Assessing the SF_6 tracer technique as an estimator of methane emissions from ruminants

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Executive Summary

This report reviews the current status of the measurement programme of methane (CH_4) emissions from NZ's farmed ruminant livestock using the "SF_6 tracer technique", including the quantitative understanding of the main determinants of CH_4 emission. The context for this study is the recent concern that under certain circumstances SF_6 (sulphur hexafluoride) may be a flawed tracer of ruminant CH_4. This report evaluates the basis for that concern and recommends investigations to better scope the applicability of SF_6 technique. The following points summarise this study.

- The SF_6 tracer technique is uniquely capable of determining methane emissions from individual ruminant animals while freely grazing.

- NZ has arguably more experience in using the SF_6 tracer technique, and more data based on its use, than any other coordinated research effort in the world.

- While most early deployments in NZ (1996 to ca 2000) of the SF_6 tracer technique used sheep and dairy cows grazing representative NZ pastures, most recent experiments have aimed to better understand determinants, mechanisms and mitigation potential of methane (CH_4) production through experiments that use housed or penned animals.

- Concerns about the SF_6 tracer technique relate to a reported correlation between the CH_4 yield (CH_4 emission per unit feed intake) estimated by that technique and the release rate of the SF_6 tracer, highlighting the need to better define any limitations on the technique's applicability.

- The purported "CH_4-SF_6 correlation" seems most pronounced for housed animals, which are distinguished by being fed distinct meals (typically twice per day). The correlation is least convincing for grazing animals, for which no alternative CH_4 measurement technique is available.

- A more detailed statistical scrutiny of available data would help to better characterise the nature and scope of the purported CH_4-SF_6 correlation, with assistance from tailored experiments designed to address ambiguities.

- Concerns with applying the SF_6 tracer technique to individual housed animals can be addressed in principle through the use of calorimetric chambers as a methane-measurement technique, but the small number of chambers available (and that can realistically be made available) constrains the experiments that can be designed to address specific questions.

- Comparisons in the literature between chamber techniques and the SF_6 tracer techniques as candidate estimators of CH_4 emission have generally demonstrated good agreement in average daily emissions. However, they also report greater day-by-day variability in CH_4 emission estimates when using the SF_6 technique (Section 4.6). The good agreement in average daily emissions gives confidence that there is no significant net systematic bias inherent in the SF_6 tracer technique. While that technique's greater variability is not fully understood, it may be

related to variability in the proportion of emissions by flatus (Section 5.1), to SF_6 being entrained into rumen gases in bursts rather than continuously (Section 5.2.1), or to variations in rumen temperature during ingestion and digestion (Section 5.3). Sections 6.3, 6.4 and 6.1 recommend approaches that could shed light on those respective possible causes of that variability.

- While the reported CH_4-SF_6 correlation is least convincing for grazing animals, more detailed statistical meta-analyses would be required to allay or confirm concerns about that correlation under grazing. Irrespective of the CH_4 measurement technique used for grazing animals, the co-determination of feed intake is notoriously unreliable and is the greatest source of uncertainty in determining CH_4 yields.

- NZ research into ruminant methane has a large stake in the SF_6 tracer technique (e.g., the large investment in research funds embodied in the so-called SF_6 database (Section 1.2)). Ruminant methane in turn is pivotally important in NZ's national inventory, with that technique providing key data (notably, the CH_4 yields, which are derived from data in the SF_6 database). For the benefit of both the science and policy development it is therefore critical to better characterize the circumstances in which the SF_6 tracer technique provides the best CH_4 data available, and to quantify any impact of the CH_4-SF_6 correlation on the full range of data in the SF_6 database.

- Recommendations in Chapter 6 seek to determine the underlying cause of the correlation between CH_4 yield and SF_6 tracer release rate through investigations which:

    - better characterize the performance of permeation tubes (the intra-ruminal SF_6 sources);
    - provide unequivocal confidence in gas analysis through further QA tests on the laboratory instrumentation and gas standards;
    - enhance understanding of the sources, pathways, and fates (exit points) of both CH_4 and SF_6 in the sheep's and cow's bodies, with a particular focus on hind-gut sources of CH_4, of flatus expulsion of both CH_4 and SF_6, and of related pathways (and dynamics where possible);
    - examine the relationship between daily patterns of CH_4 (and SF_6) eructation and daily feeding and behavioural patterns;
    - explore methods to enhance confidence in determining feed intakes during grazing by individual animals on individual days or groups of days; and
    - enhance insight into the CH_4-SF_6 correlation through more detailed statistical scrutiny.

  Many of the above investigations have value that transcends the SF_6 tracer technique through improving understanding of ruminant metabolism.

1. Introduction

1.1 Historical perspective of the SF_6 tracer technique in New Zealand

In 1994–95 the National Institute of Water & Atmospheric Research (NIWA) and AgResearch were funded to develop techniques to measure methane (CH_4) emissions from grazing ruminant livestock. This followed a recognition of the prominence of ruminant methane (CH_4) emissions (also known as "enteric CH_4" emissions) in NZ's emission profile (Hollinger & Hunt 1990, Lassey et al. 1992, Lowe 1985), and a recognition also of NZ's obligation to quantify those emissions as a ratifying party to the UN Framework Convention on Climate Change (ratified by NZ on 16 Sep 1993).

Following a period of evaluation of prospective measurement techniques, the "SF_6 tracer technique" — hereafter abbreviated "SF_6 technique" — was selected as most appropriate for NZ. This technique, which employs sulphur hexafluoride (SF_6) as a tracer, was at the time in the final stages of development at Washington State University (WSU) in Pullman WA, and had come to NIWA's attention through contacts between NIWA and the US National Center for Atmospheric Research (NCAR) in Boulder CO, where the technique was initiated. The seminal paper on the technique was published soon afterwards (Johnson et al. 1994). Using specifications supplied by the NCAR-WSU developers, NIWA fabricated gas sampling apparatus ("yokes" and "plumbed halters") and adapted a gas chromatograph (GC) for CH_4/SF_6 analysis at its then-Gracefield laboratory. This was followed by a sponsored visit by members of the SF_6 technique development team, Drs Pat Zimmerman, Hal Westberg and Kris Johnson. The combined team conducted the first trial in March 1995, with two grazing sheep and two grazing cows: Dr Garry Waghorn led the animal management at AgResearch, Palmerston North; Dr Keith Lassey led the gas analysis at NIWA's Gracefield laboratory (Lassey et al. 1995).

A major strength of the SF_6 technique was that it was uniquely capable of determining CH_4 emission rates from individual animals while grazing. That remains the case today.

Following the introduction of SF_6 technique to NZ, a joint NIWA-AgResearch team led by Drs Keith Lassey and Marc Ulyatt conducted one to two experiments annually with grazing sheep and/or cattle from 1996 to 2000. The aim of these trials was to determine CH_4 emission rates from typical NZ livestock grazing pastures representative of NZ's range of pasture types. Virtually all of this work was published in the international literature (Lassey & Ulyatt 2000, Lassey et al. 1997, Ulyatt et al. 1997, Ulyatt et al. 2002a, 2002b, 2005).

An integral requirement when measuring CH_4 emissions from livestock, no matter what technique is adopted, is to determine feed intake and feed quality. This is because the feed provides the substrate for methanogenesis in the rumen (Chapter 2), and both the quantity and quality of feed is known to be an important determinant of CH_4 emission rates. Thus, there is little to be learned about emission mechanisms or determinants unless feed intakes are measured. With such measurements, CH_4 emission can be expressed relative to feed intake (the "CH_4 yield": CH_4 emitted per unit dry matter intake, DMI, or per unit gross energy intake, GEI), a fairly robust measure that is pivotal to extrapolation to national and global emission inventories (Lassey 2007). However, the feed intake by grazing livestock is notoriously difficult to measure reliably (Lassey 2007, Ulyatt et al. 2002b) and is a major limitation to using grazing livestock in experiments designed to provide estimates of CH_4 yield.

In the late 1990s, the SF_6 technique began to be used in NZ to investigate determinants of CH_4 production, with a view to investigating emission-abatement strategies (Lassey et al. 2002). These involved: (*a*) testing novel cattle feeds for their potential to lead to reduced CH_4 without compromising productivity (Woodward et al. 2001, 2002); (*b*) investigating the relationship between CH_4 production and parameters characteristic of digestive physiology (Pinares-Patiño et al. 2003a); and (*c*) investigating the persistence of emission levels from sheep identified as relatively low emitters (Pinares-Patiño et al. 2000, 2003b). Since ca 2000 and until ca 2006, nearly all trials in NZ used the SF_6 technique to examine determinants of CH_4 production or to test CH_4-abatement strategies such as novel feeds or feed additives. The need to control and measure feed intake has required that in these trials the feed is brought to the animal rather than the animal put to pasture, necessitating that the animals be housed in crates or pens.

Since ca 2006, other issues have been identified that have cast doubt on the reliability of the SF_6 technique (subsection 1.3), causing NZ research to adopt alternative measurement strategies. Some of those doubts are the subject of this report.

1.2 The "SF_6 database"

In 2005 all available data using the SF_6 technique were assembled and entered into a Microsoft Access database, irrespective of the purpose of the experiment. For each of 21 experiments conducted between 1996 and 2003, each participating animal in each experiment represents a separate database entry, with averages as necessary over the repeat days. The database contains a field for every potentially useful datum related to animal identification and category, feed properties, estimated or measured feed intake, management regime, SF_6 "permeation rate", and inferred CH_4 emission rate.

This database is hereinafter referred to as the "SF_6 database".

1.3 Recent issues for the SF_6 technique

Since ca 2006, several practitioners of the SF_6 technique, in NZ and elsewhere, have questioned its accuracy (e.g., see Pinares-Patiño & Clark 2008). Concerns have arisen from investigations which: (*a*) compare the SF_6 technique with chamber-enclosed animals in which the CH_4 emission is inferred from analyses of the inflowing and outflowing gases; and (*b*) through statistical analyses of large datasets or through purpose-designed experiments. This report focuses on the latter set of investigations, and specifically on the claim that the inferred CH_4 emission rates may not be independent of the release rate of the SF_6 tracer (the SF_6 "permeation rate", PR). Such a claim would suggest a fundamental flaw in applying the SF_6 technique: that the intra-ruminal release of SF_6 does not ideally and conservatively trace CH_4 production and emission.

1.4 Purpose of this report

With confidence in the SF_6 technique and in the SF_6 database dented by suggestions that SF_6 is a flawed tracer of enteric CH_4, the applicability of the technique and utility of the SF_6 database are under scrutiny. Since that database reflects several NZ$M worth of research over more than 10 years, and since values for the CH_4 yield used in the NZ inventory are traceable to the database, there is merit in critically evaluating the basis for any diminution of confidence. The purpose of this report is to commence such an evaluation and recommend approaches to scope the applicability of SF_6 technique and in the SF_6 database. This report is prepared under very tight time constraints that limit the depth of the investigation.

Following an overview of "enteric" CH_4 as a by-product of ruminant digestion (Chapter 2) and an overview of the SF_6 technique (Chapter 3), the underlying evidence of SF_6 as a non-ideal tracer of CH_4 is catalogued (Chapter 4). Chapter 5 then offers contending explanations for that non-ideality, noting whether each could also account for observations reported when comparing SF_6 and enclosure techniques. In Chapter 6, experiments are proposed that could discriminate between such contending explanations, with a view to characterizing the applicability in the SF_6 technique as an estimator of ruminant CH_4 emission.

2. Methane generation in the ruminant digestive system

2.1 Enteric fermentation and methane production

A unique property of ruminants is their ability to convert cellulose, hemicellulose and non-protein nitrogen into useful products. Feed is firstly exposed to microbial digestion (fermentation) in the reticulo-rumen (forestomach), then hydrolytic digestion by the animal's enzymes takes place in the abomasum and small intestine. In the large

intestine (hindgut), undigested feed and endogenous substances are again submitted to bacterial digestion (Van Nevel and Demeyer 1996).

Fermentation in the rumen is considered an anaerobic oxidation of feed organic compounds. Fibrous feed materials are retained in the rumen for a considerable period of time (up to 72 h), where the large and diverse microbial population undertake extensive fermentation. The rumen environment provides excellent conditions for the growth of dense population of bacteria, protozoa, fungi and phage (Nolan 1999). Primary digestive microorganisms hydrolyse plant cell-wall polymers, starch and proteins, producing sugars and aminoacids, which are in turn fermented by both primary and secondary digestive microorganisms to volatile fatty acids (VFAs), hydrogen (H_2), carbon dioxide (CO_2), ammonia and heat (McAllister et al. 1996).

As a last step in rumen fermentation, methanogens reduce CO_2 to CH_4 with H_2 as energy source. The major part of the H_2 formed in the rumen is converted into CH_4 (Mills et al. 2001), whereas H_2 and CO_2 conversion to acetate (acetogenesis) is insignificant under normal rumen conditions. Thus, CH_4 formation acts as the most important ruminal electron sink into which the H_2 from all ruminal microorganisms drains (McAllister and Newbold 2008). The VFAs pass through the rumen wall into the circulatory system and after oxidation in the liver, supply a major portion of the animal's energy needs. Fermentation is also coupled to microbial growth (Figure 1) and the microbial cell protein synthesis is the major source of protein for the animal. The gaseous waste products of the fermentation (mainly CO_2 and CH_4, but also some residual H_2) are mainly removed from the rumen by eructation. Methane and heat represent a loss of dietary energy, whereas the excess of ammonia (once converted to urea) represents a loss of dietary nitrogen.

Methanogens belong to the Euryarchaeota kingdom within the domain Archaea (Nicol et al. 2003) and possess unique cofactors (e.g., coenzyme M, HS-HTP, F420) and lipids. Methanogens constitute a fundamental component of rumen microbiota, becoming established soon after birth (Morvan et al. 1994). The most common species of methanogens isolated from the rumen are strains of *Methanobrevibacter*, *Methanomicrobium*, *Methanobacterium*, and *Methanosarcina* (Jarvis et al. 2000) and studies of methanogen diversity in the rumen (Skillman et al. 2006; Nicholson et al. 2007) have indicated that new species remain to be identified.

In the rumen, methanogens are frequently found in association with protozoa. More than 50% of the ruminal biomass is comprised of ciliate protozoa (Ushida et al. 1997) and although the presence of protozoa in the rumen is not essential for the host, it is now established that they are associated with increased fibre degradation and CH_4 production (Finlay et al. 1994; van Nevel and Demeyer 1996). Ciliate protozoa are the most potent hydrogen-producing micro-organisms. Thus, the observed attachment or

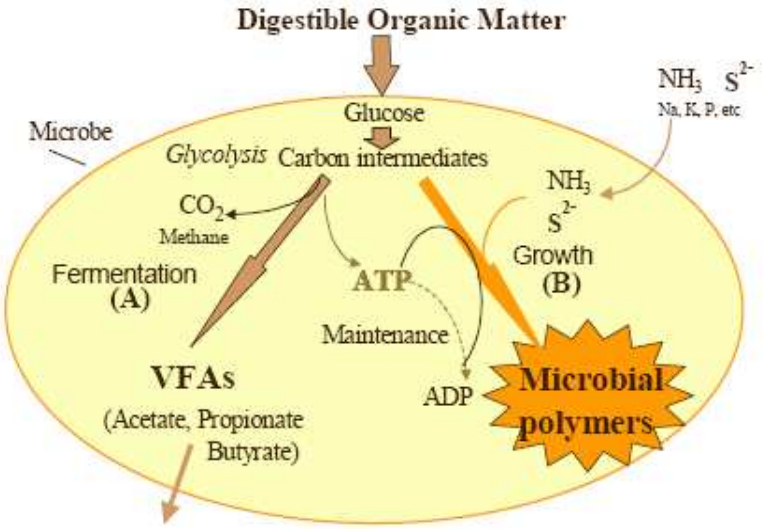

Figure 1. **A diagram describing digestion of organic matter in the rumen. Digestible feed organic matter is fermented to VFA, CO_2 and CH_4, generating adenosine triphosphate (ATP, 'the cell's energy currency') (pathway A), but intermediates are also removed as building monomers for microbial synthesis (pathway B) (from Nolan 1999, reproduced with permission).**

juxtaposition of methanogens to ciliates and even methanogens living symbiotically inside the protozoa cell (Finlay et al. 1994; Ushida et al. 1997) constitute a mechanism to make a more efficient hydrogen transfer from ciliates to methanogens. Newbold et al. (1995) and Morgavi et al. (2008) estimated that 20–25% of CH_4 production is due to the presence of protozoa. However, it has been reported that protozoa species differ in their 'methanogenic' activities (Ushida et al. 1997) and selective defaunation (e.g. of *Entodinium caudatum*) could lead to reduced CH_4 production without affecting fibre degradation (Ranilla et al. 2007).

