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Quantifying variation in estimated methane emission from ruminants using the SF_6 tracer technique

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Abstract

With the signing of the Kyoto Protocol, New Zealand must reduce its national greenhouse gas emissions. As New Zealand has a large proportion of its national emissions as methane (~31%), and methane (CH_4) has a short atmospheric lifetime, it provides a good target for mitigation strategies.

The initial aim of this research was to identify high and low CH_4-emitting cattle to assess factors that contribute to low CH_4 production. Initial studies using the SF_6 tracer technique to estimate CH_4 production could not identify consistently high and low CH_4 emitters. Research was therefore undertaken to confirm whether this was due to high variation in estimated CH_4 yields, and to quantify the within- and between-animal variation in CH_4 production when using the SF_6 technique.

This research showed considerable within- (coefficient of variation, CV = 7-10%) and between-animal (CV = 7-18%) variation in CH_4 yield (g CH_4/kg DMI) over time when using the SF_6 technique. This is larger than the within- (CV = 3%) and between-animal (CV = 10%) variation reported for calorimetry. This led to the recommendation that the SF_6 technique not be used in identifying animals for high or low CH_4 yield. A power analysis was developed based on the measured variances for the SF_6 technique. Results from this analysis provide researchers with important information on the number of animals and measurements per animal required when undertaking CH_4 experiments.

One of the sources of variation with the SF_6 technique is the SF_6 release from permeation tubes. Estimated CH_4 yield increases by approximately 8.5% when going from a release rate of 3 mg SF_6/day to a rate of 5 mg SF_6/day. Further, an *in vitro* study indicated that SF_6 release from permeation tubes is approximately 8% lower in rumen fluid than in air. While further research is required to confirm these results, they emphasise the need to allow time for the release rate to stabilise in the rumen for 4-5 days prior to undertaking measurements. It also led to the recommendation that release rates used in experiments should be within a narrow range, and balanced across experimental treatments.

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Chapter 1

Review of Literature

1.1 Introduction

The aims of this literature review are to give a basic understanding of methane production from ruminant livestock, outline implications of ruminant methane (CH_4) emissions for global warming, describe the techniques available for measuring livestock CH_4 emissions, and discuss possible ways in which emissions from livestock can be reduced. The first section considers global warming and why it occurs, followed by a section on ruminal CH_4 production and the factors affecting it. A third section is devoted to the methods available for measuring CH_4 emissions from livestock. The final section covers some of the options available for reducing CH_4 emissions from ruminant livestock.

1.2 Overview of Global Warming

1.2.1 Evidence for the Earth warming

According to Moss et al. (2000), by 2030 world temperatures will have risen between 0.5ºC and 2.5ºC, while the United Nations Framework Convention on Climate Change (UNFCCC) report an expected 1.8 to 4.0ºC warming by 2100 (IPCC, 2007). Increased mean global temperature is expected to result in a mean sea level rise of 17 to 26 cm by 2030 (Moss et al., 2000). This rise in sea level is mainly a result of thermal expansion of water in the oceans and melting Arctic and Antarctic ice.

Indications of warming include increases in atmospheric water vapour, increases in land surface (0.27ºC per decade) and ocean (0.13ºC per decade) temperatures, ice glaciers melting and shrinking in size, reduced snow coverage in the Northern Hemisphere and for most long-term records in the Southern Hemisphere, and a significant reduction in the extent of Arctic sea-ice (2.7% per decade since 1978) (Solomon et al., 2007). Increasing global surface temperature is estimated to have been approximately 0.74ºC for the 100-year period from 1906-2005 (Solomon et al., 2007).

Greenhouse gases (GHG) that contribute to global warming are present in small, but increasing concentrations in the atmosphere. These gases arise from both natural and anthropogenic (human-induced) sources, but the real concern is over the increase in GHG concentrations due to anthropogenic sources. The three main GHG and the increase in their atmospheric concentrations from pre-industrial to 2005 concentrations are carbon dioxide (CO_2; 280 to 379 parts per million, ppm), methane (CH_4; 715 to 1,774 parts per billion, ppb) and nitrous oxide (N_2O, 270 to 319 ppb). Aerosols (solid or liquid particles suspended in the air such as soil dust, sulphates, nitrates, sea salt, and carbonaceous aerosols) actually have a cooling effect (IPCC, 2001).

The UNFCCC has defined climate change as “a change in climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability” (UNFCCC, 2008). While it is accepted that the Earth is getting warmer, there is still some dispute whether this warming is due to long-term global factors, or to human contribution (Khilyuk and Chilingar, 2004).

1.2.2 Mechanisms for global warming

Warming of the Earth’s surface is caused first, by incoming radiation from the sun, and second, through absorption of outgoing radiation by molecules in the atmosphere. Incoming radiation is absorbed by gases in the atmosphere and by Earth itself. Out-going radiation is partially absorbed by GHG in the atmosphere. This radiation is then re-emitted by the gases in the atmosphere. Some of the re-emitted radiation stays within Earth’s atmosphere instead of heading out into space. Global warming is caused by extra heat being trapped and re-emitted in the Earth’s atmosphere by increasing concentrations of certain gases, including water vapour, which contributes about 14-15ºC to natural warming (Young, 2002). Increasing the concentrations of GHG will result in more of the out-going radiation being absorbed and re-emitted into Earth’s atmosphere, causing additional atmospheric warming. Concern is not focused on warming caused by natural sources of GHG, without which the earth would be too cold to support life, but on the increasing concentrations of anthropogenic (human-induced)

gases in the atmosphere that cause additional global warming and subsequently, climate change.

Each greenhouse gas (CO_2, CH_4, N_2O, and chlorofluorocarbons) absorbs and emits radiation in certain, specific bandwidths. These bandwidths are limited and different for each gas; for example, there are several absorption bands for CO_2, including one at 16 μm. In addition to absorption and re-radiation causing warming, increasing concentrations of GHG in the atmosphere cause increased opacity (IPCC, 2001). As altitude increases in the troposphere, temperature decreases, so the heat associated with the radiation that is emitted at high altitudes is less than the heat associated with emitted radiation at lower altitudes. Thus the increasing concentrations of GHG in the lower atmosphere, or troposphere, effectively trap heat in the lower atmosphere, making it warmer, but result in less heat getting to the upper atmosphere, making it cooler (Solomon et al., 2007).

1.2.3 Principal anthropogenic gases

There are many gases involved in global warming, with CO_2 being the most important anthropogenic GHG (IPCC, 2007). The six gases, or groups of gases, that have been targeted as the main GHG by the UNFCCC are CO_2, CH_4, N_2O, hydrofluorocarbons (HFC), perfluorocarbons (PFC), and sulphur hexafluoride (SF_6). Each of these gases has a different warming effect, known as its global warming potential (GWP; see Table 1.1). These GWPs provide a measure of the influence of a unit mass pulse measurement on the balance of incoming and outgoing energy in the Earth-atmosphere system, with a positive value indicating a warming effect (Solomon et al., 2007). The GWP are calculated on a weight basis relative to CO_2 over a specific time period, this typically being 100 years.

Table 1.1 Important greenhouse gases: their formulae, lifetimes and global warming potentials.

Chemical species	Formula	Lifetime (yr)[1]	100-yr GWP[2]
Carbon dioxide	CO_2	50-200[3]	1
Methane	CH_4	12	25
Nitrous oxide	N_2O	114	298
Perfluoromethane	CF_4	50,000	7,390
HFC-23	CHF_3	270	14,800
Sulphur hexafluoride	SF_6	3,200	22,800

[1] Global mean lifetime is calculated as the total atmospheric burden divided by the mean global sink of a gas in steady state.
[2] Global warming potentials expressed on a weight basis relative to CO_2 over a hundred-year timeframe.
[3]Data from IPCC, 2001, all other data from Solomon et al., 2007.

1.2.3.1 Carbon dioxide

Carbon dioxide has large natural and anthropogenic sources, as well as large sinks. Increases in atmospheric concentrations of CO_2 are due to higher levels of emissions to the atmosphere than can be removed by natural sinks. Carbon dioxide is the main anthropogenic GHG, and atmospheric concentrations have increased from about 280 ppm in 1750 AD to 379 ppm in 2005 (Solomon et al., 2007). Since 1750, increases in atmospheric CO_2 concentrations have been caused predominantly by the burning of fossil fuels (~66%) and land use change (~33%), with a small amount from cement production (IPCC, 2001; Solomon et al., 2007). Land use change is a measure of the loss of carbon from plant and soil sources due to changes in land use. This can be caused by activities such as deforestation and soil cultivation. Cement industry emissions originate from burning fossil fuels and de-carbonisation of limestone (Rehan and Nehdi, 2005). Total emissions from cement production equate to approximately 5% of total anthropogenic emissions (Humphreys and Mahasenan, 2002).

Only 50-60% of anthropogenic CO_2 emissions are removed from the atmosphere by uptake into the oceans and terrestrial biosphere (Solomon et al., 2007). However, as CO_2 can absorb radiation of only a very limited wavelength,

and most of the light of this wavelength is already being absorbed, any further warming potential due to increased atmospheric concentrations of CO_2 is restricted (Moss, 1993). While CO_2 is, and will remain, the dominant greenhouse gas in terms of global warming, other trace gases such as CH_4 and N_2O are growing in importance relative to CO_2.

1.2.3.2 Methane

Methane is released both from natural as well as anthropogenic sources. Natural sources include wetlands, wild ruminants, oceans and lakes, termites, and release from hydrates. The main anthropogenic sources are fossil fuel burning, coal mining, oil and gas drilling, rice production, ruminant livestock, landfills and waste disposal. Atmospheric concentrations of CH_4 have more than doubled from pre-industrial times (580-730 ppb) to the present (1774 ppb) (Solomon et al., 2007). However, over the last 15 years, CH_4 concentrations in the atmosphere have stabilised, so emissions now approximately equal removals. As no significant change has occurred in the removal of CH_4 from the atmosphere, and atmospheric concentrations have stabilised, it would appear that total CH_4 emissions have remained almost constant over this period (Solomon et al., 2007). Over half the CH_4 emitted into the atmosphere is anthropogenic in origin, with the main sources being ruminant livestock, rice agriculture and biomass burning (Solomon et al., 2007).

There is still a large potential for increased global warming from CH_4 (0.2°C per 1 ppm rise in atmospheric CH_4), as it can absorb light radiation in a broad spectrum of infrared light and does not compete much with other gases in its absorption range (Moss, 1993). Therefore CH_4 may play a greater role in future global warming if its atmospheric concentration increases further. Due to its high warming potential (25 times CO_2) and its short atmospheric lifetime (12 years), reducing CH_4 emissions is seen as a good option for achieving short-term reductions in global warming.

Plants can release small amounts of CH_4 (Keppler et al., 2006), which is thought to be insignificant in temperate regions, but may play a role in CH_4

emissions from tropical rainforests, although the scale and importance of these emissions are currently disputed (Lowe, 2006; Kirschbaum et al., 2006; Kelliher et al., 2006).

1.2.3.3 Nitrous Oxide

Natural sources of N_2O emissions include oceans and the soils of forests, savannas and grasslands, while anthropogenic sources include agricultural soils, urine from livestock, nylon and nitric acid production, and fossil fuel powered electricity generation (IPCC, 2001; Forster et al., 2007). Emissions of N_2O are approximately 60% from natural sources and 40% from anthropogenic sources (Solomon et al., 2007). Atmospheric concentrations have increased by 18% since pre-industrial times from 270 ppb in 1750 to 319 ppb in 2005 (Forster et al., 2007). A number of plant species can emit N_2O (Hakata et al., 2003), although the magnitude of this source does not appear to be large. According to Nevison and Holland (1997), the increased emissions of N_2O by agricultural plants are caused by increased nitrogen availability, mainly through increased fertiliser use.

1.2.3.4 Hydrofluorocarbons

Hydrofluorocarbons (HFC) have been used to replace chlorofluorocarbons and hydrochlorofluorocarbons in refrigeration, air-conditioning systems, insulating foams and metered-dose inhalers. This has led to rapidly increasing atmospheric concentrations of industrial HFC over recent years (Lindley and McCulloch, 2005; Forster et al., 2007). The HFC have atmospheric lifetimes from 1.4 to 270 years and relatively high GWP, so they are having an increasing role in global warming (Forster et al., 2007). However, large reductions of the HFC covered by the Montreal Protocol on ozone depleting gases have occurred in recent years, but due to their slow breakdown and long atmospheric lifetimes, their warming effect is reducing very slowly (Forster et al., 2007).

1.2.3.5 Perfluorocarbons

Some perfluorocarbons, such as carbon tetrafluoride (CF_4), are naturally found in very low concentrations in the atmosphere (~40 parts per trillion), while others are anthropogenic in origin (Khalil et al., 2003). Anthropogenic emissions are mainly released during aluminium manufacture, but with small and increasing quantities released from electronic chip production (Khalil et al., 2003). Research has been carried out by the aluminium industry to reduce the quantity of these gases released per tonne of aluminium produced, but this reduction is partially offset by an increase in overall production (Khalil et al., 2003). Perfluorocarbon concentrations have been increasing approximately linearly since 1960, with about 50% of the increase due to anthropogenic sources (Forster et al., 2007). Due to the long atmospheric lifetimes from 1000 to 50,000 years, and very high warming potentials, these gases make a permanent contribution to global warming (Forster et al, 2007).

1.2.3.6 Sulphur hexafluoride

Sulphur hexafluoride (SF_6) is mainly used as an insulating gas in high voltage electric equipment, as well as an inert tracer in atmospheric and oceanic studies (Forster et al., 2007). This gas is an extremely potent GHG, with a 100-year GWP 22,800 times greater than CO_2, although emissions rates are very small (Lindley & McCulloch, 2005). According to these authors, work is underway to reduce leakage of SF_6 from electric switchgear equipment, as no suitable alternative technology is available to replace SF_6 use. However, there is an approximately linear increase in atmospheric SF_6 concentrations and due to its long atmospheric lifetime, it essentially just builds up in the atmosphere (Forster et al., 2007).

1.2.4 International treaties on greenhouse gases

1.2.4.1 United Nations Framework Convention on Climate Change (UNFCCC)

As many countries are now aware of the risk of changes to Earth's climate due to global warming, the United Nations set up a framework for governments to work together to stabilise GHG concentrations in the atmosphere to prevent disastrous interference with the climate system, with this Convention being adopted in 1992 (UNFCCC, 2008). This agreement recognises that global warming and climate change are a problem, and need to be dealt with and 192 countries have now signed and ratified this convention. The main responsibilities of member countries under this convention are to gather and share information on GHG, start national strategies to address GHG emissions and adapt to their impacts, and to cooperate together to adapt to climate change (UNFCCC, 2008). When countries agree to this convention, they are agreeing to measure and take steps to limit and reduce their emissions of major GHG.

1.2.4.2 Kyoto Protocol

The UNFCCC has been recognised and agreed to as a framework, but member countries acknowledged changes were required to this basic document if faster progress was to be made on reducing GHG emissions. This led to the development of a new protocol which was drafted in Kyoto in 1997 and came into force in February 2005 (UNFCCC, 2008). Australia is the latest country to sign and ratify the Protocol, leaving the United States of America as the only major developed country that has not ratified the Protocol. The main distinction between the UNFCCC and the Kyoto Protocol is that while the UNFCCC encouraged countries to reduce their GHG emissions, the Kyoto Protocol places binding commitments on some countries.

The Kyoto Protocol distinguishes between three groups of countries. Industrialised countries that are members of the Organisation for Economic Co-

operation and Development (OECD) plus countries with economies in transition, including the Russian Federation, the Baltic States and several Central and Eastern European countries are called Annex I countries. The industrialised countries from Annex I minus the countries in transition are called Annex II. Developing countries are part of the group known as non-Annex I.

Each of these three groups have different requirements under the Kyoto Protocol. Annex I countries have an overall obligation of reducing their GHG emissions to 5% below their 1990 levels in the first commitment period of 2008 to 2012, although targets for individual countries differ (UNFCCC, 2008). Non-Annex I countries do not have extra obligations under the Kyoto Protocol besides those already stated for member countries of the UNFCCC. By ratifying the Kyoto Protocol, New Zealand has agreed to maintain its emissions of greenhouse gases during the first commitment period from 2008 to 2012 at the levels in 1990 (New Zealand Ministry for the Environment, 2007).

1.3 Greenhouse gas production in New Zealand

New Zealand has increased its annual emissions of GHG, from around 61,900 gigagrams (Gg) of CO_2 equivalent in 1990 to 77,159 Gg of CO_2 equivalent in 2005 (New Zealand Ministry for the Environment, 2007). New Zealand's GHG emissions are quite distinctive for a developed country with 48.5% of emissions originating from agriculture compared with an average of 12% for other Annex 1 countries (New Zealand Ministry for the Environment, 2007). These emissions consist predominantly of CH_4 from ruminant livestock and N_2O from animal excreta and nitrogenous fertiliser use (New Zealand Ministry for the Environment, 2007). Table 1.2 shows the breakdown of CO_2 equivalent GHG emissions for each gas for 1990 and 2005 and the percentage change over this time.

Table 1.2 Breakdown of New Zealand's greenhouse gas emissions, in CO_2 equivalents, for 1990 and 2005 for individual greenhouse gases and the percentage change between the two years.

Greenhouse gas emissions	Gigagrams CO_2 equivalent		Change from
	1990	2005	1990 (%)
CO_2 (without LULUCF)	25,462	35,880	41
CH_4	25,493	27,175	7
N_2O	10,417	13,260	27
HFC	0	742	NA
PFC	516	81	-84
SF_6	12	22	77
Emissions (without LULUCF)	61,900	77,159	25
LULUCF	18,981	24,501	29
Total emissions including LULUCF	42,919	52,658	23

LULUCF is the category: Land use, land use change and forestry and is a carbon sink. Data from New Zealand Ministry for the Environment, 2007).

It is clear from Table 1.2 that the emissions predominantly comprise CO_2, CH_4 and N_2O, while emissions of HFC, PFC, and SF_6 are low. It can be seen from Table 1.2 that while emissions of CO_2 and CH_4 were approximately equal in 1990, there has been a much larger increase in CO_2 emissions relative to CH_4 in 2005. This change has been attributed to large increases in CO_2 emissions from the energy sector compared to smaller increases in CH_4 from agriculture (New Zealand Ministry for the Environment, 2007). While total emissions increased (by 25%) from 1990 to 2005, so did the carbon sinks (by 29%). These carbon sinks accounted for approximately 32% of New Zealand's total GHG emissions in 2005 (New Zealand Ministry for the Environment, 2007).

Agriculture is currently (2005 data) responsible for 48.5% of the total national emissions; with ruminant CH_4 producing 64% and nitrous oxide 34% of the emissions from the agriculture sector (New Zealand Ministry for the Environment, 2007). Ruminant CH_4 comprises 31% of the total national CO_2-equivalent emissions, making it an important target for reducing national emissions.

1.4 Ruminant methane production

Ruminants rely on the process of microbial fermentation to ferment the plant material they ingest. They absorb the end-products of this fermentation process to meet their energy requirements and partially meet their protein requirements by digesting microbes flowing from the rumen. Symbiotic microbes in the animal's reticulo-rumen and hindgut hydrolyse plant polysaccharides to simple sugars, with the end-products of fermentation being acetic, propionic and butyric acids, CH_4 and CO_2 (McDonald et al., 1995; Hobson, 1997). This fermentation, along with some of the predominant microbial species is shown in Figure 1.1.

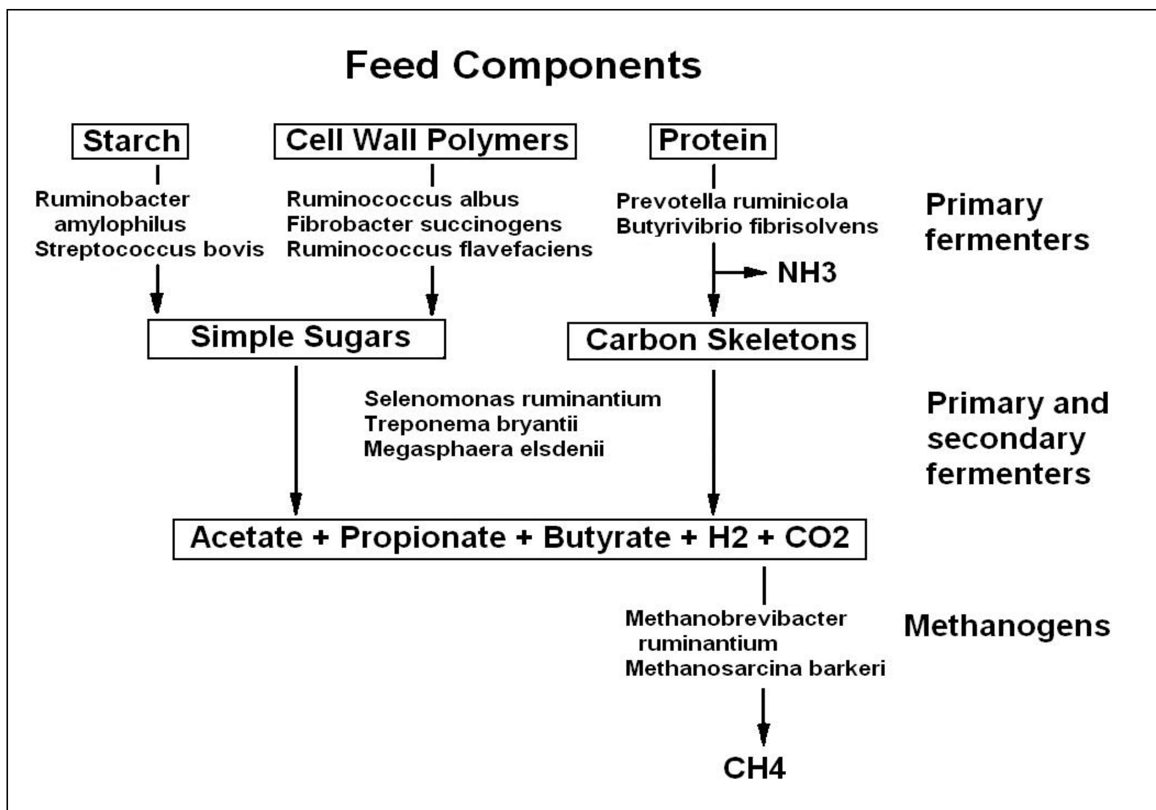

Figure 1.1 Fermentation of plant matter by rumen microbes, showing some of the major rumen bacteria involved in fermentation and methanogens which remove hydrogen gas by conversion to methane. From McAllister et al. (1996).

One of the products of microbial activity is CH_4, which is produced by microbes from the group Archaea, commonly called methanogens. Carbon dioxide and hydrogen (H_2) are combined to form CH_4 and water (H_2O) in the following equation: $CO_2 + 4\ H_2 = CH_4 + 2\ H_2O$ (Moss et al., 2000). The animal cannot

utilise CH_4 and it is eructated or exhaled with some of the CO_2 and other rumen gases that are produced in the reticulo-rumen. Emissions of CH_4 from feed can account for 2 to 12% of an animal's gross energy intake (Johnson et al., 1993).

1.4.1 Site of methane production and release

While most CH_4 production from ruminants occurs in the rumen, there is also some CH_4 production in the lower digestive tract (hindgut) of ruminants (Immig, 1996). In an isotope experiment conducted by Murray et al. (1976), sheep fed lucerne chaff produced 13% of their CH_4 in the lower digestive tract, while the study of Torrent and Johnson (1994) reported CH_4 produced in the large intestine accounted for 8-13% of total daily CH_4 production of sheep fed either cracked corn or grain based rations. From these few studies, it appears that CH_4 production is predominantly ruminal (83-94%), with smaller quantities produced in the hindgut (6-13%).

Partitioning of the routes of CH_4 release from eructation, breath and flatus has not been well studied. Murray et al. (1976) reported the routes of release were eructation (83%), breath (16%) and through the anus (1%). The latter author showed that 89% of the CH_4 produced in the hindgut was absorbed into the blood and then released into the lungs and exhaled in the breath. In another study with ewes fed chopped lucerne hay (Kempton et al., 1976), eructation and exhalation accounted for 98% of CH_4 excretion while flatus accounted for approximately 2%. These studies indicate that almost all the CH_4 produced is excreted via eructation and exhalation. As CH_4 is predominantly produced in the rumen, this will be the focus for the rest of this chapter.

1.4.2 Microbiology

There are many microbial species in the rumen, with over 200 species of bacteria, 100 species of protozoa and 8 species of fungi at concentrations of 10^9 - 10^{10} bacteria, 10^5 - 10^6 protozoa and 10^3 - 10^4 fungi per ml of rumen fluid (McAllister and Cheng, 1996). Examples of bacteria are shown in Figure 1.1,

while fungi cultured from grazing ruminants include *Neocallimastix frontalis* and *Piromyces communis* (Joblin et al., 2002).

Colonisation of the rumen by H_2-utilising microbes begins while animals are pre-ruminants consuming a milk diet. Acetogens colonise the rumen in the first 24 hours after birth (Morvan et al., 1994), while methanogens reach concentrations of approximately 10^4 per gram of rumen contents by 1-3 days after birth (Skillman et al., 2004). The concentration of methanogens increases exponentially to 10^8-10^9 per gram of rumen content by 3 weeks of age (Skillman et al., 2004). As methanogens colonise the rumen, acetogen concentrations diminish (Morvan et al., 1994).

There is great diversity in methanogen populations, with increasing numbers of new methanogens being discovered with the advent of molecular techniques (Jarvis et al., 2000; Wright et al., 2004a; Skillman et al., 2006; Wright et al., 2006; Nicholson et al., 2007). Cattle and sheep grazing fresh pasture may have greater methanogen diversity than animals fed preserved forage or grain-based diets (Jarvis et al., 2000; Wright et al., 2004a). Recent studies identified the presence of methanogens belonging to *Methanobrevibacter*, *Methanosphaera*, *Methanomicrococcus* and a large group of uncultured methanogens (Nicholson et al., 2007). The latter authors suggested that the uncultured methanogens along with *Methanobrevibacter* species may be the predominant methanogens in the rumen from cattle and sheep grazing ryegrass/white clover pastures.

1.4.3 Digestion and metabolism

The breakdown and digestion of plant material provides microbes with metabolic products that can be used to provide their energy requirements. Most rumen microorganisms use the citric acid cycle to oxidise sugar units to pyruvate, but the last step does not occur in anaerobic environments, meaning less energy is obtained relative to aerobic oxidation. During the citric acid cycle, electrons, otherwise referred to as liberated hydrogen (H_2), are taken up by the intracellular electron carrier coenzyme NAD, which is reduced to NADH (Immig, 1996). Electrons must then be transferred to an acceptor such as CO_2,

sulphate, nitrate or fumarate to regenerate the NAD and complete the fermentation of sugars (Moss et al., 2000). Fermenting microbes produce H_2 and methanogens utilise this hydrogen to reduce CO_2 to CH_4 through a series of biochemical reactions (see Figure 1.2).

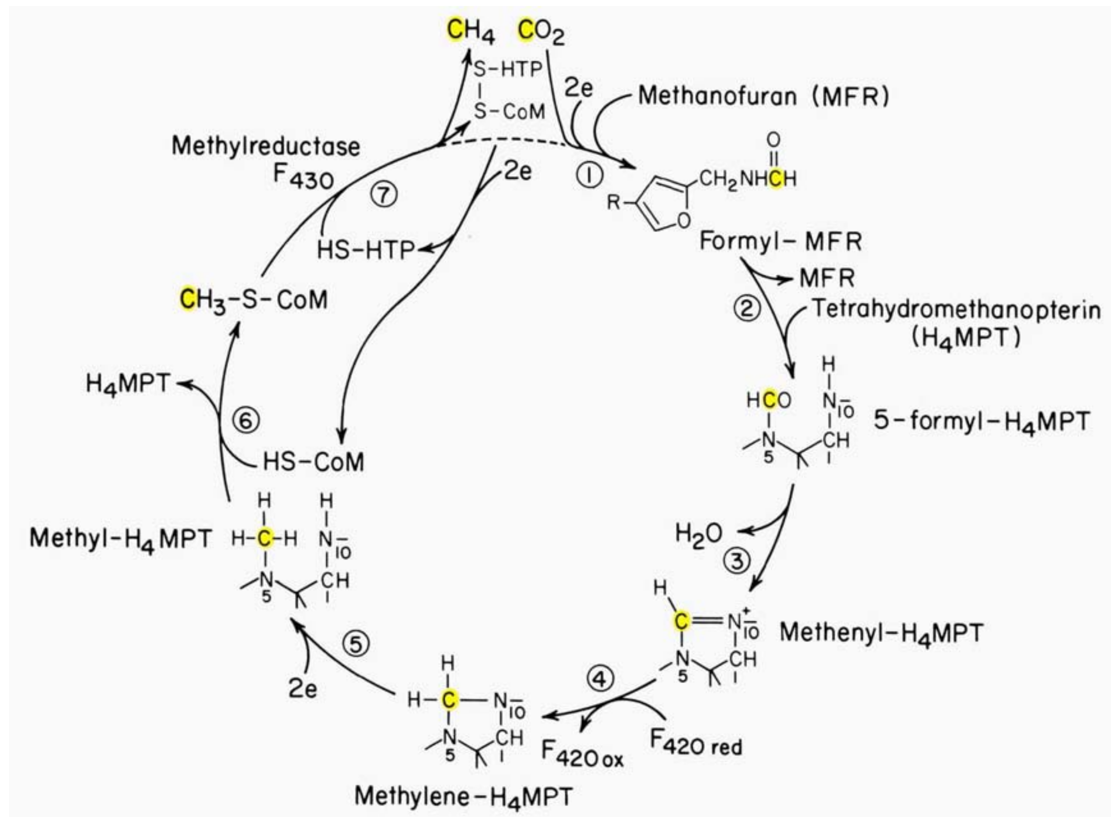

Figure 1.2 Proposed cycle for the reduction of CO_2 to CH_4. The highlighted C_1 unit is transferred through the cycle bound to coenzymes, with only the portion of the molecule bound to the C_1 unit shown for methanofuran and tetrahydromethanopterin. From Rouvière and Wolfe (1988).

