The SF_6 tracer technique for measurements of methane emission from cattle – effect of tracer permeation rate

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Land, Climate & Environment Section, AgResearch Limited, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand. *Received 30 August 2007, accepted 7 February 2008.*

Pinares-Patiño, C. S., Machmüller, A., Molano, G., Smith, A., Vlaming, J. B. and Clark, H. 2008. **The SF_6 tracer technique for measurements of methane emission from cattle – effect of tracer permeation rate.** Can. J. Anim. Sci. **88**: 309–320. Previous experiments have suggested that estimates of methane (CH_4) emissions from ruminant animals made using the sulphur hexafluoride (SF_6) tracer might be influenced by the permeation rate of SF_6 (PR). This study examined the latter issue with cattle. For this, analyses of data sets from two grazing trials involving large herds (exps. 1 and 2) and a specifically designed controlled trial (exp. 3) were conducted. Individual daily CH_4 emissions from 296 (exp. 1) and 388 (exp. 2) Friesian × Jersey cows in mid-lactation were measured with herds subdivided into four (exp. 1) or five (exp. 2) measurement groups and dry matter intake (DMI) estimated by energy metabolism algorithms. The ranges of tracer PR in exps. 1 and 2 were 2.624–5.689 and 2.214–3.594 mg d^{-1}, respectively. Experiment 3 was conducted using 12 rumen-fistulated beef steers pen-fed on lucerne silage and design arranged as a 4 × 4 Latin square with three replications. Permeation tubes with four levels of nominal PR (three tubes each): low (L), medium (M), medium-high (MH) and high (H) were randomly assigned to four rumen deployment sequences (L-M-MH-H, H-MH-M-L, MH-L-H-M and M-H-L-MH). The grazing experiments revealed a positive effect of PR on the CH_4 emission estimates (1 mg SF_6 d^{-1} accounting for 0.6–2.3 g kg^{-1} DMI), but this effect was significant ($R^2 = 0.06–0.23$, $P < 0.05$) only when there was a large range in PR (exp. 1), whereas with a narrower PR range (exp. 2) the effect was not significant ($R^2 < 0.04$, $P > 0.05$). Experiment 3 revealed that the influence of PR upon CH_4 emission estimates was linear. It is concluded that despite an influence of PR on CH_4 emission estimates, accuracy and precision of the tracer technique is warranted provided that PR are used in a narrow range and balanced between the experimental treatments.

Key words: Methane, permeation rate, SF6 tracer, cattle, variation

Pinares-Patiño, C. S., Machmüller, A., Molano, G., Smith, A., Vlaming, J. B. et Clark, H. 2008. **La technique du traceur SF_6 pour mesurer les émissions de méthane par les bovins – effet de la vitesse de diffusion du traceur.** Can. J. Anim. Sci. **88**: 309–320. Il a été montré au cours d'expérimentations antérieures que la mesure des émissions de méthane (CH_4) chez les ruminants par la technique au SF_6 peut être influencée par la vitesse de diffusion de SF_6 (PR). Cette étude a examiné la dernière question avec des bovins. Pour cela, l'analyse de l'ensemble des données de deux expérimentations effectuées au pâturage avec de grands troupeaux (Expérimentations 1 et 2) et une expérimentation en conditions contrôlées et spécialement conçue (expérience 3) a été réalisée. Les valeurs d'émissions individuelles de CH_4 sur 296 (Expérimentation 1) et 388 (Expérimentation 2) vaches Frisonnes x Jersiaises en milieu de lactation, réparties en 4 (Expérimentation 1) ou 5 (Expérimentation 2) groupes, ont été mesurées au pâturage. L'ingestion de matière sèche (DMI) a été estimée à partir d'algorithmes du métabolisme énergétique. Les gammes de variation de PR du traceur dans les Expérimentations 1 et 2 étaient de 2,624–5,689 et 2,214–3,594 mg j^{-1}, respectivement. L'Expérimentation 3 a été réalisée avec 12 bœufs nourris à l'auge avec de l'ensilage de luzerne selon un schéma expérimental en carré latin 4 × 4 avec trois répétitions. Les tubes perméables utilisés étaient répartis selon quatre niveaux de la vitesse de diffusion PR (3 tubes pour chaque niveau): faible (L), moyen (M), moyen-haut (MH) et haut (H). Les tubes étaient introduits dans le rumen des animaux selon les quatre séquences suivantes: L-M-MH-H, H-MH-M-L, MH-L-H-M et M-H-L-MH L'analyse de l'ensemble des données des expérimentations au pâturage a révèle un effet positif du PR sur les émissions de CH_4 (1 mg de SF_6 j^{-1} correspond à 0,6–2,3 g kg^{-1} DMI), mais cet effet était significatif ($R^2 = 0,06–0,23$; $P < 0.05$) seulement quand le PR varie de manière importante (Expérimentation 1). L'effet n'était pas significatif ($R^2 < 0,04$; $P > 0,05$) lorsque la gamme de valeurs du PR est plus étroite (Expérimentation 2). L'Expérimentation 3 a montré que l'influence de PR sur les mesures des émissions de CH_4 était linéaire, et qu'il n'y a pas eu de différence entre les traitements MH et H. Cette étude indique qu'une influence de PR sur les évaluations des émissions de CH_4 existe, mais que la justesse et la précision de la technique au SF_6 peuvent être assurées si les valeurs de PR sont peu différentes et sont prises en compte dans la mise en place des schémas expérimentaux.

Mots clés: Méthane, taux de diffusion, traceur SF_6, bovins, variation

Abbreviations: **ADF**, acid detergent fibre; **CHO**, soluble carbohydrates; **CP**, crude protein; **DM**, dry matter; **DMI**, dry matter intake; **LW**, liveweight; **ME**, metabolisable energy; **NDF**, neutral detergent fibre; **OM**, organic matter; **PR**, permeation rate of SF_6; **SF_6**, sulphur hexafluoride

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Ruminant livestock is the single most important source of anthropogenic emissions of CH_4 (Lassey 2007), which accounts for about 71 Tg yr^{-1}, including 44 Tg yr^{-1} from grasslands utilisation (Clark et al. 2005). In countries such as New Zealand, where livestock is a major contributor to the CH_4 emission inventory, the Kyoto Protocol commitments have prompted a renewed effort in measuring ruminant CH_4 emissions accurately and developing CH_4 abatement strategies.

In the past, measurements of CH_4 emissions from ruminants relied on the use of calorimetric methods. Although accurate in principle, these methods cannot be readily applied to large numbers of animals in a single experiment, and they impose constraints on the natural behaviour of the animals being tested. The sulphur hexafluoride (SF_6) tracer technique for CH_4 measurement was developed in the mid 1990s (Johnson et al. 1994a) and has been successfully adapted in New Zealand and other countries for use in pen-fed and grazing animals. The tracer technique is the method of choice in all situations where simultaneous measurements on large groups of animals are required (Clark et al. 2005).