Enteric CH_4 production depends on the population diversity, size and activity of the microbes in the rumen. While these are chiefly determined by dietary characteristics, they are also influenced by animal-related factors such as saliva production, rumen volume and rates of intake and passage (Pinares-Patiño et al. 2003a; Hegarty 2004) as well as by management interventions. In general, factors influencing CH_4 production interact with each other in their effects. However, the rate and extent of fermentation, fermentation pattern (type of VFAs), and hexose (e.g. glucose) partitioning between fermentation and microbial growth (Figure 1) are recognised as the main underlying mechanisms that control enteric CH_4 production rates (Monteny et al. 2006).

The intrinsic characteristics of a particular feed determine its microbial degradation rate, VFA production and hence CH_4 production rate. The rate of substrate passage through the rumen and the intrinsic degradation characteristics of that substrate

determine the extent of its degradation in the rumen before it outflows to the lower digestive tract. Production of CH_4 in the rumen is closely related to the production of VFAs, which determines the amount of excess H_2. For example, syntheses of acetic and butyric acids result in production of H_2 and CO_2, whereas propionic acid formation involves uptake of H_2 (Wolin & Miller 1988). Improved efficiency of microbial growth results in decreased rumen methanogenesis because an increased proportion of hexose is incorporated into microbial cells at the expense of fermentation into VFA and subsequent CH_4 formation (Beever 1993).

2.2 Sources of production and routes of excretion of enteric methane

In ruminants, methane is generated in both the forestomach (reticulo-rumen) and the hindgut. Experiments conducted with sheep (Murray et al. 1976; Torrent and Johnson 1994; Immig 1996) indicated that about 87% of the enteric CH_4 production takes place in the rumen, with the hindgut accounting for the remaining ~13% of total digestive tract CH_4 production. The study of Murray et al. (1976), based on four ewes fed lucerne chaff, showed that: (*a*) ~87% of CH_4 production was sourced in the rumen; (*b*) almost all (95%) of the ruminal CH_4 is excreted via eructation, with the remaining 5% being absorbed into the blood stream and subsequently excreted throughout the lungs; (*c*) about 89% of the hindgut CH_4 production was absorbed and excreted through the lungs along with the rumen-absorbed CH_4, with the residual hindgut CH_4 excreted in flatus; (*d*) that flatus therefore accounted 1–2% of the total excretion of CH_4. There is evidence (Colvin et al. 1957; Dougherty and Cook 1962; Hoernicke et al. 1965) that most (70−99%) of the eructed gases are first inhaled into the lungs, and then exhaled along with respiratory gases.

Studies with tracheostomised cattle (Dougherty and Cook 1962; Hoernicke et al. 1965) have revealed that the proportion of tracheal inhalation of eructated gases is not only variable between individuals, but it is greater when not ruminating than when ruminating. In addition, Hoernicke et al. (1965) reported that before feeding 25–94% of the total CH_4 emission (flatus not included) was via direct exhalation, whereas after feeding this pathway accounted only for 9–43% of total CH_4 emission. Furthermore, with small amounts of rumen gas, CH_4 was almost completely absorbed from the rumen, but the absorbed fraction of CH_4 decreased with increasing volume of eructated gas (Hoernicke et al. 1965). From the above it seems that in cattle rumen CH_4 absorption and subsequent exhalation is an important route of excretion, but it is highly variable between animals. Moreover, breathing frequency in cattle varies within a day, as well as differing among animals (Piccione et al. 2004).

In summary, eructation and exhalation are the major routes of excretion of digestion gases (Dougherty et al. 1964; Murray et al. 1976). In cattle, the frequency of eructation and respiration are about 0.6 and 25–40 events per min, respectively (Ulyatt

et al. 1999; Mortola and Lanthier 2005). Gas production in the rumen peaks after feeding and consequently the rate of eructation at this time is higher than at ruminating or resting (Dougherty and Cook 1962; McCauley and Dziuk 1965). While CH_4 production in the rumen and its excretion is associated with the feeding pattern (Johnson et al. 1998), the proportions released at the nose and mouth versus flatus is poorly quantified, and its determinants poorly known.

3. The SF_6 tracer technique

3.1 The underlying premises of a tracer technique

A tracer technique enables a generated or emissive flux of a fluid (liquid or gas), or of fluid-entrained particles, to be quantified even though the entire fluid efflux cannot be intercepted for measurement; instead only an undetermined fraction of that efflux can be sampled. The ideal tracer has known source strength, would be sourced alongside the source of target fluid, and would have identical behavioural characteristics (identical physics) during transit through to the sampling point. Thus, both target fluid and tracer are sampled with equal efficiencies, so that the tracer can be thought of as enabling the sampled fraction of entire fluid efflux to be quantified. This would normally require that the tracer be "conservative" (i.e., it is neither removed nor augmented during passage from source to sampling) on the basis that the target fluid also behaves conservatively, or is subjected to a known removal process.

An important characteristic of an ideal tracer is that its concentration in the sample is directly proportional to its source strength (i.e., it is scalable). Consequently, its actual source strength is unimportant (though must be known), but is generally taken to be small so that its presence has no material impact on the physical processes involved (e.g., does not increase gas pressure). This in turn would require that the tracer of choice be detectable and measurable at very low levels.

In practice, the above idealisation can only be approximated. In the case of ruminant methane, the SF_6 tracer is released in the rumen, the supposed site of almost all CH_4 production, at a rate that is presumed to match the pre-calibrated rate, and is detected in "breath samples" at the nose and mouth along with CH_4 excreted there. Once co-located with CH_4 in the rumen headspace, both gases are expected to be ejected via eructation and to disperse from the mouth and nostrils in identical fashion (the physics of these processes does not discriminate among the constituent gases) so that the eructed CH_4 and SF_6 are expected to be detected in the same proportion as their presence in the rumen headspace. Thus questions raised about the non-ideality of SF_6 as a tracer of ruminant CH_4 pertain to:

- the material importance of CH_4 pathways from production to excretion that are not mirrored by SF_6 pathways, including pathways of CH_4 from a hind-gut source, pathways that lead to excretion as flatus, and the relative importance of the bloodstream as a conduit for CH_4 and SF_6.

- whether the location of methanogenesis within the rumen matters, given that the SF_6 is released from a tube that is likely to settle gravitationally within the rumen or associated crevices or pockets whereas the rumen-sourced CH_4 is generated at the sites of digestion or microbial consumption distributed throughout the rumen

- whether any non-physical processes that discriminate between CH_4 and SF_6, such as dissolution in the rumen liquor, affect their relative efficiency of migration from rumen to exhaled breath

- whether the release rate of SF_6 in the rumen (i.e., its "permeation rate" from the pre-inserted permeation tube) is identical to its pre-calibrated permeation rate in the laboratory

- whether the SF_6 and CH_4 are released or generated at the same rate throughout the feeding cycle (ideally, the same rate as each other, but the SF_6 is released through a physical process at a rate that is presumed to be constant whereas the CH_4 is generated biologically at a rate that depends on substrate availability)

- whether background levels of CH_4 and SF_6 in the local atmosphere into which exhaled gases are entrained are correctly taken into account

3.2 The SF_6 tracer technique: operational aspects

The principles of this technique have been described many times in varying detail in the literature (e.g., Johnson et al. 1994, Lassey et al. 1997, Ulyatt et al. 1999) and will only be over-viewed here. Figure 2 provides a summary. The critical components for the purposes of this report are: (*a*) the source of SF_6 (permeation tube) and the pre-calibration of its release rate; (*b*) the location and performance of the permeation tube within the rumen; (*c*) the experimental configuration; and (*d*) the quality of laboratory determinations (by gas chromatography, GC) of CH_4 and SF_6 concentrations in "breath" samples and in background air samples. These components are considered in more detail in the following subsections.

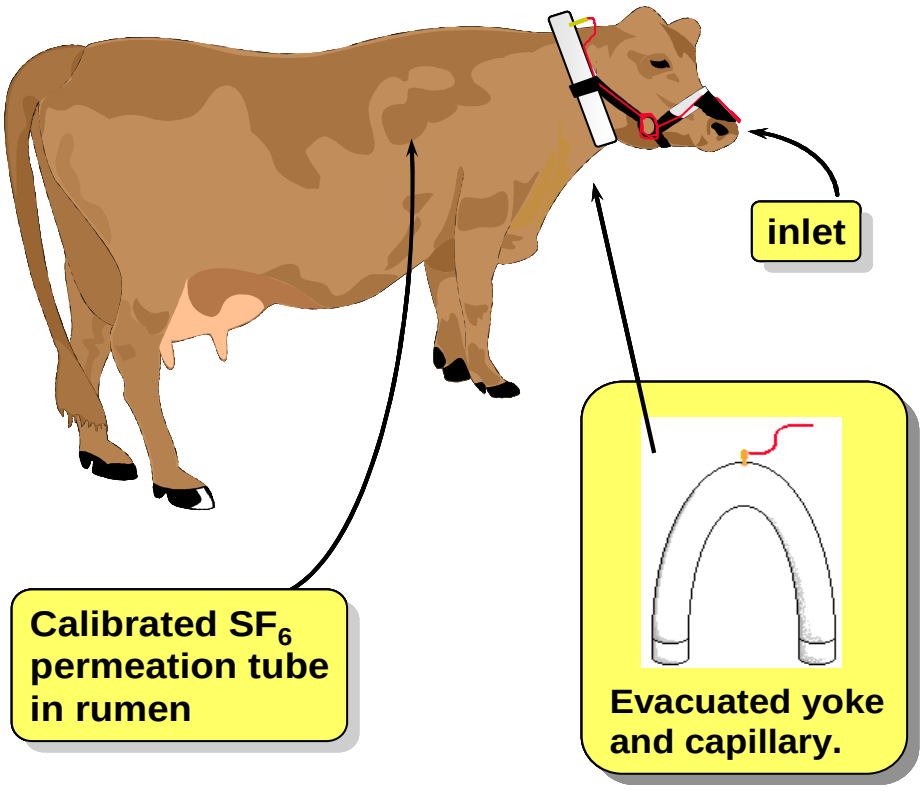

Figure 2. **Schematic diagram of animal-mounted apparatus used in the "SF_6 tracer technique". A pre-evacuated PVC canister ("yoke") draws in gas at the inlet near the nose at a rate that is limited by a length of capillary tubing (shown coiled in red), such that a 24-hour sample is collected at a near-uniform rate. The yoke contents are protected by a valve (omitted in some yokes) and a self-sealing Quick-Connect® that enables quick capillary connection and disconnection. For experiments with housed animals, the "yoke" will not usually be mounted on the animal, and may be differently shaped.**

3.2.1 The source of SF_6 tracer

The SF_6 is supplied in a pressurised "permeation tube". The tubes are fabricated out of brass to NIWA's specifications, and threaded (male) to match a Swagelok® nut. The detailed dimensions and properties of the tubes are described elsewhere (Lassey et al. 2001). It is sufficient here to note that the tubes, all individually stamped, are filled by cryogenically trapping ultra-pure SF_6 at liquid-nitrogen temperature (at which SF_6 solidifies) in a glove-box swept with dry CO_2-depleted air. Once charged, the components are held in place by a Swagelok nut tightened to a specific torque (Figure 3). The key component is a permeable Teflon® membrane, supported by a porous stainless steel frit that allows SF_6 to slowly permeate through the circular hole in the nut. The permeation rate of SF_6 is governed by the Teflon thickness (PTFE, thickness 0.24 mm is normally used) and by temperature.

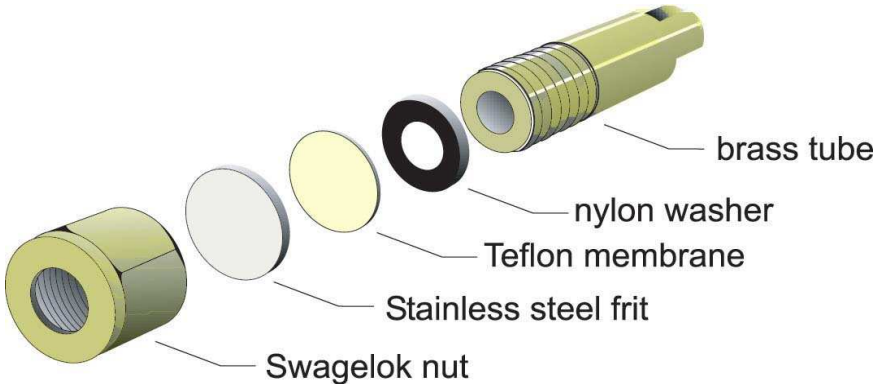

Figure 3. **Exploded view of a permeation tube, taken from Lassey et al. (2001). The nylon washer was introduced into tubes filled from Dec 1998. Larger "cattle tubes" were first introduced in April 2000, prior to which two sheep tubes had been deployed in some experiments with cattle during 1999 and 2000.**

The tubes are individually calibrated for SF_6 permeation rate through weekly weighing while maintained at 39°C (rumen temperature), for approximately 10 weeks (or longer if serial experiments are planned without intervening tube recovery). Over such a time frame weight loss is highly linear ($R^2 > 0.997$), and SF_6 is presumed to continue to permeate at that constant rate while in the rumen until only headspace SF_6 remains. However, detailed investigations have demonstrated that the permeation rate slowly changes, for reasons that are ill-understood (Lassey et al. 2001).

Two sizes of tube are used, referred to as sheep and cattle tubes. The essential differences are in permeation rate and charge capacity. While permeation rates cannot be prescribed, typical permeation rates from sheep and cattle tubes are 0.6–1.7 and 3–7 mg(SF_6) d^{-1} respectively, and respective capacities are about 0.8 and 2.2 g(SF_6). Note that 1 mg(SF_6) d^{-1} equates to 153 µL(SF_6) h^{-1}. Calibration of permeation rate is accurate to typically 0.001 mg(SF_6) d^{-1}.

3.2.2 Where does the permeation tube lodge?

A tube is inserted into the rumen of each participating animal at least 7 days prior to commencement of the experiment. The precise location within the animal's fore-stomach that the permeation tube lodges is potentially relevant. The fore-stomach is made up of two linked gastric sacs, the reticulum and the rumen, collectively, the reticulo-rumen. These two gastric sacs are the first two stomachs of a ruminant, and are joined by a large opening, allowing food to pass between the two stomachs. Swallowed food directly enters the reticulum. The food is then fermented in the reticulum and rumen, before passing to the third stomach (omasum) through the reticulo-omsal orifice. Fermentation gases, dominantly CO_2 and CH_4, are also eructed from the reticulum. The rumen, about 85% of total digestive tract volume, is therefore a "cul-de-sac" in the digesta pathway.

When tubes are orally administered ("*per os*") they always enter the fore-stomach at the reticulum. In cattle, those tubes appear to remain in the reticulum, as they are always found there when recovered through the fistula or upon slaughter. Tubes administered through a "rumen cannula", or fistula, ("*per fistula*") to cattle can lodge in the rumen instead of the reticulum. In sheep, permeation tubes administered *per os* are often relocated from the reticulum, to be usually found in the rumen upon slaughter. Very occasionally tubes have been found further along the sheep's digestive tract as far as the fourth stomach (abomasum). When administered *per fistula* to sheep, the tubes almost always lodge in the rumen.