This process of H_2 transfer from one species to another is known as inter-species hydrogen transfer and results in increased carbon turn-over, greater production of oxidised end-products, increased growth of organisms and maximal energy yield per gram of fermented organic matter (Rogers and Withman, 1991, cited by Immig, 1996).

The sole source of energy for methanogens comes from the reduction of CO_2 to CH_4 using either H_2, formate, methanol, methylamines or acetate (Rouvière and Wolfe, 1988) through four reductive intermediates: formyl-methanofuran, methenyl-tetrahydromethanopterin, methylenyl-tetrahydromethanopterin, and

methyl-tetrahydromethanopterin (McAllister et al., 1996). Methanogens possess three coenzymes which have not been found in other microorganisms: coenzyme 420 which is involved in electron transfer, coenzyme M involved in methyl transfer, and factor B involved in the enzymatic formation of CH_4 from methyl coenzyme M (Boadi et al., 2004). These specific enzymes enable methanogens to produce CH_4. The H_2 transfer from fermenting microbes to methanogens is aided by the hydrophobic property of methanogens, prompting them to stick to feed particles and to the surface of protozoa (Boadi et al., 2004), therefore allowing greater H_2 uptake and CH_4 production. This helps prevent any increase in the ruminal H_2 concentration and reduced nucleotide accumulation (e.g., NADH) and results in greater fermentation of plant material by bacteria and fungi (5-28%) than if methanogens were not present (McAllister et al., 1996).

Increased rumen retention times and lower feed intakes on long, fibrous feeds are a consequence of slower microbial degradation of structural polysaccharides in plant fibre (Hobson, 1997). This slower rate of digestion and increased ruminal retention time results in reduced feed intake and greater CH_4 production per kg dry matter intake (kg DMI) (McAllister et al., 1996). This is supported by work showing that CH_4 loss was reduced by 38% and 64% when feeding diets containing either barley or corn grain, respectively, compared with feeding barley or corn silage diets to growing beef cattle (Beauchemin and McGinn, 2005). These diets contained a very high proportion (81%) of grain to achieve this reduction in CH_4 production. Increasing fibre levels in the diet can result in higher acetate to propionate ratios and higher CH_4 production (Johnson and Johnson, 1995).

The production of propionate from pyruvate requires hydrogen, while production of acetate releases hydrogen, which is subsequently utilised by methanogens to produce CH_4 (Moss et al., 2000). Methane production is inversely related to the molar production of propionate and positively related to the acetate to propionate ratio (Johnson and Johnson, 1995). As fermentation of readily fermented carbohydrate and low pH both result in a lower acetate to propionate

ratio, and both occur with a high grain diet, less CH_4/kg DMI (CH_4 yield) is produced when feeding high levels of grain.

1.4.4 Level of feed intake

Blaxter and Clapperton (1965) demonstrated that absolute daily CH_4 production (g CH_4/day) increases with increasing intake on the diets they studied, but at a declining rate. The latter authors derived the following equation to describe this relationship:

$$CH_4 = 1.30 + 0.112*D + L(2.37-0.050D),$$

where D is the digestibility of the diet at the maintenance level of feeding and L is the level of feeding.

While the absolute amount of CH_4 produced increases with increasing feeding level, the increase is at an ever-decreasing rate in both sheep (Blaxter and Clapperton, 1965) and cattle (Coppock et al., 1964; Beauchemin and McGinn, 2006). This reduction in CH_4 production is reported to be between 0.77% to 1.6% per level of maintenance gross energy intake (Johnson and Johnson, 1995; Beauchemin and McGinn, 2006). The reduction in CH_4 production with increasing level of intake is associated with a decreased rumen residence time and decreased ruminal fermentation (Hobson, 1997; Mathison et al., 1998).

The studies mentioned in the previous paragraph were primarily undertaken with dried, conserved and\or grain diets. In contrast to this, a study with fresh forage showed no effect of level of feed intake on CH_4 yield (Molano and Clark, 2008), while a study using a mix of silage and concentrate also showed no difference in CH_4 yield with increasing intake – 20.5 versus 21.1 g CH_4/kg DMI for *ad libitum* and 65% *ad libitum*, respectively (Beauchemin and McGinn, 2006).

1.4.5 Concentrate versus forage

The forage to concentrate ratio of a diet may have an impact on rumen fermentation, with a higher grain proportion in the diet resulting in a decline in the acetate to propionate ratio and lower CH_4 production (Moss et al., 2000; Monteny et al., 2006). Energy loss as CH_4 is typically 6-7% of the gross energy intake (GEI) on forage-based diets, while very high grain diets (>90% grain) fed *ad libitum* result in CH_4 production of 2-3% of GEI (Johnson and Johnson, 1995). Van Soest (1982) indicated that a high grain diet and/or the addition of soluble carbohydrates results in an increased ruminal passage rate and lowered ruminal pH, as well as increased propionate production. These factors may combine to make methanogens less competitive in the rumen. Moss et al. (2000) estimated that increasing the level of dietary non-structural carbohydrate by 25% would result in a reduction in CH_4 production of approximately 20%.

Although very high levels of grain supplementation appear to reduce losses of CH_4, lower levels of supplementation do not seem to give pro-rata reductions. Boadi et al. (2002b) reported that supplementing 4 kg of grain to grazing steers (~30% of total DMI) had no effect on either total CH_4 production (L/day) or CH_4 per unit intake. This is supported by further work where barley silage was supplemented with a grain-based concentrate (70% barley silage: 30% grain concentrate to 30% silage: 70% corn concentrate), with no significant effect on CH_4 conversion rate (21.6 versus 19.9 g CH_4/kg DMI) (Beauchemin and McGinn, 2006). While theory would suggest that there should be a gradual change with increasing proportions of grain-based concentrate, this may not be the case in reality.

1.4.6 Animal variation

Methane production from individual animals may vary over time, even when animals are fed a constant amount of the same quality feed each day. Within-animal variation in absolute CH_4 production from day-to-day in sheep and cattle has been reported to be approximately 7% (coefficient of variation, CV) when animals were fed a constant amount of consistent quality feed (Blaxter and

Clapperton, 1965). Boadi and Wittenberg (2002) reported that the CV for day-to-day variation in CH_4 production was approximately 27% whether animals were fed *ad libitum* or on a restricted diet, while Grainger et al. (2007) reported a CV of 19% in daily CH_4 production, but this dropped to 6% when differences in intake were accounted for.

In addition to this within-animal variation, differences between animals have also been reported (Blaxter and Clapperton, 1965; Johnson et al., 1994a; Lassey et al., 1997; Boadi et al., 2002a, b; Mbanzamihigo et al., 2002). Calorimetry studies have reported between-animal differences (CV) in daily CH_4 production of 7-8% and 11.7% when animals were fed a constant diet (Blaxter and Clapperton, 1965; Boadi et al., 2002a) and 17.8% for lactating dairy cattle fed *ad libitum* (Grainger et al., 2007). In grazing studies using the SF_6 tracer technique, Lassey et al. (1997) and McNaughton et al. (2005) obtained between-animal CV for absolute daily CH_4 production of 11.5%, and 25%, respectively, when animals were fed *ad libitum* and intakes estimated using energy requirement calculations.

As many of the studies mentioned in the last two paragraphs did not have constant daily intakes, there are confounding factors that could influence the measured variation in both CH_4 production and CH_4 yield. As the level of intake can affect the CH_4 yield, it is important to have a constant daily feed intake of a consistent quality throughout the experiment. However, these studies do indicate that substantial variation can occur both between- and within-animals in daily CH_4 production. It has been suggested that this variation could be attributed to digestive tract characteristics (Boadi and Wittenberg, 2002). There are a number of variables that could influence this variation in digestive parameters, including type of feed, level and frequency of feeding, rate of intake, saliva production and composition, rumen pH, rumen capacity, and retention time of fluid and particulate matter in the rumen.

Variation in feeding behaviour or salivation characteristics could have an effect on the microbial balance in the rumen, thus affecting CH_4 production. Individual cattle appear to differ from each other in their eating and ruminating behaviour.

For instance, a study with 12 lactating cows reported a coefficient of variation in their eating time of 17% (mean 301 min/day), 16% for ruminating time (mean 457 min/day) and 24% for their water intake (mean 78 L/day) (Dado and Allen, 1994). Further, grazing animals may differ in the diet eaten by selectively grazing certain parts of the sward (Brand, 2000). Salivation rates also differ, with typical quantities of saliva produced per day of 150 litres in cattle and 10 litres in sheep (McDonald et al., 1995), although estimates vary from 38 to 190 L/day for non-lactating dairy cattle (Jacques et al., 1989). Saliva is essentially a bicarbonate-phosphate buffer with a pH around 8, and the large volumes secreted provide an aqueous medium for the rumen organisms and help to keep the rumen contents at near neutrality (Hobson, 1997). A rapid saliva secretion rate also helps to flush the rumen contents onwards to the lower digestive tract (Kay, 1987).

Feeding rate, drinking rate, and quantity of saliva produced will affect the time spent in the rumen of both fluid and particulate matter (Hegarty, 2004). This is supported by the results of Pinares-Patiño et al. (2003a), where CH_4 yield (%GEI) was negatively correlated to the particulate outflow rate and the buffering capacity of rumen fluid, but positively correlated with the quantity of rumen organic matter and rumen fill. The latter author reported that the rumen particulate outflow rate accounted for approximately 57% of the between-sheep variation.

These factors may all contribute to variance between animals in their CH_4 yield. However, as long as these characteristics are at least partially under genetic control, it may be possible to screen animals and select for animals with lower CH_4 yields.

1.4.7 Methane from excreta

It is known that CH_4 is produced both in the rumen and in the hindgut (see section 1.4.1). It is therefore conceivable that microbes, including methanogens, would be present in voided faecal material, and could continue fermentation of carbon products under certain environmental conditions e.g., warm, anaerobic

and moist. Research undertaken in temperate grazing conditions in England showed that CH_4 released by dung voided on pasture amounted to approximately 0.2% of that produced in the rumen, or at a rate of 0.603 g/cow/day (Jarvis et al., 1995). Other research in Australia with dung on pasture reported maximal CH_4 production rates of 2.74 g/cow/day during the warm summer months (Williams, 1993). In a review of CH_4 measurements from dung in New Zealand, Saggar et al. (2004) reported a range of emissions from 0.167 to 3.236 g CH_4/kg dung C. These studies show that while some CH_4 is produced from dung, this is less than 3% of that produced in the rumen (Williams, 1993) and only storage of faecal material in anaerobic lagoons is likely to produce significant CH_4 emissions from livestock manure (Lodman et al., 1993).

1.5 Measuring methane emissions from ruminant livestock

As CH_4 is a major greenhouse gas and a by-product of anaerobic fermentation resulting in a loss of energy to the animal, a considerable amount of research effort has been expended on finding ways to accurately measure CH_4 losses from ruminant livestock. Direct and indirect measurement of CH_4 emissions from ruminant livestock, both for individual animals, as well as for groups of animals, can be carried out. The main techniques for undertaking CH_4 measurements will be discussed, as well as some of their advantages and disadvantages.

1.5.1 Individual animal techniques

1.5.1.1 Enclosure techniques

The big advantage of enclosure techniques is that CH_4 production from livestock can be directly measured (Blaxter, 1967). Respiration chambers, or calorimeters, provide the most accurate and reliable measurements of CH_4 production, and are considered the ‘gold’ standard for CH_4 measurements. Closed circuit calorimetry provides the most accurate measure of CH_4 emitted over an experimental period of approximately 3 days. Indirect calorimetry

chambers or hoods can both provide an almost continuous measure (often every 4 minutes) of CH_4 emission throughout the day, while face masks provide measures of CH_4 emission over very short periods (1 hour). Another big advantage of enclosure systems is that individual animal feed intakes can be measured, providing accurate values for CH_4 emission per kg DMI. Hoods and face masks have the advantage that they are much cheaper to construct than full calorimeters (Kempton et al., 1976).

One of the important drawbacks of enclosure systems is their lack of applicability to grazing systems, such as in New Zealand (O'Hara et al., 2003). Calorimeters are also very expensive to set up and maintain, especially the closed-circuit calorimeters. Closed-circuit calorimeters are also difficult to operate (Blaxter, 1967), so most animal calorimeters constructed and operated more recently are the open-circuit type (O'Hara et al., 2003). Another drawback of calorimetry measurements is that they are labour intensive, with feed delivered to the animal and the requirement to remove faecal and urinary material and clean the calorimeter daily. Limited numbers of animals can be measured with enclosure techniques, and only for 3 to 4 days before animals need to be rested. Hoods and masks face the same disadvantages of lack of applicability to grazing, small numbers of animals, and short measurement periods, but have the added disadvantage of not measuring CH_4 released via the rectum.

Closed-circuit calorimetry was commonly used in early metabolic experiments (Blaxter and Wainman, 1961, 1964) as well as some more recent experiments (Faichney et al., 1999). Blaxter and Clapperton (1965) used data from numerous closed-circuit calorimetry experiments to develop their prediction equations for CH_4 production. In this system, an animal is placed inside an airtight chamber, with air circulated within the system (Blaxter, 1967). Air conditioning is used to maintain temperature and humidity inside the chamber. Air from within the chamber is passed through absorbents to collect CO_2 and water (H_2O), while oxygen (O_2) is released into the chamber as the animal uses it. Methane is accumulated inside the chamber over the measurement period. At the beginning and end of each experimental period, gas samples are taken

for CH_4 analysis. The barometric pressure, volume of the chamber, estimated volume of the animal, air temperature, and humidity are all measured at the beginning and end of the period. Production of CH_4 is calculated from the difference in measured concentrations in the pre- and post-experimental samples and the volume of the chamber minus the estimated volume of the animal.

Many respiration measurements undertaken with ruminants are now made with open-circuit chambers (O'Hara et al., 2003). This method involves passing outside air through a chamber housing an animal. Air flow and concentrations of O_2, CO_2 and CH_4 are measured in the air entering and exiting the chamber (Blaxter, 1967; Blümmel et al., 2005). Using the airflow through the chamber and difference in CH_4 concentrations entering and exiting the chamber, the CH_4 production can be calculated.

A ventilated hood works in the same way as an open-circuit calorimeter, except only the front part of the animal is enclosed. The hood around the animal's head must be airtight, and a type of flexible seal such as a nylon curtain (Takahashi et al., 1999) must be used around the animal's neck. A design used by Boadi et al. (2002a) allowed a housed, stall-tied animal to stand or lie down, as well as have access to feed and water. Their hood was based on that of McLean and Tobin (1987), who described the design and operation of a hood system. Gas analyses are conducted in much the same way as with calorimeters.

Another system based on the same principles as calorimetry is the facemask. This system works by fitting a mask over the animal's head for a limited period of time, typically three one-hour measurements per day, instead of using a ventilated hood (Kempton et al., 1976). Analyses are done in the same way as for the open-circuit calorimetry measurements. Animals can not eat or drink while measurements are taken. This short duration could give quite variable results, due to the within-day variation in CH_4 production (Johnson and Johnson, 1995).

1.5.1.2 Tracer techniques

Tracer techniques are useful because they do not require specialised chambers for the animals, as animals can be measured in standard animal stalls or crates, or measured in a field. The ability to use some of these techniques under grazing conditions is a big advantage in countries that employ grazing management systems (O'Hara et al., 2003). Tracer methods can be cheaper to operate than enclosure methods, but cost varies depending on the trace species and its sample concentration (Neftel et al., 2005). Intake can be measured if animals are housed in individual stalls. The ability to measure larger numbers of animals simultaneously and, therefore, to have greater replication can be a big advantage, especially when comparing sample treatments.

As tracer methods estimate, rather than measure CH_4 emission, they do not tend to be as accurate as calorimetry measurements (Boadi et al., 2002a). Tracer methods require the use of larger numbers of animals per experiment and greater replication, which can be both an advantage and disadvantage. The low concentration of sample gases can be a drawback with some of these systems due to the cost of equipment needed to accurately measure gases at trace concentrations (Neftel et al., 2005). A further challenge is to procure accurate trace gas standards in the concentration range being measured. Intake can only be estimated if animals are housed in groups or are grazing in paddocks, which mean greater inaccuracies occur in estimates of CH_4 yield due to uncertainty in individual dry matter intakes. Most tracer measurements do not include CH_4 released from the anus, so only give an estimate of the CH_4 released from the mouth and nose, although the study of Murray et al. (1976) showed that CH_4 lost in flatus was only 1% of total CH_4 emissions.

Isotopic techniques for measuring CH_4 production require the use of ^{14}C- or ^{3}H-labelled CH_4 (Murray et al., 1976). Labelled CH_4 can be continuously infused into the rumen at a known rate or dose infused, so animals must be tethered in stalls. Samples can be taken directly from the rumen, or samples of breath and/or flatus collected using calorimetry, hoods or masks. Specific activity of the

labelled CH_4 must be measured to calculate the CH_4 production. Animals must be rumen cannulated to allow re-entry into the rumen for infusing the labelled CH_4 and for gas sample collection and expensive analytical equipment is required to measure the specific activity of the labelled isotope.

The most common tracer technique used to estimate CH_4 production uses sulphur hexafluoride (SF_6) gas as a tracer for CH_4 (Johnson et al., 1994a). This technique relies on a steady release of SF_6 from a bolus administered into the rumen of an animal. Gas samples are collected from around the nose and mouth of the animal via a harness and evacuated canister and these samples are then analysed using a gas chromatograph (Lassey et al., 1997). The release rate of SF_6 from the bolus and the ratio of SF_6 to CH_4 in the breath sample are used to calculate the CH_4 emission from each animal. This method of estimating CH_4 emissions has the advantage that individual grazing animals can be measured, as well as groups of animals simultaneously. It is also a relatively cheap method for measuring CH_4 emissions, although the cost of purchasing a gas chromatograph capable of measuring SF_6 in parts per trillion is considerable.

Several other gases have been suggested as possible tracer species using similar technology and principles as the SF_6 tracer technique. Possible tracers include ethane, propane and butane, with the most promising gases being ethane and stable isotopes of CH_4 (Machmüller & Hegarty, 2005).

Ethane (C_2H_6) relies on the same principles as the SF_6 technique. Theoretically, C_2H_6 should be a good tracer for CH_4 as they are both short-chain alkanes with similar physical and chemical properties. Ethane is either dose-injected or infused directly into the rumen over a period of time (1 hour) and samples are extracted directly from the rumen for analysis by gas chromatography (Moate et al., 1997). Measurements made in this way give an instantaneous value of CH_4 production calculated from the rumen headspace and fractional clearance rate of CH_4, but do not account for gas dissolved in rumen fluid or trapped in gas bubbles, so require a compartmental model for data analysis (Moate et al., 1997). As the tracer needs to be injected or infused directly into the rumen, this

method is not suitable to measure CH_4 emissions from grazing animals, or to measure large groups of animals as claimed by Moate et al. (1997), unless ethane could be administered in a slow-release bolus and measured similarly to the SF_6 tracer technique.

1.5.2 Group techniques

Techniques are available to measure the average CH_4 emissions from groups of animals. These techniques have the advantage that they are less intrusive than either tracer or enclosure techniques. Micrometeorological techniques do not need to interfere with animals at all. However, they have the disadvantage that CH_4 measurements are not available for individual animals. Micrometeorological techniques are highly dependent on the weather, particularly stable wind strength and direction, which limits the utility of these techniques. Lack of accuracy means these techniques are not suitable to detect small differences between groups or treatments (O'Hara et al., 2003).

Measurements of CH_4 production by sheep have been undertaken using commercially available polythene-clad tunnels (polytunnels) (Lockyer and Jarvis, 1995; Lockyer, 1997). Air is sucked through the polytunnel and concentrations of CH_4 are measured in air entering and leaving the polytunnel. The polytunnel is maintained at a slight negative pressure, so any leaks in the tunnel result in background air entering the tunnel. Airflow, humidity and temperature are measured inside the polytunnel, with airflow controlled at rates of up to 0.9 m^3/s. Samples of the air flowing into and out from the tunnel are automatically collected every two minutes and analysed using a flame ionisation detector on a gas chromatograph. Methane production can be estimated using the airflow through the tunnel, and the concentrations of air entering and exiting the tunnel. This system gives a continuous measure of CH_4 production over one to several days; although if used with grazing animals, CH_4 production declines over time due to declining pasture availability and animal intakes (Lockyer and Jarvis, 1995). Daily estimates of CH_4 emissions using polytunnels appear to be lower than other estimates (Lockyer & Jarvis, 1995).

Methane emissions from grazing animals can be measured by several micrometeorological techniques, including flux-gradient (Judd et al., 1999; Griffith et al., 2002; Laubach and Kelliher, 2004), nocturnal boundary layer (Griffith et al., 2002; Harvey et al., 2002) and mass-balance (Leuning et al., 1999; Harvey et al 2002). These systems operate at the field level or larger, with air samples collected both upwind and downwind of the animals to measure CH_4 emissions. These techniques require air sampling at varying heights into the atmosphere using high towers, balloons, or aircraft to account for all of the CH_4 being emitted from the animals. Figure 1.3 shows the basic design of a mass-balance system. The difference in CH_4 concentration and the wind speed can be used to calculate the CH_4 flux.

Both mass-balance and flux-gradient techniques can use masts (Harper et al., 1999; Griffith et al., 2002; Laubach and Kelliher, 2004), while boundary layer techniques require samples to be taken at much greater heights and use tethered balloons (Harvey et al., 2002). Flux-gradient and boundary layer techniques require measurement or knowledge of wind strength and direction, temperature, gas diffusivity and humidity to calculate the CH_4 emissions. The nocturnal boundary layer technique, which is a boundary layer technique where measurements are undertaken at night, has the advantage that gas concentrations in the atmosphere generally increase at night (Griffith et al., 2002).

Harvey et al. (2002) reported the nocturnal boundary-layer technique measured CH_4 emissions to within 20% of the estimates from the SF_6 tracer technique, while Laubach & Kelliher (2004) reported two flux gradient techniques underestimated daily CH_4 production by 8-10% compared with SF_6 estimates. A more recent study showed that flux gradient, mass budget and backward-Lagrangian stochastic model measures over-estimated CH_4 emissions by 3%, 13% and 30-50% respectively (Laubach et al., 2008). However, due to the consistency of the backward-Lagrangian stochastic model, a correction could be made to this method resulting in reasonable agreement with the SF_6 estimates. Therefore, the accuracy of these techniques needs improvement before they can be reliably used for comparative CH_4 measurements.

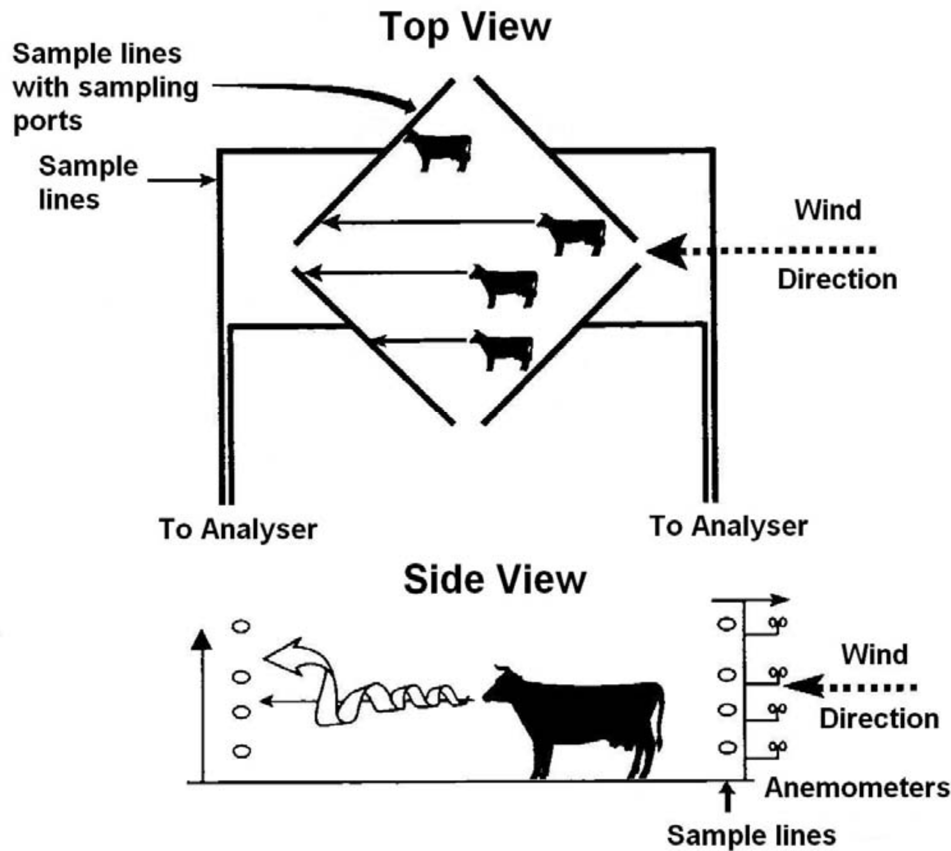

Figure 1.3 Basic design of a system for mass balance measurement of methane fluxes from grazing ruminants. Adapted from Harper et al. (1999).

1.5.3 Prediction equations

Prediction equations can be used to estimate CH_4 emissions from feed characteristics (see Blaxter and Clapperton, 1965; Moe and Tyrrell, 1979; Wilkerson et al., 1995; Pelchen and Peters, 1998) or metabolic products (see Czerkawski, 1969; Faichney et al., 1999; Blümmel et al., 2005). These will not be discussed further as they are simply synthesising the results of actual measurements of CH_4 production and the focus of this thesis is on techniques for measuring CH_4 emissions.

1.6 Estimating intake from grazing ruminants

Individual animal intakes need to be known to evaluate strategies for CH_4 reduction or mitigation. First, if CH_4 mitigation is going to be effective it needs to reduce the quantity of CH_4 produced per kg DMI. This is important, as the aim is

to reduce the quantity of CH_4 produced/kg DMI, not to reduce CH_4 production by reducing animal intakes and thus animal performance. Second, the New Zealand national CH_4 inventory uses CH_4 yield (g CH_4/kg DMI) when calculating emissions from agriculture, making it imperative to have good intake measurements. Only if the mitigation strategy reduces the CH_4 yield will it result in a reduction in CH_4 in the national inventory. If greater efficiency per unit product (g CH_4/unit meat or milk) is achieved, farmers will tend to increase production to continue to maximise use of their raw material – forage. This also stresses the importance of not reducing total intake, but reducing the amount of CH_4 produced per kg of feed eaten.

When animals are individually housed it is relatively easy to measure their feed intake, but this is not possible with grazing animals. While no method has yet been developed to directly measure grazing intake, several methods have been developed to estimate it, including the pasture mass balance technique (Ulyatt et al., 1974), total faecal collection (Ulyatt et al., 1974, Pinares-Patiño et al., 2003b), and marker techniques such as chromic oxide (Carruthers and Bryant, 1983) and n-alkanes (Dove and Mayes, 1991).

As the pasture mass balance technique gives an estimated average intake for a group of animals, not for individual animals, it will not be mentioned further. The total faecal collection method can be difficult with grazing animals, so is best used with housed animals. Marker techniques requiring only once or twice daily faecal grab sampling would be the least disruptive to normal grazing behaviour. The two marker techniques, chromic oxide and n-alkanes, can be used to estimate individual intakes from grazing animals and will be briefly outlined, along with some of their limitations.

Both the total faecal collection and chromium marker techniques rely on good *in vitro* digestibility estimates of the feed eaten. Digestibility can be measured for individual animals when they are housed, but can only be estimated for grazing animals. The estimates will only provide a group estimate, and will not account for differences between individual animals. Digestibility is normally estimated, and this can be done in several ways, including using the insoluble ash method

(Ferreira et al., 2004), in vitro estimation (Ulyatt et al., 1974, Pinares-Patiño et al., 2003b) or by iterative computer models (Macoon et al., 2003). Each of these methods has errors and these errors in intake estimates for individual animals can easily become quite large.