Experiments conducted on sheep by Murray et al. (1976) found that most (87%) of the enteric CH_4 production was accounted by the rumen and that almost all (95%) of the ruminal CH_4 was eructed, whereas about 89% of the hindgut CH_4 production was absorbed and excreted through the lungs together with the remaining ruminal CH_4. Since the majority (70-99%) of the eructed gases is first inhaled into the lungs, and then expired along with respiratory gases (Colvin et al. 1957; Dougherty and Cook 1962; Hoernicke et al. 1965) and provided that eructed and respired gases are well mixed before expiration, the SF_6 tracer technique would account for about 98% of the total production of CH_4.

The SF_6 technique involves the use of a SF_6-charged "permeation tube" dosed into the reticulo-rumen and the subsequent collection of time-integrated breath samples (usually ~22-h period) for analysis of CH_4 and SF_6 concentrations (Lassey 2007). The calculation of daily CH_4 emission is based on the CH_4/SF_6 ratio of concentrations (adjusted for background concentrations) and the specific "permeation rate" of SF_6 (PR) from the particular permeation tube. The individual PRs for tubes used in any single trial differ from each other and thus the PR becomes a potential source of variation in the calculated CH_4 emissions. In fact, there is preliminary evidence that PR contributes to the observed animal-to-animal variation in CH_4 emission estimates (Vlaming et al. 2005). The latter issue needs to be addressed before any CH_4 abatement strategy using animal-to-animal variation is pursued.

The objective of the present study was to examine the influence of SF_6 permeation rate (PR) on the estimates of CH_4 emissions from cattle. For this purpose, analyses of data sets from two grazing trials involving large herds of dairy cows (exps. 1 and 2) and a specifically designed controlled trial (exp. 3) were conducted.

MATERIALS AND METHODS

Data set from Grazing Experiments (exps. 1 and 2)

Methane emissions from 296 (exp. 1) and 388 (exp. 2) 3-yr-old F_2 Friesian × Jersey dairy cows in mid-lactation (~170 d in milk) were measured in January–February 2004 and 2005, respectively at Hawera (Taranaki, New Zealand; Lat. 39°35'S, Long. 174°16'E). The herds were subdivided into four (exp. 1) or five (exp. 2) groups balanced for calving date and milk production, and measurements were conducted on one group each week while grazing perennial ryegrass/white clover (*Lolium perenne/Trifolium repens*) pasture. Thus, trials were conducted over 4 (exp. 1) or 5 (exp. 2) consecutive weeks. The group of cows involved in the fifth week of measurement of exp. 2 was those for which sample collection was unsucesful in the previous 4 wk of measurement. Each measurement group of cows was acclimatised to the experimental procedures (grazing management and breath collection gear) during the week preceding the measurement. The cows were milked twice daily (0600–0700 and 1500–1600) and these times were used to accustom them to manipulation of the breath collection gear.

Within each experiment, paddocks were managed by rotational grazing. Paddock allocation was based on twice-a-week monitoring of herbage characteristics, which ensured similar growth stages and pre-grazing herbage masses across the weeks of measurement. Similarly, during both the acclimatisation and measurement periods, each measurement group of cows grazed pastures of a similar quantity and quality. Cows were strip-grazed and a fresh area was offered every day after the morning milking. Cows were grazed at generous herbage allowances (12–14% of liveweight, on a DM basis) to achieve ad libitum pasture intakes (Holmes et al. 1987). Samples of the herbage were collected twice daily by hand-plucking and simulating cows selective grazing. Herbage samples were oven-dried (65°C, 48 h) for both DM determination and chemical composition analysis by near infrared spectroscopy (NIRS).

Liveweight (LW) was measured automatically at each milking. Average LW was calculated as the mean of the LW at the morning milkings through the measurement week. Liveweight gain was calculated by fitting a linear regression to LW measured during the morning milkings and identified with the slope coefficient. Condition score was assessed at the start and the end of the week of measurements. Milk production was measured at each milking and samples of milk (AM and PM milking) for chemical composition analyses were taken at the mid of the measurement period. These animal data and feed quality were used to calculate the apparent feed dry matter intake (DMI) using the algorithms of the Standing Committee on Agriculture (SCA 1990).

Daily CH_4 emissions were measured during 4 (exp. 1) or 3 (exp. 2) consecutive days using the SF_6 tracer gas technique as described by Ulyatt et al (2002). Briefly, pre-calibrated permeation tubes were dosed per os into the reticulo-rumen of each animal 7 d prior to the collection of breath samples, to ensure that SF_6 concentration had reached equilibrium in the rumen. Expired gases were collected continuously (approximately 22-h periods) into evacuated PVC containers ("yokes") during the collection period. Daily background air samples were collected at four points just upwind of the pasture strip, using the same procedures as for breath samples. Breath and background samples were analysed for concentrations of CH_4 (ppm, parts per million by volume) and SF_6 (ppt, parts per trillion by volume) by gas chromatography (Shimadzu 2010, Japan) as described by Pinares-Patiño et al. (2007b). Daily CH_4 emissions were calculated from the specific SF_6 permeation rates and the CH_4/SF_6 ratio of concentrations in breath samples, after adjustment for background gas concentrations (Johnson et al. 1994a). The number of animals and their mean LW and milk yields during the measurement period, as well as the mean SF_6 permeation rate of the deployed permeation tubes are shown in Tables 1 (exp. 1) and 2 (exp. 2).

Pen Experiment (exp. 3)

This experiment was conducted in June 2005 and involved 12 well-trained 2-yr-old Hereford × Friesian steers (average LW 478 ± 41 kg) fitted with rumen cannulas and fed twice daily (0800 and 1500) on molassed-lucerne silage (ChaffHage^{TM}, The Great Hage Company, Reporoa, NZ) at 1.2 times their maintenance metabolisable energy requirements (SCA 1990). During feedings steers were kept indoors in individual stalls where drinking water was freely available. Most of the steers consumed all feed allocated within a 2-h period. At the end of feedings, steers were moved outdoors to two adjacent sawdust pads (six animals per enclosure) where only water was provided ad libitum. Stalls were allocated randomly at the start of the experiment and these allocations were maintained throughout the experiment, whereas after each feeding the allocation of steers to the outdoors pens were done arbitrarily as animals left their stalls.

A batch of SF_6 permeation tubes were manufactured and calibrated by weekly weighing over 12 wk (National Institute for Water and Atmospheric Research, Kilbirnie, Wellinton, NZ). Twelve permeation tubes with nominal four levels of SF_6 permeation rates (low, medium, medium-high and high) were selected on the basis of linearity of mass loss ($R^2 > 0.99$). The pre-calibrated PRs in each level, low (L), medium (M), medium-high (MH) and high (H) are reported in Table 3. Four sequences of permeation tube deployment were established in a cross-over manner (L-M-MH-H, H-MH-M-L, MH-L-H-M and M-H-L-MH) and randomly assigned to the experimental animals, balanced for number of replications (three animals per sequence).