3.2.3 Experimental configuration

For grazing situations, a typical physical layout of the experimental pastureland is described elsewhere (Lassey et al. 1997, Ulyatt et al. 2002b). A gas collection apparatus (Figure 2) is borne by each animal while grazing a confined paddock. An identical apparatus mounted upwind of the grazing area samples background air. A suitable means for estimating feed intake is implemented: in NZ, this is typically either (a) a whole-faeces collection bag, emptied twice daily, for male sheep; (b) an inert marker (e.g., alkane) for other animals; or (c) by calculating each animal's energy requirements. For all methods, feed digestibility is estimated through analyzing pasture samples representing the animal's diet. Feed intake estimation for grazing animals is, however, notoriously inaccurate (e.g., see Lassey 2007, Section 2.2).

For housed or penned animals, feed is delivered to the animals, and from analyses of delivered and refused feed, intake levels and quality can be determined to the required precision. The collection yoke (Figure 2) may be hung overhead to minimise the risk of its entanglement or interference. One or more background-air samplers are located within or near the confinement to represent the air inhaled by the animals and to detect concentration gradients that might result in air with different levels of CH_4 (and/or SF_6) enrichment being inhaled at different positions within that confinement. A well-ventilated environment is important to minimise such gradients.

In all cases background levels are critical to the calculation of CH_4 emission rates:

$$E_{CH4} = P_{SF6} \times \frac{16}{146} \times \frac{[CH_4]_{sample} - [CH_4]_{bkgd}}{[SF_6]_{sample} - [SF_6]_{bkgd}} \qquad (1)$$

in which E_{CH4} denotes CH_4 emission rate (g d^{-1}) calculated from P_{SF6}, the SF_6 release rate (g d^{-1}), and from the CH_4 and SF_6 mixing ratios in the sample and background air, denoted by square brackets. Mixing ratios are molar ratios relative to dried air (e.g., mmol(CH_4) $mole^{-1}$, abbreviated "ppm"; pmole(SF_6) $mole^{-1}$, abbreviated ppt), necessitating the ratio of molecular weights (16/146) to convert molar to mass units.

3.2.4 Gas chromatography: operational considerations

Analysis of methane data

The determination of CH_4 and SF_6 uses either a Hewlett Packard 5890 or Shimadzu GC 2010 Gas Chromatograph fitted with a 3m 1/8" OD, 2.2mm ID stainless steel main column packed with Molsieve 5A, 80/100 mesh, and a 0.3m pre-column of similar material (Grace Davidson, Auckland, NZ). SF_6 tracer gas is detected by an Electron Capture Detector (ECD) operating at 350°C and CH_4 by a Flame Ionisation Detector (FID) operating at 250°C. The two detectors are in series. The oven temperature is isothermal at 85°C. At 0.6 minutes, a VICI micro-electric actuator (Grace Davidson Ltd, Auckland New Zealand) is switched to allow nitrogen carrier gas to transfer a 2ml sample onto the column. The SF_6 peak elutes at 1.25min and CH_4 at 4.0min. The total run time for a duplicate sample set is 9.0 min.

Recognising the non-linear response of the ECD, a trio of gas standards (one of two trios prepared by NIWA) enable a 3-point SF_6 calibration curve to be constructed for each day's analyses. The standards in each trio, hereinafter denoted *Lo*, *Med* and *Hi*, have nominal mixing ratios for SF_6, CH_4 of (15ppt, 2.5ppm), (210ppt, 30ppm) and (1000ppt, 160ppm), respectively. A 1-point CH_4 calibration uses *Med* only, exploiting the strong linearity of the FID. As CH_4 standards, the trio are traceable to international standards (US National Institute of Standards and Technology, Boulder, CO); as SF_6 standards, sub-samples have been inter-calibrated with standards maintained by the University of Heidelberg, Germany.

At the commencement and at the end of each day's analyses, each of the trio is run in triplicate or until a coefficient of variation (CV) of <1% is achieved in the mixing ratio of each gas in each standard. Additionally, similar triplicates of *Med* are run regularly throughout the day, typically every 8–12 samples, to track any drift in instrument response and ensure reproducibility across a day and between days of measurement. Samples are run in duplicate or repeated until the CV is <1%.

Proprietary GC software analyses each chromatogram, identifying the SF_6 and CH_4 peaks (by elution time and detector) and calculating the area under each peak (see Figure 4), and uploads the analyses into an Excel® file. A customised suite of macros in that Excel file constructs calibration curves and translates chromatogram peak areas into CH_4 and SF_6 mixing ratios, either by linear interpolation between neighbouring *Med* standard runs (CH_4), or by quadratic interpolation between the commencing and ending standard-trio runs, scaled according neighbouring runs of *Med* (SF_6). File-naming conventions enable the macros to identify standards and backgrounds so that a linked Excel® macro can calculate the CH_4 emission rate for each animal for that day using Equ. 1.

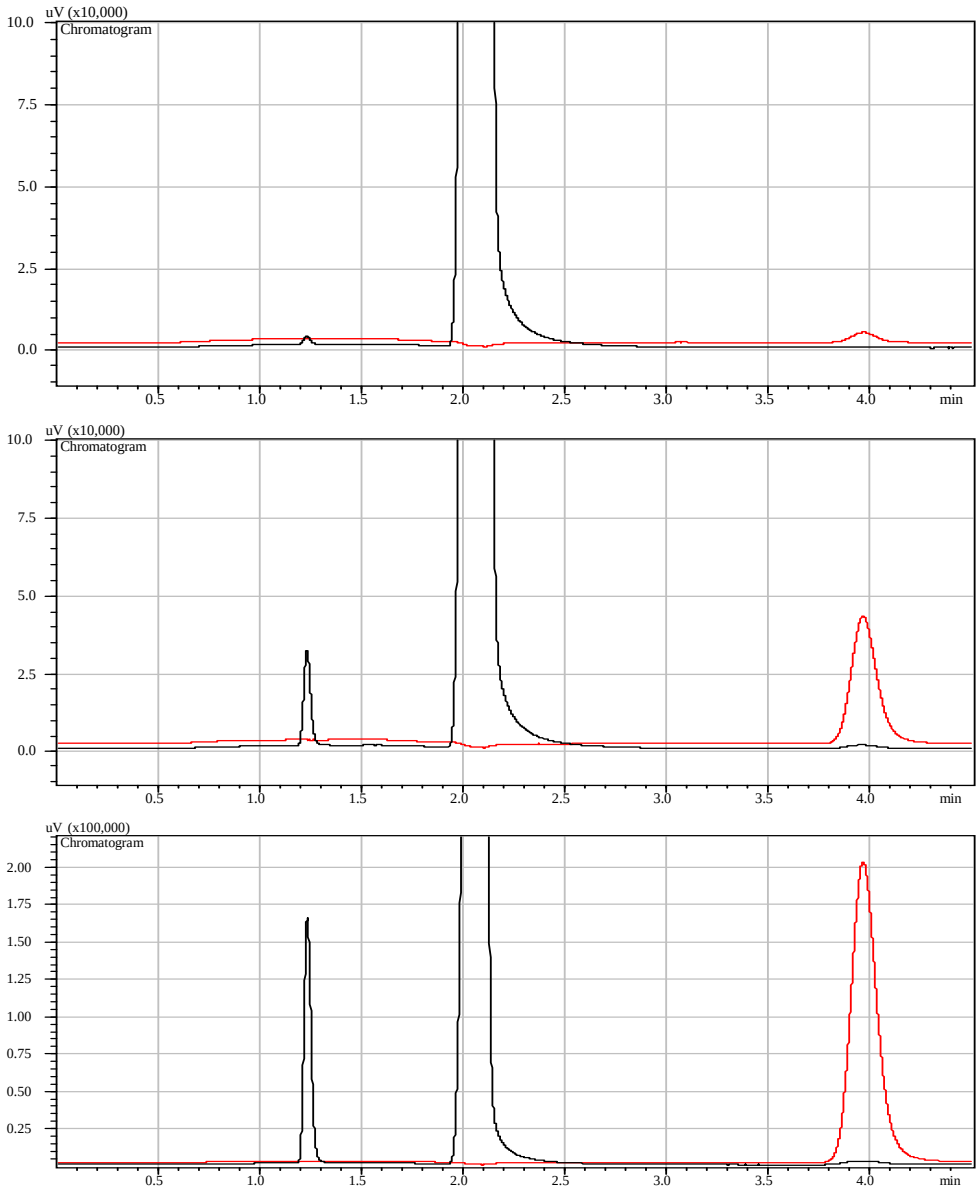

Figure 4. **Representative chromatograms for a trio of standards *Lo*, *Med Hi* (upper, middle and lower panels). Each panel plots detector response (instrument-specific units) against elution time (min) for both the flame ionisation detector (FID, red trace) and electron capture detector (ECD, black trace). The SF_6 peak elutes on the ECD trace at ~1.25 min, and the CH_4 peak on the FID trace at ~4.0 min. (The large ECD peak at ~2.1 min is oxygen). The area under each peak is a measure of mixing ratio.**

Identifying problems with sample collection

Sample canisters ("yokes") are fully evacuated prior to sample collection. Over a 24-hour collection period, the aim is to half fill a sample canister (i.e., to 50kPa absolute

pressure). This is achieved by restricting the sample flow using a short length of capillary tube (Figure 2). Each sample is pressure-checked prior to connection, and again at disconnection; samples with very low pressure (0–30kPa) or with near-atmospheric pressure (80–100kPa) indicate a problem with gas collection. Leakages in the collection equipment or water/feed blockages are the most common problems to occur, accounting for near-atmospheric or very low pressures respectively. All collection problems due to animal behaviour are recorded and taken into consideration when analysing data.

Criteria for removing sample data points

Any obvious problems due to animal behaviour or human error are recorded and addressed. These might include broken collection lines due to chewing or entanglement, halter and gas inlet dislodged from the nose or blocked, sample canister not correctly connected, or canister valves not turned on.

For all samples with abnormal pressure (below 30 kPa or exceeding 80 kPa), the collection apparatus is automatically replaced, and the sample analysed if possible. Following a review of other analyses in that day and with the same animal on other days, the results of that analysis may be accepted or rejected. Many samples at near-atmospheric pressure still have a sensible gas ratio if the leak developed late into the 24-hr collection period such as during mustering.

The concentration of both SF_6 and CH_4 in a collected sample should ideally be at least 10 times the background level (SF_6 6ppt, CH_4 2ppm). Therefore any concentration below 60ppt SF_6 or 20ppm CH_4 is carefully examined. These account for the lowest 25% of samples analysed usually corresponding to a low sample pressure, so that data point can be justifiably removed.

Daily SF_6/CH_4 ratio calculations are good indicators of how consistent the SF_6 is being recovered from the permeation tube. If that ratio for a particular animal changes markedly during a 4-day experiment, the accuracy of SF_6 recovery is questionable and therefore the calculated CH_4 is either under/over-estimated accordingly. Intra-animal CV values for a good 4-day measurement are typically less than 15.

A very low SF_6/CH_4 ratio indicates a major problem with the release of SF_6 from the permeation tube in the rumen resulting in an elevated and unrealistic estimate of CH_4 emission. Such a circumstance could be due to misplacement or relocation of the permeation tube, or a dysfunctional (or expired) tube. The useful longevity of any tube can be assessed after calibration by estimating the time before the SF_6 charge falls below a "minimal useful load" (150 µg and 600 µg for sheep and cattle tubes respectively) when non-gaseous SF_6 has been exhausted (Lassey et al. 2001).

Another approach is to calculate the amount of CH_4 produced in relation to the feed ingested. The average CH_4 production is typically 20–23 g(CH_4) per kg dry matter intake (DMI). If the amount of calculated CH_4 produced is biologically impossible for any animal to achieve the result would normally be discarded and the experimental merit of that animal questioned.

Further Quality Assurance of sample measurements

With AgResearch having two independent GC instruments, at least 1% of samples collected are re-run on the other instrument as a cross-check against instrumental error. In addition, some such cross-checking is performed at NIWA's laboratory in Wellington, though often with considerable delays. AgResearch and NIWA determinations show good inter-comparability with an R^2 value exceeding 0.97, though some discrepancies are currently under investigation.

Instrumental calibration (detector response as a function of gas mixing ratio) is cross-checked periodically by NIWA through dynamic dilution techniques in which a quantitative mixture of *Hi* (or similar) and "zero air" (synthetic air free of trace gases), traces the detector response function from ambient to in excess of *Hi* mixing ratios. NIWA's working standards and both trios of standards (*Lo*, *Med*, *Hi*) are included in the cross-check. Such cross-checking gives confidence in: (*a*) the ongoing integrity of each trio (*Lo*, *Med*, *Hi*); and (*b*) the integrity of performance of each GC and the associated chromatogram interpretation software.

Recent cross-checks have revealed some apparent discrepancies between SF_6 determinations at AgResearch and NIWA GC facilities that have yet to be resolved.

4. Evidence questioning the accuracy of the SF_6 technique

4.1 The SF_6 database: a meta-data analysis

An analytical study was conducted in 2005 to assess the possible statistical relationship between methane (CH_4) emissions as calculated using the SF_6 tracer technique and the SF_6 permeation rate (PR) (Vlaming et al. 2005). A repeat of this study is outlined below following important corrections to some entries in the SF_6 database that have subsequently been identified.

The study involves a meta-analysis of data extracted from the SF_6 database corresponding to 21 separate New Zealand experiments employing the SF_6 tracer technique conducted between 1996 and 2003. Methane emissions estimated by the technique were expressed both as emission rate (g(CH_4) d^{-1}) and as CH_4 yield (g(CH_4) kg(DMI)$^{-1}$). Experiments were categorised according to species (dairy cattle or sheep) and feeding situation (grazing or housed), with each group analysed

Table 1. **A summary of data (mean ± SD[1]) from the SF_6 database (643 observations, 1996–2003) comprising CH_4 emissions (g d^{-1} and g kg(DMI)$^{-1}$) estimated using the SF_6 tracer technique, and corresponding SF_6 permeation rates (PR), by species (dairy cattle, sheep) and feeding situation (grazing, housed). Each "observation" is based on the mean of 3–5 daily measurements for a single animal.**

Species	Feeding situation	Number of observations	CH_4 (g d^{-1})	CH_4 (g kg(DMI)$^{-1}$)	SF_6 PR (mg d^{-1})
Cattle	grazing	146	303.5±93.2	19.7±4.6	3.31±0.96
Cattle	housed	40	359.5±146.1	18.5±4.4	3.33±0.41
Sheep	grazing	248	29.2±10.5	17.8±6.2	1.42±0.77
Sheep	housed	209	22.0±5.5	18.5±4.4	1.40±0.43

[1] These are the distributions of data in the record, and do not reflect measurement uncertainty.

separately. The range of data from these experiments is summarised in Table 1. The housed dairy cattle category contained data from only two experiments with distinct PRs, so was not analysed. It should be recognised that while intakes by housed animals can be accurately determined, intakes while grazing can only be inferred indirectly, and the method of inference varies among the experiments.

Two analyses were conducted following independent statistical approaches. The first, by AgResearch statistician Dr John Koolaard, mirrored the analysis by Vlaming et al. (2005). The second, by NIWA statistician Dr Murray H. Smith offered an alternative analysis after studying the paper by Vlaming et al. (2005). Consider these in turn, referred to as the "first" and "second" analyses.

The first analysis was conducted with a linear mixed model with a fixed effect of PR (i.e., allowing for the possible linear influence of PR), and a random effect of experiment (i.e., in effect, the experiments are drawn at random from a population of experiments). In the second analysis, it was argued that a fixed effect better adjusts for differences between experiments when establishing the existence of a correlation. Simple analyses of covariance (ANCOVAs) used fixed experiment effects and a single slope for the covariate PR. Both estimated daily CH_4 emission rate (g(CH_4) d^{-1}) and estimated CH_4 yield (g(CH_4) kg(DMI)$^{-1}$) were analyzed as dependent variables, but in the first analysis only sheep data were log-transformed to account for the increasing variance with increasing estimates of CH_4 emission.