Chromic oxide can be administered to animals using several methods, such as in gelatine capsules (Carruthers and Bryant, 1983), controlled-release capsules (CRC) (Parker et al., 1990) or as chromium-mordanted fibre (Macoon et al., 2003), although some early studies used paper as a carrier for chromium sesquioxide (Corbett et al., 1960). These methods all work on the principle that chromium sesquioxide (Cr_2O_3) is indigestible and that all the Cr_2O_3 released in the rumen is voided in the faecal material. Faecal grab samples can be collected from animals once or more daily and analysed for chromium concentration by atomic absorption spectrometry (Ferreira et al., 2004). Faecal output is estimated using chromium dilution, such that the concentration of Cr_2O_3 released in the rumen is divided by the Cr_2O_3 concentration in the faeces. Intake is then calculated using the formula:

Intake = Faecal output / 1 – digestibility

There are reports that indicate chromium can work well for estimating intake (Ferreira et al., 2004), but this was with housed cattle. One of the main problems reported in the latter study was the difference between the manufacturers' stated release rate and the actual release rate of chromium from the CRC. Reports from studies with grazing cattle also indicate problems with variability in release from CRCs (Morris et al., 1993; Aranda-Osorio et al., 1996), with one study reporting that all chromium data were discarded and energy equations used to predict intakes (Lassey et al., 1997). When the Cr_2O_3 is delivered accurately, there can still be problems with variability in the data, with one study reporting a coefficient of variation of 28% for intake estimates when using Cr_2O_3 (Clark and Brougham, 1979). These studies show that while Cr_2O_3 can be used to give an indication of intake for groups of animals, this technique may not be appropriate for comparing differences in intake between individual grazing animals.

Alkanes are naturally occurring compounds found in plant cuticular waxes, and the alkanes found in plants are almost all odd-chain (Dove and Mayes, 1991). Alkanes are partially digestible, with digestibility increasing with decreasing chain length, from approximately 5% digestible for C_{35}/C_{36} to greater than 50% for C_{23} (R. Hegarty, pers. comm.) The n-alkane method of intake estimation is based on ruminal dosing of even-chain alkanes (C_{32}), followed by measurement of the ratio of dosed even-chain alkanes to natural odd-chain alkanes (C_{31} or C_{33}) in faecal samples. It is, therefore, possible to dose animals with even-chain alkanes and use the ratio of odd- to even-chain alkanes to determine daily feed intake (kg DM/day) by the following equation:

Daily feed intake = $(F_i/F_j * D_j) / (H_i – F_i/F_j * H_j)$

where F_i and H_i are odd-chain alkanes, F_j and H_j are even-chain alkanes, and D_j is the daily dose of even-chain alkanes (Dove and Mayes, 1991).

This technique relies on several assumptions. First, a representative sample of what the animals are eating must be collected, as alkane concentrations differ in different plants, and parts of the same plant (leaf versus stem versus flower (Waghorn et al., 2004).

A second assumption is that the release rate of alkanes in the rumen is accurately known (Dove et al., 2002). Some studies have reported that release rates of alkanes from controlled-release capsules are not consistent with the manufacturer's stated release rate (Ferreira et al., 2004; Swainson et al., 2005). These problems can be overcome by daily dosing of alkanes, or by measuring the release from some slow-release capsules in each batch.

A third assumption is that there is equal recovery of odd- and even-chain alkanes in faecal material (Dove and Mayes, 1991). This has not found to be the case in housed steers, with faecal recoveries of natural alkanes (C_{31} and C_{33}) from 59.5 to 77.3%, and recoveries of dosed alkanes (C_{32} and C_{36}) of 83.3-95.5% (Nia and Wittenberg, 2002). Recovery of alkanes from housed group-fed cattle was between 88% and 99% (Unal and Garnsworthy, 1999).

It has been reported that concentrations of the C_{32} alkane can be up to 217 mg or 31% of the quantity of synthetically dosed C_{32} alkane (Smit et al., 2005), while another study showed that alkane recovery can vary with the level of intake in cattle (Oliván et al., 2007).These problems with recovery rates have led to inaccuracies in estimating intakes, with one grazing experiment with 300 cows reporting highly variable results (coefficient of variation = 16%) and poor correlations (less than 0.37) with prediction equations based on energy requirements (McNaughton et al., 2005). These factors mean that while intake estimates made using the alkane technique may be useful to give an indication of intake (Ferreira et al., 2004; Smit et al., 2005), it may not be appropriate when accurate measures of individual intake are required, particularly from grazing animals.

It is possible to estimate intake from grazing animals with several techniques. However, while these techniques can give an indication of intakes, none of them provide accurate and precise estimates of intake from individual grazing animals.

1.7 Methane mitigation

Ruminant livestock produce CH_4 as an end-product of the microbial fermentation of plant material. This CH_4 is a potent GHG (25 times the warming potential of CO_2) and represents a significant energy loss (~21 g CH_4/kg DMI) to the farming system. Mitigation of CH_4 from livestock would reduce the release of this GHG into the atmosphere and potentially increase the efficiency of livestock production (Beauchemin et al., 2008).

According to Joblin (1999) it is the H_2 in the rumen that needs to be managed. The latter author stated this could be done in one of several ways, including decreasing H_2 production, reducing methanogen numbers or finding alternative pathways for H_2 to be utilised. All three pathways have been targeted, and this section will provide a brief overview of methods and that have been employed. As New Zealand livestock agriculture is based on a grazed livestock system, efficacy and applicability to the grazing system will be the focus of this section.

1.7.1 Animal performance

Any increase in individual animal performance will result in lower CH_4 production per unit of animal product (Monteny et al., 2006) due to the reduction in CH_4 loss associated with maintenance energy requirements relative to CH_4 loss from production. Animal performance can be improved in a number of ways, including better pasture management to improve the quality of pasture, feeding grain-based supplements and genetic selection for higher production (Boadi et al., 2004). One example is that high genetic merit dairy cows can produce 10,000 litres of milk per annum, instead of the more traditional 6,000 litres (Moss et al., 2000). As fewer cows would be needed to produce the same quantity of milk, the quantity of feed needed to maintain this smaller number of animals is less as is the total amount of feed consumed. According to Moss et al. (2000), this could reduce CH_4 emissions in the European Union by 20 to 30%.

In meat production systems, improving growth rates so that animals reach slaughter weight more quickly would reduce CH_4 production per kg of meat produced. Shorter lifetimes would result in less energy being required for daily maintenance due to the decreased number of days. Further, it has been reported that young animals appear to have a lower CH_4 yield than mature animals. This effect has been reported for sheep (Clark et al., 2003; Ulyatt et al., 2005), cattle (Molano et al., 2006) and deer (Swainson et al., 2007). Thus improving the efficiency of meat production systems through improved pasture quality or improved genetic merit could provide an excellent opportunity to reduce CH_4 production through the combined effects of lowered maintenance energy requirements and lower CH_4 production in young animals.

Increasing animal intakes may not only improve animal performance, but also result in lower CH_4 production per unit of feed intake as described in section 1.4.4. However, a study with sheep fed fresh pasture reported no effect of level of intake on CH_4 emissions per kg DMI (Molano and Clark, 2008). Further, a preliminary analysis of 70 CH_4 experiments conducted in New Zealand did not show any effect of level of intake on CH_4/kg DMI (H. Clark pers. comm.). These

results indicate that while reductions in CH_4 yield have been reported for concentrate diets, reductions may not occur for fresh forage diets fed in New Zealand.

The forage to concentrate ratio of a diet can have an impact on rumen fermentation as discussed in section 1.4.5. Using a modelling approach, Benchaar et al. (2001) showed that increasing the concentrate (corn plus soybean meal) proportion of an alfalfa hay diet from 0% to 20% or 50% of the diet, increased daily CH_4 production from 2.55 Mcal/day to 2.70 and 2.61 Mcal/day, respectively, while it was decreased (2.12 Mcal/day) at the highest level of concentrate feeding (70% of the diet). This is supported by the studies of Boadi et al. (2002b) and Beauchemin and McGinn (2006) who both reported no significant effect on CH_4 emissions when feeding supplementary concentrates. Supplementing grain could only be applied in the dairy industry, where animals are brought in for milking each day, but would not be suitable for the beef and sheep industries where animals are not seen for long periods. Further, the high levels of concentrate feeding required to reduce CH_4 production may make this an unsuitable method for the New Zealand situation.

The use of bovine somatotropin can enhance milk production of cattle through an increase in intake and metabolism of nutrients stimulated by the production of hormones from the liver and kidneys, which promote the uptake of nutrients into the mammary gland (Moss et al., 2000). Thus more milk is produced from individual animals meaning fewer animals are required to produce a set quantity of milk. However, bovine somatotropin is banned for use in dairy cattle in all European Union countries (Moss et al., 2000) and also in New Zealand. While chemical compounds can be used to reduce or inhibit CH_4 production, consumers are becoming wary of products from animals treated with chemicals (Moss et al., 2000).

A whole system analysis of total CH_4 emissions is required when considering increasing animal performance by methods such as increased supplementary feeds or feeding higher proportions of grain in the diet. For example, increasing the use of grain will result in an increased use of fossil fuel for chemical nitrogen

fertiliser and machinery to produce the grain, adding both nitrous oxide and fossil carbon to the GHG budget (Boadi et al., 2004). This is supported by the work of van der Nagel et al. (2003) who reported that farm studies showed GHG emissions (excluding N_2O) of 1.53 kg CO_2 equivalents/kg milk for a mixed forage and concentrate diet, but only 0.84 kg CO_2 equivalents/kg milk for a grazed pasture system. This is in contrast to the work of Lovett et al. (2006) who simulated a whole-farm system and showed that substituting forage with grain could reduce the CO_2 equivalent emissions in some farming situations.

Although increasing animal performance can result in more efficient production of livestock derived products, it is not guaranteed to reduce total CH_4 emissions at the farm level. If efficiency is increased (i.e. less feed is consumed in total to obtain a given level of output) farmers may simply make use of the extra feed available to increase animal numbers and increase overall product output. This appears to have occurred in New Zealand, with Leslie et al. (2008) reporting that the CH_4 emissions per kg milk solids for the dairy cattle industry have decreased by over 17%, but total industry emissions have increased by over 70%. For this reason, although this type of approach will result in a decrease in CH_4 per unit product produced, it will only reduce emissions if the quantity of product is held constant. Hence it may be an excellent strategy in Europe where there are quotas for milk and meat but may not work in New Zealand where producing more product is both allowable and profitable.

1.7.2 Nutritional management

1.7.2.1 Forage digestibility

As digestibility of a dried forage diet decreases, an increasing proportion of the digestion occurs in the hindgut. Methane production per mole of substrate is lower in the hindgut than in the rumen due to the increased proportion of H_2 being used by acetogens to produce acetate through reductive acetogenesis, resulting in a lower proportion of H_2 being reduced to CH_4 by methanogens (Immig, 1996), so any shift towards greater hindgut fermentation could result in decreased CH_4 emissions per kg intake.

In a series of grazing studies with lambs, Ulyatt et al. (2005) reported increasing CH_4 yield (g CH_4/kg DMI) with decreasing dry matter digestibility (DMD) of the forage, except with one group of animals that were much older (2 years vs. ~8 months). In another study, lambs fed fresh ryegrass with an OMD of either 63 or 75%, no significant difference was reported in CH_4 yields (23.7 and 22.9 g CH_4/kg DMI, respectively) (Molano and Clark, 2008). Dairy cows grazing pastures in spring produced CH_4 at 4.5-5.3% of GEI, while it increased to 6-7% when cows grazed lower digestibility pasture in summer (Robertson and Waghorn, 2002). However, part of the difference in the latter study may be attributed to differences in intake, with higher feed intakes in spring than in summer. In contrast, when feeding cattle with hay diets of differing *in vitro* organic matter digestibilities (OMD) (high = 61.5%; medium = 50.7%; low = 38.5%), no significant difference was reported in the CH_4 yields (~30 L CH_4/kg DMI) between diets (Boadi and Wittenberg, 2002).

It appears that feeding high digestibility forage may result in a decrease in CH_4 yield. However, this does not appear to be a practical mitigation strategy with current grazing systems in New Zealand.

1.7.2.2 Forage species and secondary compounds

There is some evidence that feeding legumes can result in lower CH_4 yields. Feeding increasing proportions of legume to grass decreased CH_4 production by 10% (McCaughey et al., 1999) or 16% (Lee et al., 2004). Knight et al. (2008) showed that feeding white clover as a sole diet reduced CH_4 yield compared to feeding ryegrass, while feeding Caucasian clover resulted in higher CH_4 yields than both white clover and ryegrass. The latter authors could not provide an explanation for the higher CH_4 yields when feeding Caucasian clover as a sole diet. However the latter study did report that feeding a combination of ryegrass (70%) and either white or Caucasian clover at (30%) resulted in a 17-24% reduction in CH_4 yield compared to feeding ryegrass alone. If the proportion of white clover in pastures could be increased to 30% or more, this would provide a potential mitigation option for farms on flat to rolling terrain.

Feeding some non-traditional New Zealand pastures species may also result in lower CH_4 yields from livestock. Studies with high digestibility (DMD > 70%) forages fed to sheep showed a twofold variation in CH_4 yield (Waghorn et al., 2002). Lowest CH_4 yields were reported for *Lotus pedunculatus* (11.5 g CH_4/kg DMI), followed by chicory (16.2 g CH_4/kg DMI), while highest yields were from ryegrass/white clover pasture (25.7 g CH_4/kg DMI) (Waghorn et al., 2002). Dairy cows fed sulla (*Hedysarum coronarium*) produced less CH_4 than when fed perennial ryegrass pasture (19.5 vs. 24.6 g CH_4/kg DMI) (Woodward et al., 2002). Dairy cows grazing *Lotus corniculatus* had lower CH_4 yields (19.9 vs. 24.5 g CH_4/kg DMI) than cows grazing perennial ryegrass pasture (Woodward et al., 2004), with a 13% reduction in CH_4 yield attributed to tannins in this study. This is supported by the work of Carulla et al. (2005) who reported a 13% decrease in CH_4 production by sheep fed 41 g/d *Acacia mearnsii* tannin extract. Beauchemin et al. (2008) reported that feeding forages with secondary compounds such as saponins or tannins may result in reduced CH_4 yields.

These studies indicate that feeding some forage and legume species will result in lower CH_4 yields than the typical ryegrass/white clover pasture. Further research is required to improve the agronomic performance of these plants if they are to be used as pasture species on farms (Beauchemin et al., 2008).

1.7.2.3 Fatty acids

Work by Blaxter and Czerkawski (1966) and Czerkawski et al. (1966) showed that feeding supplementary long-chain fatty acids (LFCA) depressed ruminal CH_4 production, increased faecal energy loss, but increased net energy available to the animal. This depression of CH_4 production by LCFA appears to be partially due to an inhibitory effect on methanogens (McAllister et al., 1996; Mathison et al., 1998) and partly due to decreased fibre degradation and decreased DMI (van Nevel and Demeyer, 1995; Mathison et al., 1998; McGinn et al., 2004; Beauchemin et al., 2008). The inhibition of cellulolytic bacteria by LCFA and consequent reduction in fibre degradation can be partially overcome by feeding them as calcium soaps (McAllister et al., 1996). The inhibitory effects

on CH_4 production occur with both saturated and unsaturated LCFA (Hegarty, 1999a).

The feeding of medium-chain fatty acids (MCFA) have been reported to reduce CH_4 emissions from sheep by 50% *in vitro*, and by 27% *in vivo* for a period of seven weeks, although the methane-suppressing effect was reduced with increasing fibre content of the diet (Machmüller, 2006). Mixes of sunflower and fish oils (500 g/d) containing MCFA fed as supplements to grazing dairy cows did not affect DMI or milk production, but reduced CH_4 emissions by 27% over a fourteen day period (Woodward et al., 2006), while McGinn et al. (2004) reported a 22% decrease in CH_4 production when feeding sunflower oil (5% DMI) during a 21-day period. In contrast to these positive results, Woodward et al. (2006) found no effect of either linseed or fish oils, supplemented at 300 g/d, on CH_4 emissions over a 12-week period. One explanation for this result is that adaptation to these oils by rumen microbes occurred.

In commercial diets using a range of lipid sources, reductions in CH_4 yields are likely to be from 10 to 25%, with a 5.6% reduction in CH_4 yield for each 1% addition of lipid to the diet (Beauchemin et al., 2008). However, rumen adaptation, along with the high cost and possible reduction in DMI and reduced fibre degradation mean fatty acids or refined oils are not currently a viable option for mitigation in the New Zealand pastoral system, particularly as the biggest reductions were with grain-based diets (Mathison et al., 1998; Beauchemin et al., 2008). Fat supplements are thought to be a possible option for finishing feedlot cattle, but no real options are yet available for grazing livestock (Beauchemin et al., 2008). Further, a whole system analysis would need to be conducted to account for emissions from production of these lipid sources to assess if an overall reduction in GHG emissions occurred.

1.7.3 Direct manipulation of the rumen ecological system

1.7.3.1 Alternative electron acceptors

Alternative electron acceptors work by providing an alternate sink for H_2 in the rumen. This would reduce the amount of H_2 available for methanogens to produce CH_4. There are several types of compounds that have been studied, including nitrate, sulphate and carboxylic acids as well as the microbial species known as acetogens.

Nitrate and sulphate can be administered to livestock, with either nitrate-reducing or sulphate-reducing bacteria utilising these compounds and H_2 in the rumen for growth (McAllister et al., 1996). *In vivo* studies have shown a strong suppression of rumen CH_4 production. However, toxicity can occur via the conversion of haemoglobin to methaemoglobin in the blood and an inability to transport oxygen in the blood (Sar et al., 2004). The latter authors reported that simultaneous administration of β1-4 galacto-oligosaccharides or nisin lowered rumen and plasma nitrite concentrations. However, due to the depression in DM intake and toxicity risks, these compounds cannot be practically used for ruminant CH_4 mitigation (McAllister et al., 1996; Hegarty, 1999a).

There are at least 15 potential organic or dicarboxylic acids which can be used as alternative H_2 sinks, with the two most promising from *in vitro* studies being fumarate and acrylate (McAllister and Newbold, 2008). Fumarate can be used as an electron acceptor via the fumarate reductase system (Mathison et al., 1998) or the succinate-propionate pathway (Kolver et al., 2004). McAllister and Newbold (2008) summarised the few available *in vivo* studies and found little reduction in CH_4 yield when feeding fumarate or malate at 1-3% of DMI, but reductions of 40-75% were possible for individual animals when feeding fumarate at 10% of DMI. A study with wether sheep fed dried ground lucerne showed a reduction in daily CH_4 production, but due to a reduced DMI, no effect on CH_4 yield when feeding up to 10% of DMI (Molano et al. 2008). While reductions in CH_4 production appear to occur, results can be variable between animals. Further, reductions in DMI, rumen adaptation and the prohibitive cost

of organic acids make them an unsuitable mitigation option at present (van Nevel and Demeyer, 1995; McAllister et al., 1996; Mathison et al., 1998; McAllister and Newbold, 2008).

A further possible CH_4 mitigation strategy is to replace methanogens in the rumen with acetogens which can utilise the CO_2 and H_2 to produce acetate, which is absorbed by the animal (van Nevel and Demeyer, 1995; McAllister et al., 1996). At least ten ruminal acetogens have been purified, but only two from grazing animals (Joblin, 1999). One option to do this would be to inhibit methanogenesis and then use the acetogens to keep the H_2 concentration low (Nollet et al., 1997; Joblin, 1999). However, McAllister and Newbold (2008) concluded that the challenges of increasing acetogenesis may be insurmountable at this point as judged by the fact that no peer-reviewed papers had appeared in the literature since the late 1990s.

1.7.3.2 Methane analogues

Some compounds exist that are CH_4 analogues and can specifically inhibit CH_4 formation. Methane analogues are directly toxic to methanogenic bacteria, resulting in increased ruminal H_2 and a decreased molar percentage of acetate but increased propionate (Mathison et al., 1998). The increased H_2 must be either utilised via a different pathway, such as acetogenesis, or be eructated and breathed out.

McCrabb et al. (1997) reported that daily treatment of steers with bromochloromethane complexed with alpha-cyclodextrin significantly reduced CH_4 production on both days 7 and 28 after supplementation started (treated = 0.25 mL/minute, untreated = 205 mL/minute), as well as significantly reducing the DMI (8-10%) and the acetate to propionate molar ratio (14%), but had no effect on average daily gain over a 10-12 week period. These animals were fed a combination of pollard and lucerne hay, so is not representative of a typical grazing situation in New Zealand, and research to assess the effect for forage fed animals is still required.

Another analogue which can be used to suppress CH_4 production is chloroform (Bauchop, 1967). Chloroform can be administered in small quantities (1.4 mL/day) and achieve large reductions in CH_4 production (>100 g CH_4/day) by the second day of treatment (Chaves et al., 2007). The latter authors also reported that ruminal propionate concentrations nearly doubled, ruminal acetate concentrations decreased, and that DM digestibility dropped from 71% to 53%. However, after 2-3 days of chloroform treatment, CH_4 production gradually returned to pre-treatment quantities (Chaves et al., 2007).

Alpha-bromoethanesulfonic acid (BES) inhibits methanogenesis because it is a structural analogue of the co-factor, mercaptoethanesulfonic acid, used by methanogenic bacteria in the formation of CH_4 (Mathison et al., 1998; Nollet et al., 1997). It is a very effective CH_4 inhibitor, reducing CH_4 emissions from 3.9% to 0.6% of gross energy intake immediately after administration (McAllister and Newbold, 2008). However, microbes can adapt to BES within four days of continuous administration, making it unsuitable for reducing ruminant CH_4 production (Mathison et al., 1998).

Another enzyme that has been targeted is hydroxymethylglutaryl~ScoA reductase. As this enzyme is specific to methanogens, inhibiting substances appear to have excellent potential for CH_4 mitigation, and *in vitro* studies have shown promising results (Miller and Wolin, 2001). However, no *in vivo* data are available to confirm the *in vitro* results.

While some of these compounds appear to give strong suppression of CH_4 production, their effects are only transitory and the move by consumers away from food products from animals treated with chemical additives means these are unlikely to provide useful mitigation options (McAllister and Newbold, 2008).

1.7.3.3 Antibiotics

Benefits from antibiotics such as ionophores, other than CH_4 reduction, include increased feed efficiency and decreased feed intake (Mathison et al., 1998) as well as improved animal productivity of approximately 8% (Moss et al., 2000).

This effect may be due to the inhibitory effect of antibiotics on gram-positive bacteria, which produce lactic, acetic, butyric and formic acids and H_2, changing fermentation towards gram-negative bacteria that produce succinate and propionate (Chen and Wolin, 1979; van Nevel and Demeyer, 1995; Tedeschi et al., 2003).

Beauchemin et al. (2008), in a summary of several experiments, reported that supplementing monensin to cattle typically resulted in a 5-10% reduction in CH_4 production, while van Nevel and Demeyer (1995) reported that supplementing monensin to fattening beef animals resulted in a 25% decrease in CH_4 production. However, the reduction appears to be diet dependent, with Guan et al. (2006) reporting that larger reductions in CH_4 yield are possible with high (30%) levels of grain feeding than lower levels (27%). An *in vivo* study with forage-fed cattle supplemented with 320 mg sodium monensin/day (as a slow release bolus) reported variable reductions in CH_4, with a reduction of 9% from 16.9 to 15.3 g CH_4/kg DMI for indoor forage-fed cows, but no reduction for cows grazing ryegrass/white clover pastures (van Vugt et al., 2005). An experiment feeding fresh forage to sheep reported a 33% reduction in CH_4 yield when monensin was dosed at 15 mg/day (Swainson et al., 2008). Other studies have shown small, non-significant reductions from monensin (McGinn et al., 2004), or no effect on CH_4 yield (Waghorn et al., 2008).

Some challenges with using monensin to reduce CH_4 production include inadequate performance of slow-release boluses for use in grazing animals (Waghorn et al., 2008), and a lack of persistence in reductions in CH_4 production over time, with CH_4 production returning to pre-treatment values within two weeks in some studies (Johnson et al., 1994b; van Nevel and Demeyer, 1995; Johnson and Johnson, 1995) and within two months in another study (Guan et al., 2006). The lack of a consistent and long-term reduction in CH_4 yield, along with increasing resistance to using antibiotics in animal feeds makes their value as a mitigation tool questionable (Beauchemin et al., 2008).

Some rumen bacteria produce anti-bacterial peptides (bacteriocins) that can inhibit other bacterial species (McAllister and Newbold, 2008). Kalmakoff et al.

(1996) showed that 50% of the rumen bacteria they tested had bacteriocin-like activity. Klieve and Hegarty (1999) reported that one of these bateriocins, nisin, reduced CH_4 production by up to 36% *in vitro*. Studies to date have focussed on *in vitro* testing, and the *in vivo* efficacy of these compounds still needs to be tested, including stability in the rumen milieu (McAllister and Newbold, 2008). Bacteriocins hold potential as a non-chemical CH_4 mitigation option that may be suitable for use in grazing animals if new bacteriocins that are specific to methanogens can be identified (McAllister and Newbold, 2008).

1.7.3.4 Bacteriophages

Another option to reduce CH_4 production would be to utilise bacteriophages (microbial viruses) to lyse methanogens in the rumen (Klieve and Hegarty, 1999, Walker et al., 2004). Newbold et al. (1996) reported the occurrence of methanogen lysis in the rumen and that this lysis appeared to be primarily mediated through the action of bacteriophages. An advantage of bacteriophages is that they are naturally occurring, and have the potential to survive in the rumen, providing a mitigation option for grazing livestock. However, no phage specific to ruminal methanogens have been reported, and due to their host-specificity, may pose a challenge due to the diversity of methanogens (McAllister and Newbold, 2008).

1.7.3.5 Immunisation

A new line of research has developed in recent years considering the possibility of vaccinating ruminant livestock against methanogens. The aim would be to stimulate the animal to produce antibodies against known ruminal methanogens. If a vaccine could be developed this would have the potential to be a cost-effective mitigation tool for grazing livestock, as animals could be vaccinated once, with no routine handling or on-going costs and no impact on grazing management. A methanogen vaccine has been developed in Australia with a reported 7.7% reduction in CH_4 yield, although there was no effect on total CH_4 production (Wright et al., 2004b). Further work in New Zealand with the same vaccine formulation reported no effect on daily CH_4 production, CH_4

yield or animal performance when tested in sheep (Clark et al., 2004). Research is underway in New Zealand to identify defined antigens, with preliminary laboratory studies showing some promising results (Buddle et al., 2008).

1.7.3.6 Defaunation

It has been shown that there is a symbiotic relationship between some ciliate protozoa and rumen methanogens (van Nevel and Demeyer, 1995; Ushida and Jouany 1996; Mathison et al., 1998). Methanogenesis associated with hydrogen transfer from protozoa contributes approximately 37% of the rumen CH_4 production (Hegarty, 1999b). Protozoa can be removed from the rumen in a process known as defaunation. There are several factors associated with the decrease in CH_4 production in defaunated animals. First, protozoa produce predominantly acetate, butyrate and H_2, so defaunation causes a shift in VFA production towards more propionate (Hegarty, 1999a), as well as a reduced methanogen population (Hegarty, 1999b). Second, defaunation results in lower ruminal fibre digestibility (van Nevel and Demeyer, 1995). It has also been suggested that there may be an increase in the partial pressure of oxygen in the rumen when protozoa are not present (Hegarty, 1999b).

Hegarty (1999b) reported that defaunation of the rumen reduced CH_4 production by an average of 13%. Recent work with ciliate-free lambs by McAllister and Newbold (2008) showed a 26% reduction in CH_4 yield compared with faunated lambs, while Morgavi et al. (2008) showed a similar decrease (~20%) in both daily CH_4 production and CH_4 per kg metabolic live weight and reported that this effect lasted for up to 2 years. In contrast, another study reported no effect of defaunation on CH_4 production (Bird et al., 2008). The latter study fed animals a forage-only diet (dried lucerne) while both the McAllister and Newbold (2008) and Morgavi et al. (2008) studies fed mixed forage and concentrate diets. This is in agreement with reports by Mathison et al. (1998) and Hegarty (1999b) that defaunation can reduce methanogenesis by 20 to 50%, but the greatest reductions occur with high starch diets.

Although defaunation does show some promise the lack of availability of practical defaunation methods mean defaunation can not be practically used to reduce CH_4 production at the present time (Mathison et al., 1998; Hegarty, 1999b).

1.7.4 Exploiting animal variance

It is known that variation exists between animals in their CH_4 production as discussed in Section 1.4.6. Evidence from New Zealand studies using the SF_6 tracer technique to estimate CH_4 emissions indicate that for any group of animals measured, ~10% are high emitters and ~10% are low emitters with a 40% difference between the two groups (O'Hara et al., 2003). This inter-animal variation in CH_4 yield applies to several animal species, periods in time, and diet types (Lassey et al. 1997; Pinares-Patiño et al., 2003a, b; Goopy and Hegarty 2004). There are two approaches currently being used to select for low CH_4-emitting animals: selection by identifying animals with a low CH_4 yield and selection for animals with a low residual feed intake.