After a 10-d acclimatisation to feeding and management conditions, measurements were carried out during four consecutive periods (1, 2, 3, and 4) each lasting 7 d (days 1–7). During each measurement period, the permeation tubes were inserted into the reticulum via the rumen cannula on day 1 and retrieved on day 7. At retrieval, the tubes were rapidly transferred to other animals following the sequence shown in Table 3. The swapping of permeation tubes between sequences of deployment was conducted randomly for any of the three animals within each sequence.

Within each period, breath samples from individual animals were collected over days 5–7 using the SF_6 tracer procedures as described for exps. 1 and 2. Permeation tubes were recovered at the end of the experiment, and post-experiment PRs determined through serial weighings, from which individual PRs could be determined for each measurement period by interpolation (Lassey et al. 2001). The mean specific PR at each of the measurement periods are shown in Table 3. Concentrations of CH_4 and SF_6 gases and the ratio of these gases in the breath and background samples were determined as for exps. 1 and 2, whereas the daily CH_4 emissions were calculated using the specific PR instead of the pre-calibrated PR. Throughout the trial, the daily background air samples were collected at two points facing the predominant wind direction inside the building (doors) or the upwind of the

Table 1. Cow numbers (*n*), mean (±SD) liveweight (LW) and milk yield, and mean (±SD) permeation rate of SF_6 (PR) from permeation tubes for the four measurement groups, exp. 1

	Measurement group				
	1	2	3	4	Overall
Cows					
n	75	65	79	77	296
LW (kg cow^{-1})	421 ± 33	417 ± 38	415 ± 41	422 ± 38	419 ± 37
Milk yield (L d^{-1})	14.0 ± 2.5	12.6 ± 2.6	12.5 ± 2.6	12.0 ± 2.2	12.8 ± 2.6
SF_6 permeation tubes[z]					
PR (mg d^{-1})	3.84 ± 0.61	3.86 ± 0.69	3.77 ± 0.75	3.80 ± 0.67	3.81 ± 0.68

[z] The number of permeation tubes corresponded to the number of cows, one tube per cow.

Table 2. Cow numbers (*n*), mean (± SD) liveweight (LW) and milk yield, and mean (±SD) permeation rate of SF_6 (PR) from permeation tubes for the five measurement groups, exp. 2

	Measurement group					
	1	2	3	4	5	Overall
Cows						
n	93	90	85	90	30	388
LW (kg cow^{-1})	418±40	421±34	423±35	425±37	439±37	423±37
Milk yield (L d^{-1})	13.5±2.0	14.3±2.3	13.7±2.9	13.0±3.1	12.8±2.5	13.6±2.6
SF_6 permeation tubes[z]						
PR (mg d^{-1})	2.74±0.23	2.80±0.31	2.81±0.28	2.81±0.30	2.82±0.35	2.79±0.29

[z] The number of permeation tubes corresponded to the number of cows, one tube per cow.

sawdust pads. The background collection yokes were moved from indoors to outdours and vice versa when the animals were moved. The range of concentrations of CH_4 and SF_6 in background samples were 1.9–2.9 ppm and 3.1–4.7 ppt, respectively.

Feed supply for the entire experiment was bought as a single batch. Individual feed allocations were weighed daily and samples of feed offered were collected daily and oven-dried (65°C, 48 h). Feed refusals amounted to only a few grams and were considered negligible. Chemical composition of feed on offer was analysed on within-period pooled samples according to the procedures described by Pinares-Patiño et al. (2007b). Mean daily DMI for each animal was averaged over the entire measurement period (7 d).

At all the measurement periods the steers belonging to each of the four sequences of permeation tube deployment were neither physically grouped nor differently managed. Thus, the experimental design was a replicated 4 × 4 Latin square with steers as rows and periods as columns (Table 3).

The experimental protocols for both the grazing and pen experiments were approved by the Animal Ethics Committee of AgResearch Limited.

Calculations and Statistical Analysis

Mean daily CH_4 emissions by individual animals were calculated by averaging their daily measurements. Methane emissions were expressed on an absolute daily basis (g d^{-1}) and per unit of DMI (g kg^{-1}) (CH_4 yield). The term "CH_4 emission", is a generality referring to both "daily CH_4 emission" and "CH_4 yield", but in all cases is calculated based on measured CH_4/SF_6 ratios (background adjusted) and individual PR values. Since the terms "daily CH_4 emission" and "CH_4 yield" represent calculated values, they refer to "apparent" rather than actual CH_4 emissions.

Preliminary analysis of variance of CH_4 emission data from the grazing experiments (1 and 2) including groups of measurement, PR, DMI and their interactions showed that there were small but significant effects of measurement groups on the daily CH_4 emission in both

Table 3. Sequences of SF_6 permeation rate (PR) treatments and mean (±SD) PR applied to the groups of steers throughout the periods of measurement, exp. 3

	Periods of measurement[z]			
	1	2	3	4
Sequence A (n = 3 steers)				
Treatment	L	M	MH	H
PR (mg d^{-1})	1.911±0.055	3.616±0.053	5.263±0.217	10.904±0.278
Sequence B (n = 3 steers)				
Treatment	H	MH	M	L
PR (mg d^{-1})	11.340±0.278	5.303±0.212	3.615±0.054	1.898±0.063
Sequence C (n = 3 steers)				
Treatment	MH	L	H	M
PR (mg d^{-1})	5.342±0.206	1.906±0.057	11.049±0.278	3.614±0.055
Sequence D (n = 3 steers)				
Treatment	M	H	L	MH
PR (mg d^{-1})	3.618±0.052	11.194±0.278	1.902±0.060	5.223±0.223

[z] Permeation rates (PR) of tubes at the second, third and fourth measurement periods were calculated by interpolation from the post-recovery calibration of mass loss.