4.1.1 First analysis

Results of the first analysis for estimated daily CH_4 emission rates by grazing cattle are shown in Figure 5. A positive correlation between estimated emission rate and PR is evident, and P=0.023 suggests that this correlation is significant. A similar

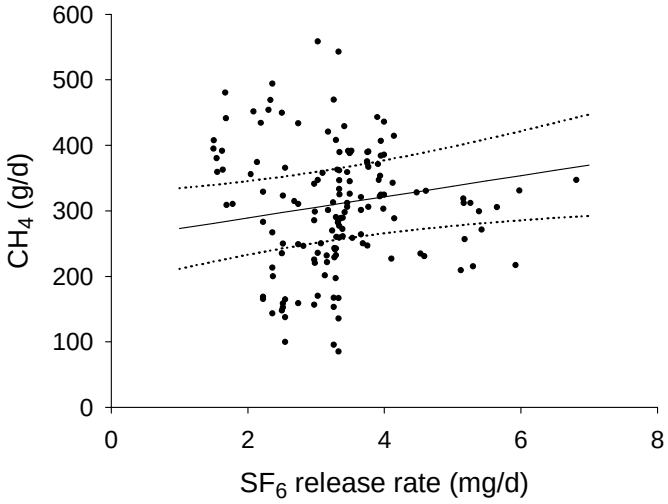

Figure 5. **Methane data for grazing dairy cattle showing the fitted model regression (with 95% confidence intervals of the modelled line) for estimated daily CH_4 production on SF_6 release rate (PR) (slope = 16.14 g(CH_4) mg(SF_6)$^{-1}$, *P* = 0.023).**

correlation was evident for housed sheep (slope = 2.06 g(CH_4) mg(SF_6)$^{-1}$, *P* = 0.035), but was not evident for grazing sheep (*P*=0.15).

The statistical entity *P* has the following interpretation. For the "null hypothesis" that in the underlying population the dependent variable *y* is uncorrelated with a particular independent variable *x*, *P* is the probability that a sample of the size under study drawn at random from that population exhibits a correlation between *x* and *y* that exceeds that found in the study. But see comments in Section 4.1.2 on the reliability of using *P* to reject the null hypothesis.

Counterpart results of the first analysis for estimated CH_4 yield indicated non-significant relationships with PR for all three analyzed categories: grazing dairy cattle (*P*=0.165), grazing sheep (*P*=0.370), and housed sheep (*P*=0.153).

The first analysis demonstrates that a significant positive relationship can occur between the SF_6 PR and estimated daily CH_4 emission, but that this relationship weakens or disappears when that emission is scaled with DMI. This suggests that DMI and PR may co-vary as a result of their separate variation with experiment. There are several explanations for DMI varying with experiment:

a) DMI is assessed in different ways in different experiments, including; direct measurement (housed or penned animals only); whole faeces collection (male sheep only); the use of inert markers (increasingly rarely due to unreliability); and calculated using an energy-requirements model. The assessment of DMI

for individual grazing livestock over individual days is especially uncertain, and its accuracy undetermined.

b) DMI differs among experiments due to using animals of different bodyweights, such as juvenile versus mature animals. This would be most obvious in the sheep dataset where lambs of a range of ages, or lactating ewes, are used in different experiments.

c) DMI varies with level of productivity, such as lactation. This is most obvious when cows at different levels of lactation are used in different experiments. Energy requirements models (not always the same model) are commonly used to calculate the DMI of grazing dairy cows, taking account of productivity levels.

d) Many experiments involve use of novel feeds or additives, each diet having a characteristic digestibility that can affect DMI as calculated using an energy requirements model.

e) Many experiments with housed animals involve supplying feed at different levels relative to maintenance requirements.

In addition, while CH_4 yield is believed a fairly robust concept for a given diet across a range of animal classifications, it does appear to strongly differ between juvenile and mature sheep (Clark et al. 2003; Ulyatt et al. 2005), a distinction that remains equivocal for other species (Lassey 2008).

The range of PR is observed to differ markedly among experiments (e.g., Figure 6), either by design for some experiments, due to changes in permeation tube construction (e.g., introduction of the nylon washer in Dec 1998 systematically reduced SF_6 PRs culminating in designing and deploying a larger "cattle tube": see Figure 3) or in permeation tube materials (e.g., different batches of Teflon in use from time to time), or simply for no apparent reason.

Therefore, addressing the CH_4 yield instead of daily CH_4 emission removes (or reduces) DMI as an obvious covariate for this meta-analysis. According to this first analysis, the null hypothesis that CH_4 yield is uncorrelated with PR cannot be rejected on the basis of data in the SF_6 database. Nevertheless, with the *P* value approaching significance in some categories, notably grazing dairy cattle and housed sheep, purpose-designed experiments to further test the "null hypothesis" would be merited.

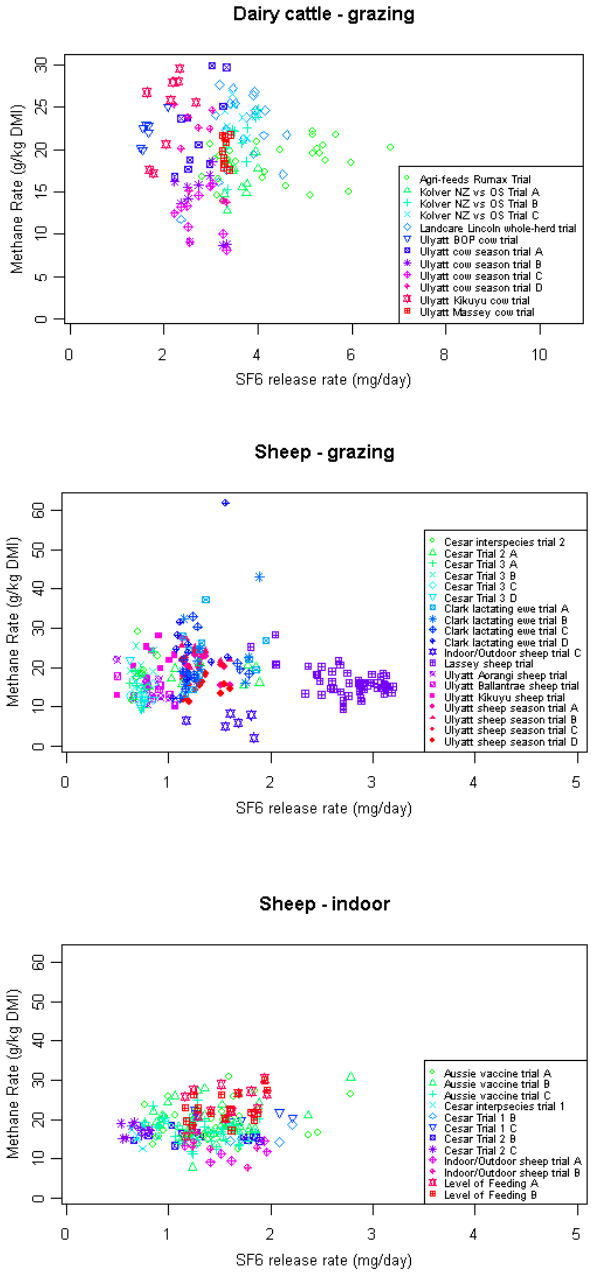

Figure 6. Plots of estimated CH_4 yield against SF_6 release rate (PR) by experiment, for grazing dairy cattle grazing sheep, and housed sheep (upper, middle, and lower panels). The labels for each experiment are as recorded in the SF_6 database.

4.1.2 Second analysis

The second analysis focuses on only the grazing dairy cattle and housed sheep categories. The simple ANCOVA with fixed experimental effect and a single slope for

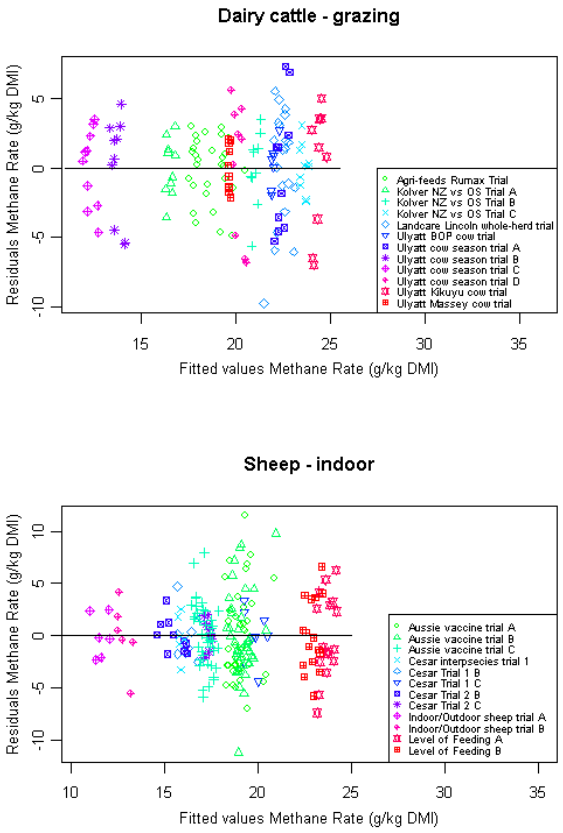

Figure 7. **Plot of residuals (actual values less fitted values) against fitted values (values predicted by the regression fit) by experiment, for the fitted ANCOVA models.**

the PR as covariate effectively assumes a constant variance for all units, but allows this assumption to be tested.

For the two categories assessed as significant in the first analysis, estimated daily CH_4 emission for grazing cattle and for housed sheep, only the former is significant in the second analysis (2-sided *P* values 0.016 and 0.207, respectively). More detailed ANCOVA results are reported for estimated CH_4 yield as the dependent variable.

Figure 6 reports the data under analysis from the SF_6 database (the same underlying data as in Figure 5). With the identification of different experiments, the grouping by experiment is immediately obvious. To put another way, there is an obvious association between experiment and either or both of estimated CH_4 yield and SF_6 PR for some experiments. From an ANCOVA on the grazing cattle and housed sheep categories, 2-sided *P* values are 0.106 and 0.066, respectively, neither of which are significant. To add further analysis, Figure 7 reports the residuals by experiment against the corresponding fitted values. The fitted values refer to CH_4 yields

"predicted" by the fitted regression line for each experiment, and the residuals are the difference between those and the recorded CH_4 yield. The residuals necessarily have zero mean. Dr Smith's interpretation (personal communication to KRL, 2008) is that there is no evidence that variance scales with mean (and therefore that log-transformation is warranted), but there is evidence that variance varies with experiment. A follow-up analysis might therefore fit a linear model with fixed experimental effects, a single PR effect, and different variances for each experiment. There is also sufficient reason to carry out a carefully designed experiment to verify the reality of any relationship between CH_4 emission and SF_6 PR.

Dr Smith cautions against using *P* uncritically as a discriminator of the null and non-null hypothesis. This is demonstrated in a simple simulation (Sellke et al. 2001) in which it is known, *a priori*, that the underlying population has a 50% chance of having negligible correlation and a 50% chance of a non-negligible correlation (these proportions are not critical). Then, of those tests of the null hypothesis for which $P \approx 0.05$, "at least 23% (and typically close to 50%)" will have negligible correlation. Sellke et al. (2001) conclude that "for testing 'precise' hypotheses, *P*-values should not be used directly, because they are too easily misinterpreted".

4.2 Tailored experiment: May 2004

When early analyses of the SF_6 database suggested a significant positive relationship between daily CH_4 emission and SF_6 PR (Vlaming et al. 2005), a more careful experiment was designed and carried out using a modified cross-over design (Vlaming et al. 2007) that we now describe.

Twelve steers divided into two groups of six were given either one or two permeation tubes (mean PRs 2.878 and 7.336 mg(SF_6) d^{-1}, respectively) and offered either energy maintenance (M) or 2×M levels of feed intake to determine the effect of both PR and intake on calculated CH_4 emissions. There were thus four sub-groups of three steers on four treatments in each measurement period: M with low PR, M with high PR, 2×M with low PR, and 2×M with high PR. All animals remained on the same feeding level (offered either M or 2×M) for the duration of the experiment. Animals were fed a lucerne silage diet, supplied twice daily at 08:00 and 16:00 hours. Feed not eaten by the animals was collected and weighed prior to next feeding.

Tubes were inserted *per fistula* on Day 1, then following a 14-day acclimatisation to the diet four 24-hr samples were collected on Days 16–19. On Day 19 tubes were recovered and immediately reallocated *per fistula* so that steers that had a low PR treatment for the first measurement period had a high PR treatment for the second measurement period, and vice versa. The second measurement period commenced 3 days later with 24-hr samples collected on Days 23–26.

Table 2. **Estimated CH_4 emission (g d^{-1}) for two groups of six steers offered maintenance (M) and 2×M feed and given either a single ("Low SF_6") or two ("High SF_6") permeation tubes, May 2004. Data are mean ± SEM.**

SF_6 release rate	Feeding level M	Feeding level 2×M	Mean	Significance of Feeding level
Low SF_6 PR	110.8 ± 5.6	157.6 ± 5.1	134.2 ± 7.9	
High SF_6 PR	129.1 ± 3.0	192.5 ± 7.1	160.8 ± 10.2	
Mean	119.9 ± 4.1	175.0 ± 6.7		*F*<0.001
Significance of SF_6			*F*<0.001	Feed×SF_6, *F*=0.041

Table 3. **Estimated CH_4 yield (g kg(DMI)$^{-1}$) for two groups of six steers offered maintenance (M) and 2×M feed and given either a single ("Low SF_6") or two ("High SF_6") permeation tubes, May 2004. Data are mean ± SEM.**

SF_6 release rate	Feeding level M	Feeding level 2×M	Mean	Significance of Feeding level
Low SF_6 PR	18.9± 1.1	17.7 ± 0.2	18.3± 0.6	
High SF_6 PR	22.3 ± 0.6	21.2 ± 0.6	21.8 ± 0.4	
Mean	20.6 ± 0.8	19.5 ± 0.6		*F*=0.199
Significance of SF_6			*F*<0.001	Feed×SF_6, *F*=0.923

While steers on the M feeding level unsurprisingly refused less feed than animals on the 2×M level, the former group still consumed significantly less feed per day (5.85 ± 0.11 (SEM) kg(DM)) than the group at 2×M (8.98 ± 0.16 (SEM) kg(DM)) (F < 0.001). Both feeding level and SF_6 PR were significantly correlated with estimated daily CH_4 production (F < 0.001, Table 2), although the effect of PR was greater at the 2×M feeding level (22% increase) than at the M feeding level (16.5% increase).

The F statistic on which the probability F in Tables 2–3 is based is the ratio of the between-treatment variance to the within-treatment variance. The larger that ratio the more evidence there is that the treatment means are distinct. The probability value, F, is the probability of obtaining (by chance alone) an F statistic greater than the treatment value when the null hypothesis of no effect of treatment is true. Results are considered significant when F < 0.05.

