and Fig. 2 ($R^2 = 0·24$) is very weak. The difference recorded in the CH_4 production estimated by IVGPT with that of RC was attributed to the fibre fractions of the diets. Average ADF content (g/kg) from D_1–D_9 was 292, whereas it was 247 from diets D_{10}–D_{19}. Similarly, coefficients of digestibility for DM, OM and TDN of D_1–D_9 were 0·69, 0·71 and 0·679, respectively, whereas they were 0·72, 0·78 and 0·774 in D_{10}–D_{19}, respectively. The fermentation of diets (D_1–D_9) was comparatively slow to start with, due to higher structural carbohydrate content that resulted in lower gas volume and subsequently lower methanogenesis in the initial stages of fermentation; whereas it was opposite in the case of D_{10}–D_{19}. The *in vitro* gas volume indicated that in those diets containing low fibre and with high digestibility, onset of fermentation was quick resulting in higher total gas production and hence CH_4 output, whereas the reverse was true in diets containing higher ADF with lower digestibility. However, after 48 h of incubation the CH_4 estimated by IVGPT was higher from D_{10}–D_{19} as compared with that measured in RC (Fig. 3). This was due to the degradation of fibre components and subsequent methanogenesis. To confirm this, an analysis using ADF as covariate was performed. The covariate analysis indicated that ADF was statistically significant ($P < 0·001$) $R^2 = 71·0$ and ($P < 0·001$) $R^2 = 62·2$ after 24 and 48 h IVGPT, respectively.

The output of CH_4 from D_1–D_9 ranged from 118 to 165 ml/l (mean 134) of total gas produced after 24 h of *in vitro* incubation and 131 to 187 (mean 161) after 48 h; whereas it ranged from 154 to 175 ml/l (mean 164) after 24 h and 165 to 210 (mean 196) after 48 h from D_{10}–D_{19}.

DISCUSSION

It has been recognized that CH_4 is a potent greenhouse gas and CH_4 production from ruminants contributes about 0·15 to global atmospheric CH_4 flux (Moss *et al.* 1994). Since CH_4 is a loss of biological energy to rumen microbes as well as the host ruminant, reducing CH_4 energy losses would in turn increase feed conversion into animal products such as milk, meat and wool. Results of the current RC study showed considerable variation in the proportional CH_4 production in adult goats fed diets varying in nutritional composition. Quantity and quality of feed consumed by animals have a major effect on the CH_4 production because individual VFA proportion in the rumen is influenced by roughage: concentrate ratio (Fahey & Berger 1988), nature and rate of fermentation of carbohydrates (Johnson *et al.* 1996). Roughage-based diets favour acetate production and increase CH_4 production per unit of fermentable OM (Johnson & Johnson 1995). Methane production

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Table 6. *Comparison of CH_4 production in Japanese goats measured in RC with that estimated by IVGPT at two intervals*

Diets	RC	IVGPT 24 h	IVGPT 48 h	Chamber *v.* IVGPT 24 h mean differences	Chamber *v.* IVGPT 48 h mean differences
			Methane (ml/g DM)		
D_1	36	29	37	6·6	−1·2
D_2	28	24	29	4·1	−0·8
D_3	33	27	32	5·8	0·5
D_4	39	35	38	4·2	1·0
D_5	37	33	38	3·9	−1·2
D_6	37	34	39	4·0	−1·8
D_7	26	21	26	4·5	−0·3
D_8	27	26.	31	1·2	−4·1
D_9	23	20	23	3·1	−0·2
D_{10}	39	37	45	1·8	−6·2
D_{11}	38	37	43	1·3	−4·2
D_{12}	35	37	46	−2·0	−10·9
D_{13}	28	36	40	−7·9	−12·2
D_{14}	25	32	41	−7·2	−16·4
D_{15}	26	29	39	−3·2	−12·9
D_{16}	28	28	37	−0·3	−9·3
D_{17}	37	34	43	2·7	−6·1
D_{18}	30	33	41	−2·5	−10·9
D_{19}	25	30	38	−4·6	−13·1
			Methane as proportion of GE (MCR) (e^{-1})		
D_1	7·8	6·3	8·0	1·5	−0·2
D_2	6·1	5·2	6·2	0·9	−0·2
D_3	7·1	5·9	7·0	1·3	0·1
D_4	8·2	7·4	8·1	0·8	0·1
D_5	7·6	6·9	8·0	0·7	−0·4
D_6	7·7	7·0	8·5	0·7	−0·8
D_7	5·8	4·6	5·7	1·2	0·1
D_8	6·0	5·7	6·9	0·3	−0·9
D_9	5·1	4·4	5·2	0·7	−0·1
D_{10}	8·2	7·8	9·4	0·4	−1·3
D_{11}	8·0	7·8	8·9	0·2	−0·9
D_{12}	7·3	7·7	9·6	−0·4	−2·3
D_{13}	6·0	7·7	8·6	−1·7	−2·6
D_{14}	5·0	6·6	8·6	−1·6	−3·5
D_{15}	5·4	6·1	8·2	−0·7	−2·8
D_{16}	5·8	5·9	7·8	−0·1	−2·0
D_{17}	7·8	7·2	9·0	0·6	−1·3
D_{18}	6·4	6·9	8·7	−0·5	−2·3
D_{19}	5·1	6·1	7·7	−1·0	−2·6
S.E.M. (D.F. = 54)	1·32	1·20	1·42	0·99	1·29