Table 4. Dry matter (DM) content and chemical composition of diets during the grazing and pen experiments (mean ± SD)

	Experiment 1 (grazed pasture)	Experiment 2 (grazed pasture)	Experiment 3 (lucerne silage)
DM (%)	20.2 ± 3.2	22.5 ± 22.3	35.0 ± 0.4
OM (% of DM)	89.3 ± 0.6	88.6 ± 1.1	93.2 ± 1.0
CP (% of DM)	20.2 ± 2.6	21.0 ± 1.4	17.8 ± 0.3
NDF (% of DM)	54.0 ± 3.4	59.0 ± 3.1	54.8 ± 1.9
ADF (% of DM)	28.8 ± 1.2	31.0 ± 1.0	28.1 ± 1.6
CHO (% of DM)	7.3 ± 0.7	5.0 ± 1.9	7.5 ± 0.9
OMD (%)	66.4 ± 2.0	65.5 ± 1.5	65.1 ± 1.7
ME (MJ kg^{-1} DM)	10.5 ± 0.6	10.5 ± 0.2	10.4 ± 0.3

Abbreviations: DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; CHO, soluble carbohydrates; OMD, organic matter digestibility; ME, metabolisable energy.

exp. 1 (partial $R^2 = 0.034$, $P = 0.008$) and exp. 2 (partial $R^2 = 0.075$, $P = 0.001$), whereas a large proportion of the overall variation in CH_4 emission had between-animal sources ($R^2 = 0.81$ and 0.72 in exps. 1 and 2, respectively). Thus, considering that weather and grazing conditions likely changed over the experimental periods, and given that the sole objective of the grazing experiments was to assess the contribution of PR to the overall variation in CH_4 emission estimates, statistical analyses of the grazing data were conducted within each group of measurement. Multiple regression analyses using the forward selection mode of Proc Reg of SAS software (SAS Institute, Inc. 1999–2001) were conducted in order to assess the relative importance of PR (mg d^{-1}) and apparent DMI (kg d^{-1}) in accounting for the total variation in daily CH_4 emission (g d^{-1}). No other variables were used in the multiple regression analysis. Given that CH_4 emission from pasture-fed animals is closely related to feed intake (Pinares-Patiño et al. 2003, 2007b) and that apparent DMI in this study was highly variable between animals (coefficient of variation ~11%), statistical analysis within groups focused on single effects of PR upon the apparent CH_4 emission per unit of intake (CH_4 yield). This analysis was conducted using the Proc GLM of SAS software. Comparisons of linear regressions (common, separated or parallel lines) were carried out using Proc GLM of SAS software.

Data for the pen experiment were analysed in a 4 × 4 Latin square with three replications. Effects of the nominal SF_6 permeation rate (PR) upon concentrations in breath samples of CH_4, SF_6, CH_4/SF_6 ratio, absolute daily CH_4 emission (g d^{-1}) and CH_4 yield (g kg^{-1} DMI) were analysed using the model:

$$Y_{ijk} = \mu + \text{steer}_i + \text{period}_j + PR_k + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, steer_i is the effect of steer, period_j is the effect of period of measurement, PR_k is the effect of the nominal SF_6 permeation rate ($k = L, M, MH, H$) and ε_{ijk} is the residual. Comparisons of means were made using the least significant difference of means option of Genstat (2006). The Proc GLM procedure of SAS software was used to test linear and quadratic contrasts of PR, using specific PR interpolated at the time of the treatments application. Unless otherwise stated, results are presented as means and standard deviations (± SD).

RESULTS

Grazing Experiments (exps. 1 and 2)

Herbages offered during the grazing experiments were of medium quality (Table 4). Average organic matter digestibility (OMD) and metabolisable energy (ME) contents (66% and 10.5 MJ kg^{-1} DM, respectively) were similar between the two experiments. Within each experiment, chemical compositions and quality of herbages offered varied little with the measurement periods, except for the relatively large variation (coefficient of variation, CV) in crude protein (CP) (13%) during exp. 1 and soluble carbohydrates (38%) in exp. 2.

Table 5. Mean (± SD) daily dry matter intake (DMI), as calculated using an energy requirements model, and apparent CH_4 emissions by the four measurement groups of cows, exp. 1

	Measurement groups				
	1	2	3	4	Overall
Number of cows	75	65	79	77	296
DMI (kg cow^{-1})	17.0 ± 1.8	16.5 ± 2.5	17.4 ± 1.8	18.5 ± 1.8	17.4 ± 2.1
CH_4 emission					
g d^{-1}	320.1 ± 40.4	333.0 ± 41.2	338.5 ± 46.5	336.4 ± 37.9	332.1 ± 42.1
g kg^{-1} DMI	18.9 ± 2.8	20.5 ± 3.0	19.7 ± 3.4	18.3 ± 2.3	19.3 ± 3.0

Table 6. Mean (±SD) daily dry matter intake (DMI), as calculated using an energy requirements model, and apparent CH_4 emissions by the five measurement groups of cows, exp. 2

	Measurement groups					
	1	2	3	4	5	Overall
number of cows	93	90	85	90	30	388
DMI (kg cow^{-1})	17.2±1.9	17.1±1.6	15.7±1.7	17.0±1.9	17.4±1.4	16.8±1.9
CH_4 emission						
g d^{-1}	297.2±36.0	294.0±31.2	278.3±32.6	278.6±35.3	318.7±26.2	289.7±35.2
g kg^{-1} DMI	17.4±2.1	17.2±1.8	18.0±3.0	16.6±2.6	18.3±1.3	17.4±2.4

Mean DMI and apparent CH_4 emission estimates for exps. 1 and 2 are shown in Tables 5 and 6, respectively. In exp. 1, the mean DMI was 17.4 kg cow^{-1} and the mean apparent CH_4 emission was 332 g d^{-1} or 19.3 g kg^{-1} DMI (CH_4 yield). The corresponding estimates for Experiment 2 were 16.8 kg cow^{-1}, 290 g d^{-1} and 17.4 g kg^{-1} DMI. Thus, both mean feed intake and CH_4 emission estimates during exp. 2 seemed to be slightly lower than during exp. 1. Within both grazing experiments, DMI as well as CH_4 emissions were similar across measurement groups (Tables 5 and 6).

Results of the multiple regression analyses of apparent daily CH_4 emission upon PR (mg d^{-1}) and estimated DMI (kg cow^{-1}) for each measurement group of cows in exp. 1 (four groups) and exp. 2 (five groups) are shown in Tables 7 and 8, respectively. All measurement groups in Experiment 1 exhibited a positive and significant association ($P < 0.01$) between PR and the apparent daily CH_4 emission, PR explaining between 6 and 21% of the total variance (partial R^2) in apparent daily CH_4 emission. Similarly, except for Group 3, there was positive and significant association ($P < 0.05$) between the estimated DMI and the apparent daily CH_4 emission, the former explaining between 5 and 36% of the total variance of the latter. For all the measurement groups but Group 2, PR had relatively higher importance than the estimated DMI in explaining the variance in apparent daily CH_4 emission.

In contrast to exp. 1, in exp. 2 the association between PR and the apparent daily CH_4 emission was significant ($P < 0.05$) only for Group 2, with PR explaining less than 4% of the overall variance in CH_4 emission (Table 8). In this experiment (exp. 2), the estimated DMI and apparent daily CH_4 emissions had positive and significant ($P < 0.0001$) associations for Groups 1, 2 and 5 only, with DMI explaining between 22 and 44% of the total variance in daily CH_4 emission (Table 8).