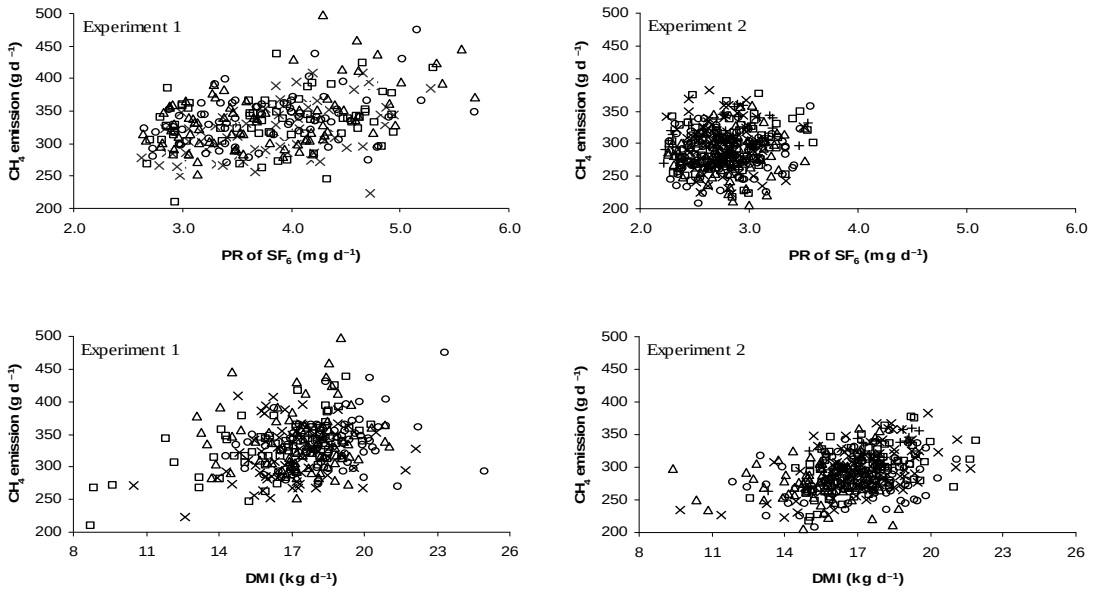

Figure 8. **Relationships between estimated CH_4 emission (g d^{-1}) and permeation rate (PR) of SF_6 from permeation tubes (mg d^{-1}), and dry matter intake (DMI, kg d^{-1}) for each measurement group during the large-herd grazing experiments 1 and 2. Labels ×, □, Δ, o, and + represent groups 1, 2, 3, 4 and 5, respectively.**

There was a significant relationship (F = 0.041, Table 2) between feeding level and SF_6 PR for daily CH_4 emission, indicating that the two may positively co-vary. However, there was no such relationship between feeding level and SF_6 PR (F = 0.92) for CH_4 yield, implying that scaling CH_4 emission rate with DMI has removed the co-variation. This is analogous to a similar co-variation noted in Section 4.1.1, and does not imply a direct influence of PR upon feeding level.

Feeding level had no effect on estimated CH_4 yield (F > 0.05; Table 3). However, PR was still positively related to estimated CH_4 yield (F < 0.001) whose values were 19% higher when based on a high PR (21.8 g(CH_4) kg(DMI)$^{-1}$) than on the low PR (18.3 g(CH_4) kg(DMI)$^{-1}$).

The SF_6 PR can affect the calculated CH_4 yield from animals when employing the SF_6 tracer technique. This experiment with stall-fed cattle suggests that the difference in estimated CH_4 yield between PR values of 3 and 5 mg(SF_6) is approximately 8.5%.

4.3 Two large-herd grazing experiments

Methane emissions from 296 (Experiment 1) and 388 (Experiment 2) three-year-old Friesian × Jersey dairy cows in mid-lactation were measured, using the SF_6 technique,

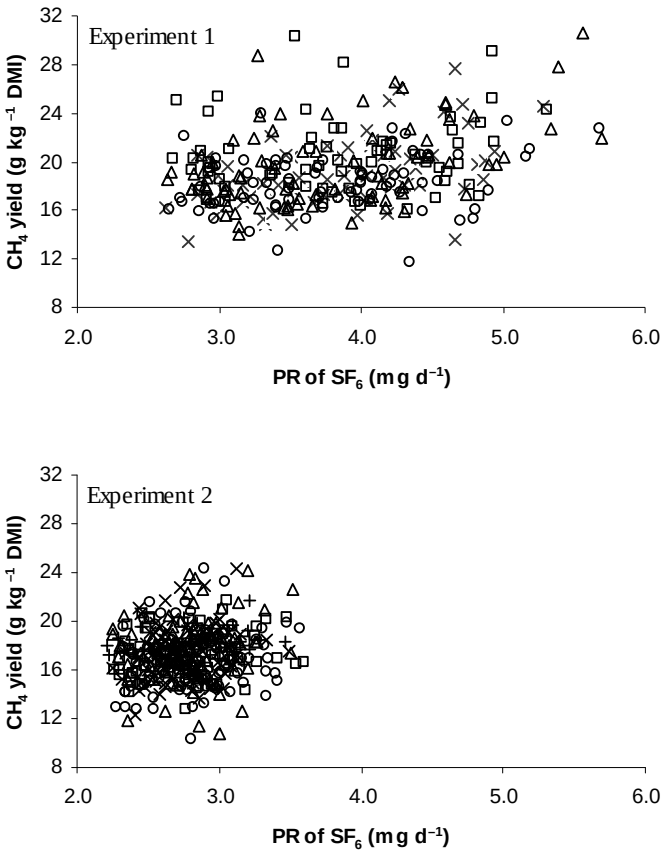

Figure 9. **Relationships between estimated CH_4 yield ($g(CH_4)\ kg(DMI)^{-1}$) and SF_6 PR ($mg(SF_6)\ d^{-1}$) for each measurement group during grazing experiments 1 and 2. Labels ×, □, Δ, o, and + represent groups 1, 2, 3, 4 and 5, respectively.**

in January–February 2004 and 2005, respectively, at Hawera, Taranaki, NZ (Pinares-Patiño et al. 2008b). The herds were subdivided into four (Experiment 1) or five (Experiment 2) groups balanced for calving date and milk production, and measurements conducted in one group each week while grazing perennial ryegrass/white clover pasture at generous herbage allowances. Thus, trials were conducted over 4 (Experiment 1) or 5 (Experiment 2) consecutive weeks.

Daily CH_4 emissions were measured during 4 (Experiment 1) or 3 (Experiment 2) consecutive days using the SF_6 technique, with pre-calibrated permeation tubes administered *per os* into the reticulo-rumen of each animal seven days prior to commencing collection of breath samples. The SF_6 PRs from the permeation tubes used with each of the measurement groups (1, 2, 3 and 4) during Experiment 1 were (mean ± standard deviation among the tubes): 3.84 ± 0.61, 3.86 ± 0.69, 3.77 ± 0.75,

and 3.80 ± 0.67 mg(SF_6) d^{-1}, respectively, whereas the PRs used during Experiment 2 were 2.74 ± 0.23, 2.80 ± 0.31, 2.81 ± 0.28, 2.81 ± 0.30, and 2.82 ± 0.35 mg(SF_6) d^{-1} for groups of measurement 1, 2, 3, 4 and 5, respectively. The range of PRs in Experiment 2 was much smaller than in Experiment 1.

The cows were milked twice daily (0600–0700 and 1500–1600 h). Milk production was measured at each milking and samples of milk (AM and PM milking) were taken for chemical composition analyses midway through the measurement period. 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. Condition score was assessed at the start and the end of the week of measurements.

The above animal data together with feed quality were used to estimate the feed dry matter (DM) intake (DMI) using energy-requirement algorithms developed by the Australian Standing Committee on Agriculture (SCA, 1990). This approach was judged to be the most reliable way to estimate DMI, given the difficulty in measuring it directly or indirectly (Section 1.1). Effectively, the GEI and associated DMI as estimated provide the energy necessary for the cow to both maintain body condition and sustain milk production, taking account of the various efficiencies of energy conversion. The error incurred by using such an algorithm for indiviual animals on individual days or groups of days is undetermined.

The range of PRs in Experiments 1 and 2 were 2.624–5.689 and 2.214–3.594 mg(SF_6) d^{-1}, respectively. In Experiment 1, the mean estimated DMI was 17.4 kg cow^{-1} day^{-1}, the mean estimated CH_4 emission rate was 332 g cow^{-1} d^{-1}, and the mean estimated CH_4 yield 19.3 g $kg(DMI)^{-1}$. The corresponding mean estimates for Experiment 2 were 16.8 kg(DMI) cow^{-1} day^{-1}, 290 g(CH_4) $cow^{-1}d^{-1}$ and 17.4 g $kg(DMI)^{-1}$. Relationships between estimated daily CH_4 emission (g d^{-1}) and both PR (mg d^{-1}) and estimated DMI (kg day^{-1}) for each measurement group of cows in Experiments 1 (four groups) and 2 (five groups) are shown in Figure 8. Experiment 1 showed a positive relationship between estimated CH_4 emissions and PR, whereas Experiment 2 showed no significant relationship. All measurement groups in Experiment 1 exhibited a positive and significant association ($P<0.01$) between PR and estimated daily CH_4 emission, with PR explaining between 6 and 21% of the total variance. In contrast for Experiment 2 the association between PR and the apparent daily estimated CH_4 emission was significant ($P<0.05$) only for Group 2, with PR explaining less than 4% of the overall variance.

Figure 8 also shows a positive relationship between estimated daily CH_4 emission and estimated DMI for both Experiment 1 and 2, which was expected as DMI is the most

important determinant of CH_4 emission. In Experiment 1, except for Group 3, that relationship was positive and significant ($P<0.05$), with estimated DMI explaining between 5 and 36% of the total variance of the estimated daily CH_4 emission. However, for all groups except Group 2 in Experiment 1, PR had relatively higher importance than the estimated DMI in explaining that total variance. In Experiment 2, the estimated DMI was positively and significantly ($P<0.0001$) related to estimated daily CH_4 emissions, explaining between 22 and 44% of the total variance.

The relationships between PR and the estimated CH_4 yield for each measurement group in Experiments 1 and 2 are shown in Figure 9. In Experiment 1, there was a positive and significant ($P<0.04$) relationship between these variables for all groups except Group 2 ($P=0.27$). In this experiment, each mg(SF_6) d^{-1} increase in PR was associated with an increase in estimated CH_4 yield of 0.6–2.2 g kg(DMI)$^{-1}$, explaining between 6 and 23% of the total variance. In Experiment 2, the same relationship only approached statistical significance ($P<0.07$) for Groups 1, 2 and 3. Further, each mg(SF_6) d^{-1} increase in PR was associated with a similar increase in estimated CH_4 yield as observed in Experiment 1, but the proportion of total variance explained by PR was very small (<5%).

In conclusion, these grazing experiments revealed a positive effect of PR on the CH_4 emission estimates (1 mg(SF_6) d^{-1} associated with 0.6–2.3 g kg(DMI)$^{-1}$), but this effect was significant (R^2=0.06–0.23, $P<0.05$) only when there was a large range in PR (Experiment 1), whereas with a narrower PR range (Experiment 2) the effect was not significant ($R^2<0.04$, $P>0.05$). It should also be noted that the estimation of individual DMIs is fraught with uncertainty, making no allowance for individual feed conversion efficiencies that depart from that of the "standard cow" represented in the energy requirement algorithm.

4.4 Tailored experiment: June 2005

A pen experiment was conducted to examine a dependence of estimated CH_4 emission of SF_6 PR (Pinares-Patiño et al. 2008b). Twelve well-trained 2-year-old Hereford × Friesian steers (live-weight 478 ± 41 kg) fitted with rumen cannulae were fed twice daily (0800 and 1500 h) on molassed-lucerne silage at restricted feeding levels. 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.

Twelve permeation tubes with nominal four levels of SF_6 PR (low, medium, medium-high and high) were selected from a batch of newly charged tubes on the basis of linearity of mass loss (R^2 >0.99). The high-PR tubes were fabricated with Teflon® of lower thickness to achieve the high PR. The pre-calibrated permeation rates in each

Table 4. Effect of SF_6 permeation rate (PR) upon mean concentrations of gases in the breath samples and estimated CH_4 for the "tailored experiment" of June 2005.

	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 (x 10^{-3})	455.1[a]	265.6[b]	225.4[c]	105.3[d]	13.8	0.001	0.100
Estimated 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 $kg(DMI)^{-1}$	18.1[a]	19.9[a]	23.3[b]	22.1[b]	1.0	0.001	0.151

[y] Refers to molar ratios (mol(trace gas) mole(dry sample)$^{-1}$), in excess of background concentrations
[z] Probability value for orthogonal contrast for linear or quadratic effect of SF_6 permeation rate. Values > 0.05 are statistically not significant.
[a–c] Means in row with different letters are significantly different (P < 0.05).

level, low (L), medium (M), medium-high (MH) and high (H) were (mean ± standard deviation): 1.91±0.05, 3.62±0.05, 5.34±0.21 and 11.34±0.28 mg(SF_6) d^{-1}, respectively.

Four sequences of permeation tube deployment (four "treatments") 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 animals, balanced for number of replications (three animals per sequence). Thus, the experimental design was a replicated 4×4 Latin square. After acclimatisation to feeding and management conditions, measurements were carried out during four consecutive periods (1–4) each lasting 7 days (Days 1–7). During each measurement period, the permeation tubes were inserted *per fistula* into the reticulum on Day 1 and retrieved on Day 7. At retrieval, the tubes were rapidly transferred *per fistula* to other animals following the sequence of deployment. The swapping of permeation tubes between sequences of deployment were 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. Permeation tubes were recovered at the end of the experiment and post-experiment permeation rates determined through serial weighing, from which individual permeation rates could be determined for each measurement period by interpolation (Lassey et al. 2001). The mean permeation rates specific to each of the measurement periods were used to calculate the daily CH_4 emissions at each measurement period.

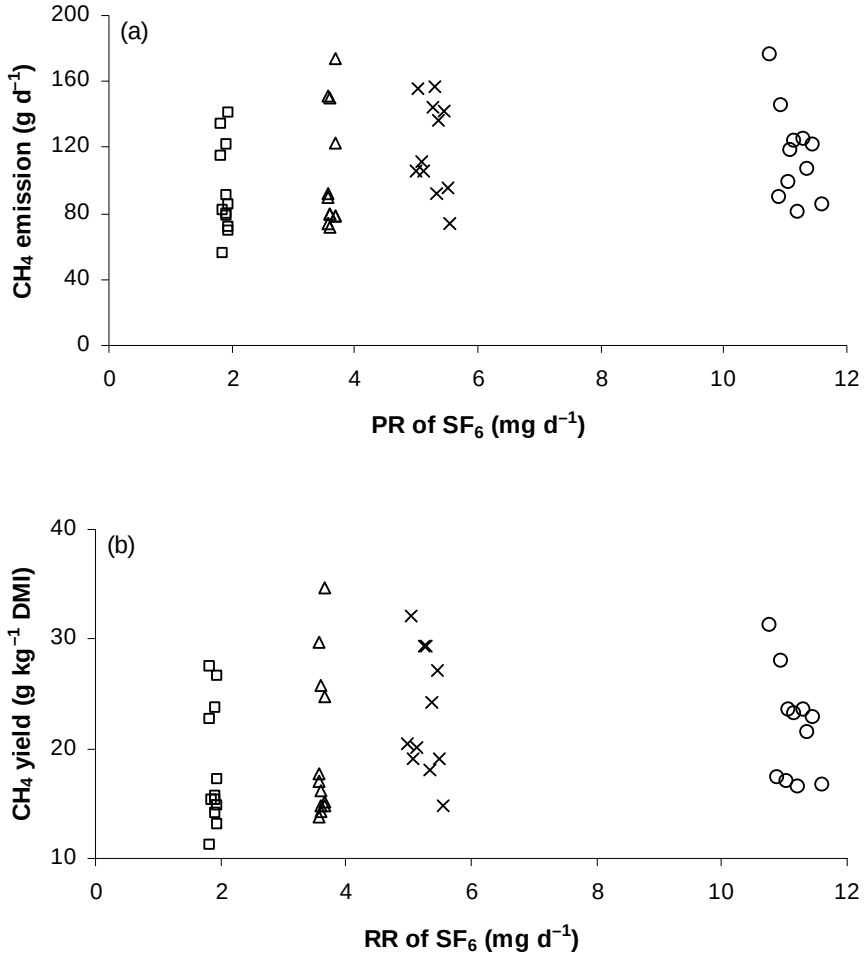

Figure 10. **Estimated daily CH_4 emission (g d^{-1}) (upper panel) and CH_4 yield (g kg(DMI)$^{-1}$) (lower panel) as functions of SF_6 PR (mis-labelled RR in lower panel) (mg d^{-1}) for individual animals for each PR treatment (low, □; medium, Δ; medium-high, ×; high, o) in the "tailored experiment" of June 2005.**

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. Feed refusals accounted only for few grams and were considered negligible. Dry matter contents of feed on offer was analysed on within-period pooled samples. Mean daily DMI for each animal was averaged over the entire measurement period (7 days).

Table 4 presents the effects of PR treatment upon the mean concentration of gases in breath samples and the calculated CH_4 emissions. Bearing in mind that breath sampling efficiency (i.e., its dilution with entrained air) will vary according to the

detailed halter and inlet configuration (Figure 2), the concentration of CH_4 (ppm) nevertheless did not differ ($P>0.05$) among PR treatments. As expected, there was a significant linear ($P<0.05$) effect of PR treatment upon both the concentration of SF_6 (ppt) and the CH_4/SF_6 ratio. (These tests do not contradict the algebraic result that for SF_6 concentration that vary linearly with PR, then the CH_4/SF_6 ratio would vary as its reciprocal).