If animals can be found that are consistently high or low in their CH_4 emissions, research could be undertaken to find and exploit the factors responsible. Several studies have identified high and low CH_4 emitters (Pinares-Patiño et al., 2000; Pinares-Patiño et al., 2003b; Goopy and Hegarty, 2004; Pinares-Patiño et al., 2005). However, in each of the latter studies some of the animals changed rank when they were re-measured at a later date. While exploitation of animal variance for low CH_4 production offers a path to reduce ruminant CH_4 emissions without the use of chemicals or feed additives, the reasons for the apparent lack of consistency in estimated CH_4 yield must first be identified.

In Australia, research has been undertaken to improve the feed efficiency of beef animals by selection for low residual feed intake (Hegarty et al., 2007). Animals selected for a low residual feed intake also had significantly lower daily CH_4 production (25%), tended to have a lower CH_4 yield per kg average daily gain (~24%), but there was no difference in their CH_4 yield per kg DMI.

Both these methods of using animal variation to select for animals with low CH_4 yields have the opportunity to be used with grazing livestock, are open to continuous improvement, and have the ability to provide an additive-free, cost-effective mitigation option. If animals have higher production efficiency, coupled with reduced CH_4 production, a higher likelihood of uptake by the agricultural sector could be expected.

1.8 Conclusions and the need for future research

Global warming is occurring, apparently due to the increasing concentrations of greenhouse gases in the atmosphere, mainly CO_2, CH_4, and N_2O. Atmospheric concentrations of these GHG need to be brought to a steady state to avoid any dangerous changes to earth's climate.

New Zealand has a unique emissions profile for a developed country due to its agriculturally based economy, with CH_4 and N_2O from the agricultural sector constituting over half its estimated annual emissions on a CO_2 equivalent basis. A large proportion of these emissions are from ruminant livestock.

Methane is produced as a by-product of the fermentation of ingested feed in the rumen, with H_2 and CO_2 being released by bacteria, protozoa and fungi, and utilised by methanogens to produce CH_4. Energy loss as CH_4 accounts for approximately 6% of the gross energy ingested by ruminant livestock or 21 g CH_4/kg DMI. Methane yields (g CH_4/kg DMI) are greater on the pasture diets fed in the New Zealand grazing system than for grain-based systems.

There are a number of methods to measure CH_4 production from livestock, both individual and group methods. Of these methods, the SF_6 tracer technique is the most commonly used method to estimate CH_4 production from individual grazing livestock, and from large groups of individual animals.

There are a number of alternative methods to reduce CH_4 emissions from livestock, but their applicability *in vivo*, cost, long-term efficacy and acceptance by consumers mean that, at present, no method can be recommended. Another

challenge is the application of a mitigation strategy to the New Zealand grazing system. One strategy is to select for animals with low CH_4 emissions. Individual ruminant livestock vary in their CH_4 yield, and if this has a genetic basis, animals could be screened and selected for low CH_4 emissions. All such experiments would need to be carried out with housed cattle due to the inability to accurately estimate animal intakes and CH_4 production while grazing.

The author was involved in grazing experiments using the SF_6 tracer technique to estimate CH_4 production from large numbers of dairy cattle to obtain relative high and low CH_4 emitters (McNaughton et al., 2005; Cavanagh et al., 2008). From the first experiment, high and low emitters were selected and re-measured to confirm their high or low CH_4 yields and their individual rankings. These latter measurements were conducted when the cows were housed, so feed intakes could be accurately measured. However, cows did not maintain consistent ranking over time, but substantially changed their ranking between each of the three measurement periods (Pinares-Patiño et al., 2005).

Studies to date indicate a need to confirm and determine the cause of the exaggerated variance in estimated CH_4 yield when using the SF_6 tracer technique. This variance may be due to greater variance from the SF_6 technique than from calorimetry, greater animal variance when measured in stalls or at grazing compared to animal chambers, or a combination of both these two factors. Identification of the sources of variance will allow researchers to minimise any variance from the technique, and maximise the potential of selecting animals for low CH_4 yield when using the SF_6 tracer technique.

Chapter 2

The effect of SF_6 release rate, animal species and feeding conditions on estimates of methane emissions from ruminants

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2.1 Abstract

In New Zealand, methane (CH_4) emissions from ruminants are estimated using the sulphur hexafluoride (SF_6) tracer technique. This technique does not measure CH_4 production directly, but involves inserting a calibrated source of SF_6 gas into the rumen and estimating emissions from the ratio of CH_4 to SF_6 in breath samples collected continuously from above the nose of each animal and the known release rate of SF_6 from the calibrated source. Methane estimates obtained using this method are more variable than measurements obtained using respiration calorimetry. The absolute release rate of SF_6 from the calibrated source inserted into the rumen could contribute to this variation.

Data were extracted from a database containing details of 21 separate New Zealand experiments where the SF_6 technique was used to estimate CH_4 emissions. Experiments were divided into four groups according to species (dairy cattle or sheep) and feeding location (grazing or housed), with each group analysed separately. The housed dairy cattle category contained data from only two experiments with distinct SF_6 release rates, so was not analysed. Data were analysed using a linear mixed model with a fixed effect of SF_6 release rate, and random effect of experiment.

Daily CH_4 production (g CH_4/day) was positively related to SF_6 release rate for both grazing cattle ($P < 0.05$) and housed sheep ($P < 0.05$) but not for grazing sheep experiments. Methane yield (g/kg DMI) was positively, but not significantly related to SF_6 release rate for grazing cattle and housed sheep, but not for grazing sheep.

While mean CH_4 production values obtained using the SF_6 technique are similar to those obtained using direct methods, the effect of SF_6 release rate on estimated CH_4 production identified here may contribute to the greater variability of the SF_6 tracer technique.

2.2 Introduction

2.2.1 Uncertainty in estimated methane yield

Methane (CH_4) emissions arising from enteric fermentation (31%) and animal waste (2%) make up 33% of New Zealand's national GHG emissions (New Zealand Ministry for the Environment, 2007). Total livestock CH_4 emissions are calculated by multiplying total estimated animal intake by the CH_4 yield (g CH_4/kg DMI) for each livestock class (New Zealand Ministry for the Environment, 2007). Total intake for each livestock class is estimated by multiplying the total animal population by the estimated dry matter intake (DMI) of the 'average' animal from each class. The CH_4 conversion rate for each class is based on measurements of CH_4 yield from experiments carried out in New Zealand, with CH_4 loss values of 16.8 – 21.6 g CH_4/kg DMI being adopted for livestock categories in the national inventory (New Zealand Ministry for the Environment, 2007).

A Monte Carlo simulation, whereby numbers are repeatedly sampled from within a probability distribution for each data input category, aggregated, and analysed to indicate which category has the greatest uncertainty, has been carried out for New Zealand's enteric CH_4 emissions (Clark et al., 2003). The simulation indicates that the biggest influence on the level of uncertainty in New Zealand's national CH_4 inventory is uncertainty surrounding the quantity of CH_4 emitted per kg of dry matter intake or CH_4 yield (Clark et al., 2003).

The principle reason for this is the large animal-to-animal variation in CH_4 yield found when any group of animals have their CH_4 yields estimated using the SF_6 tracer technique (Lassey et al., 1997). The reasons for this variation have not been well defined, but are known to be common across animal species (Lassey et al., 1997). In addition to this, animals are not consistent in their CH_4 yield across time and diets (Boadi et al., 2002a; Pinares-Patiño et al., 2003b; Goopy and Hegarty, 2004), and measurements made using the SF_6 tracer technique tend to be more variable than measurements made using calorimetry (Johnson

et al., 1994a; Boadi et al., 2002a; Grainger et al., 2007; Pinares-Patiño et al., 2007a).

2.2.2 Background to the sulphur hexafluoride technique

The SF_6 technique is the only non-calorimetric technique widely used for estimating CH_4 production from individual grazing animals (O'Hara et al., 2003). This technique has been widely used in New Zealand for the development of emission factors for the national inventory, for comparing mitigation treatments and to select animals that appear to have naturally low CH_4 emission per unit of feed digested. This section will look in more detail at how the technique works and the underlying assumptions behind the technique.

Estimating CH_4 production using the SF_6 tracer technique involves placing a pre-calibrated source of SF_6 into the rumen of an animal. In New Zealand, the SF_6 is placed inside a small brass tube (hereafter referred to as a permeation tube). The SF_6 permeates out of the permeation tube as a gas, and is released from the rumen via eructation and exhalation.

Breath samples are continuously collected from around the nose and mouth by putting a modified harness, or halter, on each animal for a 24 h sampling period (Lassey et al., 1997). Each halter has tubing attached which delivers the air sample via a QuickConnect® valve to a pre-evacuated polyvinyl chloride (PVC) canister (yoke) on the animal's back or neck. A piece of small-diameter metal tubing, referred to as capillary tubing, in this line restricts the airflow to half-fill the yoke over the 24 h period. By collecting this way, both the expired air from the lungs and any eructated gases are sampled. Yokes are fitted or exchanged once during each twenty-four hour period for four to five days. Background air samples are also collected to correct for the background concentration of both CH_4 and SF_6.

Samples are analysed with flame ionisation and electron capture detectors for CH_4 and SF_6, respectively, using a gas chromatograph (Lassey et al., 1997). In New Zealand, sample analysis involves partial extraction of gas sample from

the yoke and pressurising this sample using a pump system prior to chromatograph analysis. A set of three standards, containing both CH_4 and SF_6, are used to make a calibration curve against which experimental concentrations of both CH_4 and SF_6 are compared. These standards are manufactured by the New Zealand Institute of Water and Atmospheric Research. Standards are analysed daily before and after experimental samples are analysed, and the mid-range standard is analysed after every 10th experimental sample.

The release rate of the tracer gas, SF_6, and the ratio of SF_6 to CH_4 in the breath are used to calculate the CH_4 emissions (g/day) of each animal (Q_{CH4}) by the following equation:

$$Q_{CH4}=Q_{SF6} \times [CH_4]/[SF_6]$$

where $[CH_4]$ and $[SF_6]$ denote the yoke concentrations (parts per million and parts per trillion, respectively) above the background concentrations of these gases, and Q_{SF6} is the release rate of SF_6 (mg/day) from the permeation tube.

The first assumption for any tracer technique is that the tracer is inert, with no detrimental effects or interactions with substances in the experimental environment. Studies have demonstrated that SF_6 gas does not appear to affect the rumen biota (Johnson et al., 1992).

A second assumption is that the tracer gas must behave in a similar way to the gas being measured. As gas transport from the rumen is predominantly through eructation (Murray et al., 1976), the gas exiting should be thoroughly mixed by turbulent diffusion (Johnson et al., 1994a). While CH_4 is eructed from the rumen, it is also exhaled through the breath (Ulyatt et al., 1999). Any CH_4 that is exhaled should be well mixed in the breath sample. It has been reported that SF_6 is extremely stable in the atmosphere, and is soluble at trace levels in water and seawater with respective solubility coefficients 0.16 and 0.12 mol/L/atmosphere at 39°C (Bullister et al. 2002). As the partial pressure of SF_6 in the rumen is extremely low (2-5 mg SF_6 (New Zealand) in ~2200 L of carbon dioxide and ~700 L CH_4 per cow per day (Moate et al., 1997)), very little would

tend to be solubilised. On this basis, it is assumed in animal studies that there is no interaction between SF_6 and rumen contents, supporting the assumption that SF_6, along with CH_4 and other rumen gases are eructated and released via the mouth and nose.

One issue when conducting indoor experiments using the SF_6 technique is that SF_6 gas is about six times heavier than CH_4 gas (Ulyatt et al., 1999). This can result is SF_6 settling in places such as feed bins or in rooms with very poor ventilation. In practice, this has never been an issue, although some layering has been measured (M. Tavendale pers. comm.). This effect does not occur with grazing experiments and can be easily avoided with housed animals if there is sufficient ventilation.

A third assumption is that the tracer gas, SF_6, is released at a constant, known rate from the rumen. Permeation tubes are manufactured with computer-controlled lathes (K. Lassey pers. comm.). Figure 2.1 shows an expanded view of an assembled permeation tube, and the various components of it.

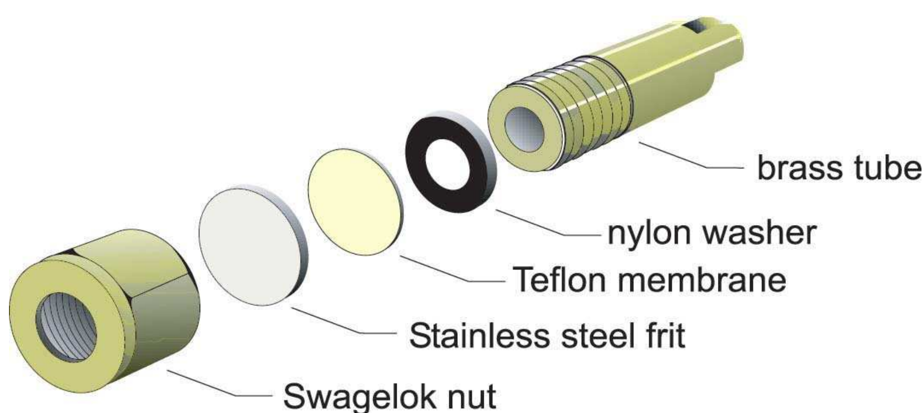


Figure 2.1 An expanded view of an assembled permeation tube. From Lassey et al., (2001).

The body of the tube is made of brass, and has a brass Swagelok® nut fitted on the end. The Teflon® membrane restricts the permeation of SF_6 gas from the tube, and the steel frit and nylon washer help protect the Teflon® from internal pressure and distortion during tightening of the nut. Permeation tubes for cattle are charged with 2 to 3 grams of liquid SF_6. The charged tubes are then kept in a 39°C environment and individually weighed weekly; with masses recorded to

0.05 mg. Weighing is undertaken for approximately 100 days before use in an experiment. Linear regression fits of these weights against time are used to give an SF_6 release rate for individual tubes. Only tubes that show linear rates of SF_6 release during this monitoring phase are used *in vivo*, and must have a regression fit of $R^2 > 0.998$ when the first two weeks of data are ignored. (Lassey et al., 2001)

Although release rates from the permeation tubes are linear over the pre-insertion monitoring period, release rates are not linear over long periods (Lassey et al., 2001). This led to the use of quadratic equations to determine the SF_6 release rates for experiments over longer periods of time (Lassey et al., 2001). Where possible, permeation tubes are collected post-experiment for further serial weighing. These data can then be used in conjunction with the pre-experimental data to provide the quadratic equation. Lassey et al. (2001) have suggested that an interaction between SF_6 and the Teflon® membrane causes the slower release of SF_6 over time.

The SF_6 technique, which collects breath samples from above the nose/mouth area, relies on the vast majority of CH_4 exiting the mouth and nose. However, few studies have been undertaken looking at partitioning of CH_4 excretion between breath and flatus. One experiment with three sheep (Murray et al., 1976) indicated that 99% of CH_4 was excreted through the nose and mouth, and only 1% through the anus. Assuming this is correct, and that sheep and cattle partition CH_4 excretion in the same way, then the SF_6 technique should estimate CH_4 emissions at approximately 99% of calorimetric measurements.

Representative sampling of respired and eructated air is critical to the accuracy of the technique. Sampling equipment must be positioned just above the nose of the animal, and the tubing must be intact to get good collection. In dry dusty environments, or when animals are grazing wet pasture, the capillary tubing can become partially or completely blocked with dust or water. Blockages are less frequent when the halter is correctly adjusted to fit individual animals. As the capillary tubing has the narrowest diameter, with a flow rate of around 1 ml/min for cattle, it is usually the first place to be blocked. The capillary restricts the

amount of collected sample, so that the yoke is half filled over the 24-hour collection period, resulting in a collected sample of about 1.2 L, as the yokes used for cattle have an average volume of 2.5 L.

Once the sample has been collected, it must be analysed via a gas chromatograph as described above. Accurately measuring gases at such trace concentrations (parts per trillion for SF_6 and parts per million for CH_4) requires expensive analytical equipment – namely a gas chromatograph with both a flame ionisation detector and electron capture detector. Obtaining accurate gas standards at these low concentrations is difficult as few laboratories have the facilities to make them (K. Lassey pers. comm.). In New Zealand, standards are manufactured by the New Zealand National Institute of Water and Atmospheric Research. Further, because SF_6 is a 'sticky' gas (K. Lassey, pers. comm.) it is important to ensure good ventilation and laboratory practice to prevent contamination of samples or equipment by high SF_6 concentrations in the laboratory.

2.2.3 Background to and aims of the study

In a study with cloned cattle, CH_4 emissions were estimated using the SF_6 tracer technique before and after rumen contents were swapped between two groups of 'high' and 'low' CH_4-emitting animals (M. Krause and H. Clark, unpublished data). Results from this experiment showed that swapping rumen contents did not change the 'high'/'low' status of the animals; 'high' animals remained 'high', and 'low' animals remained 'low'. This may indicate that the host animal has a major influence on its CH_4 production. However, as permeation tubes were not swapped during this experiment, the hypothesis was proposed that the permeation tube may have an influence on estimated CH_4 production for individual animals.

AgResearch Ltd, in conjunction with the New Zealand National Institute for Water and Atmospheric Research, has carried out CH_4 experiments using the SF_6 tracer technique in New Zealand since 1996. Results of experiments conducted from 1996 up to and including 2003, have been entered into a

database. This includes data on animal species (dairy cattle, sheep), feeding location (housed, grazing), SF_6 release rate, herbage intake, and diet quality. The aim of this study was to conduct a meta-analysis of these data to ascertain what, if any, relationship may exist between absolute SF_6 release rate and estimated CH_4 production and yield.

2.3 Materials and methods

The database of CH_4 experiments comprised information from twenty-one experiments, covering a total of 643 observations from both sheep and dairy cattle in either a housed or grazing environment (Tables 2.1 and 2.2). In some experiments, repeated measurements were conducted on the same animals. However, these measurements were conducted during different seasons, or with different feed types or locations. For this reason, these measurements were assumed to be independent. This accounts for the larger number of measurements than animals reported in Table 2.2.

Data from these experiments were divided into four groups according to species (dairy cattle or sheep) and feeding location (grazing or housed) and were analysed using Genstat software (Payne et al., 2006b). Each group was analysed separately because of the difference in CH_4 output between sheep and cattle and because intake can be accurately measured indoors but only estimated at grazing. Methane output was analysed as both production (g/day) and yield (g/kg DMI), using a linear mixed model with a fixed effect of SF_6 release rate, and random effect of experiment. A Wald test (Payne et al., 2006a) was used to indicate the significance of the effect of SF_6 release rate.

There was a large range in both CH_4 emissions (g/day and g/kg DMI) and SF_6 release rates (mg/day) (see Table 2.1). For example, the range in CH_4 emissions for dairy cattle was 82.1 to 558.2 g CH_4/day, and the SF_6 release rate was 1.5 to 6.8 mg SF_6/day, while for sheep the range in CH_4 emissions was 10.5 to 70.8 g CH_4/day and the SF_6 release rate was 0.5 to 3.2 mg SF_6/day. Diets were mainly fresh perennial ryegrass-based pasture, although one

housed dairy experiment used ensiled pasture, and a mixed grain and forage diet was fed in another dairy experiment. Several of the housed sheep trials used lucerne chaff or forage crops. The CH_4 emissions for housed dairy cattle come from only two experiments.

Table 2.1 A summary of methane emissions (g/day and g/kg DMI) and SF_6 release rates for dairy cattle and sheep measured at grazing or housed using the SF_6 tracer technique. Data comprise a total of 643 observations taken between 1997 and 2003. Data are mean ± s.d.

Species	Feeding location	Number of observations	CH_4 (g/day)	CH_4 (g/kg DMI)	SF_6 Release (mg/day)
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

The permeation tubes represent a range of measured release rates with non-arbitrary values and non-zero means, making them suitable as a covariate (fixed effects factor) rather than a random effect. Experiment was included as a random effect in the model due to the large variation (CV=48%) in the data between experiments. For the sheep experiments, natural logarithms of both daily CH_4 production and yield were used for analyses in order to stabilize the variance, which increased with the mean. Genstat software (Payne et al., 2006b) was used to fit the mixed model and estimate the effect of SF_6 release rate on CH_4 output. Straight lines were fitted to predictions of CH_4 output (averaged over the different experiments) for various SF_6 release rates to obtain the fitted model for each group. The fitted model regression together with 95% confidence intervals of the modelled lines were plotted on the raw data (the fitted values for grazing and housed sheep were back-transformed before plotting) for each group (see Figure 2.2 and 2.3). The housed cattle category had only two experiments with quite distinct SF_6 release rates so there were insufficient data for this analysis.

Table 2.2 Experiments from the methane database used in these analyses, including reference, experiment date, location, animal type, number of animals measured and mean ± s.d. values.

Reference	Experiment date	Location	Type	Number	DMI (kg/day)	Methane (g/day)	SF_6 release (mg/day)	Intake Estimation
Ulyatt et al., 1997	Mar 1996	grazing	cattle	10	13.37 ± 0.83	262.85 ± 28.12	3.30 ± 0.06	Own model
Ulyatt et al., 2002 (b)	Mar 1997	grazing	cattle	9	15.63 ± 1.91	375.94 ± 62.60	2.09 ± 0.34	SCA, 1990*
Ulyatt et al., 2002 (a)	Sep '97-Jun '98	grazing	cattle	10	14.94 ± 4.98	252.14 ± 127.69	2.75 ± 0.37	SCA, 1990
Ulyatt et al., unpublished	Mar 2000	grazing	cattle	8	18.86 ± 1.83	413.30 ± 40.70	1.65 ± 0.19	SCA, 1990
Waghorn et al., 2001	Sep '00-Mar '01	grazing/housed	cattle	20	19.42 ± 4.11	387.84 ± 73.44	3.55 ± 0.22	Alkane
Waghorn et al., 2003b	Oct 2002	grazing	cattle	20	15.46 ± 1.96	345.25 ± 69.54	3.66 ± 0.53	SCA, 1990
Waghorn et al., 2003a	Oct 2002	grazing	cattle	30	14.52 ± 1.59	270.34 ± 42.86	4.31 ± 1.14	Alkane
Krause et al., unpublished	May 2003	housed	cattle	10	9.47 ± 1.05	124.82 ± 26.49	2.80 ± 0.46	Measured
Lassey et al., 1997	Mar 1996	grazing	sheep	47	1.28 ± 0.18	20.61 ± 4.47	2.77 ± 0.33	Total faecal collection
Pinares-Patiño, 2000	Aug '96-Jan '97	housed	sheep	8	1.16 ± 0.12	21.14 ± 4.96	1.66 ± 0.36	Measured
Ulyatt et al., 2002 (b)	Mar 1997	grazing	sheep	10	0.79 ± 0.15	16.12 ± 3.09	0.83 ± 0.16	Total faecal collection

Table 2.2 continued.

Ulyatt et al., 2005	Mar 1997	grazing	sheep	10	1.33 ± 0.22	19.08 ± 4.44	0.83 ± 0.16	Total faecal collection
Ulyatt et al., 2005	Apr 1997	grazing	sheep	10	1.65 ± 0.26	22.58 ± 3.62	0.83 ± 0.16	Total faecal collection
Ulyatt et al., 2002 (a)	Sep ’97-Jul ‘98	grazing	sheep	12	1.57 ± 0.30	29.79 ± 5.69	1.31 ± 0.15	Alkane
Pinares-Patiño et al., 2003 (c)	May 1998	housed	sheep	10	1.21 ± 0.21	20.16 ± 4.02	1.11 ± 0.48	Measured
Pinares-Patiño et al., 2003 (a)	Oct 1999	housed	sheep	6	1.40 ± 0.31	24.95 ± 8.26	0.74 ± 0.08	Measured
Pinares-Patiño et al., 2003 (b)	Oct-Dec 1999	grazing	sheep	7	2.14 ± 0.32	32.73 ± 7.12	0.73 ± 0.07	Total faecal collection
Clark et al., unpublished	Sep ’01-Apr ‘02	grazing	sheep	12	1.97 ± 0.60	41.41 ± 11.75	1.33 ± 0.25	Alkane/herbage mass
Clark et al., unpublished	Nov 2002	grazing/housed	sheep	18	2.60 ± 1.15	23.28 ± 5.81	1.56 ± 0.27	Alkane/measured
Molano et al., 2004	Dec 2002	housed	sheep	16	1.00 ± 0.32	23.42 ± 8.52	1.56 ± 0.28	Measured
Clark et al., 2004	May-Jul 2003	housed	sheep	45	1.21 ± 0.17	20.94 ± 6.78	1.41 ± 0.42	Measured

*SCA is the Standing Committee on Agriculture, 1990: Ruminants, Feeding standards for Australian livestock

2.4 Results

Daily CH_4 production was positively related to SF_6 release rate in grazing dairy cattle with a slope significantly different to zero (Slope = 16.14, $P < 0.05$) (Figure 2.2a). Daily CH_4 production was positively related to permeation tube release rate (Slope = 2.06, $P < 0.05$) in housed sheep, but not in grazing sheep (Slope = -2.08, P = 0.147) (Figure 2.2b and c).

(a)

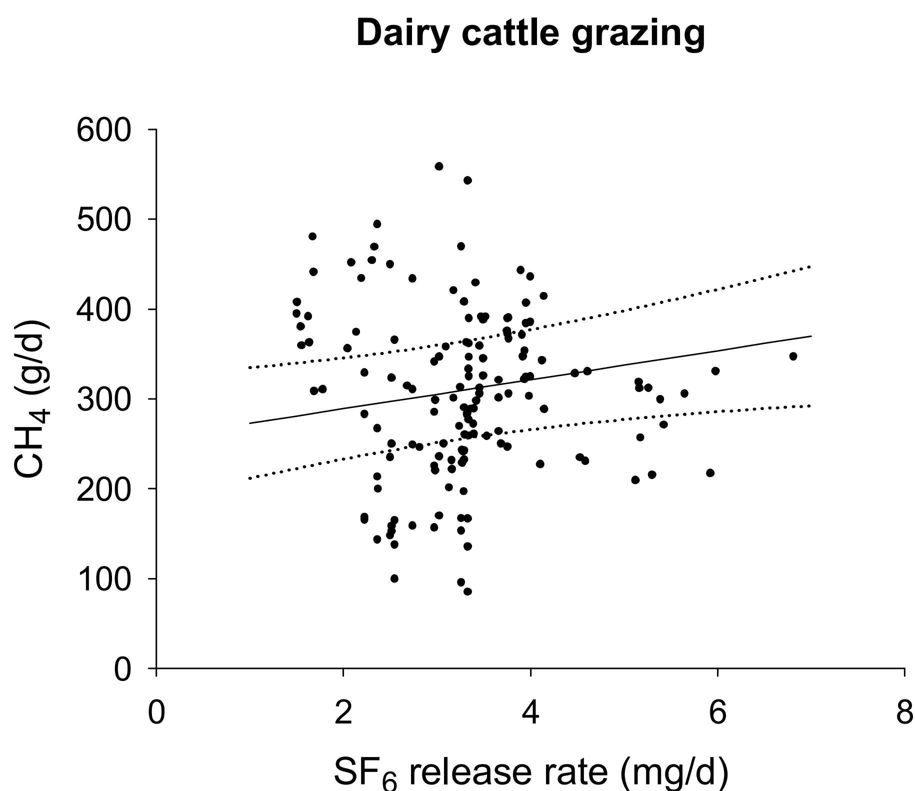
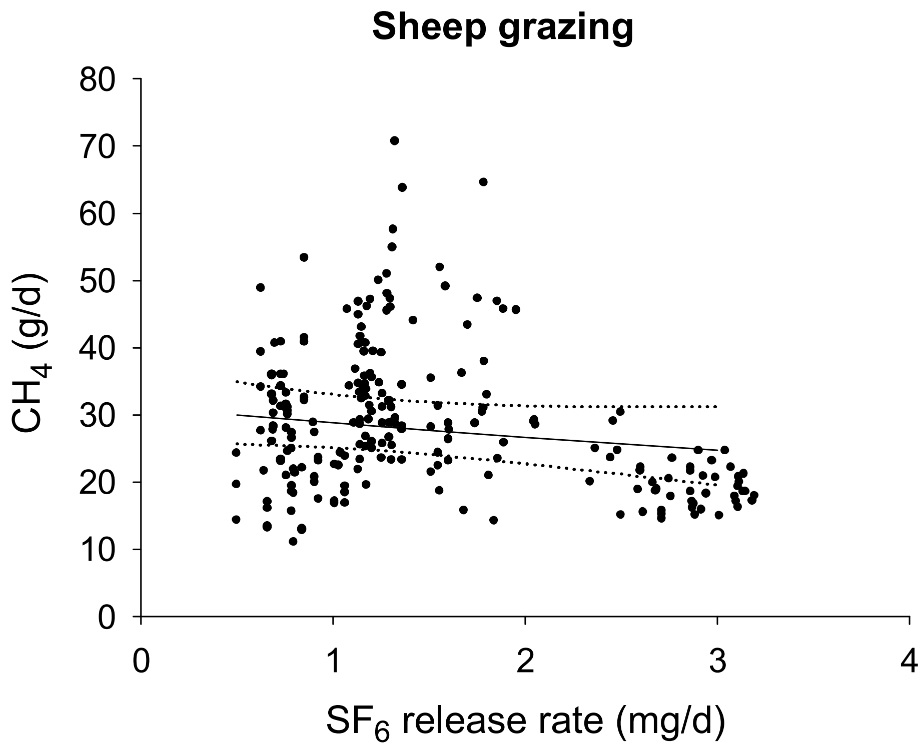


Figure 2.2 Methane data with fitted model regression (with 95% confidence intervals of the modelled line) of estimated daily CH_4 production on SF_6 release rate in grazing dairy cattle (a) and sheep for either grazing (b) or housed (c) feeding locations.