Fig. 1 shows the general relationships between apparent daily CH_4 emission and PR, and DMI for exps. 1 and 2. In exp. 1, there is a positive relationship between PR and apparent CH_4 emissions; however, exp. 2 shows no typical scatter. It should be noted that permeation tubes used in exp. 2 had a narrower range of PR than those used in exp. 1 (2.214–3.594 vs. 2.624–5.689 mg d^{-1}; see Tables 1 and 2). Fig. 1 also shows a positive spread of DMI and apparent daily CH_4 emission data for both exps. 1 and 2.

The simple linear relationships between PR (mg d^{-1}) and the apparent CH_4 yield (g kg^{-1} DMI) for each measurement group in exps. 1 and 2 are shown in Table 9 and Fig. 2. In exp. 1, there was a positive and significant ($P < 0.04$) relationship between these variables for Groups 1, 3 and 4, but not for Group 2 ($P = 0.27$). In this experiment, each mg d^{-1} increase in PR accounted for an increase of between 0.6 and 2.2 g in apparent CH_4 yield (per kg of DMI) and PR explained between 6 and 23% of the total variance in CH_4 yield. In exp. 2, the relationship between PR and apparent CH_4 yield only approached statistical significance ($P < 0.07$) for groups 1, 2 and 3. Further, each mg d^{-1} increase in PR accounted for a similar increase in apparent CH_4 yield as observed in exp. 1, but the proportion of total variance explained by PR was very small (<5%).

Table 7. Multiple regression analysis of the apparent daily CH_4 emission (g d^{-1}) for each measurement group based on permeation rate of SF_6 (PR, mg d^{-1}) and daily dry matter intake (DMI, kg cow^{-1}) in exp. 1

Variable entered (in order)^z	Partial R^2	Model R^2	P value
Group 1			
PR	0.214	0.214	<0.0001
DMI	0.074	0.288	0.0080
Model	$y = 91.9 + 32.2$ PR $+ 6.2$ DMI		<0.0001
Group 2			
DMI	0.357	0.357	<0.0001
PR	0.062	0.419	0.0124
Model	$y = 120.8 + 14.9$ PR $+ 9.4$ DMI		<0.0001
Group 3			
PR	0.205	0.205	<0.0001
DMI	0.013	0.217	0.2734
Model	$y = 176.8 + 29.1$ PR $+ 3.0$ DMI		<0.0001
Group 4			
PR	0.128	0.128	0.0014
DMI	0.053	0.181	0.0322
Model	$y = 176.8 + 18.8$ PR $+ 4.8$ DMI		0.0006

^{z}Variables (PR and DMI) are listed in order of selection in the model.

Table 8. Multiple regression analysis of the apparent daily CH_4 emission ($g\ d^{-1}$) for each measurement group based on permeation rate of SF_6 (PR, $mg\ d^{-1}$) and daily dry matter intake (DMI, $kg\ cow^{-1}$) in exp. 2

Variable entered (in order)^z	Partial R^2	Model R^2	P value
Group 1			
DMI	0.237	0.237	<0.0001
PR	0.008	0.245	0.3191
Model	$y = 92.9 + 14.4\ PR + 9.6\ DMI$		<0.0001
Group 2			
DMI	0.222	0.222	<0.0001
PR	0.037	0.259	0.0407
Model	$y = 84.3 + 19.4\ PR + 9.1\ DMI$		<0.0001
Group 3			
PR	0.020	0.020	0.1924
DMI	0.013	0.033	0.2954
Model	$y = 194.0 + 17.9\ PR + 2.2\ DMI$		0.2478
Group 4			
DMI	0.030	0.030	0.1056
PR	0.014	0.044	0.2684
Model	$y = 185.3 + 13.7\ PR + 3.2\ DMI$		0.1469
Group 5			
DMI	0.437	0.437	0.0001
PR	0.021	0.458	0.3332
Model	$y = 72.3 + 12.6\ PR + 12.1\ DMI$		0.0005

^{z}Variables (PR and DMI) are listed in order of selection in the model.

Table 9. Estimates of intercept and slope, proportion of variance explained (R^2) by model, and probability value for the simple linear regression of apparent CH_4 yield ($g\ kg^{-1}$ DMI) upon the permeation rate of SF_6 (PR, $mg\ d^{-1}$) for each measurement group during exps. 1 and 2 (grazing)

	Intercept	Slope	R^2	P value
Experiment 1				
Group 1	10.6	2.2	0.23	<0.0001
Group 2	18.2	0.6	0.02	0.2728
Group 3	12.0	2.0	0.20	<0.0001
Group 4	15.2	0.8	0.06	0.0359
Experiment 2				
Group 1	12.7	1.7	0.03	0.0762
Group 2	14.0	1.1	0.04	0.0600
Group 3	11.7	2.3	0.05	0.0509
Group 4	14.1	0.9	0.01	0.3447
Group 5	16.6	0.6	0.02	0.4582

Comparison of simple linear regression lines revealed that separate lines fitted best the data in exp. 1, whereas parallel lines (common slope = 1.36 ± 0.42) fitted best the data in exp. 2. The intercept values for groups 1, 2, 3, 4 and 5 in exp. 2 were 13.6, 13.4, 14.2, 12.7 and 14.5, respectively.

The test of comparison of linear regression lines showed that separate lines fitted best the data in exp. 1, whereas parallel lines fitted best the data in exp. 2. In the latter experiment, the common slope was 1.36 $g\ kg^{-1}$ DMI per $mg\ SF_6\ d^{-1}$.