The within-treatment variations in CH_4 concentration were similar across 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. 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; $P<0.0001$). The within-treatment variation in the CH_4/SF_6 ratio decreased with increase in PR as would be expected.

There were significant effects ($P<0.05$) of PR treatments upon both the estimated daily CH_4 emission (g d^{-1}) and CH_4 yield (g kg(DMI)$^{-1}$) (Table 4) and although L and M, and MH and H treatments, taken in pairs, did not differ either in estimated daily CH_4 emission or in estimated CH_4 yield, the overall pattern of response to PR was better captured by a linear ($P=0.001$) than a quadratic ($P=0.15$) relationship. Thus, for example, each 1 mg(SF_6) d^{-1} increase in PR accounted for 0.36 g kg(DMI)$^{-1}$ increase in estimated CH_4 yield. The within-treatment variation in estimated CH_4 emission (both g d^{-1} and g kg(DMI)$^{-1}$) seemed to be relatively smaller at the higher PR treatments (Figure 10).

This experiment reinforced observations made of the grazing experiments that both the daily CH_4 emission and the CH_4 yield, as estimated with the SF_6 technique, increased with increasing PR. This effect was more linear than quadratic, with each 1 mg(SF_6) d^{-1} associated with a 0.36 g(CH_4) kg(DMI)$^{-1}$ increase in estimated CH_4 yield. However, H permeation tubes, with PR values twice those of MH tubes, led to estimated CH_4 emissions similar to those for MH tubes. The set of H permeation tubes were fabricated using Teflon material different from that of the other sets in order to achieve the high PR. With H treatment excluded from calculations, each 1 mg d^{-1} increase in PR was associated with an increase of 1.40 g kg(DMI)$^{-1}$ in estimated CH_4 yield, which is consistent with the association found in the grazing experiments of Section 4.3.

However, it could be noted that permeation tubes were administered (*per fistula*) only two days prior to commencing breath sampling. This is an unusually short equilibration period that may not assure a steady SF_6 distribution in key pathways of the host's body.

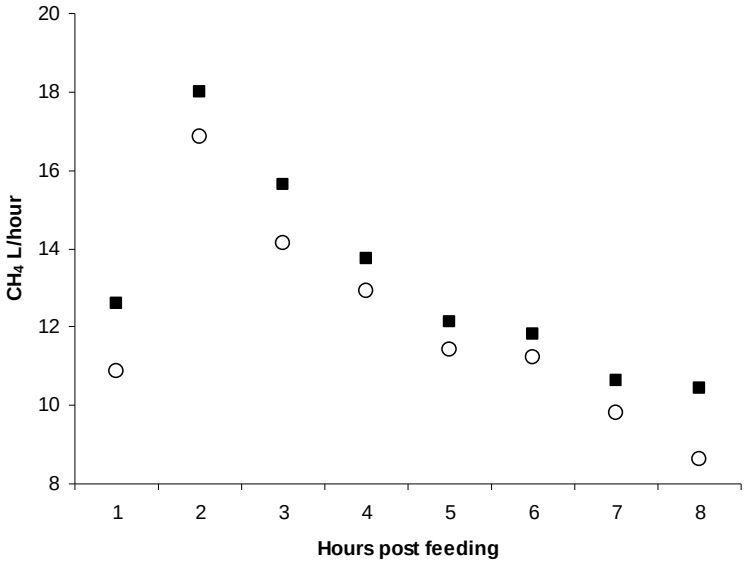

Figure 11. **Calculated hourly rates of CH_4 production in the rumen of cows deployed with permeation tubes with low (○) or high (■)SF_6 PR The rumen headspace gases were collected unobtrusively through rumen cannulae. Each data point represents mid-points of consecutive sample collections, the first just prior to feeding. (1 g(CH_4) occupies 1.4 L at standard temperature and pressure).**

4.5 Rumen headspace sampling

An experiment to sample rumen headspace gases directly in cattle equipped with ruminal cannulas (fistulas) and *in situ* SF_6 permeation tubes was conducted at INRA-Clermont Ferrand (France) (Pinares-Patiño et al. 2008c). We report here the effect of SF_6 permeation rate (PR) (the "treatment") on the level of SF_6 in rumen headspace gases.

Six adult non-lactating Holstein cows were used, each fitted with permanent ruminal cannulas equipped with stoppers, allowing collection of rumen head space gas samples without having to open the cannula (Jouany and Senaud 1979). The experiment lasted 39 days, which included 21 days of acclimatisation, followed by two periods (P1 and P2) of gas measurements over days 23–25 and 37–39, respectively.

Cows were randomly subdivided into two groups of 3 animals each, and the groups randomly assigned to permeation tube deployments with low SF_6 PR (Lo-PR, 1.57±0.28 mg d^{-1}) or high PR (Hi-PR, 3.14±0.56 mg d^{-1}) in a crossover design over days 17–25 and 31–39. (Ranges are mean ± standard deviation. among the 3 tubes, and do not reflect calibration uncertainty). Tubes were inserted *per fistula* 7 days before P1 and P2, while during days 25–31 tubes were maintained in the laboratory at

39°C. The cows were kept in individual stalls and fed maize silage at 80% of their *ad libitum* intake, delivered in two equal meals at 0800 and 1600 h. Rumen gases (50 mL) were sampled immediately before the morning feeding and then hourly over 8 hours. Mixing ratios of CH_4 and SF_6 in rumen gas head space were determined by gas chromatography after quantitative dilution with nitrogen gas.

Unsurprisingly, there was no effect of treatment (P=0.80) upon mean CH_4 concentration in each group (305 and 291 ppm for Hi-PR and Lo-PR, respectively). Despite the two-fold difference in SF_6 permeation rate between Lo-PR and Hi-PR permeation tubes, treatment effects on mean SF_6 concentration only approached statistical significance (P=0.09) (381 and 212 ppt for Hi-PR and Lo-PR, respectively). As expected, the mean CH_4/SF_6 ratio of molar concentrations differed significantly (P=0.001) between the treatments (0.651×10^6 and 1.197×10^6 for Hi-PR and Lo-PR, respectively). When the CH_4/SF_6 ratios in rumen headspace gas and pre-calibrated SF_6 permeation rate were used to calculated CH_4 production rates, the Hi-PR treatment yielded consistently higher hourly CH_4 production rates than the Lo-PR tubes (Figure 11). The mean CH_4 production calculated for cows bearing the Hi-PR tubes were 8.5% higher than for those bearing the Lo-PR tubes (221 *vs* 204 $g(SF_6)\ d^{-1}$), although this difference was not significant (P=0.34).

4.6 Comparison between SF_6 and enclosure techniques

Comparisons to date between estimations of mean CH_4 emission rates based on enclosure in chambers and based on the SF_6 technique have generally displayed good agreement (Grainger et al. 2007, McGinn et al. 2006, Pinares-Patiño et al. 2008a). This suggests that there is no systematic error made using the SF_6 technique that is material, unless such a systematic error coincidentally compensated for the failure of the SF_6 technique to trace flatus CH_4.

However, it does appear that CH_4 estimates using the SF_6 technique display more variability, either between animals or between days for the same animal, than when using chamber techniques, and accordingly that comparisons between SF_6 and chamber techniques do not always agree well for individual animals (e.g., Grainger et al. 2007). This situation, over-viewed by Pinares-Patiño & Clark (2008), is the subject of ongoing investigation. However, there is merit in examining possible explanations for the effect of SF_6 PR for their ability to also account for an enhanced variability in CH_4 emission as calculated using the SF_6 technique.

5. Evaluation of the evidence

The experiments summarised in Chapter 4 strongly suggest that the SF_6 PR can influence the methane emission rate ($g(CH_4)\ d^{-1}$) and its counterpart CH_4 yield ($g(CH_4)\ kg(DMI)^{-1}$) as calculated using the SF_6 technique. We refer below to this

influence in general, and to the influence on estimates of CH_4 yield in particular, as the "CH_4-SF_6 correlation". Such an influence is a surprising result that calls into question the adoption of SF_6 as a tracer and/or the level of tracer employed. The influence of tracer is much less convincing for grazing animals than for housed animals (ie, when the animals spend many hours each day feeding than when the feed is brought to the animal and, generally, eaten quickly), though can nevertheless still be significant (e.g., Section 4.3).

The surprising CH_4-SF_6 correlation result has prompted a search for an explanation. Different potential explanations are explored in the following subsections, which largely mirror, but not with 1:1 correspondence, the "questions raised" in Section 3.1.

5.1 Site differences between exit points for CH_4 and SF_6

As noted in Chapter 2, a single definitive experiment (Murray et al. 1976) demonstrated that 1–2% of excreted CH_4 is expelled as flatus, and would thereby not be detected by the SF_6 technique. However, this experiment was conducted with four ewes fed a single diet throughout (lucerne chaff). It is pertinent to ask:

- how much inter-animal variation is there in this "flatus proportion"?
- does the flatus proportion apply across different species (notably cattle)?
- is the flatus proportion dependent upon feed quantity and quality (and thence potentially on the site of digestion), and/or upon the daily feeding pattern?
- as a means to answer the above questions, do surgically modified animals or invasive techniques replicate the real gas transactions?

If the flatus proportion were to vary appreciably among cohort animals, this could cause a greater variation in CH_4 emission estimated using the SF_6 technique than for the same estimated from chamber experiments, as has been reported (Section 4.6).

McGinn et al. (2006) have reported that CH_4 estimates using the SF_6 tracer technique shows closer agreement with those using chamber techniques when the animals (cattle) are fed high-forage diets than when fed high-grain (corn and barley) diets, and closer also when feed intakes are restricted than when unrestricted. McGinn et al. "hypothesize that greater differences between the techniques would exist when cattle are fed diets that are extensively fermented post-ruminally compared to diets that are extensively fermented in the rumen". More post-ruminal digestion "would provide a greater opportunity for CH_4 release through the rectum". McGinn et al. provide evidence that a corn-based diet has a greater degree of post-ruminal digestion, and argue also that unrestricted feeding levels shorten the feed-retention time in the rumen, enabling greater post-ruminal digestion than for restricted intakes. Thus, McGinn et al.

conjecture that the 1–2% of flatus CH_4 reported by Murray et al. (1976) would underestimate the actual flatus proportion when cattle are fed diets with a greater degree of post-ruminal digestion.

A logical extension of the findings by McGinn et al. (2006) is that the SF_6 technique would underestimate actual CH_4 emission, even if without statistical significance, especially for diets or intakes with more extensive post-ruminal digestion, because SF_6 almost certainly fails to trace CH_4 released at the rectum. (The "almost certainly" arises because SF_6 release at the rectum has not been confirmed, and in principle if the flatus proportion of both CH_4 and SF_6 were identical, then SF_6 would ideally trace these exit points even though the SF_6 technique does not detect flatus gases.)

Experiments conducted by AgResearch in collaboration with NIWA have detected traces of SF_6 in urine that correspond to a negligible exit point for that gas ($\sim 10^{-7}$ of the source strength). Similar minute traces have been extracted from faecal material under vacuum (but probably accounting for interstitial gases rather than fully-absorbed gas, and not accounting for flatus gas).

Thus, while flatus emissions have the potential to explain discrepancies between CH_4 emissions as estimated using chamber and SF_6 techniques, and the purported greater variability of the latter technique, it is not clear how such discrepancies could discriminate according to the SF_6 permeation rate.

5.2 Differential intra-ruminal transport of CH_4 and SF_6

5.2.1 Physical discrimination

As noted in Section 3.2.2, the permeation tubes almost always lodge in the rumen of sheep, and the reticulum of cows. Because gases are eructed directly from the reticulum, SF_6 released from rumen-located tubes are one step removed from eructation. Noting that a typical tube releases only ~5 (for sheep tubes) or ~20 (for cattle tubes) $\mu L(SF_6)$ hourly, it is potentially possible during periods of no or low digestion for such small gas releases to be collected and retained for long periods in crevices or pockets in the rumen (or attached to particulate material), especially while reposing. Indeed, hour-by-hour monitoring of exhaled gases from sheep kept in metabolism crates has noted that SF_6 can be absent in breath samples for hours at a time, especially while reposing (Martin et al. 2007), only to be released in bursts that may coincide with a resumption of physical activity. While the cause of this absence is unknown, it could be related to the temporary capture of SF_6 that has no counterpart for CH_4 partly because of the far greater volumes of the latter (by $\sim 10^5$) and partly because of its more distributed source.

While the above suggests that SF_6 may not ideally trace CH_4 sources in and eructed from the reticulo-rumen due to differential transport into eructed gases, this is probably more likely to be influential on the sub-day time scale, and may introduce a source of variability in consecutive-day breath sampling. It may also suggest that permeation rates can be "too low" by enhancing the ability of crevices or pockets to temporarily intercept SF_6. However, it does not suggest why the SF_6 technique should not be reliable for average emissions over multi-day measurement periods, irrespective of SF_6 permeation rate, other than to introduce a source of day-to-day variability.

5.2.2 Non-physical discrimination

Both CH_4 and SF_6 dissolve in aqueous solutions, albeit to minor levels (CH_4 at 39°C: 21 mg L^{-1} or 1.3 mmole L^{-1}; SF_6 at 39°C: 29 mg L^{-1} or 0.20 mmole L^{-1}). The amount of the day's production of CH_4 and the day's release of SF_6 that could dissolve in rumen liquor and be swept down the digestive tract depends upon water and saliva throughput. While the proportion of CH_4 removed from the rumen this way is negligible, the proportion of SF_6 can approach 10–15% — or even higher if SF_6 bubbles can be swept along with the rumen liquor. This proportion would appear too large to account for observation in the event that all the dissolved SF_6 were eventually expelled as flatus. Moreover, if a fixed daily amount of SF_6 (i.e., limited by solubility, irrespective of SF_6 PR) were expelled this way a correlation between estimated CH_4 emission rate and SF_6 PR would be induced, but it would be in the wrong direction (viz, a negative correlation!). Furthermore, it is commonly accepted that most SF_6, as well as CH_4, in the hindgut is absorbed into the bloodstream from which it outgases in the lungs.

The above would apply to any hypothesized mechanism that prevents a fixed daily amount of SF_6 from being eructed: it would induce a correlation between estimated CH_4 emission rate and pre-calibrated SF_6 PR that was in the opposite direction from that observed.

As noted in Section 2.2, some rumen-generated CH_4 is absorbed into the bloodstream, though most of that is re-routed to the breath via the lungs. It is not known how much SF_6 is similarly absorbed, but it is unlikely to ideally trace this pathway. Nevertheless, one need only be concerned about gases that are rumen sourced and subsequently exhaled, irrespective of the pathway (via eructation or via absorption and respiration), and whether or not SF_6 traces CH_4 from rumen to exhalation via either pathway. However, one caveat is that the transit time of the longest SF_6 pathway, from release to exhalation, should be appreciably shorter than the duration of permeation tube residence in the host's rumen, in order to be assured that SF_6 distribution is steady. Thus the tube should be inserted some days in advance of breath sampling; seven days has become the norm (but was not followed in the experiments of Sections 4.2, 4.4).

5.3 The pre-calibrated and intra-ruminal SF_6 release rates: A mismatch?

All permeation tubes are calibrated while held at 39°C, which temperature characterizes internal temperature of the ruminant animal. However, as PRs are known to increase with temperature, estimates of CH_4 emission rate could be systematically in error if that internal temperature differs systematically from 39°C, and will have a variation induced by a variable temperature. Moreover, the temperature of importance is that of the reticulum or rumen contents. Whereas blood temperature may be confined within very tight bounds, rumen contents could be expected to vary as food and (cold) water are ingested and as fermentation takes place. This is confirmed by Dr Gerald Cosgrove (AgResearch, personal communication to KRL, 2008) who has deployed recently-developed temperature sensors in the animal rumen; Dr Cosgrove reports that rumen temperatures vary by up to 2°C below 39°C and that an indicative average would be less than 39°C. This suggests that the actual intra-ruminal release rate of SF_6 could be less than the pre-calibrated rate (and with some variability during the feeding cycle that might depend on the feeding pattern of the animal concerned), and the real daily CH_4 emission rate would therefore be over-estimated by Equ. (1).