(b)



(c)

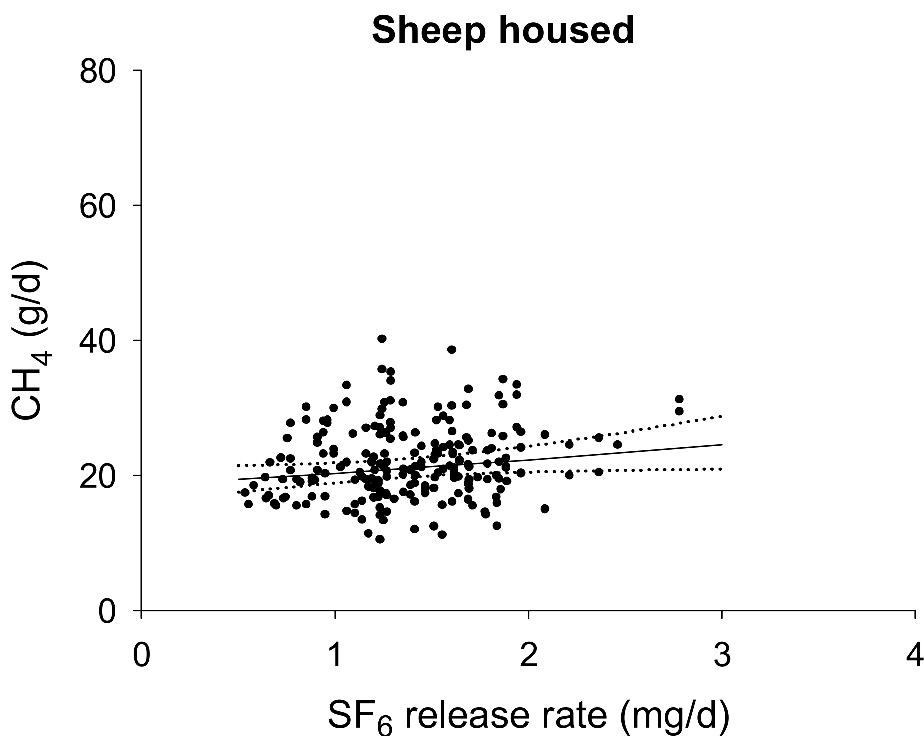


Figure 2.2 continued. Methane data with fitted model regression (with 95% confidence intervals of the modelled line) of estimated daily CH_4 production on SF_6 release rate in grazing dairy cattle (a) and sheep for either grazing (b) or housed (c) feeding locations.

Methane yield had a small positive, but non-significant, relationship with SF_6 release rate for grazing dairy cattle (Slope = 0.64, P = 0.165) (Figure 2.3a). Neither grazing sheep (Slope = -0.06, P = 0.370), nor housed sheep (Slope = 0.05, P = 0.153), displayed a significant relationship between SF_6 release rate and estimated CH_4 yield (Figure 2.3b and c).

(a)

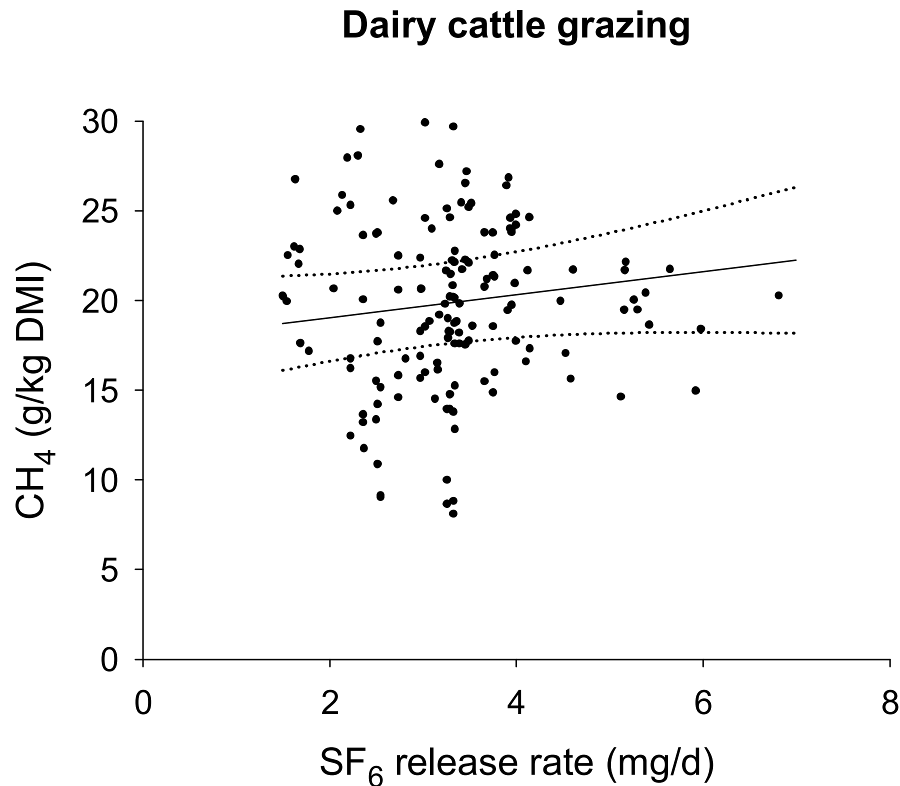
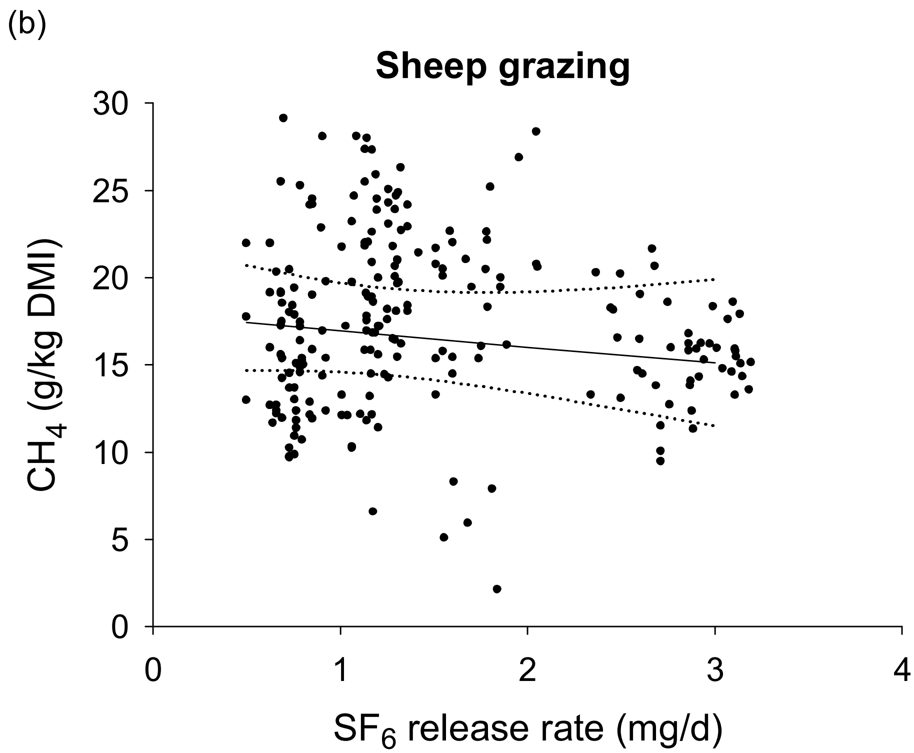


Figure 2.3 Methane data with fitted model regression (with 95% confidence intervals of the modelled line) of estimated CH_4 yield on SF_6 release rate in grazing dairy cattle (a) and sheep for either grazing (b) or housed (c) feeding locations.

(b)



(c)

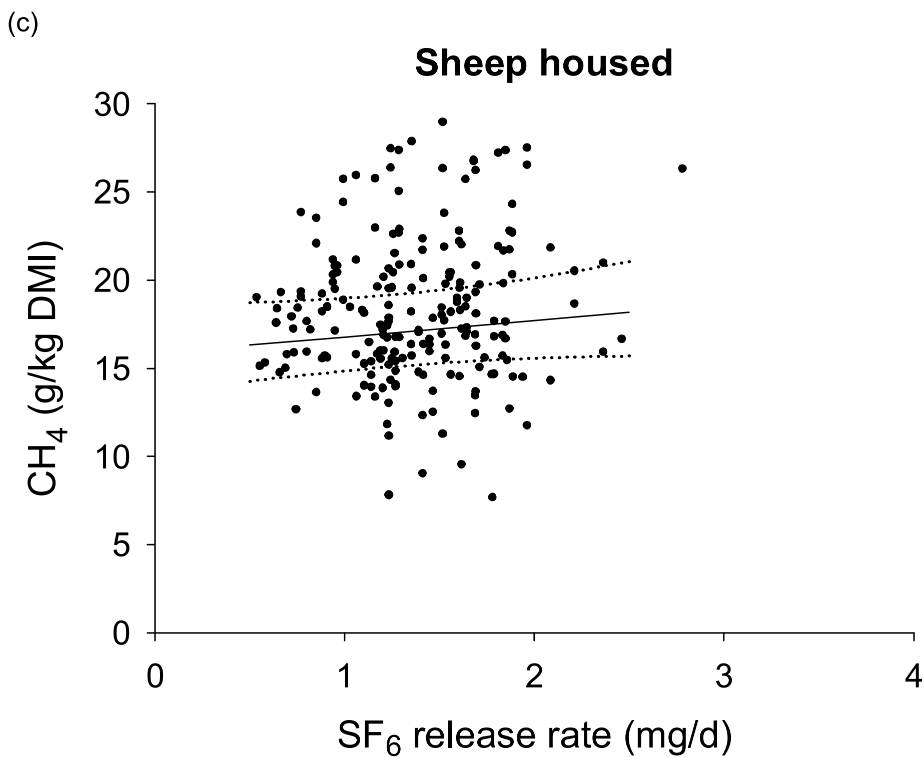


Figure 2.3 continued. Methane data with fitted model regression (with 95% confidence intervals of the modelled line) of estimated CH_4 yield on SF_6 release rate in grazing dairy cattle (a) and sheep for either grazing (b) or housed (c) feeding locations.

2.5 Discussion

The hypothesis tested was that SF_6 permeation tube release rate does not influence estimates of CH_4 emissions obtained using the SF_6 tracer technique. However, the present study suggests that in some circumstances this hypothesis should be rejected, because significant positive relationships between the SF_6 permeation tube release rate and absolute daily CH_4 production were found. Results from absolute daily CH_4 production estimates in the grazing cattle and housed sheep datasets indicate that SF_6 release rate may not be fully accounted for in the equation: CH_4 emission = SF_6 release rate x CH_4:SF_6 ratio in the breath sample.

A positive relationship between CH_4 emissions and SF_6 release rate implies that the ratio of CH_4:SF_6 in the collected breath samples rises as the SF_6 release rate increases. This is counter-intuitive as it implies a lower efficiency of SF_6 recovery as the rate of SF_6 release increases. Sulphur hexafluoride is slightly soluble in fresh water and seawater, with respective solubility coefficients 0.16 and 0.12 mol/L/atmosphere at 39°C (Bullister et al. 2002) and it has been found to partition to liquid organic phases in laboratory experiments (Wilson and Mackay, 1996). However, as the level of solubility is low, and the partial pressure in the rumen is very low, little SF_6 should be lost via this route. Further, loss of SF_6 with rumen liquor would probably have a proportionally greater effect with low rather than high SF_6 concentrations.

Permeation tube behaviour may not be constant in different milieu; in particular, they may be influenced by temperature and pressure (Bullister et al. 2002). While tubes are kept at 39ºC (rumen temperature) before deployment, they are in a dry air environment. However, the rumen contains solid, liquid and gas phase material, and is quite different to the environment in which the permeation rates are measured prior to insertion in the rumen. It is possible that by measuring release rates in dry air at 39ºC, we are not measuring the 'true' release rate of the permeation tube in the rumen. When tubes are recovered from animals, they are often encrusted with rumen matter, which may restrict flow. It has been reported that the Teflon® is not consistent across sheets, and

that SF_6 can interact with Teflon® (Lassey et al., 2001). This interaction between the SF_6 and the Teflon® membrane may account for some of the variation between tubes, and possibly some of the variation found in CH_4 estimates made using the SF_6 tracer technique. The crucial issue raised by the results presented here is why the SF_6 release rate from higher release permeation tubes may be affected to a greater extent than permeation tubes with lower SF_6 release. Although it seems plausible that release rates can be influenced through the interaction of SF_6 with the Teflon® membrane, microbial deposits on the membrane or rumen fluid entering the permeation tube it is difficult to explain why this would cause a greater reduction in SF_6 release from permeation tubes with higher release rates.

The lack of a significant relationship between CH_4 yield and SF_6 release rate for housed sheep is puzzling since there was a significant effect of permeation tube release rate on CH_4 production per day. One explanation is that the CH_4 yield in sheep seems to increase with age (Clark et al., 2003; Ulyatt et al., 2005) and the data for sheep used in these analyses included both mature and immature animals, in contrast to the cattle which were all mature animals. This extra variability in CH_4 yield due to age differences could mask any possible effect of SF_6 release rate. The data appear to support this, with greater variability in CH_4 yield for the sheep datasets than for the dairy cattle datasets even though they contain many more measurements (see Table 2.2).

Error due to inaccuracy in estimating individual feed intakes will result in extra variance in estimated CH_4 yields and this may mask any effect of SF_6 release rate on estimated CH_4 emissions. In the grazing experiments summarised here, DMI was estimated by a number of different, imperfect, methods varying in accuracy and precision (e.g. alkane internal marker, pasture mass balance, and calculations based on energy requirements) and was unlikely to have been as accurate as direct measurements carried out with housed animals.

The positive relationship reported here between SF_6 release rate and daily CH_4 production and positive trend between SF_6 release rate and estimated CH_4 yield is supported by subsequent work. In perhaps the largest study ever undertaken

with grazing cattle (McNaughton et al., 2005), CH_4 measurements were made on 300 grazing dairy cattle over a period of four weeks. Permeation tube release rates in the study ranged from 2.62 to 5.69 mg SF_6/day and there was a significant, positive relationship between SF_6 release rate and CH_4 production.

This examination of a range of experiments in New Zealand where SF_6 has been used to estimate CH_4 emissions suggests that SF_6 release rate can influence estimated CH_4 emissions. In New Zealand, a large effort has been put into quantifying CH_4 emissions from ruminants using the SF_6 tracer technique to underpin the national CH_4 inventory and anything that casts doubt on the accuracy of the technique is a cause for concern. Comparative studies carried out to date suggest that any effect of SF_6 release rate is relatively small under most circumstances, as mean values obtained using the SF_6 technique are similar to those obtained using hood calorimetry (Boadi et al., 2002a) and open-circuit calorimetry (McGinn et al., 2006; Grainger et al., 2007). However, the effect of SF_6 release rate identified here may make a significant contribution to the greater variability in CH_4 estimates made using the SF_6 tracer technique.

These results at the very least indicate that further research is required to (1) confirm, quantify and explain the relationship between SF_6 release rate and estimated CH_4 production and yield when using the SF_6 tracer technique and (2) assess whether absolute SF_6 release rate can at least partially explain why estimates obtained using the SF_6 tracer method are more variable than those obtained using calorimetry.

Chapter 3

The possible influence of intra-ruminal sulphur hexafluoride release rates on calculated methane emissions from cattle

Most of this chapter has been published in: *Canadian Journal of Animal Science* (2007) 87: 269-275.

3.1 Abstract

Estimates of methane (CH_4) production from grazing animals are routinely made using the sulphur hexafluoride (SF_6) tracer technique. While this technique is generally regarded as useful, some investigators report a higher variability in measurements when compared with calorimetry. The SF_6 technique is a marker dilution method in which a known release rate of SF_6 from an intra-ruminal permeation tube is used to calculate CH_4 emissions from the ratio of SF_6:CH_4 in expired breath. The release rate of SF_6 is unique for each tube, and although calculated CH_4 emissions should be independent of SF_6 release rate, an analysis of research conducted in New Zealand has suggested a possible influence of SF_6 release rate upon calculated CH_4 emissions (Chapter 2).

A modified cross-over design, with two groups of six steers given either one (2.878 mg SF_6/d) or two (7.336 mg SF_6/d) permeation tubes and offered either energy maintenance (M) or 2 x M levels of feed intake was undertaken to determine the effect of SF_6 release rate and intake on calculated CH_4 emissions.

A high SF_6 release rate elevated the calculated CH_4 production per day (P < 0.001) and per kg dry matter intake by 19% (P < 0.001) irrespective of the level of intake. Level of intake had no effect on estimated CH_4 yield.

Release rate of SF_6 can affect the calculated CH_4 emissions from animals when employing the SF_6 tracer technique, with the difference in estimated CH_4 yield between a 3 and a 5 mg SF_6 release rate approximately 8.5%. It is recommended that SF_6 release rates used in experiments are within a narrow range and are balanced across experimental treatments.

3.2 Introduction

Inter-animal variation in the quantity of CH_4 emitted per kg dry matter intake (kg DMI) has been observed for several animal species, time periods and diets (Lassey et al., 1997; Pinares-Patiño et al., 2003b; Goopy and Hegarty, 2004). Measurements made using the SF_6 tracer technique appear to be more variable than measurements obtained using calorimetry. For example, Lassey et al. (1997), McNaughton et al. (2005), and Boadi and Wittenberg (2002) obtained coefficients of variation (CV) of 11.5%, 15.5% and 25%, respectively from SF_6 studies whereas Blaxter and Clapperton (1965) quote values of 7-8% for calorimetry studies. Exploiting animal-to-animal variation in CH_4 emissions may provide a potential route for reducing CH_4 emissions from ruminants, but it is important to clearly separate any variation associated with the measurement technique from actual variation in CH_4 production.

The SF_6 tracer technique for calculating CH_4 emissions was developed by Johnson et al. (1994a) and is the method of choice for grazing studies in NZ (Lassey et al., 1997). It is the only method able to assess CH_4 emissions from individual ruminants under grazing conditions and for screening large groups of individual animals simultaneously.

A well-calibrated source of SF_6 released into the rumen headspace is critical to the efficacy of the technique. Permeation tubes that release the SF_6 are individually manufactured and assembled, and each tube has a unique release rate (Lassey et al., 2001). Release rates are measured accurately (to 0.00005 g) through weekly weighing for at least 3 months before insertion into an animal. Typical release rates from the permeation tubes in cattle are in the range 2-5 mg SF_6/day in New Zealand. It is not yet possible to manufacture permeation tubes to a specified SF_6 release rate, possibly due to non-uniformity of the Teflon® membrane through which gas emerges from the permeation tube (Lassey et al., 2001).

It is well documented that CH_4 production is related to the level of feed intake (Blaxter and Clapperton, 1965), but there are no data to ascertain whether the level of intake, and hence rumen outflow rate, affects the release of SF_6 in the breath. The SF_6 technique relies on the assumption that all SF_6 released from the tube is well mixed with other rumen gases and expelled with those gases in the breath. Wilson and Mackay (1996) found that SF_6 did not adhere to solid organic phases of media in a laboratory experiment, but that it could partition to liquid organic phases. It has been reported that SF_6 is extremely stable in the atmosphere, and is soluble at trace levels in fresh water and seawater, with respective solubility coefficients 0.16 and 0.12 mol/L/atmosphere at 39°C (Bullister et al., 2002). As the partial pressure of SF_6 in the rumen is extremely low, very little would tend to be solubilised. On this basis, it is assumed in animal studies that there is no interaction between SF_6 and rumen contents.

This study was undertaken because evaluations of CH_4 production from grazed cattle and housed sheep experiments (see Chapter 2) and a cow grazing experiment (McNaughton et al., 2005) demonstrated a positive relationship between SF_6 release rate and estimated daily CH_4 production in some circumstances. Any relationship of this nature would increase between-animal variance and have consequences for both experimental design and the estimated uncertainty in the national ruminant CH_4 emission inventory. The primary aim of the experiment reported here was to measure the extent to which the SF_6 release rate might influence calculated CH_4 production from individual animals. A secondary objective was to determine whether there was an interaction between the level of feed intake and SF_6 release rate on CH_4 production.

3.3 Materials and methods

3.3.1 Experimental design

An experiment was conducted at AgResearch Grasslands in Palmerston North, New Zealand (NZ) with 12 rumen-fistulated Hereford x Friesian steers in May 2004. Methane emissions were estimated using the SF_6 tracer technique developed by Johnson et al. (1994a) and adapted for NZ grazing conditions by Lassey et al. (1997). Either one or two permeation tubes were administered to animals to estimate CH_4 emissions and to assess if there was an influence of absolute SF_6 release rate on calculated CH_4 emissions. Permeation tubes were chosen to cover the range in SF_6 release rates used in cattle experiments in NZ. The experiment ran for 4 weeks, with the first two weeks consisting of an 'adjustment' period to the diet, facilities and the CH_4 measuring equipment, and the second two weeks the 'measurement' period. The research was approved by the AgResearch Animal Ethics Committee.

The twelve steers were allocated to two groups for the duration of the experiment and were offered either a low (maintenance) or high (twice maintenance) feeding allowance throughout the experiment; the quantity of feed offered was calculated using the Australian Feeding Standards system (Standing Committee on Agriculture, 1990).

Within each feeding level group, the animals were randomly assigned to two SF_6 release rate groups. The mean SF_6 release rates (mean $\pm$ SEM) were 2.878 ± 0.051 and 7.336 ± 0.050 mg SF_6/d, achieved by administering either one or two permeation tubes to each animal. Consequently, there were four groups of three steers in each measurement period: low intake with low SF_6 release rate, low intake with high SF_6 release rate, high intake with low SF_6 release rate, and high intake with high SF_6 release rate. All tubes were recovered *per fistula* on day 20 and reallocated *per fistula,* so that steers that had a low release rate for the first measurement period had a high release rate for the second measurement period, and vice versa. Swapping of the

permeation tubes was carried out within feeding level group and all animals remained on a high or low feeding level for the duration of the experiment.

3.3.2 Animals and feeding

The steers had a mean weight of 409 kg (SD ± 25 kg) at the beginning of the 26-day experimental period. For the 14-day diet adjustment period the animals were group-fed in their feeding level groups on outdoor feed pads. Animals received the maintenance and twice maintenance feeding regimes during the 'adaptation' period. During the measurement period (days 15 to 26) they were housed in individual stalls in a well-ventilated barn.

Steers had *ad libitum* access to fresh drinking water throughout the experiment. The diet (dry matter, DM, basis) consisted of 70% ensiled lucerne (*Medicago sativa*), which contained small, but unknown, quantities of molasses (ChaffHage™, The Great Hage Company, Reporoa, NZ), and 30% poor quality meadow hay (predominately *Lolium perenne*). Feed was weighed and offered twice daily at 0900 and 1600 h in two equal quantities. Feed refused was collected before the morning feeding each day, weighed, and sub-sampled for DM determination to calculate DM intakes. Feed refused for all animals was hay, apart from one animal on one day that did not consume all of its lucerne silage.

Chemical composition of feed offered and refused was determined by Near-Infrared Reflectance Spectrometry (NIRS) (Corson et al., 1999). Chemical composition by NIRS was calibrated using a database of wet chemistry analyses. Organic matter digestibility for the hay was calibrated against values from *in vivo* digestibility experiments, while *in vitro* DM digestibility for the silage was calibrated against an *in vitro* method using cellulase enzyme solution and silage reference standards as controls (Corson et al., 1999). Composition of the diet eaten was calculated using the proportion of DMI from each dietary constituent and the chemical composition of those constituents.

3.3.3 Methane measurements

Permeation tubes were administered to the animals on day 9 of the experiment. Methane emissions were measured using the SF_6 tracer technique (see chapter 2 for detailed description) between days 15-19 (period 1), and again for days 22-26 (period 2) following the exchange of the permeation tubes.

Gas samples were collected in a pre-evacuated PVC canister (yoke) mounted on the animals back via a tube mounted about 5 cm dorsal to the nostrils that was held in place by a halter. A capillary tube restricted airflow from the tube mounted adjacent to the nostrils to about 1 ml/min. The yokes were approximately half-filled over the 24-hour collection period and were fitted or exchanged at approximately 0900 hour daily during CH_4 measurements. Two background air samples were collected during each day from the barn.

Gas samples collected in the yokes were measured with flame ionisation and electron capture detectors for CH_4 and SF_6, respectively, using a gas chromatograph (Hewlett Packard 5890 Series II). The NZ National Institute of Water and Atmospheric Research independently calibrated the gas chromatograph prior to the start of the experiment. The analysis procedure involved a partial extraction of gas from the yoke and pressurising the sample with a pump system to facilitate transfer to the gas chromatograph. A set of three standards containing known concentrations of both SF_6 and CH_4 were used for calibration against which both CH_4 and SF_6 were measured. The standards were run before and after the analysis of the 54 samples (yokes and background samples) from each measurement period, and one standard was included after every tenth experimental sample. Standards were analysed in triplicate, and samples in duplicate.

The release rate (mg SF_6/day) of the SF_6 tracer gas and the ratio of SF_6 to CH_4 in the breath were used to calculate the CH_4 emissions (g CH_4/day) of each animal (Q_{CH4}):

$$Q_{CH4} = Q_{SF6} \times [CH_4]/[SF_6]$$

where $[CH_4]$ and $[SF_6]$ denote the concentrations (in ppm and ppt, respectively) in the yokes after background corrections, and Q_{SF6} is the release rate of SF_6 (mg/day) from the permeation tube(s).

3.3.4 Permeation tubes

Permeation tubes were manufactured and calibrated by the NZ National Institute of Water and Atmospheric Research. These tubes were charged with SF_6 in August and September 2003 with an average of 2.97 g SF_6 per tube. The tubes were kept in a 39°C environment (rumen temperature) and individually weighed at weekly intervals until placement in the steers for this experiment. Gravimetric weighing was used to calculate the SF_6 release rate from the tubes using 10 weights obtained prior to insertion into the steers as recommended by Lassey et al. (2001). Release of SF_6 from the permeation tubes was very constant over time, with linear regression of weight loss showing an $R^2 > 0.995$ for all tubes.

Permeation tubes were recovered *per fistula* at the completion of the experiment and weighed for a further 5 weeks to determine post-experiment release rates. The 10 pre- and 5 post-experiment weights were used to calculate a 'corrected' SF_6 release rate during the experiment using the method described by Lassey et al. (2001). This method uses the weights from pre- and post-experimental weighing and applies quadratic curvature to infer the release rate during the experimental period.

3.3.5 Statistical analysis

The experimental design was a modified cross-over; a cross-over for SF_6 release rate, but with animals on a set level of feeding for the duration of the experiment. Data were analysed using a general analysis of variance (ANOVA) to determine the effect of SF_6 release rate and feeding level on calculated CH_4 emissions (both g/d and g/kg DMI), using Genstat version 9 (Genstat Committee, 2002). Treatment structure included the effect of feeding level, SF_6 release rate, and the interaction between feeding level and SF_6 release rate. Block structure included the effects of steer, time and the interaction between steer and time. Four-day mean DMI and CH_4 emissions for each steer were used in all analyses. Failure to collect a representative breath sample occurred on a single day for four different animals and in these cases the CH_4 estimate is the mean of three daily samples.

3.4 Results

3.4.1 Feed intake

Steers fed at maintenance ate 95% of feed offered (5.85 kg DM $\pm$ SEM 0.11) compared to 73% of feed offered in the high intake group (8.98 kg DM $\pm$ SEM 0.16) ($P < 0.001$). The high intake group refused mainly hay, so their diet differed slightly from those fed maintenance (Table 3.1), with a lower neutral detergent fibre and higher crude protein concentration in the DM. Intakes were similar for the low and high intake groups in both measurement periods (Table 3.1).

3.4.2 SF_6 release rate

Release rates calculated using 10 pre-experimental weights (low 2.878, high 7.336 mg SF_6/d) were slightly higher than release rates calculated with 10 pre-experimental and 5 post-experimental weights (low 2.800, high 7.182 mg SF_6/d). Methane yields calculated using the two different release rates were similar (pre-experimental weights 20.0±0.5 g CH_4/kg DMI, pre-experimental and

post-experimental weights 19.5 ± 0.5 g CH_4/kg DMI). The correlation between the two sets of results was 0.991 and the recommendation of Lassey et al. (2001) to use 10 pre-experimental weights for calculating SF_6 release rates for experiments within 4 weeks of permeation tube insertion was followed.

Table 3.1 Chemical composition of ChaffHage™ and meadow hay offered during the experiment and of the diets eaten by steers offered maintenance (M) and 2 x maintenance (2xM) intakes estimated by near-infrared reflectance spectrometry.