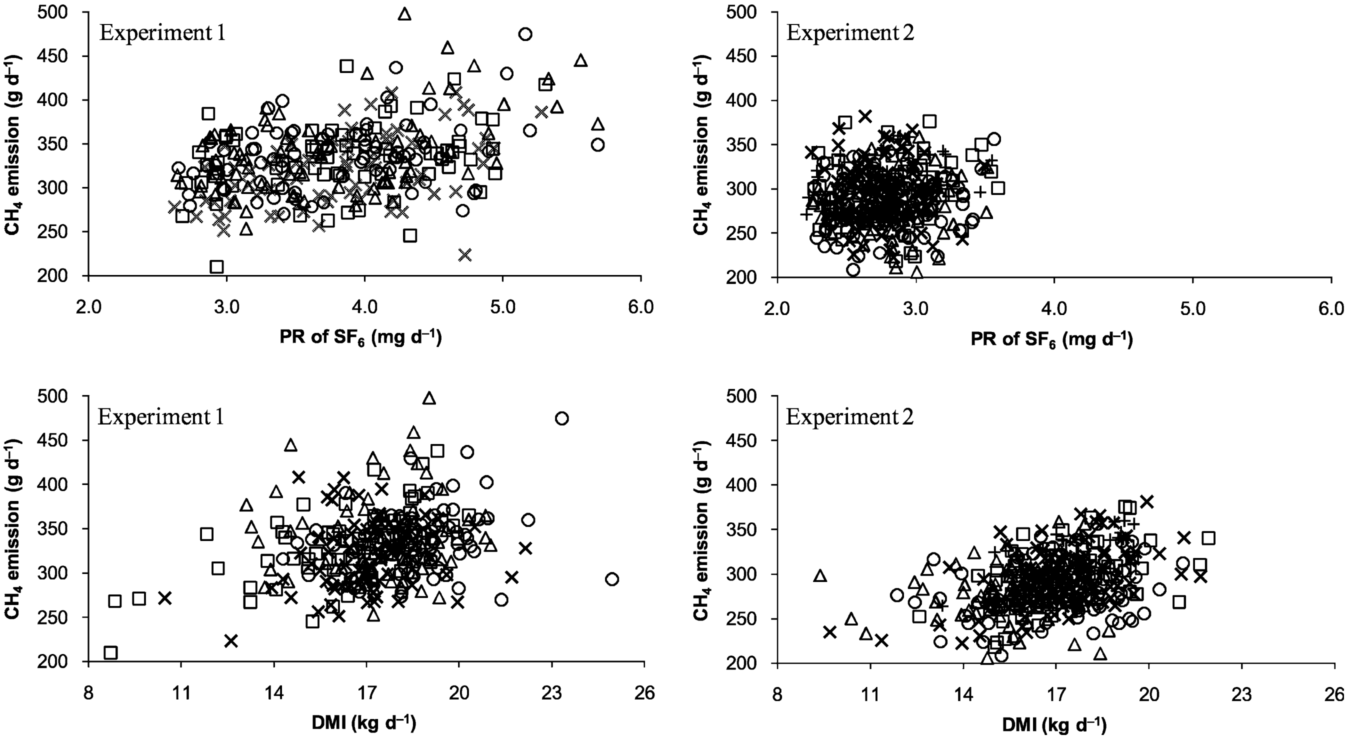

Fig. 1. Relationships between apparent CH_4 emission ($g\ d^{-1}$) and permeation rate (PR) of SF_6 from permeation tubes ($mg\ d^{-1}$), and dry matter intake (DMI, $kg\ d^{-1}$) for each measurement group during exps. 1 and 2. Labels ×, □, Δ, ○, + represent groups 1, 2, 3, 4 and 5, respectively.

Pen Experiment (exp. 3)

Table 10 presents the effects of the nominal SF_6 permeation rate (PR) treatments upon the mean concentration of gases in breath samples and the calculated CH_4 emissions. The concentration of CH_4 (ppm) did not differ ($P > 0.05$) between the nominal PR treatments. As expected, there was a significant linear ($P < 0.05$) effect of PR treatments upon both the concentration of SF_6 (ppt) and the CH_4/SF_6 ratio. Nevertheless, the lack of significant difference ($P > 0.05$) between M and MH treatments on the SF_6 concentration was unexpected.

Figure 3 shows the within-treatment values for CH_4 and SF_6 concentrations and the ratio of their concentrations. Variations in CH_4 concentration were similar between treatments. The within-treatment variations in SF_6 concentrations were also relatively similar for L, M and MH treatments, but variation for the H treatment was larger than those for the other treatments. There were some influential data points for CH_4 and SF_6 concentrations, which corresponded to the same individuals, i.e., a particular individual having high CH_4 and SF_6 concentrations. Within PR treatments, the concentrations of CH_4 and SF_6 correlated highly ($r = 0.93$, 0.94, 0.98, and 0.94 for L, M, MH, and H, respectively; $P < 0.0001$). One important feature shown by Fig. 3 is the fact that the within-treatment variation in the CH_4/SF_6 ratio decreased with increase in PR.

There were significant effects ($P < 0.05$) of PR treatments upon both the apparent daily CH_4 emission (g d^{-1}) and CH_4 yield (g kg^{-1} DMI) (Table 10), and although L and M, and MH and H treatments did not differ either in apparent daily CH_4 emission or CH_4 yield, the overall pattern of response to PR was linear ($P = 0.001$) rather than quadratic ($P = 0.15$). Thus, for example, each 1 mg d^{-1} increase in PR accounted for 0.36 g kg^{-1} DMI increase in apparent CH_4 yield. The within-treatment variation in apparent CH_4 emission (both g d^{-1} and g kg^{-1} DMI) seemed to be relatively smaller at the higher PR treatments (Fig. 4).

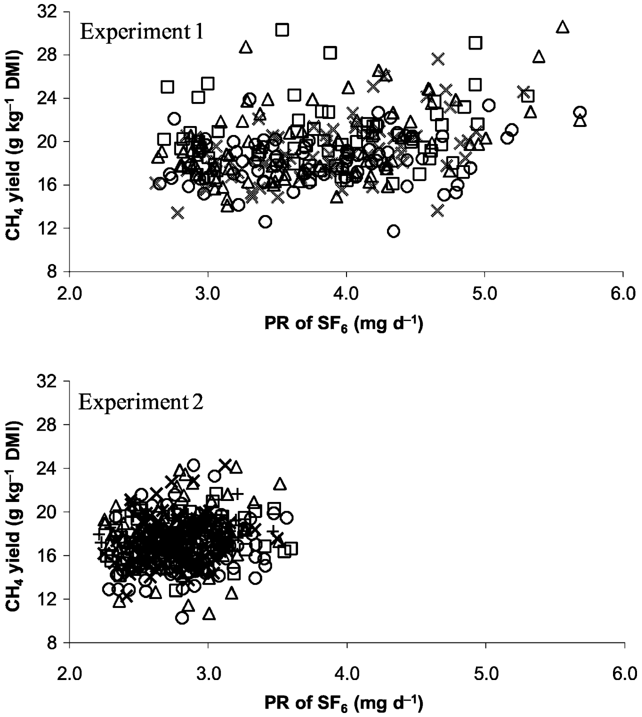

Fig. 2. Relationships between apparent CH_4 yield per unit of feed intake (g kg^{-1} DMI) and permeation rate (PR) of SF_6 from permeation tubes (mg d^{-1}) for each measurement group during exps. 1 and 2. Labels ×, □, Δ, ○, + represent groups 1, 2, 3, 4 and 5, respectively.