D_1–D_{19}, diets 1–19 fed to goats during the RC studies.
IVGPT 24 h, IVGPT conducted up to 24 h; IVGPT 48 h, IVGPT conducted up to 48 h.

recorded from the sole alfalfa (D_9) was the lowest among all the diets. According to Varga *et al.* (1985) CH_4 yield from leguminous forages were lower than from grass forages due to the type of carbohydrate present. When mixed diets were fed to animals (as in the present study) quantity of CH_4 produced depends on the level and type of grain in the diet and fermentation that result. Diets D_4–D_6 contained 0·50 of concentrate; D_{17}–D_{19} contained 0·68 and D_{10}–D_{12} contained 0·82 of concentrate. The composition of the concentrate mixture of different diets is given in Table 2.

The corresponding CH_4 output (average ml/g DMI) from these groups was 26·5, 37·9, 30·9 and 37·3, respectively. Increased concentrate portion in the diet also resulted in increased digestibility

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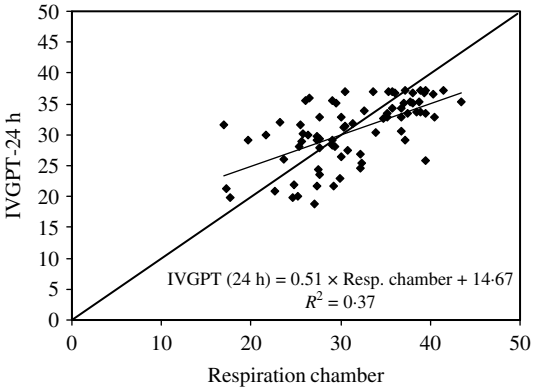


Fig. 1. Regression graph showing the comparison of methane output measured in respiration chamber (RC) with that estimated after 24 h IVGPT. 24 h IVGPT: *in vitro* gas production test carried out for 24 h. Covariate analysis: 24 h IVGPT *v.* respiration chamber, with acid detergent fibre (ADF) as covariate. ADF was statistically significant ($P<0·001$) $R^2=0·71$.

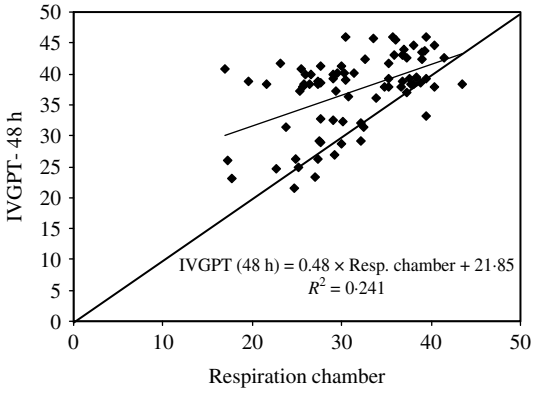


Fig. 2. Regression graph showing the comparison of methane output measured in RC with that estimated after 48 h IVGPT. 48 h IVGPT: *in vitro* gas production test carried out for 48 h. Covariate analysis: 48 h IVGPT *v.* respiration chamber, with ADF as covariate. ADF was statistically significant ($P<0·001$) $R^2=0·622$.