	Composition of feed offered (% of DM)		Composition of feed eaten (% of DM)	
Chemical Component	ChaffHage™	Meadow hay	M	2xM
Crude protein	18.0	9.1	15.4	16.6
Lipid	5.0	1.6	4.0	4.5
Ash	10.3	7.3	9.4	9.8
Acid detergent fibre	38.6	42.7	39.8	39.3
Neutral detergent fibre	45.9	70.0	53.1	49.8
Soluble carbohydrate	4.5	6.1	5.0	4.8
DM Digestibility[1]	66.5	56.9	63.7	65.1
ME (MJ/kg DM)	10.6	8.5	10.0	10.3
DM intake (kg/steer/d)				
Period 1			5.91	8.86
Period 2			5.78	9.09

Abbreviations. DM, dry matter; ME, metabolisable energy; MJ, megajoule.
[1]Meadow hay value is reported as organic matter digestibility, and converted to DMD using DMD = 0.939*OMD + 3.67 (MAFF, 1984) for the composition of diet eaten.

3.4.3 Background gas concentrations

Background concentrations of both CH_4 and SF_6 were low relative to sample concentrations. Measured background values of both gases are shown in Table 3.2. Average concentrations of CH_4 and SF_6 in collected samples for both the high and low SF_6 release groups are shown in Table 3.3. The concentration of

SF_6 in the collected breath sample was higher for the high SF_6 release group than for the low SF_6 release group, while CH_4 concentrations were similar.

Table 3.2 Daily background concentrations of SF_6 (ppt) and CH_4 (ppm) measured from two locations in the barn during the two measurement periods.

		Day 1		Day 2		Day 3		Day 4	
		CH_4	SF_6	CH_4	SF_6	CH_4	SF_6	CH_4	SF_6
Period 1	Location 1	8.17	26.80	8.59	32.11	6.94	21.95	7.52	23.77
	Location 2	10.38	33.74	11.53	38.93	13.33	43.22	11.07	36.07
Period 2	Location 1	8.86	33.43	9.12	40.67	7.82	26.00	10.06	34.57
	Location 2	10.87	34.51	10.70	39.28	10.84	33.24	14.38	41.30

Table 3.3 Average concentrations of CH_4 (ppm) and SF_6 (ppt) in gas samples collected from steers with either a single (low SF_6 release) or two (high SF_6 release) permeation tubes during period 1 and period 2 of measurement[1].

	Period 1		Period 2	
	CH_4	SF_6	CH_4	SF_6
Low SF_6 release	130.3 ± 15.5	320.7 ± 26.3	110.8 ± 7.4	263.7 ± 16.5
High SF_6 release	134.0 ± 8.2	630.2 ± 31.4	138.2 ± 9.9	704.0 ± 44.6

[1]Data are mean ± SEM.

3.4.4 Methane

Both feeding level and SF_6 release rate significantly affected calculated CH_4 production (g/d; $P < 0.001$; Table 3.4), although the effect of SF_6 release rate was greater at the high level of feeding (22% increase) than at the low level of feeding (16.5% increase). When CH_4 emissions were expressed per kg DMI (CH_4 yield), feeding level no longer had an effect on calculated CH_4 yield ($P >$ 0.05; Table 3.5) (for raw data see Appendix 1). However, there remained a significant effect of SF_6 release rate on CH_4 yield ($P < 0.001$) with values 19% higher when based on a high SF_6 release (21.8 g CH_4/kg DMI) compared to the low rate (18.3 g CH_4/kg DMI). Estimated energy loss of CH_4 as a proportion of the metabolisable energy (ME) intake was higher for the high SF_6 release rate

(~12% of ME) than the low SF_6 release rate (~10% of ME), although the significance of this difference was not tested in the analysis. There was a significant interaction ($P < 0.05$) between CH_4 production and SF_6 release rate, but no significant interaction between CH_4 yield and SF_6 release rate ($P = 0.92$). No significant period effect was observed.

Table 3.4 Methane production (g/d) for two groups of six steers offered maintenance (M) and 2 x M feed and given either a single (Low SF_6 release) or two (High SF_6 release) permeation tubes[1].

	M	2xM (g/d)	Mean	Significance
Low SF_6 release	110.8 ± 5.6	157.6 ± 5.1	134.2 ± 7.9	Feeding level F = 0.001
High SF_6 release	129.1 ± 3.0	192.5 ± 7.1	160.8 ± 10.2	SF_6 release F < 0.001
Mean	119.9 ± 4.1	175.0 ± 6.7		Feed x SF_6 F = 0.041

[1]Data are mean ± SEM.

Table 3.5 Methane yield (g/kg DMI) for two groups of six steers offered maintenance (M) and 2 x M feed and given either a single (Low SF_6 release) or two (High SF_6 release) permeation tubes[1].

	M	2xM (g/kg DMI)	Mean	Significance
Low SF_6 release	18.9 ± 1.1	17.7 ± 0.2	18.3 ± 0.6	Feeding level F = 0.199
High SF_6 release	22.3 ± 0.6	21.2 ± 0.6	21.8 ± 0.4	SF_6 release F < 0.001
Mean	20.6 ± 0.8	19.5 ± 0.6		Feed x SF_6 F = 0.923

[1]Data are mean ± SEM.

3.5 Discussion

The data presented here provide direct empirical evidence of a positive relationship between SF_6 release rates and calculated CH_4 emissions (g/d and g/kg DMI) and confirms previous indications of positive relationships (McNaughton et al., 2005; Chapter 2). McNaughton et al. (2005) demonstrated

a positive relationship in a grazing experiment involving 301 lactating dairy cows (R^2 = 0.15; P < 0.001), while Chapter 2 reported regressions between SF_6 release rates and absolute daily CH_4 production that were different from zero in experiments with grazed cattle (P = 0.023) and housed sheep (P = 0.035). Results from the present experiment further suggest that the influence of SF_6 release rates on CH_4 yield does not depend upon the level of feeding.

The 19% difference in calculated CH_4 yields, when using an SF_6 release rate of 7 mg/d versus 3 mg/d, is not easily explained. The positive relationship between SF_6 release rate and calculated CH_4 emissions, expressed either as production or yield, implies that for an increasing SF_6 release rate the ratio of SF_6:CH_4 in the collected breath sample increases more slowly than the SF_6 release rate. This would indicate a lower SF_6 recovery at higher SF_6 release rates, which is not expected for a conservative tracer. It is not clear why this lower recovery would occur, and the opposite may be expected if SF_6 recovery was associated with adsorption onto digesta fractions in the rumen. However, adsorption onto digesta fractions seems unlikely as SF_6 does not adsorb onto solid organic phases of media (Wilson and Mackay, 1996) and the very low concentration of SF_6 in rumen headspace gas (2-5 mg SF_6 (New Zealand cattle release rates) in ~2200 L of carbon dioxide and ~700 L CH_4 per cow per day (Moate et al., 1997)) should have a negligible solubility (Bullister et al., 2002).

Background concentrations of both SF_6 and CH_4 were elevated relative to values obtained in grazing experiments and it is possible that this affected the results. Background levels of SF_6 and CH_4 are normally in the region of 11 parts per trillion (ppt) and 2 parts per million (ppm), respectively (Tavendale and Klein, 2003), compared with the 34 ppt and 10 ppm recorded here. However, previous work with sheep using SF_6 permeation tubes with release rates < 2.5 mg/d has shown that elevated backgrounds similar to those reported here had no significant effect on CH_4 estimates (Tavendale and Klein, 2003). In our experiment, although background concentrations of SF_6 and CH_4 were elevated, the collected breath sample concentrations were 7 to 10 fold higher than background concentrations and although an inadequate estimation of background concentrations cannot be entirely ruled out, it seems unlikely that

these elevated backgrounds had an influence on the estimated CH_4 emissions. Further, issues with background concentrations cannot explain the relationship between SF_6 permeation tube flow rate and estimated CH_4 production reported in a grazing experiment by McNaughton et al. (2005).

To test the sensitivity of CH_4 estimates to lower or higher background gas concentrations, calculations were carried out for each CH_4 measurement day using the measured breath sample concentrations and background concentrations that were either half or double the measured concentrations. It was assumed that if one gas was lower or higher in the background sample, the other gas would follow the same pattern. Either halving or doubling the daily background concentrations of CH_4 and SF_6 made a mean difference of less than 1% to the estimated CH_4 yield for the first set of measurements in this experiment (Figure 3.1).

High background concentrations had a greater influence than low concentrations, but this effect was still small. It is interesting to note that the high background concentrations (~20 ppm CH_4 and ~60 ppt SF_6) plus high SF_6 release rate (7 mg/day) resulted in consistently lower estimates of CH_4 yield, whereas high backgrounds plus low SF_6 release rate (3 mg/day) resulted in higher estimated CH_4 yield. During the second measurement period this same phenomenon was again observed. This result is due to the higher proportion of the background SF_6 (~30 ppt) relative to the sample concentration from the low SF_6 release rate (~ 300 ppt) compared with the high release rate (~650 ppt). This finding indicates that if both gases are not well mixed, with similar behaviour and collection efficiency, the SF_6:CH_4 ratio may be affected. While this effect was small, it shows the importance of adequate ventilation, gas mixing, and equal collection efficiency of the gases. Further, issues with background concentrations cannot explain the relationship between SF_6 permeation tube flow rate and estimated CH_4 production reported in a grazing experiment by McNaughton et al. (2005).

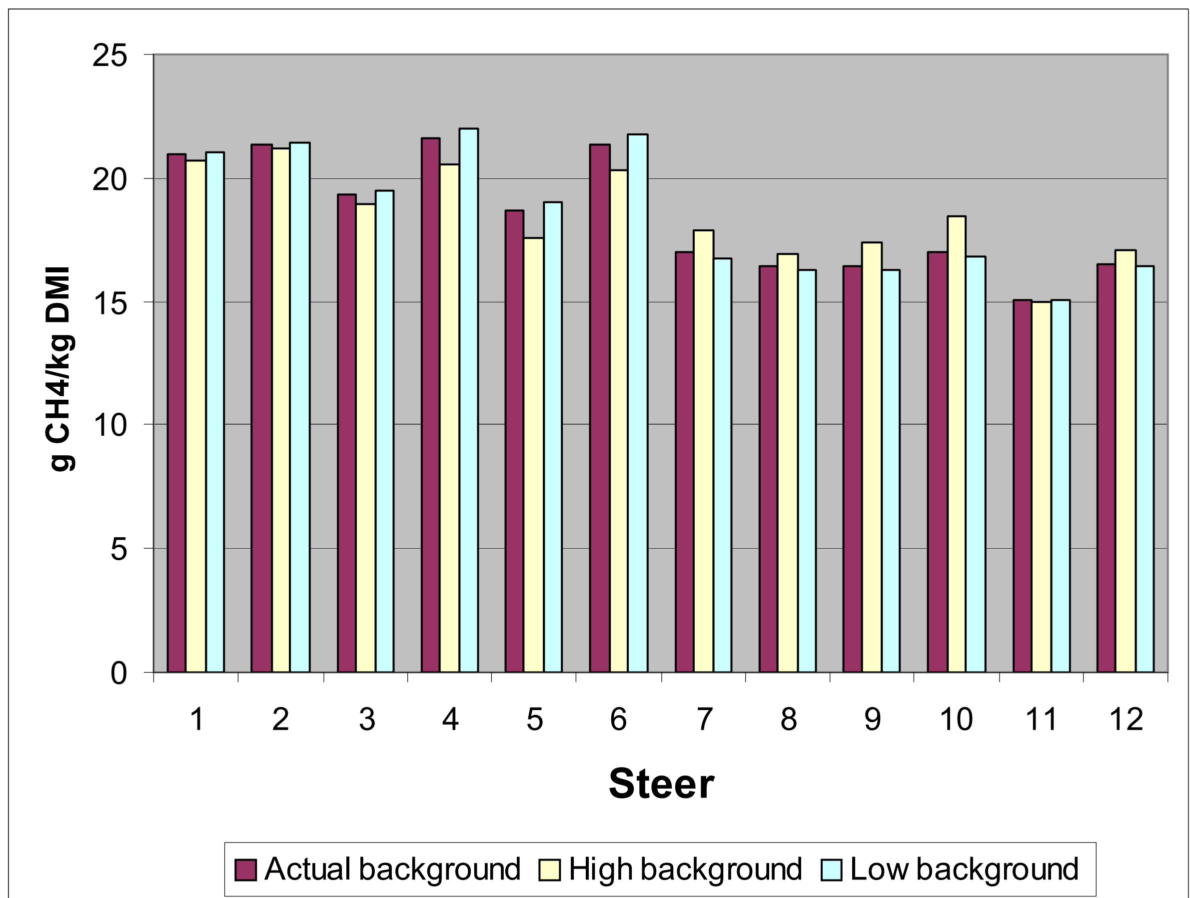


Figure 3.1 Estimated CH_4 yield from 12 steers during the first week of measurement, calculated with actual, high (double) and low (half) background concentrations of CH_4 and SF_6.

An alternative explanation for the influence of SF_6 release rates is that SF_6 may be released from the rumen via another route. This could occur through losses to the lower digestive tract by SF_6 being either directly washed out of the rumen as gas, in solution, or adhering to feed particles. While SF_6 can dissolve into some body tissues, this SF_6 will diffuse into the bloodstream and will be released into the lungs and out in the breath (Schrikker et al., 1989; Schimmel et al., 2004). It is possible that SF_6 could be lost in excrement, and this should be tested. However, it is hard to imagine why proportionally higher SF_6 loss would occur at higher release rates.

It is also possible that the explanation for the results reported here lies with the actual measurement of SF_6 from the breath samples. Calibration of standards and the linearity of the standard curve are important for converting sample concentrations to quantities of SF_6. If the calibration was not linear this could

cause a systematic bias due to the fact that the samples were measured at different points along the calibration curve. However, three standards at concentrations of 15 to 1000 ppt are used, and there is a log relationship across the standards. Figure 3.2 shows the log relationship of the detector response across the range of standard gases using the measured concentrations from the analyses of this particular experiment.

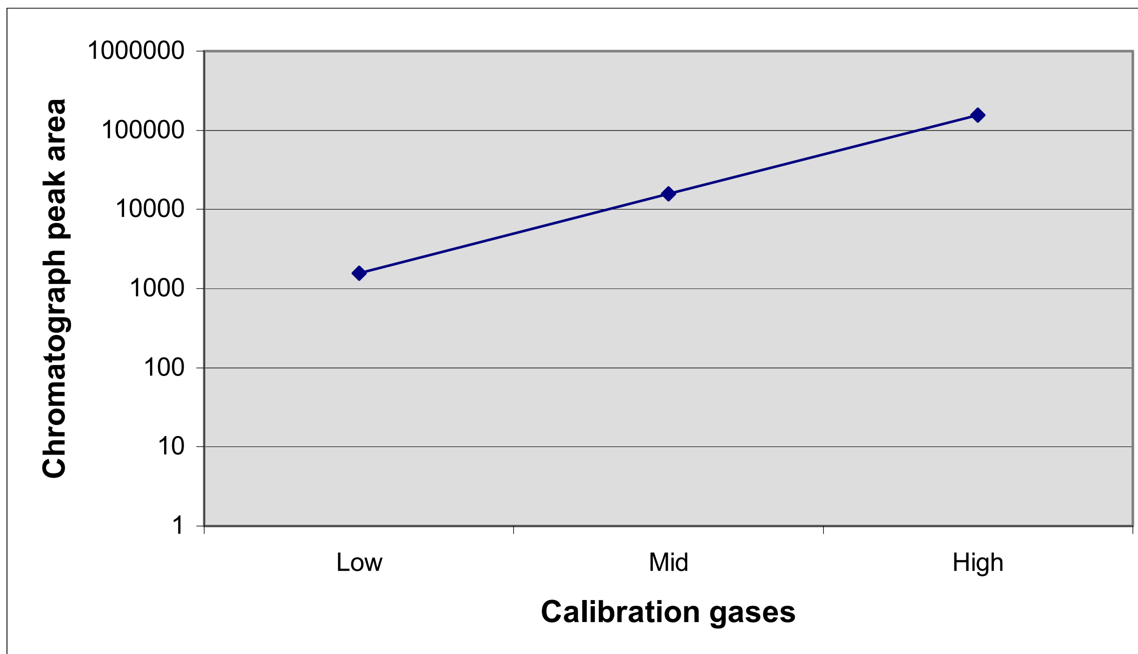


Figure 3.2 Measured SF_6 chromatograph peak area (logarithmic scale) for the three standard gases (15, 150 and 1000 ppt) used to calibrate collected samples against.

It can be seen that there is a logarithmic response over the concentration range of the standard gases. Further, the gas chromatograph was independently calibrated by the NZ National Institute of Water and Atmospheric Research prior to this experiment. This was done to give confidence that the results would not be biased by measurement error. These factors strongly suggest that the difference in estimated CH_4 yields between the low and high SF_6 release rate cannot be attributed to any systematic bias in the measurement of SF_6 concentrations in the collected breath samples.

The findings presented here demonstrate the need for diligence in the use of markers and may explain some of the additional variance associated with the

SF_6 tracer technique (CV = 15.5%, McNaughton et al., 2005) compared to calorimetry (CV = 7-8%, Blaxter and Clapperton, 1965). However, the variability in CH_4 emissions attributable to using SF_6 permeation tubes with differing SF_6 release rates may, in practice, account for only a small proportion of the between-animal variation in calculated CH_4 emissions that is a feature of all CH_4 estimates obtained using the SF_6 technique (see Chapter 2). This is simply because the 2.5-fold difference in SF_6 release rates used in our experiment is far higher than the range used in typical experiments carried out in New Zealand. In one of the largest CH_4 experiments (300 cows) undertaken in New Zealand to date, a single batch of 355 permeation tubes was utilised (McNaughton et al., 2005) and Figure 3.3 shows the distribution of SF_6 release rates from this batch of permeation tubes.

It is clear from Figure 3.3 that the vast majority (76%) of permeation tubes in this batch have a release rate between 3.0 and 5.0 mg SF_6/day although a small number of tubes have release rates outside this range, with the distribution skewed towards a higher SF_6 release rate. Using the difference in estimated CH_4 yield between the 3 and 7 mg SF_6/day release rates and assuming a linear relationship between these two points, the difference in estimated CH_4 yield between a 3 and a 5 mg SF_6 release rate would be approximately 8.5%. If the influence of SF_6 release rate on calculated CH_4 is linear across the range of tubes used in practice, there will be no effect of SF_6 release rate on average per animal emissions estimated for a group of animals. However, the effect is important when comparing emissions from selected individual animals. Ulyatt et al. (2002c) report differences of up to 40% in CH_4 emissions from individual animals estimated using the SF_6 tracer technique and suggest that exploiting these differences is a possible way of mitigating enteric CH_4 emissions. If our findings are applicable in all situations some of these differences between individuals could be somewhat smaller once the influence of SF_6 release rate is taken into account.

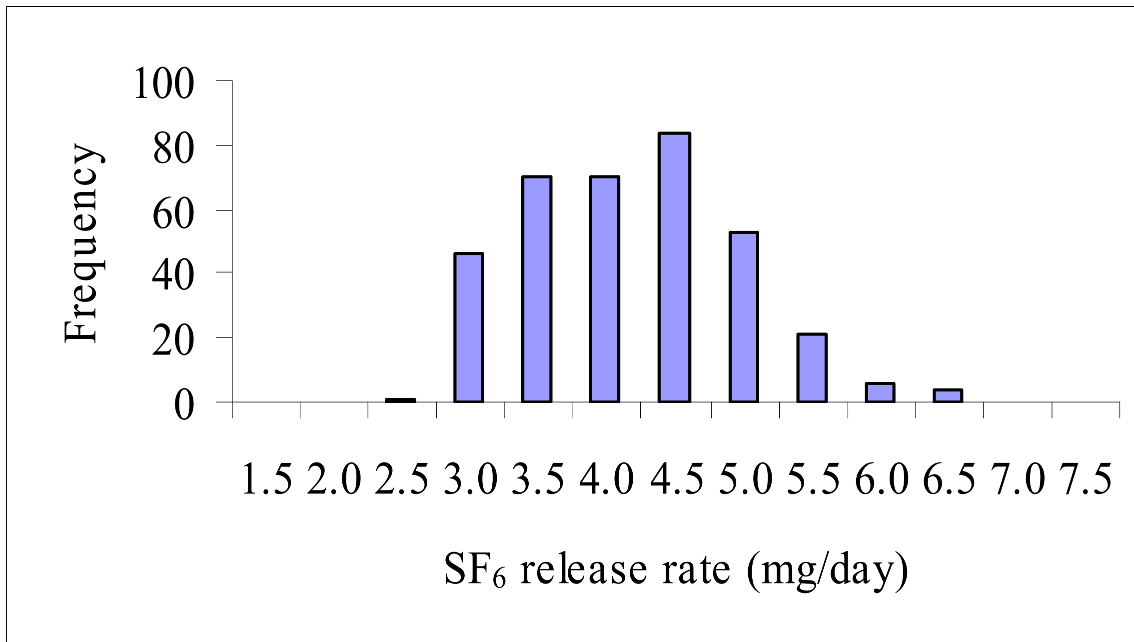


Figure 3.3 Distribution of SF_6 release rates from a single batch of 355 permeation tubes used in one experiment (see McNaughton et al., 2005).

Between-animal variation becomes of practical importance when comparing groups of animals or treatments, as it increases the number of animals required to detect treatment differences. A further implication of the influence of SF_6 release rate on CH_4 estimates is that considerable care needs to be taken when comparing values obtained using calorimetry with those obtained using the SF_6 tracer technique, since these comparisons, especially for individual animals, will not be independent of SF_6 release rate.

Further research is needed to confirm the findings presented here, establish the causes of the influence of SF_6 release rate and to define the range in SF_6 release rates that are able to provide robust and defensible estimates of CH_4 production from ruminants.

Chapter 4

In vitro assessment of the sulphur hexafluoride (SF_6) release rate in three media and daily loss of SF_6 in urine and faecal gas

4.1 Abstract

Greater variation can occur in estimated methane yield (g CH_4/kg DMI) when the values are obtained using the sulphur hexafluoride (SF_6) tracer technique compared with animal calorimetry measurements. One possible source of variation is the permeation tube release rate. Release rates of permeation tubes are currently determined in dry air, and it is not known whether the release rate stays constant when permeation tubes are placed in the rumen milieu. An *in vitro* experiment was conducted to measure release rates from six permeation tubes in air, water and strained rumen fluid.

Results indicate that SF_6 release is constant in dry air, with a slightly lower (~8%) daily release rate using the technique developed in this chapter than gravimetric weighing. The mean release rate from all six tubes in air (3.51 mg SF_6/day) was significantly higher (p=0.001) than in water (2.55 mg SF_6/day) or rumen fluid (2.34 mg SF_6/day). However, results from 10 to 12 consecutive hourly measurements with a single tube indicate that the reduction in SF_6 release from tubes placed in water (6%) and rumen fluid (11%) is not as great as that found for the three one-hour measurements (water = 27%, rumen fluid = 33%). An experiment was conducted to assess whether SF_6 was lost via urine or in gas trapped in faecal material. Total daily loss, corrected for background concentration, from both sources was approximately 7.46 x 10^{-5} mg SF_6/day, compared with a mean permeation tube release of 4.6 mg/day. This was 0.0016% of the quantity of SF_6 released from a permeation tube per day.

This research suggests that a lower than expected release rate, ~10%, may occur when permeation tubes are placed in the rumen. Analyses of urine and faecal gas indicate that insignificant quantities of SF_6 are released via either of these routes.

4.2 Introduction

It has been reported in Chapters 2 and 3 that there is larger variation in estimated daily CH_4 production and yield using the SF_6 tracer technique than measurements carried out with animal calorimetry. One possible cause of increased variation in estimated CH_4 production is a relationship between SF_6 release rate and estimated CH_4 yield. Chapter 3 showed that CH_4 yields were approximately 19% higher when using a release rate of about 7 mg SF_6/day compared to 3 mg SF_6/day. However, Chapter 3 concluded that variation in SF_6 release rate would account for only a small proportion of this extra variance in CH_4 production when using the SF_6 technique.

Having a well-calibrated source of SF_6 released into the rumen headspace is critical to the efficacy of this technique. One of the underlying assumptions is that the SF_6 is released at a constant, known rate from a permeation tube inserted into the animal's rumen. Permeation tubes are currently calibrated by serial, weekly weighing in dry air at 39ºC. Weekly values are then translated to daily release rates by looking at the weight loss of the tube over time. This method gives an accurate measure of SF_6 release from each individual tube in air. It is assumed that the permeation tube release rate remains constant when the permeation tube is administered to an animal.

The rumen milieu is not the same as dry air, as the tube is in a fluid environment, with various solutes and particulate matter. Two possible things could affect the reliability of SF_6 release from the tube. First, the tube may not have the same release rate in the rumen as in dry air. Second, the release rate may be more variable in the rumen than in dry air. While there is no apparent explanation why this would occur, it is known that there is a discrepancy between the actual and expected weights of permeation tubes that have been administered and retrieved from animals (Lassey et al., 2001). This weight discrepancy was attributed to rumen contents on the permeation tube. Another possible explanation is that the release rate of SF_6 may have been lower in the rumen, resulting in the higher than expected weights of retrieved tubes.

A study was undertaken to test the hypotheses that the SF_6 release rate from permeation tubes will not differ when placed in air, water or strained rumen fluid. Data from this study should help to confirm the assumption that the permeation tube release rate is the same in the rumen milieu as in dry air.

Another underlying assumption is that SF_6 released from the permeation tube is well mixed with rumen gases and eructated with those gases. While SF_6 appears to be extremely stable in the atmosphere, it is soluble at trace levels in fresh water and seawater, with respective solubility coefficients 0.16 and 0.12 mol/L/atmosphere at 39°C (Bullister et al. 2002). While SF_6 has been shown not to adhere to solid organic phases of media in groundwater studies (Wilson and Mackay, 1996), SF_6 could partition to liquid organic phases. As the partial pressure of SF_6 in the rumen is extremely low (2-5 mg SF_6 (New Zealand) in ~2200 L of carbon dioxide and ~700 L CH_4 per cow per day (Moate et al., 1997)), the hypothesis is that very little would tend to be solubilised. On this basis, it has been assumed in animal studies that SF_6 does not adhere to rumen particulate matter and is not solubilised in rumen fluid.

A second study was conducted to test whether SF_6 is released via either urine or gas trapped in faecal material. Ideally SF_6 would also be measured in flatus, but the facilities did not allow this. Data from this study would help to confirm the hypothesis that all SF_6 is released via the mouth and nose.

4.3 Materials and methods

4.3.1 *In vitro* tube experiment

A laboratory study was undertaken in April 2007 to assess the release rate of SF_6 from six individual permeation tubes in each of 3 different media – air, water and strained rumen fluid. All tubes were measured first in air. Three permeation tubes were measured in water, followed by rumen fluid, while the other three were measured in rumen fluid, followed by water. This was done to account for any possible effect of the fluid treatments on subsequent SF_6

release rate from the permeation tubes. All measurements were carried out in an incubator set to 39°C to match rumen temperature.

Zero grade air was utilised for the air treatment. Reverse osmosis treated water was used for the water treatment (hereafter referred to as water). The rumen fluid was collected from a pasture-grazed beef cow before the start of the experiment. The rumen fluid was strained through two layers of cheesecloth and frozen in single batch quantities. Water and rumen fluid measurements were conducted using 200 mL of fluid and these were warmed to 39°C inside the incubator prior to use in measurements.

4.3.1.1 Permeation tubes

The SF_6 permeation tubes were manufactured and filled by the New Zealand Institute of Water and Atmospheric Research (NIWA) in March 2006. Tubes were stored at AgResearch Limited in a 39°C room and gravimetrically weighed once a week until the time of the experiment. Weekly tube weights were not available for the whole period from filling to the time of this study. However, weight data from the twenty-eight weeks prior to the beginning of the experiment showed a constant weight loss, with straight-line regressions showing an $R^2 > 0.999$ for all tubes. Release rates ranged from 3.5 to 4.2 mg SF_6 per day as calculated from the weight loss from the last 28 pre-experimental weights in dry air at 39°C. If only the last 10 weights were used as suggested by Lassey et al. (2001), this made a difference of only 0.01 mg SF_6/day to the release rate for any particular tube, so the 28-week values were utilised. Tubes were maintained at 39°C throughout the experimental period.

4.3.1.2 Measuring system

A system was set up to collect diluted gas samples over 1 hour periods. A single SF_6 permeation tube was placed inside an Agee® preserving jar (approximately 1 L volume) fitted with a Perfitseal® dome and screw band (Figure 4.1). A small fan was bolted to the bottom of the dome lid to circulate the air inside the jar. The air inside the jar was diluted with zero grade air, via a

copper tube inserted through the lid, at a rate of 1 L/min using a Matheson mass flow controller. The mass flow controller was calibrated by NIWA prior to the experiment. A second copper tube was inserted as a vent and to collect gas samples. The jar was placed inside an incubator (Gallenkamp orbital incubator, Cat. No. IOC400.XX1.C, UK) set to 39 ± 0.5 °C to match rumen temperature. A magnetic stirrer was placed under the jar inside the incubator to allow slow stirring of fluid in the bottom of the jar. This stirrer was also used during air measurements for consistency.