The temperature sensitivity of SF_6 permeation rates has not been established experimentally. Nevertheless, according to a laboratory catalogue (Analytical Instrument Development, Inc, PA, USA, ca 1980) supplied by R.J. Martin (personal communication to KRL, 2008), PRs in general conform to the following empirical (Arrhenius-like) relationship:

$$\log \frac{\mathrm{PR}_2}{\mathrm{PR}_1} = \alpha \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \qquad (2)$$

in which PR_i is the PR at absolute temperature T_i, and α is an empirical constant which varies with permeant and with permeable material within 10–20% of 2950°K. Thus:

$$\frac{1}{\mathrm{PR}} \frac{\partial \mathrm{PR}}{\partial T} = \frac{\alpha}{T^2} \qquad (3)$$

which implies a PR sensitivity at 39°C (312°K) within the range 3.0±0.5% per °C.

Thus, a variable rumen temperature averaging between 38 and 39°C implies an intra-ruminal SF_6 PR that can vary and having a daily average that is lower than calibrated by less than ~3%. This in turn provides daily CH_4 emission estimates that are over-estimated by less than ~3%. The over-estimate could differ among days and among animals depending on ingestion and digestion patterns, which could potentially account for some of the variability reported in Section 4.6.

5.4 Within-day variability of CH_4 production

It would be expected that CH_4 generation varies throughout the day concordantly with the feeding pattern (e.g., Grainger et al. 2007, Fig. 3). Thus a constant tracer release rate cannot ideally trace a variable CH_4 generation rate. The variability in CH_4 generation rate would likely be at its greatest (most 'spikey') in housed experiments where the animals are fed twice daily (a typical frequency) and are observed to consume each meal within ~1 h, and at its least where animals graze continuously during most of the daylight hours. This is fully consistent with the observation that the CH_4-SF_6 correlation is more convincing for housed than for grazing animals. Moreover, it is also consistent with experiments performed by NIWA personnel in cooperation with AgResearch (Martin et al. 2007) which revealed large inter-hour variations in both CH_4 and SF_6 concentrations in breath samples that appeared to be associated with the feeding pattern.

The above hypothesis — that the SF_6 technique works best when the CH_4 production rate throughout the day is as uniform as achievable, and most closely approached during grazing — is also consistent with the finding by McGinn et al. (2006) that "the SF_6 tracer technique is most reliable for the grazing system". This is also the system for which the SF_6 technique is uniquely applicable.

5.5 Accounting for background levels of CH_4 and SF_6

Corrections for background levels of CH_4 and SF_6 (see Equ. (1)) are critical wherever: (*a*) breath collection efficiencies are low so that sample concentrations of either CH_4 or SF_6 are within a factor of ~10 of background levels; or (*b*) background levels have the potential to vary markedly due to the possibility of large concentration gradients (spatial or temporal) in CH_4 or SF_6. (see Section 3.2.3). The latter possibility is of importance mainly in housed situations, and can be addressed by deploying multiple background samplers to detect time-integrated gradients (Lassey 2007, Section 2.3).

As long as sufficient background samples are collected, and are appropriately located, QA/QC procedures should recognise samples that might be problematic (Section 3.2.4). Nevertheless, it is possible, even if unlikely, that "background issues" could bias the result and lead to a CH_4-SF_6 correlation, because such issues would be at their most significant where PRs are low. Such a bias persisting across multiple experiments is implausible.

5.6 Uncertainties in estimating feed intake while grazing

As reasoned in section 4.1.1, the uncertainty and inaccuracy in estimating feed intakes by grazing animals can result in an apparent or accidental association between PR and DMI (e.g., see Figure 6), arising because each can vary with experiment. Actual DMI

will vary among experiments due to the animals having different bodyweights and lactation levels, and estimating DMI using inevitably-imprecise approaches introduces further variation. The association is likely to be at its most apparent for grazing cows whose DMI is estimated on the basis of an energy requirements model, and whose energy requirements for maintenance can be multiplied ~2.5-fold by the demands of lactation. The extent to which grazing behaviour is affected by constraints on the animal's "lifestyle" imposed by the mounting of breath sampling apparatus (Figure 2) and by other experimental logistics (e.g., frequent mustering) is unknown, but these effects are usually minimised by acclimatizing the animals to wearing the apparatus prior to commencing measurements. If these impositions result in diminished feed intake, then CH_4 emission is likely to be concomitantly diminished, while productivity (lactation or growth) will respond more slowly. Thus GEI and DMI will be over-estimated when based on the productivity during the few days of measurements, leading to an under-estimated CH_4 yield.

Employing a feed requirements model presumes also that daily DMI of the individual animal is fully predictable on the basis of the energy required to maintain the animal and sustain its measured productivity, together with the properties of the feed on offer. Neither may fully determine actual DMI, due to individuality in energy conversion efficiency and in feed selection, as well as to the animal's reaction to imposed changes in its "lifestyle", as noted above.

While the above reinforces the perennial problem of determining feed intakes by grazing animals, any systematic errors incurred would be independent of PR, so that any correlation between estimated CH_4 yield and PR would be "accidental" rather than systematic.

6. Recommendations for future research

This section identifies some questions that that could be resolved through experiments, and proposes specific or general experiments to achieve this. The intent is that such experiments could identify the CH_4-SF_6 correlation and/or show how to correct for it.

6.1 Permeation tube performance

One explanation for the CH_4-SF_6 correlation is that permeation tubes, once located in the rumen, do not perform as expected or as they did during laboratory calibration. Already, some unexpected behaviours have been documented (Lassey et al. 2001) for the idealised situation of tubes maintained in a dry isothermal environment. Realising that the rumen is neither dry nor isothermal (Section 5.3), our knowledge of tube performance would be appreciably enhanced by experiments which:

a) determine the temperature sensitivity of SF_6 PR from both sheep and cattle tubes and for a range of SF_6 permeation rates of both to confirm the sensitivity of Equ. (3) (see Section 5.3).

b) determine the SF_6 PR of permeation tubes while immersed in water and/or simulated rumen liquor: do they permeate at the same rate as in air during calibration? Preliminary tests done so far by J.B. Vlaming and M. Tavendale (AgResearch) are equivocal, but hint at a lower PR while immersed. Much earlier tests by K.R. Lassey and C.F. Walker (NIWA) could not detect any significant "abnormal" mass loss during several weeks of immersion.

c) assess whether permeation tube location (rumen *vs* reticulum) influences SF_6 concentration in the rumen headspace or collected breath sample and thence on the estimated CH_4 emission, especially for sheep where the permeation tube usually lodges in the rumen (Section 3.2.2).

6.2 Gas chromatography performance

As noted in Section 3.2.4 (sub-section "Further Quality Assurance of sample measurements"), some recent QA cross-checks on GC analyses have revealed discrepancies between AgResearch and NIWA GC determinations that have yet to be explained. These need to be addressed urgently, not only to resolve the discrepancies, but also to establish if those discrepancies introduce a bias with SF_6 level in part explanation for the CH_4-SF_6 correlation.

Any mis-calibration or compromised calibration of a high-SF_6 standard (denoted *Hi* in Section 3.2.4) could account for a CH_4-SF_6 correlation. Such a calibration error would lead to an erroneous "calibration curve" used to translate GC-ECD response to SF_6 mixing ratio with greatest error at high SF_6 values. This would provide a bias in CH_4 emissions estimated for high-PR permeation tubes. It is therefore critical to maintain confidence in working standards through regular cross-checking against laboratory primary standards, especially in the event of surprises such as the CH_4-SF_6 correlation. All GC determinations used in the meta-analyses of Section 4.1, including any done externally (e.g., at DPI Ellinbank, Vic, Australia), should have their associated standards similarly cross-checked regularly against recognised or common standards. Such cross-checking between AgResearch and NIWA standards has been the practice, albeit with limited frequency.

6.3 Internal pathways and fates of CH_4 and SF_6

Our knowledge about CH_4 generation within the digestive tract, the determinants of such generation, and the dynamics and fates of the generated CH_4, is quite limited and derives from alarmingly few experiments with a narrow diversity of animal species

(and animal numbers), feeds, and feeding patterns (Section 2.2). In addition, we have minimal knowledge of the dynamics of SF_6 in the animal's body, including its redistribution from the digestive tract, the dynamics of that redistribution, and SF_6 fates. Taken together, we have little confirmation or verification for the assumption that SF_6 pathways and dynamics mimics those of CH_4 — or at least of rumen-sourced CH_4 — and therefore that SF_6 is an adequate tracer of (rumen-sourced) CH_4. Some imperfections in that mimicry may not matter (for example, different combinations of parallel pathway from rumen source to exhalation), provided that each gas is close to steady state during the experiment.

To enhance confidence in SF_6 as a tracer of enteric CH_4, the following experimental objectives requiring conceptually and ethically complex experimental designs and procedures, would add valuable and relevant knowledge, not only to issues surrounding the SF_6 technique, but to digestive metabolism generally:

a) to differentiate and quantify emissions of CH_4 and SF_6 via breath and flatus, at different feeding levels, feeding patterns, and diets, for both sheep and cattle. Experiments utilising chambers could be designed without the need to intervene surgically or invasively. This would require isolating the "front half" of the animal in the chamber from the "rear half" and having separate and separately-sampled air flows in each half. The isolation could be via a suitable curtain, or it could require that the animal be astride two chambers with front and rear halves in different chambers (and different flow rates in each chamber optimised to the different front and rear emission rates).

b) to examine fates of CH_4 and SF_6 other than via gaseous pathways (i.e., in urine, faeces, milk), and to enhance understanding of the pathways to these fates by examining CH_4 and SF_6 content in blood and other tissues. The overall goal of both this and Objective (*a*) would be to establish detailed budgets for SF_6 and CH_4 in the sheep's (and ideally the cow's) body.

c) to investigate the dynamics of the processes quantified in Objective (*a*), with the specific aim of determining how long it takes for SF_6 to achieve equilibrium after inserting the permeation tube — or, at the least, of verifying that 7 days is long enough, noting that this is the "normal" protocol but that the logistics of some experiments have required a shorter time (e.g., the tailored experiments of Sections 4.2, 4.4).

6.4 Daily emission profiles of CH_4 and SF_6 under different feeding regimes

To better understand how within-day CH_4 emissions relate to feeding and behavioural patterns as well as how well SF_6 traces these emissions for different feeding patterns (Section 5.2.1, 5.4), it would be valuable to:

a) undertake real-time continuous analysis of breath samples in calorimetry chambers to clarify the daily profile of CH_4 and SF_6 emissions under different feeding regimes. This can be done with permeation tubes of different PR in order to check any dependence of SF_6 profile upon PR.

b) investigate the "meal effect": that the utility of the SF_6 technique might vary with the frequency of meals, from two meals per day to continuous supply throughout the day as a simulation of grazing (subdividing the daily nutritional requirement accordingly). This should be done in conjunction with Objective (*a*) using calorimetry chambers, though automated breath sampling from metabolic crates would be an alternative.

To the extent that SF_6 entrainment into eructed gases may be in bursts rather than continuous (Sections 5.2.1, 5.4), these investigations would explore reasons for discontinuous bursts, and whether or not those discontinuities might contribute to the greater variability in CH_4 emission estimated by the SF_6 technique than estimated by chamber techniques, as has been reported by some experimenters (Section 4.6).

6.5 Verification of DMI estimation under grazing

The SF_6 technique seems to work best while grazing, but the big difficulty with grazing is in the assessment of DMI. For cows in particular, DMI is commonly assessed using an energy-requirements model, and more confidence is needed in the reliability of such an assessment when applied to individual animals on individual days or groups of days. To enhance such confidence:

a) compare measured DMI with calculated DMI (using various energy-requirement formulations) under "simulated grazing conditions" of Objective 6.4(*b*)

b) for the many housed experiments that have already been conducted, retrospectively calculate the DMI for each animal (where the necessary data are available) using one or more energy-requirement formulations to cross-check against the measured DMI.

6.6 Independent cross-checks of emissions under grazing

While chambers offer an opportunity to verify or cross-check emission estimates using the SF_6 technique, the comparison is less than ideal because the chamber does not provide an ideal environment in which to deploy the SF_6 technique concurrently. In a grazing situation, micrometeorological techniques provide an opportunity for independent cross-check. Again the comparison is not ideal even if the measurements are concurrent because the micrometeorological approach determines the emissive flux averaged across the flock or herd (or from a "footprint" within it). Furthermore, the precision that can be achieved for the emissive flux estimates depends on the prevailing weather (ideally, uniform light winds from a direction without obstacles to wind flow), and can rarely be better than ~15% with available technologies. Nevertheless, with freedom to select appropriate weather, the herd-scale measurements can be useful for providing unbiased estimates of paddock-scale methane fluxes (Lassey 2007, Section 3) as an independent cross-check on per-animal emission estimates or sufficiently-large emission reduction estimates (e.g., Denmead et al. 2000, Laubach & Kelliher 2004, Laubach et al. 2008, McGinn et al. 2008).

6.7 The SF_6 database

There appears to be one or more experiments absent from the SF_6 database. Specifically, the experiment with grazing cows reported by Lassey et al. (1997) appears to be absent. Noting also the necessity for some post-entry corrections to data (see Section 4.1), and with much of the earlier data (to ca 2003) having been manually entered into the database, an automated cross-check against the original data would be warranted.

In view of the critical importance of gas standards in assuring reliable gas analysis (Section 6.2), it would be valuable to also record in the database the suite of standards used in the analysis (or individual standards if suites are not kept intact).

6.8 Statistical analyses

All experiments reported in Chapter 4 draw conclusions on the basis of certain statistical tests, so that the purported CH_4-SF_6 correlation that is the subject of this report owes its existence to statistical inference. There is a suggestion that such statistical inference techniques (e.g., using *P*-values) may be prone to misinterpretation (Sellke et al., 2001). In order to ensure the robustness of such an inference:

a) All non-confidential data reported in Chapter 4, and appropriate data not reported there should be subjected to detailed scrutiny by two independent statisticians, who should strive to reach consensus on whether:

- a non-negligible CH_4-SF_6 correlation is proven;
- additional experiments should be designed and undertaken both to further examine the hypothesis of a negligible correlation, and if necessary and possible to quantify the correlation so as to enable the "real" CH_4 emission to be inferred from experimental data.

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Statistical analyses were performed by J. Koolaard and F. Potter (AgResearch, Palmerston North), or by M.H. Smith (NIWA, Wellington).

References cited

Beever, D.E. 1993. Rumen function. Pp. 187-215 *in*: Quantitative aspects of ruminant digestion and metabolism, Forbes, J.M.; France, J. *eds.* CAB International, Oxon, UK.

Clark, H.; Brookes, I.; Walcroft, A. (2003) Enteric methane emissions from New Zealand ruminants 1990–2001 calculated using an IPCC Tier 2 approach. A Report prepared for the NZ Ministry of Agriculture and Forestry by AgResearch Ltd.

Colvin, H.W.; Wheat, J.D.; Rhode, E.A.; Boda, J.M. (1957). Technique for measuring eructated gas in cattle. *Journal of Dairy Science 40*: 492–502.

Denmead, O.T.; Leuning, R.; Griffith, D.W.T.; Jamie, I.M.; Esler, M.B.; Harper, L.A.; Freney, J.R. (2000). Verifying inventory predictions of animal methane emissions with meteorological measurements. *Boundary-Layer Meteorology 96*: 187–209.

Dougherty, R.W.; Cook, H.M. (1962). Routes of eructed gas expulsion in cattle — a quantitative study. *American Journal of Veterinary Research 23*: 997–1000.

Dougherty, R.W.; Allison, M.J.; Mullenax, C.H. (1964). Physiological disposition of C^{14}-labeled rumen gases in sheep and goats. *American Journal of Physiology 207*: 1181–1188.