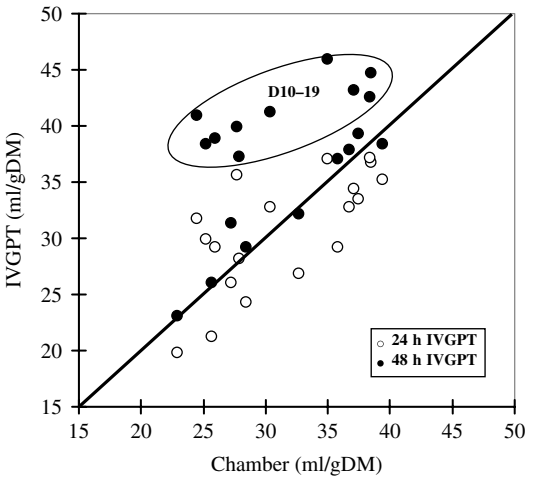


Fig. 3. Comparison of CH_4 production (ml/g DM) by goats measured in RC with that of CH_4 estimated after 24 and 48 h IVGPT. IVGPT: *in vitro* gas production test. D10–19: diets 10–19.

(Table 4); hence, there was no significant ($P>0·05$) reduction in the CH_4 output at higher concentrate level (0·82 in D_{10}–D_{12}) when compared with D_1 (Table 5). However, lower CH_4 production from diets containing 0·68 of concentrate was attributed to the reduced ADF digestibility, which could be possibly due to the fat contents in D_{18} ($P=0·087$) to D_{19} ($P=0·029$). Fat content (g/kg) in these diets ranged from 61 to 75 and hence ADFD was also comparatively lower. Dietary fats were reported to have a suppressing influence on CH_4 emission (Lee *et al.* 2003). However, quantity, degree of unsaturation and chain length of the lipid affects CH_4 output (Blaxter & Wainman 1964).

The correlation between DMD and CH_4 production was 0·8 among all the diets. Blaxter & Clapperton (1965) calculated that the relationship between CH_4 emission and digestibility was dependent on intake level. When feed was given at low levels of intake, CH_4 emission increased with digestibility (similar to current observations), whereas with high intakes CH_4 emission fell as digestibility increased. Thus, while the weight of evidence supports reduction of CH_4 by increasing the ratio of cell wall to soluble carbohydrate in the diet, the impact of carbohydrate type on CH_4 production was complex. Absence of any significant effect of carbohydrate source on CH_4 production in the current study was attributed to the lower level of feeding (1·1 M).

Addition of by-products SPVS (D_2) ($P<0·016$) and pumpkin (D_{13}) ($P<0·052$) contributed to a reduced CH_4 emission in goats when compared with that of D_1. The reduction was on average 7·5% (range 5·9–9·1) with SPVS and 8·2% (range 6·5–9·9) with pumpkin as compared with that of D_1 (Table 5). Although there was no adverse effect ($P>0·05$) of these compounds on DMD, NDF and ADF digestibility were reduced in D_{13}. There were no earlier reports about the effect of these by-products on methanogenesis in goats. The exact reasons/factors responsible for CH_4 suppression are yet to be identified. Further studies are required to determine the specific factors/moiety responsible for anti-methanogenic property in these compounds, so that they could be incorporated as cheaper dietary supplements to inhibit methanogenesis in ruminants.

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Incorporation of brewers' grains in the diet (D_{14}–D_{16}) reduced CH_4 emission ($P<0{\cdot}01$) from goats compared with D_1. Maximum CH_4 suppression of 12·0% (range 10·2–13·8; $P=0{\cdot}025$) was recorded in freeze-dried material (D_{14}) when compared with those dried at 60 °C (10·0%; range 8·90–11·1 in D_{15}; $P=0{\cdot}044$) or 130 °C (8·0%; range 6·80–9·20 in D_{16}; $P=0{\cdot}079$). Although there was no ($P>0{\cdot}05$) adverse effect of brewers' grains addition on DMD, NDF digestibility was reduced. Wainmann *et al.* (1984) observed that fermentation of brewery and distillery products containing readily available fibre resulted in half to one-third of CH_4 production compared with that seen with common feedstuffs of comparable digestibility. However, Sullivan & Martin (1999) and Lila *et al.* (2004) failed to find any effect of live yeast preparation on CH_4 production. Newbold & Rode (2006) reported that yeast did have an effect on rumen methanogenesis (an average decrease in CH_4 of 27·0%); effects were dependent on the yeast used and were highly variable (ranging from no effect to a 5·0% decrease in daily CH_4 output). Beever (1993) suggested that the partitioning of degraded DM between microbial synthesis and fermentation products was responsible for the alteration in the pattern of hydrogen production and hence methanogenesis.