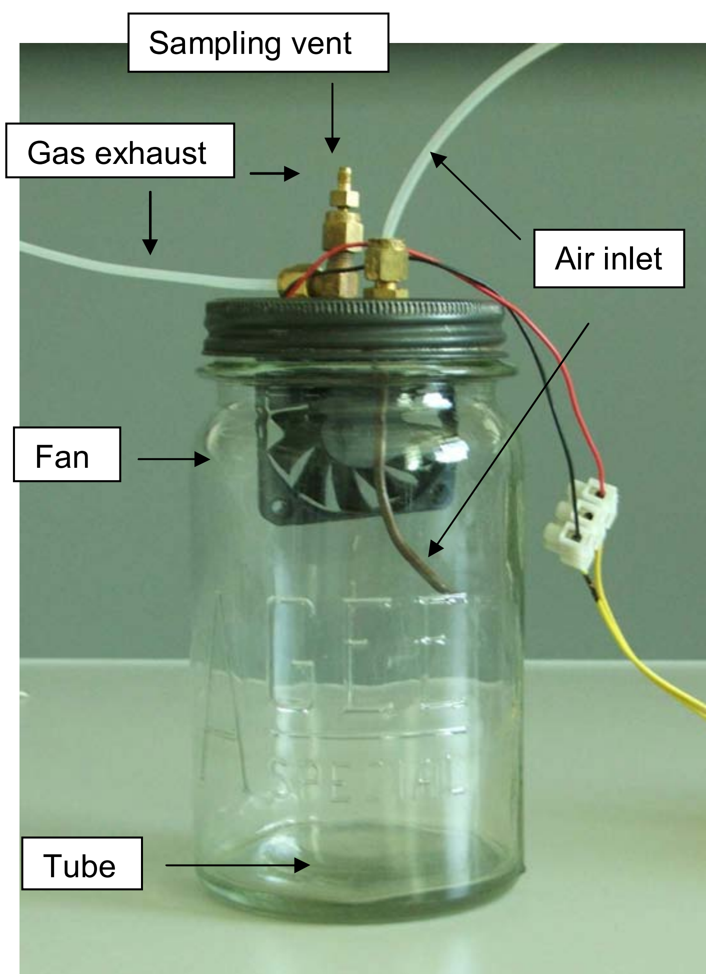


Figure 4.1 Vessel used for sampling SF_6 released from permeation tubes during the experiment, showing the gas inlet and exhaust and where the permeation tube would be placed.

Preliminary measurements with dry air showed good agreement between calculated daily release from samples taken over 10-minute, 1-hour and 24-hour sampling times (Table 4.1). Similar measurements taken with the same

permeation tube in water indicated that 10-minute sampling was unreliable, but that 1-hour sampling gave a good comparison with gravimetric weighing.

The permeation tube used for the sampling data presented in Table 4.1 had a release rate of 3.23 mg SF_6/day as measured by gravimetric weighing. Sampling in air resulted in slightly higher estimates, while sampling in water resulted in slightly lower estimates. From these results it was decided to undertake the experiment using 1 hour sampling periods.

Table 4.1 Preliminary measurements of permeation tube SF_6 release rate measured in both dry air and with the permeation tube submerged in water for periods of 10 and 60 minutes and 24 hours. Data are mean ± SE.

	Air		Water	
Sampling time	*n*	mg SF_6/day	*n*	mg SF_6/day
10 minutes	6	3.40 ± 0.05	10	0.13 ± 0.03
60 minutes	3	3.46 ± 0.02	5	3.17 ± 0.02
24 hours	3	3.30 ± 0.13	2	3.05 ± 0.20

Permeation tubes were allowed to stabilise in the incubator for a 1 hour period before sampling began. Evacuated containers (approximately 2.5 L) were used to sample gas leaving the vent of the bottle at a rate of 14 mL/min for a period of 1 hour. Three consecutive 1-hour samples were taken for each measurement. One SF_6 tube was measured over a longer period in each medium to determine when stabilisation was reached, with 10 consecutive 1-hour samples in air and 11 consecutive 1-hour samples in water and rumen fluid.

4.3.1.3 Sample analyses

Samples were analysed using gas chromatography (Shimadzu 2010, Shimadzu Corp., Kyoto, Japan). Gas samples were extracted and pressurised prior to analysis using a pump system. A 50 µL sample loop and a 3.3 m molecular sieve column (Alltech Associates, Auckland, New Zealand) were utilised for the analyses. The column temperature was set to 85°C and the electron capture detector to determine SF_6 concentration was set to 250°C. Samples were

compared to a 1 part per million SF_6 standard prepared by NIWA to determine the SF_6 concentration. Each sample was analysed in triplicate. The average of the three samples was divided by the measured concentration of the standard gas to get a concentration in parts per million. Concentrations were multiplied up to give a daily value, which was subsequently converted from a concentration to a mass, thus providing a daily release rate in mg per day.

4.3.1.4 Statistical analysis

There were three treatments for each permeation tube (air, water and rumen fluid), and two treatment sequences (air, water, rumen fluid; air, rumen fluid, water) were utilised. Data were analysed with a general analysis of variance (ANOVA) to determine if treatment had any effect on SF_6 release rate, and to test if treatment sequence had any effect on subsequent measurements of release rate. Analysis was carried out using Genstat version 9 (Genstat Committee, 2002). Treatment structure included the effects of treatment and treatment sequence, while the block structure included the effect of permeation tube and the interaction between permeation tube and treatment sequence.

4.3.2 Alternate SF_6 release sites investigation

Faecal and urine samples were collected prior to administration of SF_6 permeation tubes and 24 days after administration from 9 cross-bred dairy cattle. Permeation tubes had release rates of ~4.5 mg SF_6/day. This research was carried out as part of a larger experiment looking at digestion characteristics related to CH_4 production as reported by Pinares-Patiño et al. (2007b). Briefly, nine Friesian x Jersey dairy cattle were fed fresh perennial ryegrass/white clover pasture. Individual animal measurements included daily feed intake, two periods of CH_4 measurement using the SF_6 tracer technique, two days measurement of feeding behaviour, 5 days measurement of total faecal and urinary output and digestibility. Faecal and urinary samples for this study were collected immediately after the morning feeding at 07:00 hours and just prior to the afternoon feed at 17:00 hours on both days. Faecal samples were collected via rectal grab sampling, while urine samples were collected in a

bucket when cows urinated. Cows were fed *ad libitum* when the first set of samples were collected, and 95% of their *ad libitum* intake during the second sampling period. As one cow had to be removed from the experiment due to lameness, complete samples were only available from 8 cows.

4.3.2.1 Sample measurement

Faecal samples were immediately taken to the laboratory and ~150 g of fresh faeces were transferred to glass laboratory bottles for gas extraction, while between 150 to 200 g of fresh faecal material was dried in a forced-air oven (65ºC for 48 hours) to assess the dry matter content of the faeces. Each bottle had a tube with a quick-connect fitting attached to the lid. This tube was connected to an evacuated canister for 2 hours to extract any gas trapped in the faecal material (see Figure 4.2). It was assumed that by this time the faecal gas was evenly dispersed under the vacuum.

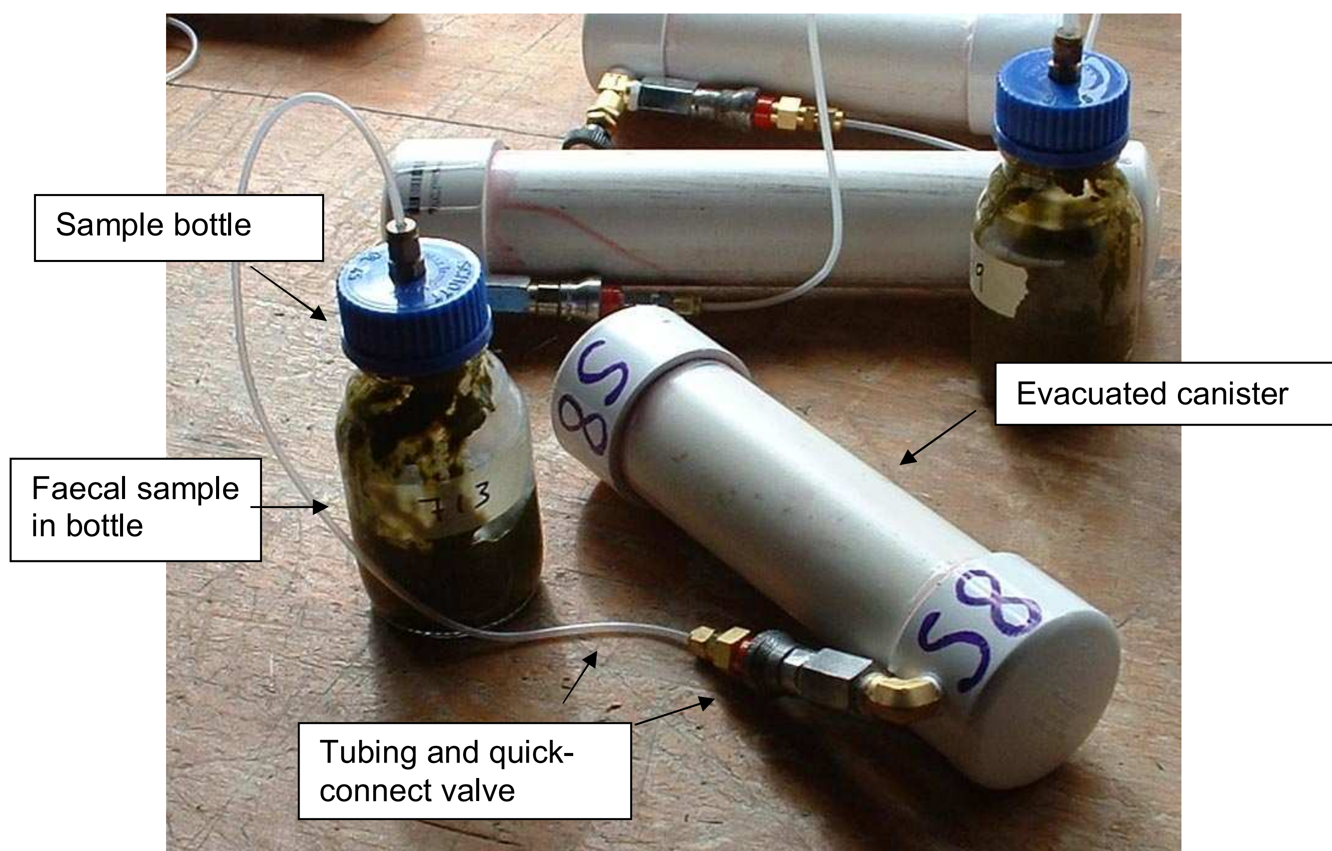


Figure 4.2 Laboratory bottle containing ~150 g fresh cattle faeces connected to an evacuated canister to extract gas trapped in the faecal material for analysis of CH_4 and SF_6 concentrations.

Dry matter intake was measured daily during the experiment. Individual animal digestibilities were measured during a five-day period in the experiment, which included measurements of total faecal and urinary output. Faecal output for the sampling days was calculated using the measured daily intake and mean digestibility for individual animals measured during the digestibility period. Mean wet faecal output was 30 kg during the adaptation period and 33 kg during the measurement period. Each cows total urinary output (mean = 24 kg) on the sampling days was assumed to be the same as that measured during the digestibility period when there was no cross-contamination between faecal and urinary excretions.

4.3.2.2 Sample analyses

Extracted faecal gas was analysed using the normal gas chromatography methods used for CH_4 measurements as reported in Chapter 2. During the background sampling period, canisters with a volume of approximately 1,700 mL were used. Due to the low sample volume, samples were diluted with nitrogen prior to analyses on the gas chromatographs. These canisters were not available for the sampling period after administration of the permeation tubes, so different canisters were used. The sampling canisters used during treatment sampling had an approximate volume of 600 mL. Due to the smaller canister size, these samples did not require dilution prior to analysis. The dilution of the background samples was accounted for in the calculations of total CH_4 and SF_6 from faecal samples.

Collected urine samples were immediately transferred to ~250 mL glass bottles with ground-glass stoppers. Glass was used to prevent any SF_6 diffusion or loss from the urine sample. For this reason, glass bottles were completely filled to avoid loss of SF_6 into headspace gas in the bottle. Duplicate samples (two sample bottles) were filled with urine from each cow for both sampling times. Background samples were collected on November 24, sampling after permeation tube insertion occurred on December 18. Samples were transported to NIWA on December 20 for analysis of SF_6 and CH_4 concentrations by

chromatographic methods. Analyses were completed by Dec 24. This gave a maximum of one month between sample collection and analyses.

Concentrations obtained from chromatography were converted to quantities in g and mg, for CH_4 and SF_6 respectively. The calculations accounted for dilution in the bottle and yoke, change in sample pressure and conversion from a concentration to a mass. The mass of gas was then scaled up from the sample size to total release using daily faecal and urinary outputs.

4.4 Results

4.4.1 *In vitro* tube experiment

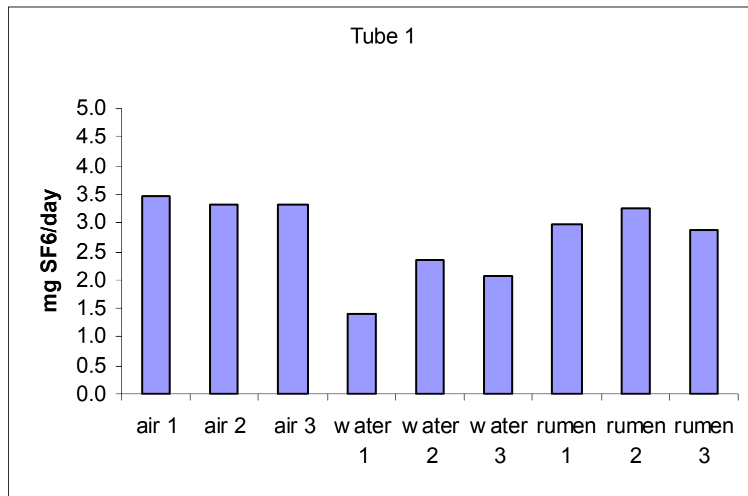
Treatment sequence (air, water, rumen fluid or air, rumen fluid, water) had no effect on release rate ($p = 0.98$), and there was no interaction between treatment sequence and treatment effect ($p = 0.16$). As treatment sequence had no effect, data are presented in a standard format of air, water, rumen fluid.

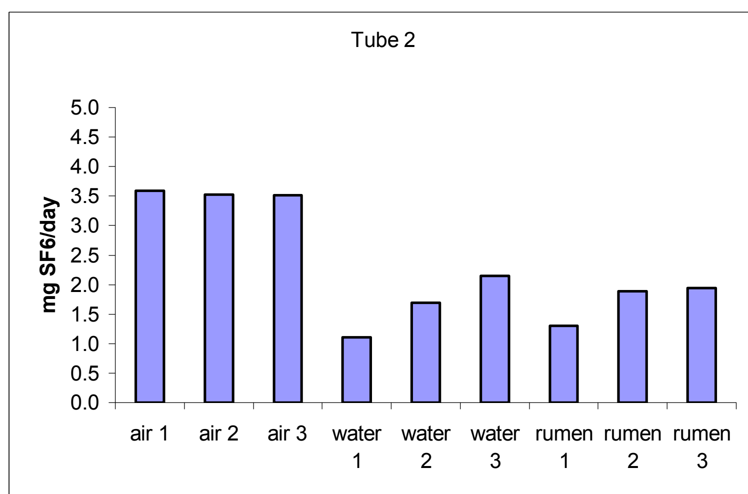
The mean release rate of SF_6 from three one-hour measurements from all six permeation tubes in air (3.51 mg SF_6/d) was significantly higher ($p = 0.001$) than in water (2.55 mg SF_6/d) or rumen fluid (2.34 mg SF_6/d). The least significant difference for treatment effect was 0.501, indicating that the water and rumen fluid treatment release rates were not different. Compared to air, measuring in water resulted in a 27% reduction and in rumen fluid a 33% reduction in SF_6 release rate (Table 4.2).

Table 4.2 Calculated mean (± SE) SF_6 release rates (mg/day) for six different permeation tubes when measured by gravimetric weighing, or by three consecutive one-hour samples of SF_6 release in air, water or strained rumen fluid.

	Treatment			
SF_6 tube	Weighing	Air	Water	Rumen fluid
1	3.81	3.36 ± 0.04	1.93 ± 0.28	3.04 ± 0.11
2	3.83	3.54 ± 0.02	1.65 ± 0.30	1.71 ± 0.21
3	4.16	3.92 ± 0.11	3.25 ± 0.53	2.75 ± 0.81
4	3.51	3.19 ± 0.06	2.65 ± 0.26	1.81 ± 0.14
5	3.84	3.59 ± 0.05	2.90 ± 0.68	2.50 ± 1.18
6	3.87	3.47 ± 0.04	2.91 ± 0.10	2.21 ± 0.57

Measurements from all six permeation tubes showed a stable (SD = 0.10) calculated daily release rate from consecutive 1-hour samples in air, but calculated release rates were more variable in water (SD = 0.62) and rumen fluid (SD = 0.87). The measurements conducted in air were in reasonable agreement with the values from gravimetric weighing, although they were 8.5% lower on average. Measured release rates in water (34%) and rumen fluid (39%) were much lower than release rates measured by gravimetric weighing. Five out of the six permeation tubes had a lower calculated daily release rate in water and rumen fluid during the first 1-hour measurement than during the second and third hour measurements (Figure 4.3).





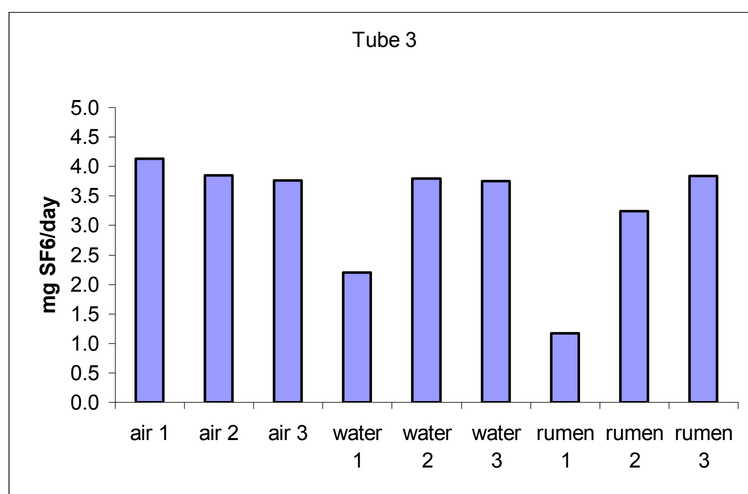
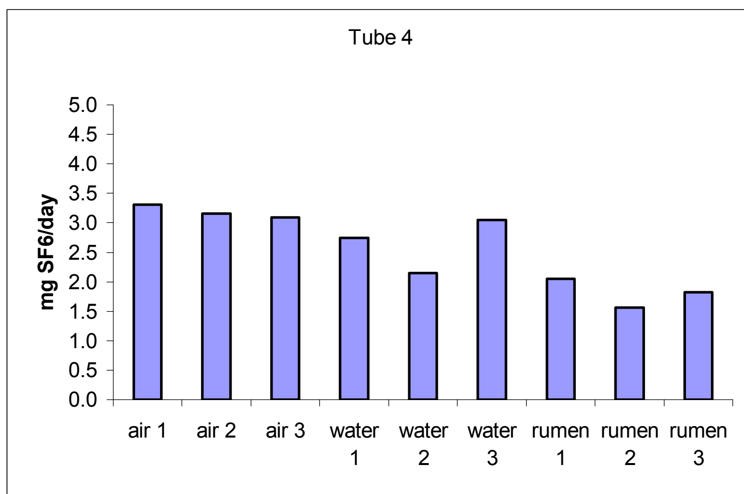
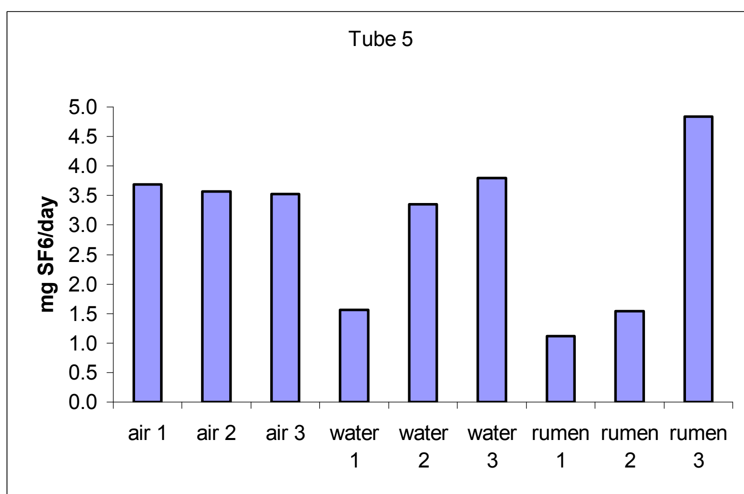


Figure 4.3 Daily permeation tube release rates (mg SF_6/day) calculated from three consecutive 1-hour measurements of SF_6 release rate for six different permeation tubes measured in air, water and rumen fluid.





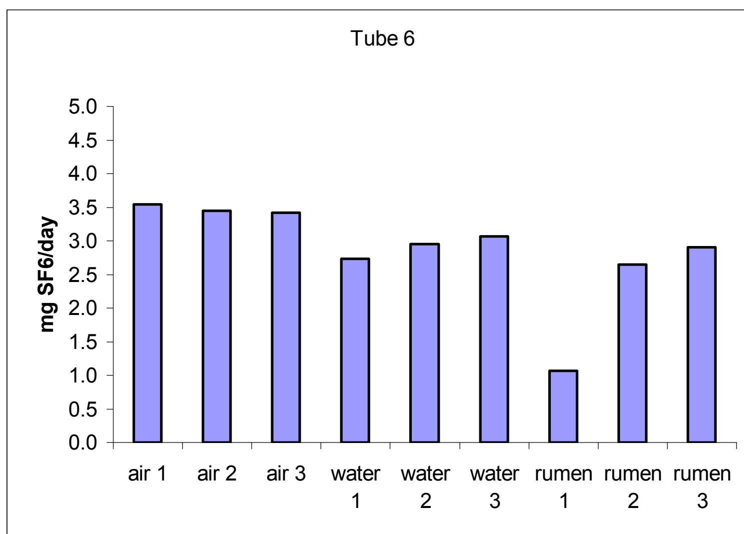


Figure 4.3 continued Daily permeation tube release rates (mg SF_6/day) calculated from three consecutive 1-hour measurements of SF_6 release rate for six different permeation tubes measured in air, water and rumen fluid.

A series of consecutive hourly measurements were taken for 10 to 12 hours in each media using tube 3. These results showed a relatively stable hourly release rate in air (mean ± SD, 3.81 ± 0.13 mg SF_6/day), but a more variable release in water (3.41 ± 0.69 mg SF_6/day) and rumen fluid (3.17 ± 1.18 mg SF_6/day) (Figure 4.4). However, measured release rates remained below the release rate of 4.16 mg SF_6/day measured by gravimetric weighing. Measurements in air continued to be stable over time, but hourly measurements in water and rumen fluid did not reach stability over this time frame, but continued to fluctuate.

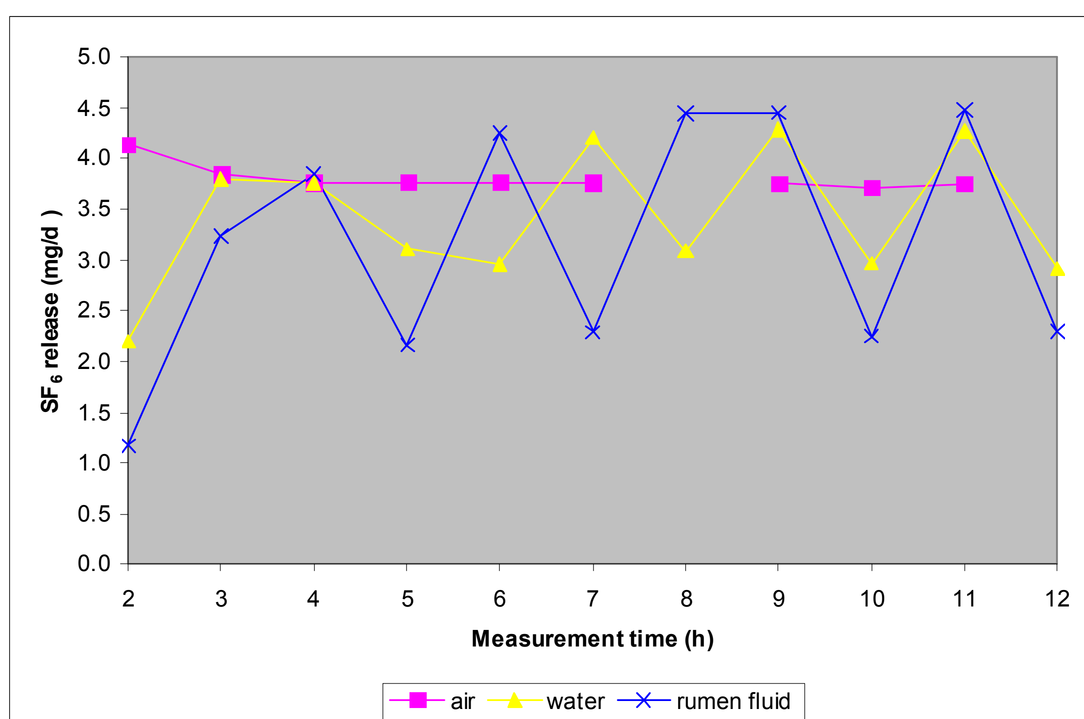


Figure 4.4 Calculated daily SF_6 release rate (mg/day) from consecutive 1-hour samples measured in air, water and rumen fluid from an individual permeation tube (tube 3).

4.4.2 Alternate SF_6 release sites

Small quantities of SF_6 were detectable in gas trapped in faecal material, both before and after the administration of permeation tubes to the cows (see Table 4.3). Faecal samples collected prior to the administration of the permeation tubes had similar concentrations (4.22 ppt) to samples of background air (6.4 ppt) collected using the same method as for the collection of trapped faecal gas.

Concentrations of SF_6 in faecal gas (10.29 ppt) were approximately 2.5 times higher after administration of the permeation tubes.

Table 4.3 Measured concentrations of SF_6 from background air or gas trapped in faecal material (mean ± SEM) and calculated daily quantities of SF_6 released by gas trapped in the faecal material of cattle pre- and post- SF_6 permeation tube administration.

	Background	Faecal gas		
	ppt	ppt	mg/day	% daily SF_6 tube release
Pre-SF_6 tube	6.4	4.22 ± 0.30	4.5×10^{-6}	1.0×10^{-6}
Post-SF_6 tube	4.5	10.29 ± 2.92	8.1×10^{-6}	1.7×10^{-6}

It can be seen that only minute quantities of SF_6 (~6×10^{-6} mg/day) were detected in faecal gas compared with the mean daily SF_6 release rate (4.6 mg/day). The concentration of SF_6 in urine was extremely low, and was a small proportion of the daily release from the permeation tubes (Table 4.4). Total loss of SF_6 via faecal gas (minus background losses) and urine totalled 7.46×10^{-5} mg SF_6/day, or 0.0016% of the daily quantity of SF_6 released from the permeation tubes.

Table 4.4 Mean (± SEM) concentration and calculated quantities of SF_6 from cattle urine samples measured pre- and post-SF_6 permeation tube administration.

		SF_6	
	fmol/L[1]	mg/day	% daily SF_6 tube release
Pre-SF_6 tube	ND[2]	-	-
Post-SF_6 tube	493.8 ± 40.6	$7.1 \times 10^{-5} \pm 5.6 \times 10^{-6}$	0.0015 ± 0.0002

[1]femtomols [2]ND, not detected

Although not an integral part of this experiment, CH_4 concentrations were also measured in both faecal gas and urine samples. Results indicate that small quantities of CH_4 are lost via both these routes (Table 4.5). Total daily release of CH_4 via faecal gas and urine from these animals was approximately 0.1 g/day, which is miniscule compared to the estimated mean daily emission of 327 g/day (Pinares-Patiño et al., 2007b). The large difference in CH_4 concentration between the pre- and post-SF_6 tube measurements was likely

caused by the difference in sampling canister size for the pre- (1700 mL) compared with the post-SF_6 tube (600 mL) measurement.

Table 4.5 Mean (± SE) concentration and calculated daily quantities of CH_4 released via cattle faecal gas and urine as measured using chromatography prior to and 24 days after SF_6 permeation tube administration.