DISCUSSION

Johnson et al. (1994a) examined the validity of the SF_6 tracer technique for CH_4 measurement by comparing 55 measurements made using the tracer (with cattle in pens) to those obtained from 25 measurements using open circuit respiration chambers. They found that the mean tracer estimates were 93% of those in the chambers. These differences were, however, not significant. A similar validation test with sheep (Pinares-Patiño et al. 2007a) found that tracer estimates were 95% of those in

Table 10. Effect of permeation rate of SF_6 (PR) upon mean concentrations of gases in the breath samples and apparent CH_4 emissions in exp. 3

	PR of SF_6					Effect[z]	
	L	M	MH	H	SEM	Linear	Quadratic
Mean concentration of gases[y]							
CH_4 (ppm)	47.5*a*	51.1*a*	48.2*a*	45.2*a*	6.24	0.735	0.598
SF_6 (ppt)	119.5*a*	238.2*b*	278.8*b*	524.0*c*	52.1	0.001	0.736
CH_4/SF_6 ratio (× 1000)	455.1*a*	265.6*b*	225.4*c*	105.3*d*	13.8	0.001	0.100
Apparent CH_4 emission							
g d^{-1}	93.8*a*	103.4*a*	121.2*b*	115.4*b*	5.1	0.001	0.148
g kg^{-1} DMI	18.1*a*	19.9*a*	23.3*b*	22.1*b*	1.0	0.001	0.151

[z] Probability value for orthogonal contrast for linear or quadratic effect of SF_6 permeation rate.
[y] Refers to molar ratios (volume/volume) adjusted for background concentrations.
a–c Means in row with different letters are significantly different ($P < 0.05$).

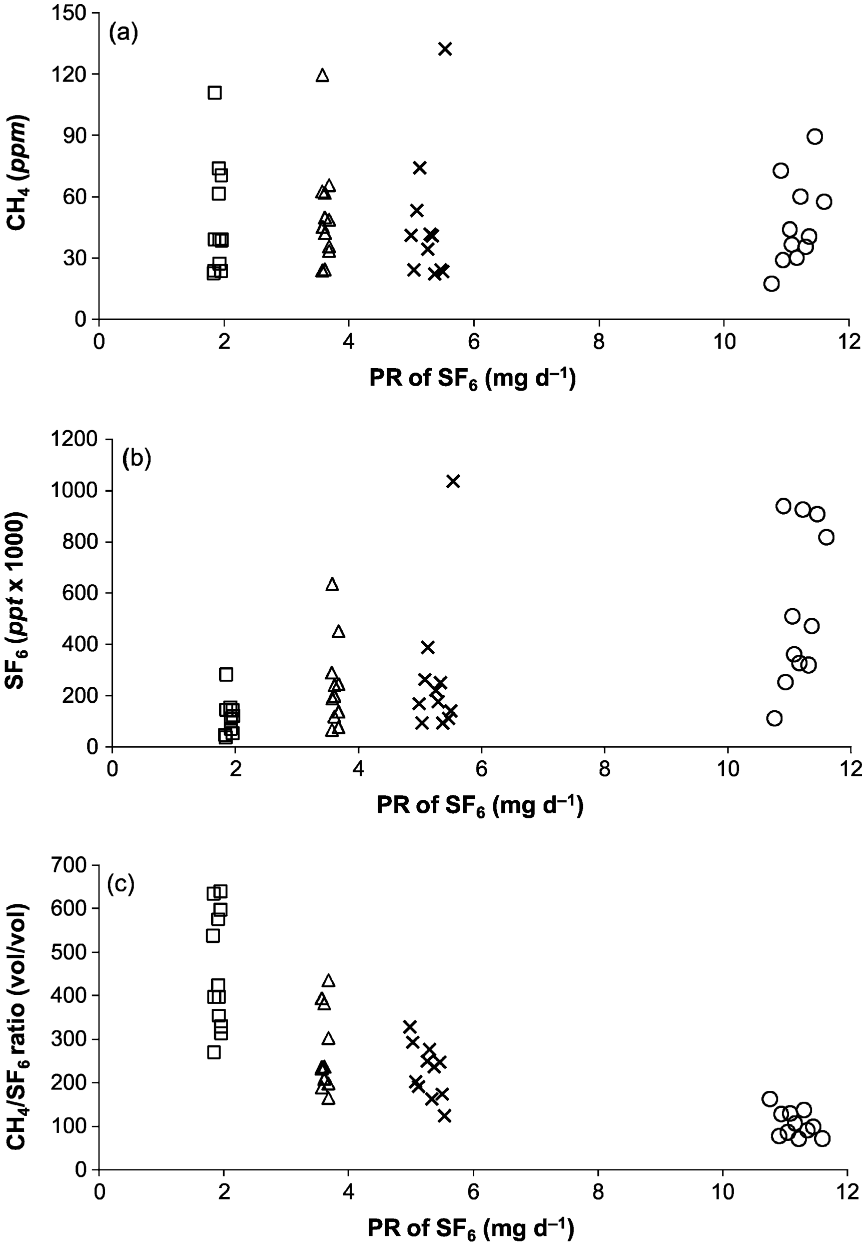

Fig. 3. Relationships between the actual permeation rate (PR) of SF_6 from permeation tubes (mg d^{-1}) and individual animal's mean concentrations of CH_4 (a) and SF_6 (b) gases, and CH_4/SF_6 ratio of concentrations (c) on breath samples for each of the PR treatments (low, □; medium, Δ; medium-high, ×; high, ○) in exp. 3. Within low, medium and medium-high treatments the highest concentrations of both CH_4 and SF_6 correspond to single influential individuals.

calorimetric chambers. Tracer estimates of CH_4 emissions slightly smaller than those in respiration chambers are expected as flatus CH_4 excretion, which accounts for approximately 2% of the total emission of CH_4 (Murray et al. 1976), is not detected by the SF_6 tracer technique. However, other studies with cattle using hoods (Boadi et al. 2002) or chambers (Grainger et al. 2007) reported tracer estimates slightly higher (by 2–5%) than calorimetric estimates, but not significantly different.

Results of the grazing experiments (1 and 2) of this study showed that PR had positive affect upon the calculated CH_4 emission values, but this effect depended upon the range of PR used. For example, in exp. 1, in which PR ranged from 2.624 to 5.689 mg d^{-1}, the effect of PR on the apparent daily CH_4 emission was more important than that of DMI, accounting for between 6 and 21% of the overall variation. However, when PR of permeation tubes used