The relationships between CH_4 emission measured in RC (ml/g DM) and DMI, organic matter intake (OMI) (−0·60), GE intake (GEI) (−0·64) and digestible organic matter intake (DOMI) (−0·57) were negative. The trend was similar when expressed as proportion of GE (−0·60 DMI, −0·61 OMI, −0·60 GEI and −0·57 DOMI). Johnson & Johnson (1995) noted that CH_4 loss as per cent of GEI declined by 0·02 for each multiple increase of intake; caused mainly by the rapid passage of feed out of the rumen (Mathison *et al.* 1998; Hegarty 2001). However, the extent to which intake levels affect passage rate of roughages is proportionally less than with concentrate or mixed diets (Mathison *et al.* 1998). Benchaar *et al.* (2001) reported an increase in CH_4 with increasing DMI, irrespective of the diet type (100 alfalfa *v.* 30:70 alfalfa/concentrate mixture). However, when CH_4 was expressed relative to GEI or digestible energy (DE), CH_4 yield decreased when DMI was increased, similar to the findings in the current study.

In earlier studies, Shibata *et al.* (1992) recorded 27·1 ml CH_4/g DMI from Japanese male goats at around 1·5 maintenance feeding level. Moss (2002) reported 31·0 ml/g DMI in sheep. IPCC (2000) recommendation for estimate of methane emission is 7% GE in mature sheep. The CH_4 produced from goats in the present study fed a range of diets was 14·4 litres/day or 30·8 ml/g DMI or 38·4 ml/g DOMI. The variations recorded (range 23 ml/g to 39 DMI or 0·05–0·08 of GE) in CH_4 production clearly render prediction equations that assume constant relationships of CH_4 production less useful. A similar observation was also made recently by Blummel *et al.* (2005) after comparing CH_4 emission from straw-fed sheep in open circuit RC with that predicted by fermentation characteristics measured by IVGPT.

Comparison of CH_4 production measured in RC with that estimated by IVGPT

It is well established that goats (browser) are more resistant to dietary intake of tannins than sheep/cattle (grazer) (Silanikove *et al.* 1996) and tannins are reported to suppress methane emission in ruminants (Hess *et al.* 2006). Since none of the diet samples contained any known source of tannin, it was presumed that source of rumen inoculum (cattle in the current study) was not a contributing factor for the variation in the methane estimated by IVGPT when compared with that produced by goats in RC.

The proportion of CH_4 increased as the incubation time increased, suggesting that slowly digestible fraction of feed was associated with higher CH_4 production. Although the proportion of CH_4 in the total gas increased with time of incubation, the actual amount of CH_4 produced was much higher during the first 24 h of incubation. It was 26·2 ml/g from D_1–D_9 after 24 h of incubation and 36·9 ml/g from D_{10}–D_{19} with average being 31·6 ml/g DM. However, CH_4 produced after 24 h incubation averaged 0·81 of the total CH_4 produced after 48 h of incubation from all the diets (D_1–D_{19}). There were two salient observations, in those diets containing higher proportions of easily fermentable carbohydrates (non-fibrous carbohydrate and hemi-cellulose); CH_4 output estimated after 48 h IVGPT was higher than that measured in the RC, whereas in diets containing higher proportions of structural carbohydrates, CH_4 estimated after 48 h-IVGPT was very close to that measured in chamber (Fig. 3). After comparing the CH_4 emission measured by SF_6 technique in cattle with that estimated by IVGPT Bhatta *et al.* (2006) also reported similar findings.

In the current study, goats were fed at maintenance level (1·1 M) and since a large part of the ruminant population would be fed at above maintenance levels, future experiments are recommended on the comparative studies of those diets with IVGPT. Further, the present study focused on mixed diets, results might differ with very high concentrate/roughage diets when the VFA profile changes substantially.

CONCLUSIONS

The CH_4 production measured in the open circuit RC from goats fed at 1·1 M diets differing in nutrient composition ranged from 22·9 to 39·4 with the average being 14·4 litres/day or 30·8 ml/g DMI or 42·3 ml/g DDMI. When it was expressed as a proportion of GE it ranged from 0·0502 to 0·0819, with

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an average of 0·0665. Addition of by-products like SPVS, pumpkin and brewers' grains in the diet suppressed CH_4 emission. Although composition of the diets had a significant effect on methane emission, methane output estimated by IVGPT was very close to that measured in the chamber demonstrating that this system could be used to estimate the CH_4 production potential from diets to generate a database and also to plan mitigation strategies in small ruminants to improve their performance as well as to reduce the greenhouse gases. However, further *in vitro* studies are recommended in feed samples from small ruminants fed at higher levels of intake.

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