Finlay, B.J.; Esteban, G.; Clarke, K.J.; Williams, A.G.; Embley, T.M.; Hirt, R.P. (1994). Some rumen ciliates have endo-symbiotic methanogens. *FEMS Microbiology Letters 117*: 157-162.

Grainger, C.; Clarke, T.; McGinn, S.M.; Auldist, M.J.; Beauchemin, K.A.; Hannah, M.C.; Waghorn, G.C.; Clark, H.; Eckard, R.J. (2007). Methane emissions from dairy cows measured using the sulphur hexafluoride (SF_6) tracer and chamber techniques. *Journal of Dairy Science 90*: 2755–2766.

Hegarty, R.S. (2004). Genotype differences and their impact on digestive tract function of ruminants: a review. *Australian Journal of Experimental Agriculture 44*: 459–467.

Hoernicke, H.; Williams, W.F.; Waldo, D.R.; Flatt, W.P. (1965). Composition and absorption of rumen gases and their importance for the accuracy of respiration trials with tracheostomized ruminants. *In:* Blaxter, K.L. (ed). Energy metabolism, pp. 165–178. Academic Press, London, UK.

Hollinger, D.Y.; Hunt, J.E. (1990). Anthropogenic emissions of carbon dioxide and methane in New Zealand. *Journal of the Royal Society of New Zealand 20*: 337–348.

Immig, I. (1996). The rumen and hindgut as source of ruminant methanogenesis. *Environmental Monitoring and Assessment 42*: 57–72.

Jarvis, G.N.; Strömpl, C.; Burgess, D.M.; Skillman, L.C.; Moore, E.R.B.; Joblin, K.N. (2000). Isolation and identification of ruminal methanogens from grazing cattle. *Current Microbiology 40*: 327–332.

Johnson, K.; Huyler, M.; Westberg, H.; Lamb, B.; Zimmerman, P. (1994). Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. *Environmental Science and Technology 28*: 359–362.

Johnson, K.A.; Westberg, H.H.; Lamb, B.K.; Kincaid, R.L. (1998). The use of sulphur hexafluoride for measuring methane production by cattle. *In:* McCracken, K.J.; Unsworth, E.F.; Wylie, A.R.G. (eds). Energy metabolism of farm animals, pp 189–192. CAB International, Oxon, UK.

Jouany J.P.; Senaud J. (1979) Description d'une technique permettant d'effectuer des prélèvements répètés de gaz dans le rumen. *Annales de Biologie Animale Biochimie. Biophysique. 19*: 1007–1010.

Lassey, K.R. (2007). Livestock methane emission: From the individual grazing animal through national inventories to the global methane cycle. *Agricultural and Forest Meteorology 142*: 120–132.

Lassey, K.R. (2008). Livestock methane emission and its perspective in the global methane cycle. *Australian Journal of Experimental Agriculture 48*: 114–118.

Lassey, K.R.; Ulyatt, M.J. (2000). Methane emission by grazing livestock: A synopsis of 1000 direct measurements. *In*: van Ham, J.; Baede, A.P.M.; Meyer, L.A.; Ybema, R. (eds). Non-CO_2 Greenhouse Gases: Scientific Understanding, Control and Implementation, pp. 101–106. Kluwer, Dordrecht, The Netherlands.

Lassey, K.R.; Lowe, D.C.; Manning, M.R.; Waghorn, G.C. (1992). A source inventory for atmospheric methane in New Zealand and its global perspective. *Journal of Geophysical Research 97*: 3751–3765.

Lassey, K.R.; Martin, R.J.; Brailsford, G.W.; Waghorn, G.C.; Ulyatt, M.J.; Zimmerman, P.R.; Westberg, H.H.; Johnson, K.A. (1995). Ruminant methane measurements: Preliminary trials. *NIWA Science & Technology Series No. 22*. 12p.

Lassey, K.R.; Ulyatt, M.J.; Martin, R.J.; Walker, C.F.; Shelton, I.D. (1997). Methane emissions measured directly from grazing livestock in New Zealand. *Atmospheric Environment 31*: 2905–2914.

Lassey, K.R.; Walker, C.F.; McMillan, A.M.S.; Ulyatt, M.J. (2001). On the performance of SF_6 permeation tubes used in determining methane emission rates from grazing livestock. *Chemosphere — Global Change Science 3*: 367–376.

Lassey, K.R.; Pinares-Patiño, C.S.; Ulyatt, M.J. (2002). Methane emission by grazing livestock: Some findings on emission determinants. *In*: van Ham, J.; Baede, A.P.M.; Guicherit, R.; Williams-Jacobse, J.G.F.M. (eds). Non-CO_2 Greenhouse Gases: Scientific Understanding, Control Options and Policy Aspects, pp. 95–100. Millpress, Rotterdam, The Netherlands.

Laubach, J.; Kelliher, F.M. (2004). Measuring methane emission rates of a dairy cow herd by two micrometeorological techniques. *Agricultural and Forest Meteorology 125*: 279–303.

Laubach, J.; Kelliher, F.M.; Knight, T.W.; Clark, H.; Molano, G.; Cavanagh, A. (2008). Methane emissions from beef cattle—comparison of paddock- and animal-scale measurements. *Australian Journal of Experimental Agriculture 48*: 132–137.

Lowe, D.C. (1985). Are New Zealand's sheep causing an increase in atmospheric methane? *Weather and Climate 5*: 13–18.

Martin, R.; Brailsford, G.; Lassey, K. (2007). Report on investigation by NIWA of ethane as an 'alternative tracer' for methane, 2006–07. NIWA report to AgResearch, Oct 2007.

McAllister, T.A.; Newbold, C.J. (2008). Redirecting rumen fermentation to reduce methanogenesis. *Australian Journal of Experimental Agriculture 48*: 7–13.

McAllister, T.A.; Okine, E.K.; Mathison, G.W.; Cheng, K.-J. (1996). Dietary, environmental and microbiological aspects of methane production in ruminants. *Canadian Journal of Animal Science 76*: 231–243.

McCauley, E.H.; Dziuk, H. E. (1965). Correlation of motility and gas collection from goat rumen. *American Journal of Physiology 209*: 1152–1154.

McGinn, S.M.; Beauchemin, K.A.; Iwaasa, A.D.; McAllister, T.A. (2006). Assessment of the sulphur hexafluoride (SF_6) tracer technique for measuring enteric methane emissions from cattle. *Journal of Environmental Quality 35*: 1686–1691.

McGinn, S.M.; Chen, D.; Loh, Z.; Hill, J.; Beauchemin, K.A.; Denmead, O.T. (2008). Methane emissions from feedlot cattle in Australia and Canada. *Australian Journal of Experimental Agriculture 48*: 183–185.

Mills, J.A.N.; Dijkstra, J.; Bannink, A.; Cammell, S.B.; Kebreab, E.; France, J. (2001). A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application. *Journal of Animal Science 79*: 1584–1597.

Monteny, G.-J.; Bannink, A.; Chadwick, D. (2006). Greenhouse gas abatement strategies for animal husbandry. *Agriculture, Ecosystems and Environment 112*: 163–170.

Morgavi, D.P.; Jouany J.-P.; Martin C. (2008). Changes in methane emission and rumen fermentation parameters induced by refaunation in sheep. *Australian Journal of Experimental Agriculture 48*: 69–72.

Mortola, J.P.; Lanthier, C. (2005). Breathing frequency in ruminants: a comparative analysis with non-ruminant mammals. *Respiratory Physiology & Neurobiology 145*: 265–277.

Morvan, B.; Dore, J.; Rieulesme, F.; Foucat, L.; Fonty, G.; Gouet, P. (1994). Establishment of hydrogen-utilizing bacteria in the rumen of newborn lamb. *FEMS Microbiology Letters 117*: 249–256.

Murray, B.R.; Bryant, A.M.; Leng, R.A. (1976). Rates of production of methane in the rumen and large intestine of sheep. *British Journal of Nutrition 36*: 1–14.

Newbold, C.J.; Lassalas, B.; Jouany, J.P. (1995). The importance of methanogens associated with ciliate protozoa in ruminal methane production *in vitro*. *Letters in Applied Microbiology 21*: 230–234.

Nicholson, M.J.; Evans, P.N.; Joblin, K.N. (2007). Analysis of methanogen diversity in the rumen using temporal temperature gradient gel electrophoresis: identification of uncultured methanogens. *Microbial Ecology 54*: 141–150.

Nicol, G.W.; Glover, A.L.; Prosser, J.I. (2003). Molecular analysis of methanogenic archaeal communities in managed and natural upland pasture soils. *Global Change Biology 9*: 1451–1457.

Nolan, J.V. (1999). Stoichiometry of rumen fermentation and gas production. *In:* Reyenga, P.J.; Howden, S.M. (eds). Meeting the Kyoto Target, Implications for the

Australian Livestock Industries, pp 21–28. Bureau of Rural Sciences, Kingston, Canberra, Australia.

Piccione, G.; Caola, G.; Mortola, J.P. (2004). Day/night pattern of arterial blood gases in the cow. *Respiratory Physiology & Neurobiology 140*: 33–41.

Pinares-Patiño, C.S.; Clark, H. (2008). Reliability of the sulfur hexafluoride tracer technique for methane emission measurement from individual animals: an overview. *Australian Journal of Experimental Agriculture 48*: 223–229.

Pinares-Patiño, C.; Ulyatt, M.J.; Holmes, C.W.; Barry, T.N.; Lassey, K.R. (2000). "Inter-sheep variation in methane emission, a tool for mitigation?" Presented at the Second International Conference on Methane Mitigation, Novosibirsk, Russia.

Pinares-Patiño, C.S.; Ulyatt, M.J.; Lassey, K.R.; Barry, T.N.; Holmes, C.W. (2003a). Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay. *Journal of Agricultural Science, Cambridge 140*: 205–214.

Pinares-Patiño, C.S.; Ulyatt, M.J.; Lassey, K.R.; Barry, T.N.; Holmes, C.W. (2003b). Persistence of differences between sheep in methane emission under generous grazing conditions. *Journal of Agricultural Science, Cambridge 140*: 227–233.

Pinares-Patiño, C.S.; Holmes, C.W.; Lassey, K.R.; Ulyatt, M.J. (2008a). Measurement of methane emission from sheep by the sulphur hexafluoride tracer technique and by calorimetric chamber: failure and success. *Animal 2*: 141–148.

Pinares-Patiño, C.S.; Machmüller, A.; Molano, G.; Smith, A.; Vlaming, J.B.; Clark, H. (2008b). The SF_6 tracer technique for measurements of methane emission from cattle — effect of tracer permeation rate. *Canadian Journal of Animal Science 88*: 309–320.

Pinares-Patiño, C.S.; Koolaard, J.; Clark, H.; Rochette, Y.; Jouany, J-P.; Martin, C. (2008c). Effect of SF_6 tracer permeation rate upon the calculated ruminal methane production rates using rumen head space gas composition. Proceedings of 3rd Conference on Greenhouse Gases and Animal Agriculture, Christchurch, New Zealand. Available at http://www.publish.csiro.au/?act=view_file&file_id=EAv48n2posters.pdf.

Ranilla, M.J.; Jouany, J.-P.; Morgavi, D.P. (2007). Methane production and substrate degradation by rumen microbial communities containing single protozoal species in vitro. *Letters in Applied Microbiology 45*: 675–680.

Sellke, T.; Bayarri, M.J.; Berger, J. (2001). Calibration of *P*-values for testing precise null hypotheses. *The American Statistician 55*: 62–71.

Skillman, L.C.; Evans, P.N.; Strömpl, C.; Joblin, K.N. (2006). 16S rDNA directed PCR primers and detection of methanogens in the bovine rumen. *Letters in Applied Microbiology 42*: 222–228.

Standing Committee on Agriculture. (1990). Feeding Standards for Australian Livestock — Ruminants. CSIRO Publishing, Victoria, Australia. 266 pp.

Torrent, J.; Johnson, D.E. (1994). Methane production in the large intestine of sheep. *In*: Aguilera, J.F. (ed). Energy metabolism of farm animals, pp. 391–394. EAAP Publ. 76, CSIC, Servicio de Publicaciones, Mojacar, Spain.

Ulyatt, M.J.; Lassey, K.R.; Martin, R.J.; Walker, C.F.; Shelton, I.D. (1997). Methane emission from grazing sheep and cattle. *Proceedings of the New Zealand Society of Animal Production 57*: 130–133.

Ulyatt, M.J.; Baker, S.K.; McCrabb, G.J.; Lassey, K.R. (1999). Accuracy of SF_6 tracer technology and alternatives for field measurements. *Australian Journal of Agricultural Research 50*: 1329–1334.

Ulyatt, M.J.; Lassey, K.R.; Shelton, I.D.; Walker, C.F. (2002a). Methane emission from dairy cows and wether sheep fed sub-tropical grass-dominant pastures in mid summer in New Zealand. *New Zealand Journal of Agricultural Research 45*: 227–234.

Ulyatt, M.J.; Lassey, K.R.; Shelton, I.D.; Walker, C.F. (2002b). Seasonal variation in methane emission from dairy cows and breeding ewes grazing ryegrass/white clover pasture in New Zealand. *New Zealand Journal of Agricultural Research 45*: 217–226.

Ulyatt, M.J.; Lassey, K.R.; Shelton, I.D.; Walker, C.F. (2005). Methane emission from sheep grazing four pastures in late summer in New Zealand. *New Zealand Journal of Agricultural Research 48*: 385–390.

Ushida, K.; Tokura, M.; Takenaka, A.; Itabashi, H. (1997). Ciliate protozoa and ruminal methanogenesis. *In*: Odonera, R.; Itabashi, H.; Ushida, K.; Yano, H.; Sasaki, Y. (eds). Rumen microbes and digestive physiology in ruminants, pp. 209–220. Japan Scientific Society Press. S. Karger, Basel, Tokyo.

Van Nevel, C.J.; Demeyer, D.I. (1996). Control of rumen methanogenesis. *Environmental Monitoring and Assessment 42*: 73–97.

Vlaming, J.B. (in prep.). Quantifying variation in estimated methane emission from ruminants using the SF_6 tracer technique. Ph.D. thesis, Massey University, Palmerston North, New Zealand [incomplete].

Vlaming, J.B.; Clark, H.; Lopez-Villalobos, N. (2005). The effect of SF_6 release rate, animal species and feeding conditions on estimates of methane emissions from ruminants. *Proceedings of the New Zealand Society of Animal Production 65*: 4–8.

Vlaming, J.B.; Brookes, I.M.; Hoskin, S.O.; Pinares-Patiño, C.S.; Clark, H. (2007). The possible influence of intra-ruminal sulphur hexafluoride release rates on calculated methane emissions from cattle. *Canadian Journal of Animal Science 87*: 269–275.

Wolin, M.J.A.; Miller, T.L. (1988). Microbe-microbe interactions. *In:* Hobson, P.M. (ed). The rumen microbial ecosystem, pp 343–359. Elsevier Applied Science, London, UK.

Woodward, S.L.; Waghorn, G.C.; Ulyatt, M.J.; Lassey, K.R. (2001). Early indications that feeding *Lotus* will reduce methane emissions from ruminants. *Proceedings of the New Zealand Society of Animal Production 61*: 23–26.

Woodward, S.L.; Waghorn, G.C.; Lassey, K.R.; Laboyrie, P.G. (2002). Does feeding sulla (*Hedysarum coronarium*) reduce methane emissions from dairy cows? *Proceedings of the New Zealand Society of Animal Production 62*: 227–230.

Annex: Abbreviations and Acronyms

ANCOVA	analysis of covariance
CH_4	methane
CO_2	carbon dioxide
DM	dry matter
DMI	dry matter intake
GC	gas chromatography, or gas chromatograph
GEI	gross energy intake
H_2	hydrogen
LW	live (body-)weight
MAF	NZ Ministry of Agriculture and Forestry
NIWA	National Institute of Water & Atmospheric Research Ltd
NZ	New Zealand
PR	permeation rate (of SF_6 from permeation tube)
SF_6	sulphur hexafluoride
UN	United Nations
VFA	volatile fatty acids