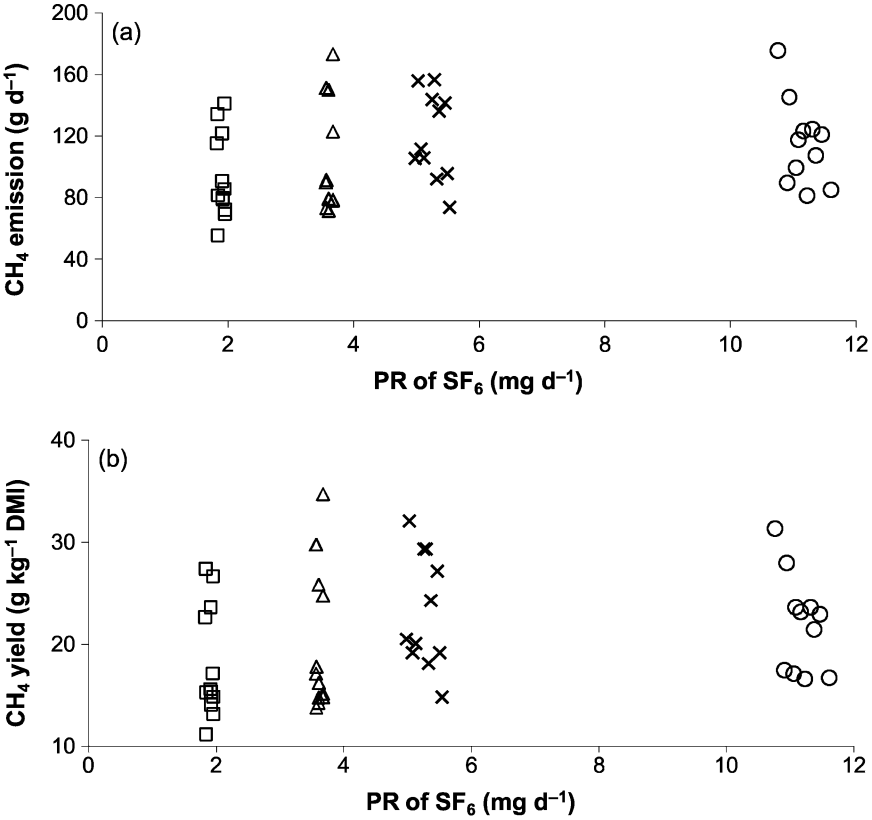

Fig. 4. Relationships between the actual permeation rate (PR) of SF_6 from permeation tubes (mg d^{-1}) and individual mean apparent daily CH_4 emission (g d^{-1}) (a) and apparent CH_4 yield per unit of feed intake (g kg^{-1} DMI) (b) for each of the PR treatments (low, □; medium, Δ; medium-high, ×; high, ○) in exp. 3.

in exp. 2 were in a narrower range (2.214–3.594 mg d^{-1}), the effect of PR on the apparent daily CH_4 emission was not significant (explaining $<4\%$ of the overall variation in apparent daily CH_4 emission) and less important than DMI.

Methane production in ruminants originates from the process of digestion of feed matter and it is more pertinent to express the CH_4 emission per unit of feed intake, i.e., CH_4 yield. Experiments 1 and 2 of this study showed that PR had a positive effect upon the apparent CH_4 yield (g kg^{-1} DMI). This effect, however, was only discernible with a sufficiently large (approximately twofold) range of PR for the permeation tubes used. In practice, permeation tubes deployed in any single trial are found in a range similar to those used in exp. 2 and under these conditions the contribution of PR to the overall variation in CH_4 yield would be very small ($<5\%$) and animal-to-animal source of variation remain the largest.

Experiment 3 was designed to evaluate, in a more controlled way, the extent of the PR effect on the apparent CH_4 emission. This experiment confirmed the observations in the grazing experiments that both the apparent daily CH_4 emission and CH_4 yield increased with increasing PR. This effect was linear rather than quadratic, with each 1 mg d^{-1} resulting in a 0.36 g kg^{-1} DMI increase in CH_4 yield. However, H permeation tubes, despite having PR values twice those of MH tubes, yielded apparent CH_4 values similar to those of MH tubes. The latter needs to be interpreted with caution as in order to obtain exceptionally high PR, the set of H permeation tubes was manufactured using Teflon material different to that of the other sets. When H treatment was excluded from calculations each 1 mg d^{-1} increase in PR resulted in an increase in an apparent CH_4 yield of 1.40 g kg^{-1} DMI, which is within the range of increase found in the grazing experiments (1 and 2). In practice, cattle permeation tubes are used in the range represented by L and M tubes. Although apparent CH_4 yields by these two treatments did not differ significantly from each other, precision of the tracer technique might become compromised under circumstances where only few animals per treatment are used or interest is on CH_4 emissions from individual animals as the tracer technique seems to be associated with extra animal-to-animal variability (Johnson et al. 1994a,b; Ulyatt et al. 1999; Boadi et al. 2002; Grainger et al. 2007; Pinares-Patiño et al. 2007a).

Experiment 3 of this study revealed that although the H treatment was associated with the largest variability in SF_6 concentrations, it resulted in the lowest variability in CH_4/SF_6 ratio, suggesting that precision in CH_4 estimation may be gained with increasing PR. The validation studies of the SF_6 tracer technique conducted by Johnson et al. (1994a) and Boadi et al. (2002) used permeation tubes with PR in the range 0.36–1.44 mg d^{-1}, i.e. much lower than those commonly used in our laboratory (mean 2.8 mg d^{-1}) for measurements in cattle. A recent parallel CH_4 emission measurement by the tracer and calorimetric methods conducted in cattle housed in calorimetric chambers (McGinn et al. 2006) found that the tracer technique, which employed permeation tubes with PR in the range 4.30–4.93 mg d^{-1}, was more precise than the calorimetric technique, especially when forage diets were used, whereas a similar comparison of the techniques (all in chambers) conducted with dairy cows fed on fresh pasture forage (Grainger et al. 2007) indicated an extra 8.2% variation associated with the tracer technique for tracer PR of 3.7 ± 0.7 mg d^{-1}.

This study confirms preliminary observations in our laboratory (Vlaming et al. 2007) of a direct relationship between PR and calculated CH_4 emission. Consequently, it warns that in order to obtain precision in CH_4 measurement by the tracer technique, the permeation tubes to be deployed in any single experiment should not only have a narrow range of PR, but permeation tubes should be balanced between treatments on the basis of their PR. Nevertheless, further research is necessary to determine the optimum PR level necessary to achieve the most accurate estimate of CH_4 emissions. Parallel measurements by both calorimetric and tracer techniques, using different PR levels, similar to that described in exp. 3 of this study, are necessary to determine optimum PR. In addition, given that CH_4 emissions are influenced by multiple factors (e.g., feeding level and feed quality), the effect of these factors on PR and apparent CH_4 emissions must also be established.

CONCLUSIONS

This study supports preliminary observations in our laboratory that there is a positive effect of PR on the corresponding estimates of CH_4 emission by the SF_6 tracer technique. This effect, however, is significant only when the range in PR of permeation tubes used at any single trial is large. Thus, accuracy and precision of the technique is warranted if the PR is used in a narrow range and PR is balanced between experimental treatments. The reasons for the effect of PR upon the calculated CH_4 emission are unknown and remain to be understood for the major ruminant species and under different feeding conditions. Once understood, an optimum PR may be determined in order to obtain the most accurate and precise estimations of CH_4 emissions.

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