	Faecal gas		Urine	
	ppm	g/day	µmol/L	g/day
Pre-SF_6 tube	809.8 ± 263.5	0.10 ± 0.022	1.16 ± 0.11	0.02 ± 0.002
Post-SF_6 tube	145.8 ± 45.2	0.01 ± 0.004	1.75 ± 0.09	0.03 ± 0.002

4.5 Discussion

Calculated daily release rates of SF_6 from permeation tubes in air from the three 1-hour measurements were slightly lower using this technique than when using gravimetric weighing. The average release rate from the six tubes in air was 8.5% lower than release rates from gravimetric weighing, with a range from 5% to 12% lower. As this technique measured the concentration of gas being released, it relied on accurate calibration of the measuring equipment. Any bias in the calibration would result in a bias in the measurements and, although unlikely, this could provide one hypothesis why the gas measurements gave a lower release than gravimetric weighing. However, the mass flow detector was calibrated prior to this experiment to provide a constant air flow and there was good mixing of the air and fluids inside the sampling vessel. Analyses were conducted using a gas chromatograph that was regularly used for measurement of SF_6, and all samples were compared with a 1 ppm SF_6 standard prepared by NIWA. These factors indicate that the measurements should have been accurate.

Results from this preliminary study indicate that the release rate of SF_6 from permeation tubes over three 1-hour periods may be lower in fluids, such as water or rumen fluid, than in air. This has implications for the standard practice of measuring release rates by gravimetric weighing from tubes stored in dry air at 39°C.

Hourly measurements from permeation tube number 3 over ten to twelve hours indicated that the release rate was much more variable in water (coefficient of variation, CV = 20%) and rumen fluid (CV = 37%) than in air (CV = 3%). It can been seen in Figure 4.4 that measured release rates obtained from tube 3 when suspended in water and rumen fluid were lower (38% and 65%, respectively) in the first hour than those obtained in subsequent measurements. This is perhaps an indication that SF_6 was being solubilised in the fluid and possibly adhering to the organic matter in the rumen fluid (Wilson and Mackay, 1996; Bullister et al., 2002). This indicates the need for a longer stabilisation period. Mean release rate data from the twelve one-hour measurements indicate that removing the first measurement value reduces the difference in measured release rates between air and fluids, as well as the within-treatment error (Table 4.6).

Table 4.6 Mean (± SE) calculated daily SF_6 release rate from 10-12 consecutive 1-hour samples measured in air, water and rumen fluid from an individual permeation tube (tube 3) with or without the value from the first measurement (M1).

	Air	Water	Rumen fluid
With M1	3.81 ± 0.04	3.41 ± 0.21	3.17 ± 0.36
Without M1	3.77 ± 0.01	3.53 ± 0.19	3.37 ± 0.33

Mean calculated daily release rates from the twelve consecutive measurements with tube 3 showed lower release rates in water (10%) and rumen fluid (17%) than in air. Excluding the first measurement from the mean results in a smaller reduction in release rates when tubes are submerged in water (6%) or rumen fluid (11%) compared with measurements in air. This suggests that when hourly measurements of release rate are taken over 12 hours, the difference in measured release rate in fluids is lower than in air by 6-11%.

A feature of the hourly measurements over the 12-hour timeframe is the continued variation as seen in Figure 4.4 and Table 4.6. One possible explanation is that when permeation tubes are placed in air, SF_6 gas can permeate directly out in to the surrounding air, but when placed in fluid a small amount of back-pressure will be exerted on the Teflon® membrane. While any back-pressure would be low, and very small compared to the pressure inside a

permeation tube (about 32 bar, Lassey et al., 2001), it may still affect SF_6 release rate. This could be caused by the low partial pressure of water or other gases inside the tube resulting in water entering the permeation tube and interfering with the ability of SF_6 to permeate out. Another possible hypothesis is that the fluid may cause bubbling of SF_6 gas, as bubbles have been seen by the author on the membrane of permeation tubes during the laboratory experiment and by researchers in Canada (T. McAllister, pers. comm.) and New Zealand (R. Martin, pers. comm.). Bubbles of SF_6 would periodically break free and float to the surface of the fluid. Depending on how often these bubbles are released, this could have a significant impact on shorter-term measurements. Sampling for longer periods (24 hours for example) would reduce the possible variation from intermittent release, while the short-term studies may provide some useful insight into how SF_6 is released from permeation tubes.

Greater variability in sample concentration of SF_6 occurs when using sample collection periods of 15 minutes or 3 hours compared with the normal 22-24 hour sampling period, resulting in greater variability in calculated daily CH_4 emissions (Martin et al., unpublished). Results indicated that while greater variability existed in estimates from 3-hour sampling, the mean of the 3-hour samples was similar to the 24-hour estimate. This effect was possibly due to trapping of gas inside the rumen and only periodical release via eructation (Martin et al., unpublished). An alternative explanation is that as well as intermittent release of gas due to eructation, there may be an intermittent release of SF_6 from the permeation tube.

Sulphur hexafluoride is slightly soluble in water and, therefore, possibly in rumen fluid. If the solubility coefficient of Bullister et al., (2002) of 0.16 mol/L/atmosphere is used, and the partial pressure of SF_6 in the sampling vessel is assumed to be equal to its concentration in the sample collected, then the amount of SF_6 that could potentially be solubilised in 200 mL of water is equal to approximately 0.1 µmol. This would represent 8% of the amount released in the first hour from a permeation tube releasing 4 mg SF_6/day. Little SF_6 should be solubilised into the water, especially after the stabilisation period. Data from this experiment indicate that it took two hours for the release rate to

stabilise in 200 mL of fluid. This emphasises the need to allow time for permeation tubes to stabilise in the rumen environment before beginning CH_4 measurements.

It is possible that more SF_6 was trapped in solution with the rumen fluid, as Wilson and Mackay (1996) showed that it could adhere to liquid organic phases. This might help to explain the slightly lower measured release rate from tubes immersed in rumen fluid than in water. While SF_6 is a relatively heavy gas, both the fluid and air portions in the sampling vessel were assumed to be well mixed by the magnetic stirrer and fan, so settling of SF_6 to the bottom of the sampling vessel should not have occurred.

While SF_6 release from permeation tubes appears to be very constant in air, it appears to be more variable in fluids. These results indicate that when permeation tubes are placed in fluid, the release of SF_6 may be intermittent rather than truly continuous. In addition, while four of the six permeation tubes exhibited similar behaviour, the other two (tubes 1 and 2) exhibited quite different behaviour (Figure 4.3). Differences in behaviour between permeation tubes are problematic, as they cannot be easily corrected for. These differences in permeation tube behaviour may account for some of the extra variation reported for the SF_6 technique when compared with calorimetry, due to using an estimate of the SF_6 release rate that is not consistent with the actual release rate in the rumen. While more research needs to be carried out to confirm the differing behaviour between permeation tubes, it may be necessary to find a suitable replacement to Teflon® as a permeable membrane due to the likelihood of it interacting with SF_6.

Results from this experiment indicate that the higher than expected weight of permeation tubes recovered from animals may be partly due to a lower SF_6 release rate and hence a lower weight loss. A lower release rate would result in over-estimation of daily CH_4 production. Using the value from the twelve consecutive 1-hour measurements, SF_6 release rate may be over-estimated in the rumen by approximately 10%. Assuming this is correct, then the estimated CH_4 yield (g CH_4/kg DMI) would be 10% too high. Grainger et al. (2007)

reported that the SF_6 technique over-estimated daily CH_4 production by 3% compared with measurements from calorimetry. This over-estimation may be explained by a lower than expected release of SF_6 from permeation tubes.

It appears that SF_6 can be absorbed into the body and released in urine. While SF_6 was only detected after administration of permeation tubes, the quantities were miniscule (~0.00007 mg SF_6/day) compared with actual release rates (~4.5 mg SF_6/day). This validates the assumption that SF_6 is not appreciably lost via release in urine. Studies using SF_6 as a tracer in lungs indicate that SF_6 quickly transfers from blood to the lungs and is released in the breath (Schrikker et al., 1989; Schimmel et al., 2004). It follows that any SF_6 absorbed into the body would not be trapped in body tissue, but would be released in the breath. This would then follow the same route of excretion, through the blood, lungs and breath, as some of the CH_4 (Murray et al., 1976).

Low concentrations of SF_6 were detected in faecal gas before permeation tubes were administered (~4 ppt). This was presumably related to the background atmospheric concentrations of SF_6, which are normally about 4 ppt. Concentrations increased after permeation tube administration to ~10 ppt. The calculated daily quantity released via faecal gas would be approximately 8.2 x 10^{-6} mg/day; which would be insignificant compared to the actual release rates (~4.5 mg/day). It is possible that SF_6 could be released via flatus, or in solution or adhering to faecal material. Possible losses of SF_6 via these pathways still need to be assessed. These results indicate that the total loss via both urine and faecal gas would add up to approximately 0.000015 mg SF_6/day.

Results from this experiment indicate that there may be a lower than expected release rate from permeation tubes when immersed in water and rumen fluid. These results contradict the current assumption of the SF_6 technique that the release rate from permeation tubes measured by gravimetric weighing is the same as the release rate in the rumen. Analyses of urine and faecal gas indicate that SF_6 is only released in minuscule quantities via these routes, thus supporting the assumption that SF_6 is almost exclusively released via the mouth and nose. Further study is required to determine whether and if so, in what

quantities, SF_6 may be released in flatus or in solution with faecal material. The differential release of SF_6 in fluids compared with air needs to be confirmed and quantified in future studies. This should lead to possible changes or adjustments in the methods used to ensure CH_4 estimates made using the SF_6 technique are as accurate as possible.

Chapter 5

Within- and between-animal variance in methane emissions in non-lactating dairy cows

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5.1 Abstract

A number of studies of ruminant methane (CH_4) emissions have focused on selecting high and low CH_4-emitting animals. One challenge faced by this work is the lack of consistency, or repeatability, in animal rankings over time. A possible explanation for the lack of repeatability is a relatively high within-animal variation in daily CH_4 emissions. An experiment was undertaken with four non-lactating dairy cattle to assess the within- and between-animal variation in CH_4 emissions when measured using the SF_6 tracer technique over a period of at least three weeks on each of two contrasting diets. The two diets were fed to the cattle at maintenance energy levels; first lucerne silage (lucerne), followed by a cereal+lucerne silage+straw mixed diet (TMR). Daily CH_4 measurements were undertaken for 23 days on diet 1 and 30 days on diet 2.

Daily CH_4 production was significantly ($P < 0.001$) lower when feeding the lucerne diet (124.3 g CH_4/d, SEM = 11.1) than when feeding the TMR diet (169.8 g CH_4/d, SEM = 11.0). Lower CH_4 yields (g CH_4/kg DMI) were recorded for the lucerne (22.8 ± 2.0) than the TMR (32.0 ± 2.0) diet. Cows differed significantly from one another ($P < 0.05$) in daily CH_4 yield. Estimates of repeatability (variation between animals/total variation) for daily CH_4 yield were 47% and 73% for the lucerne and TMR diets, respectively. Coefficients of variation in average daily CH_4 yield per animal in this experiment ranged from 8 to 18%, despite the fact that each animal received the same quantity and quality of feed each day. Variances for daily CH_4 yield were smaller for the lucerne (within animal = 6.91, between animals = 6.23) than for the TMR (within animal = 10.09, between animals = 27.79). While further research is required to confirm the results presented here, the high within-animal variability in CH_4 yield measured using the SF_6 tracer technique may explain why there has been difficulty in obtaining consistent animal rankings in CH_4 yields when animals are measured on multiple occasions. These results also suggest that the SF_6 tracer technique may exaggerate apparent between-animal differences in CH_4 yields.

5.2 Introduction

Mitigation of ruminant methane (CH_4) has become an important area of research as CH_4 emissions have been linked to global warming, as discussed in Chapters 1 and 2. One particular approach that could provide a cheap, longer-term reduction is the use of natural variation in CH_4 production between animals to breed for animals with lower CH_4 yields (g CH_4/kg DM intake). A number of studies have been undertaken to select high and low CH_4-emitting animals using the SF_6 tracer technique (Pinares-Patiño et al., 2003b; Goopy and Hegarty, 2004; Pinares-Patiño et al., 2005). While high and low CH_4 emitters, in terms of CH_4 yield, were identified in all of these studies after a single set of measurements, these animals did not maintain the high/low emission trait in subsequent measurement periods. The lack of repeatability in CH_4 yield rankings between animals may be due to natural variation in emissions from individual animals, variation in the measurement technique, or a combination of both.

Day-to-day, or within-animal, variation in daily CH_4 production from measurements made with cattle fed a constant quantity of feed each day was reported to be 7% of absolute daily CH_4 production when measured over four to five days in calorimetry chambers (Blaxter and Clapperton, 1965). More recently, Grainger et al. (2007) reported a coefficient of variation (CV) in daily CH_4 production of 4.3% for open-circuit calorimetry chambers and 6.1% for the SF_6 tracer technique from animals fed a varying quantity of feed and measured over three days using both techniques.

Blaxter and Clapperton (1965) reported that between-animal variation for cattle was 7-8% of absolute daily CH_4 production when animals were fed a constant quantity of feed and measured over four to five days. Grainger et al. (2007) reported a higher between-animal variation (CV) of 17.8% of absolute daily CH_4 production for calorimetry measurements and 19.6% for SF_6 estimates from animals with varying levels of intake. Between-animal CV in absolute daily CH_4 production from grazing studies using the SF_6 tracer technique have been

reported to be between 11 and 25% (Lassey et al., 1997; Boadi and Wittenberg, 2002; McNaughton et al., 2005).

Some of the greater variance for the grazing experiments reported in the previous paragraph will be due to a combination of variance in estimated intake and variance in estimated CH_4 emission. Daily dry matter intake of animals cannot be accurately estimated (Ulyatt et al., 2002a; Lassey, 2007) and can, therefore, result in large variations in CH_4 yield estimates. It is important to note that most of these studies had varying levels of feed intake and this can also result in greater variation in CH_4 yields, both between days and between animals.

These studies indicate that substantial within- and between-animal variation occurs in absolute daily CH_4 production. Other researchers have described problems with poor repeatability over time, with animals changing their ranking within a group when estimating daily CH_4 yields using the SF_6 tracer technique (Pinares-Patiño et al., 2003b; Goopy and Hegarty, 2004; Pinares-Patiño et al., 2005). However, some of the latter studies were with grazing animals and error due to the difficulty of measuring intake at grazing may partially explain this lack of repeatability. There has been little quantification of the variation in either absolute daily CH_4 production or CH_4 yield in the published literature from studies that have used the SF_6 technique, and no proportioning of this variance to either within- or between-animal factors.

An assumption behind studies that are seeking to identify low- and high-emitting animals is that daily CH_4 yield is constant when feeding a constant quantity and quality of feed and hence a measurement at one time point in is an accurate reflection of overall CH_4 yield potential of an individual animal. The focus of this study was to assess the within- and between-animal variation in daily CH_4 yield when feeding a diet of constant quantity and quality and using the SF_6 tracer technique to estimate CH_4 production. The aim was to test the hypothesis that large within-animal variation in CH_4 yield is a factor in the lack of repeatability found in studies that have tried to identify consistently high and low CH_4 emitting animals when using the SF_6 technique.

5.3 Materials and methods

5.3.1 Experimental design

Two contrasting diets were fed to four Friesian x Jersey dairy cows; first a conserved forage diet (lucerne silage), followed by a cereal+lucerne silage+straw diet (TMR). Methane emissions from each cow were measured daily using the SF_6 tracer technique. Intake was restricted to maintenance energy levels for indoor cattle calculated using the equation, ME_m (MJ/day) = 8.3 + 0.091 x live weight (ADAS, 1984). The experiment ran for 74 days, with the first 9 days for adaptation to the lucerne diet, 23 days of measurement for the lucerne diet, 12 days of measurement and adaptation to the TMR diet, and 30 days of measurement on the TMR diet.

5.3.2 Animals and feeding

Cows had an average weight (± s.d.) of 417 ± 24 kg at the beginning of the experiment. Housing was in individual stalls in a well-ventilated barn, with free exercise periods twice daily on an outdoor sawdust pad for 30 to 60 minutes. Cows had *ad libitum* access to fresh drinking water throughout the experiment. Feed was individually fed in equal quantities twice daily at 0800 and 1600 hours. Feed samples were collected for dry matter (DM) determination (oven drying for 48 hours at 60°C) and chemical composition analysis by wet chemistry. The cows ate all the feed offered each day, so no refusals were collected.

The lucerne diet consisted of ensiled lucerne (*Medicago sativa*), which contained small, but unknown, quantities of molasses (ChaffHage™, The Great Hage Company, Reporoa, NZ). The silage came as three separate batches, so individual cows were fed from single batches within dietary periods. Transition to the TMR diet was carried out progressively in 3-day periods, with cows receiving 30, 50, 80 then 100% of the concentrate pellet as a proportion of that fed during the TMR period. The TMR included a concentrate-based pellet, barley straw, and the same lucerne silage as the lucerne diet. On a DM basis,

the TMR diet comprised 60% pellet, 17% straw and 23% lucerne silage. The composition of the pellet is shown in Table 5.1.

Table 5.1 Constituents of the concentrate pellet (% of pellet mixture on dry matter basis) fed to the cows as a part of the TMR diet.

	% of pellet
Maize meal	29.90
Barley meal	16.33
Wheat bran	41.56
Soybean meal	4.82
Molasses	3.90
Calcium carbonate	1.73
Sodium bicarbonate	1.51
Vitamin and mineral premix	0.26

5.3.3 Methane Measurements

Administration of SF_6 permeation tubes to the animals was undertaken on day 7 of the experiment, with CH_4 measurements beginning on day 9. Release rates from the SF_6 permeation tubes (mg SF_6/day) for each cow were: cow 1 = 3.677, cow 2 = 3.975, cow 3 = 3.547, cow 4 = 4.228. The permeation tubes were selected to minimise differences in absolute SF_6 permeation rate. This followed from the study reported in Chapter 3, which showed that the absolute release rate of SF_6 from permeation tubes can influence estimates of CH_4 emission when using the SF_6 tracer technique; high permeation tube release rates result in higher estimated CH_4 yield. Using the relationship between permeation tube release rate and calculated CH_4 yield suggested in Chapter 3, the difference in estimated CH_4 yield between the highest (4.228) and lowest (3.547) release rate used in this experiment would be a maximum of 0.5 g CH_4/kg DMI. Methane emissions were measured daily from day 9 until the completion of the experiment on day 74 using the SF_6 tracer technique as described in Chapters 2 and 3.

Permeation tubes were recovered from the animals at the conclusion of the experiment and re-entered the weighing programme in the laboratory. As the experiment ran for almost 11 weeks, the method of Lassey et al. (2001) was used to obtain a corrected weekly SF_6 release rate for each permeation tube. The only correction resulting from this procedure was an increase in the release rate of the tube inserted into cow 4 (4.228 to 4.229 mg SF_6/day) from week 5 onwards.

5.3.4 Rumen sampling

A small grab sample was collected from the middle of the rumen of each animal *per fistula* on the Thursday of each week, and for four consecutive days during the second week of each dietary period. Samples were collected immediately prior to the morning feed (0 hour), and at 1, 2, 4, and 6 hours after the morning feed. Rumen samples were strained through two layers of cheesecloth to obtain a rumen fluid sample. Rumen fluid was immediately measured for pH (RHM210, Radiometer, Denmark) and samples acidified and stored at -18°C for ammonia (NH_3) and volatile fatty acid (VFA) analyses.

Measurements of rumen volume were undertaken on the last day of each dietary period prior to the morning feed. The rumen was emptied *per fistula* and the contents weighed, hand-mixed and sub-sampled (~3kg wet weight), with contents returned to the cow within 20 minutes. Wet rumen samples were weighed, oven dried at 60°C for 48 hours in a forced-air oven and then re-weighed for DM determination.

5.3.5 Laboratory analyses

Feed samples were dried (48 hours at 60ºC) and ground through a 1 mm mesh sieve (Wiley Mill, USA), then submitted for chemical analysis to the analytical laboratory at AgResearch Limited Grasslands Campus (Palmerston North, New Zealand). Ash concentration was determined by burning in a muffle furnace at 600°C for 8 hours (AOAC International, 2005). Lipid was determined as the ether extract from repeat washing of the feed sample with petroleum ether,

followed by extraction with aqueous 80% ethanol and enzymatic extraction of the starch using the phenol sulphuric method (AOAC International, 2005). Nitrogen was measured using a Carlo Erba NA1500 nitrogen analyser (Carlo Erba Strumentazione, Milan). An in-house methodology was used for analyses of *in vitro* DM digestibility using neutral detergent solubilisation, followed by cellulytic enzyme break-down of the fibrous component. Digestibility values were validated with coefficients derived from animal experiments with sheep and cattle fed maintenance level diets of similar feed types (D. Corson, pers. comm.). Neutral detergent fibre (NDF) was determined by treating samples with heat-resistant amylases to prevent enzymatic digestion, followed by digestion with a neutral detergent solution, washing, drying and weighing the NDF residue (van Soest et al., 1991; Golding et al., 1985; AOAC International, 2005). Acid detergent fibre (ADF) was determined by digestion of the feed sample with acidified quaternary solution, followed by washing, drying and weighing of the ADF residue (AOAC International, 2005). Soluble sugar was determined by extraction with aqueous 80% ethanol and the phenol sulphuric method, while starch was measured using the residue of 80% ethanol extraction and enzyme treatment, followed by the phenol sulphuric method and calorimetry with absorbance read at 490 nm (AOAC International, 2005; Southgate, 1976; Batey, 1982; Du Bois et al., 1956). Gross energy of the feedstuffs was measured using a Leco Automatic bomb calorimeter AC-350 (Leco Corporation, St Joseph, USA) at the Massey University Nutrition Laboratory (Palmerston North, New Zealand).

Rumen fluid samples were analysed for volatile fatty acid (VFA) and ammonia (NH_3) concentrations by the Nutrition Laboratory at Massey University. Rumen fluid samples were deproteinised using metaphosphoric acid and the supernatant was injected directly into a gas chromatograph (Carlo Erba 5380, capillary column Alltech ATTM-1000, 15m x 0.53mm ID, 1.00µm film) and measured with an FID detector, using iso-caproic acid as an internal standard following the method of Wronkowska et al. (2006). Ammonia samples were analysed using enzymatic determination kits (Roche 11 112 732035, UV test). Only the pre-feeding (0 hour) and 1 and 4 hour post-feeding samples from the

second and third Thursdays of each dietary period were analysed for VFA and NH_3 concentrations.

5.3.6 Statistical analysis

Both absolute daily CH_4 production and CH_4 yield were analysed with a repeated measures analysis using the MIXED procedure of SAS (SAS, 2002). The model included the fixed effect of diet and day, the diet by day interaction and the random effect of animal. Estimates of variances within- and between-animals were obtained within each dietary period and using the combined data from both dietary periods. Repeatability of CH_4 emission was calculated as the proportion of variance between animals to the total variance, which was estimated as the sum of the within- and between-animal variances. A Fisher test (P=0.05) was used to test whether repeatability measures differed significantly between dietary periods. The Fisher test indicates whether there are significant differences between animals, but does not indicate if individual animals are different from one another.

Analyses of VFA data were conducted using the MIXED procedure with the fixed effect of diet and day, the diet by day interaction and the random effects of animal and day.

5.4 Results

5.4.1 Feed intake

The nutrient analyses of the three batches of lucerne silage, the barley straw and the concentrate pellet are shown in Table 5.2. Small differences in intake occurred between animals due to differences in DM content of the batches of silage. The DM intake of the cows was between 5.3 and 6 kg DM/cow/day for the lucerne diet and 5.2 to 5.4 kg DM/cow/day for the TMR diet. Daily intakes of nutrient components by individual cows were calculated by multiplying the proportion of the dietary components by the dietary chemical composition and are shown in Table 5.3.

Table 5.2 Chemical composition (g/100g DM) of the three batches of lucerne silage, the barley straw and the concentrate pellet offered to cows during the experiment.

	Silage 223	Silage 173	Silage 093	Straw	Pellet
DM Dig %	65.49	66.48	68.80	51.59	87.44
Ash	9.56	9.34	9.94	5.47	5.91
ADF	42.38	42.77	41.28	52.49	8.18
NDF	50.86	48.79	47.26	83.42	24.87
Lipid	1.69	1.62	1.33	1.54	3.24
Nitrogen	3.69	3.60	3.53	0.77	2.43
Sol. Sugar	1.53	1.72	1.77	1.44	4.83
Starch	0.00	0.00	0.00	0.00	43.93
GE (kJ/g)	19.56	19.44	19.11	18.14	18.36

Abbreviations. Dig, digestibility; DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre; Sol. Sugar, ethanol soluble sugar, GE, gross energy.

Table 5.3 Daily dietary chemical composition (g/100g DM) for each cow, by diet, calculated by multiplying the proportion of each dietary component by its nutrient content.

Chemical component	Cow 1		Cow 2		Cow 3		Cow 4	
(g/100g DM)	lucerne	TMR	lucerne	TMR	lucerne	TMR	lucerne	TMR
Ash	9.34	6.85	9.56	6.68	9.94	6.68	9.34	6.85
NDF	48.79	40.09	50.86	40.73	47.26	40.73	48.79	40.09
ADF	42.77	23.76	42.38	23.53	41.28	23.53	42.77	23.76
Lipid	1.62	2.48	1.69	2.59	1.33	2.59	1.62	2.48
Nitrogen	3.60	2.43	3.69	2.44	3.53	2.44	3.60	2.43
Sol. Sugar	1.72	3.51	1.53	3.50	1.77	3.50	1.72	3.51
Starch	0.00	25.70	0.00	26.40	0.00	26.40	0.00	25.70
GE (kJ/g)	18.13	17.68	18.21	17.77	17.83	17.77	18.13	17.68

Abbreviations. NDF, neutral detergent fibre; ADF, acid detergent fibre; Sol. CHO, ethanol soluble sugar; GE, gross energy.

5.4.2 Methane

Absolute daily CH_4 production from cows differed ($P < 0.001$) between diets, with mean (± SEM) production of 124.3 (± 11.1) g CH_4/day from the lucerne diet and 169.8 (± 11.0) g CH_4/day from the TMR diet (Table 5.4). Lower CH_4 yields (g CH_4/kg DMI) were also recorded on the lucerne diet (22.8 ± 2.0) than the TMR diet (32.0 ± 2.0) (for raw data see Appendix 2). Cows differed significantly ($P < 0.05$) from one another in both CH_4 production (Table 5.4) and CH_4 yield (Table 5.5).

Table 5.4 Mean ± SD daily CH_4 production (g CH_4/day) of four cows fed either lucerne silage (23 days) or TMR (30 days).

	Cow 1	Cow 2	Cow 3	Cow 4
Lucerne	102.0 ± 8.8	125.5 ± 22.3	134.9 ± 23.9	133.9 ± 10.4
TMR	139.5 ± 14.4	153.3 ± 21.1	190.2 ± 20.6	196.6 ± 20.6

Table 5.5 Mean ± SD daily CH_4 yields (g CH_4/kg DMI) of four cows fed either lucerne silage (23 days) or TMR (30 days).

	Cow 1	Cow 2	Cow 3	Cow 4
Lucerne	19.4 ± 1.7	23.2 ± 4.1	22.2 ± 4.0	25.4 ± 1.9
TMR	26.0 ± 2.7	29.3 ± 4.0	36.4 ± 3.9	36.6 ± 3.8

Daily CH_4 energy loss as a proportion of gross energy intake (GEI) follows the same pattern as CH_4 yield (Table 5.6). Average energy lost on the lucerne diet was 6.1% of GEI and on the TMR diet was 10.1% of GEI.

Table 5.6 Mean ± SD daily CH_4 loss as a percentage of dietary gross energy from four cows fed either lucerne silage (23 days) or TMR (30 days).

	Cow 1	Cow 2	Cow 3	Cow 4
Lucerne	5.9 ± 0.5	7.1 ± 1.3	6.9 ± 1.2	7.8 ± 0.6
TMR	8.2 ± 0.8	9.2 ± 1.3	11.4 ± 1.2	11.5 ± 1.2

Background SF_6 and CH_4 concentrations (parts per trillion, ppt, and parts per million, ppm, respectively) remained low and relatively constant throughout the

experimental period with values of 12.9 ± 1.9 ppt (lucerne) and 11.2 ± 2.0 ppt (TMR) for SF_6 and values of 4.6 ± 0.9 ppm (lucerne) and 5.2 ± 0.9 ppm (TMR) for CH_4.

Within-animal variation (CV) in absolute daily CH_4 production ranged from 8% to 18% for individual cows. Between-animal CV in absolute daily CH_4 production were 12% and 16% for the lucerne and TMR diets, respectively. Variation (CV) in daily CH_4 yield by individual cows in this experiment ranged from 8 to 18% despite the fact that each animal received the same quantity and quality of feed each day. Between-animal CV in CH_4 yield was 11% for the lucerne and 16% for the TMR diet. Variances for daily CH_4 yield were smaller for the lucerne diet (within animal = 6.91, between animals = 6.23) than for the TMR (within animal = 10.09, between animals = 27.79); absolute daily CH_4 production followed a similar pattern (Table 5.7).

Estimates of repeatability (variation between animals/total variation) for daily CH_4 yield were 47% and 73% for the lucerne and TMR diets, respectively. The mean repeatability for daily CH_4 production across both diets was 59%, and the mean for daily CH_4 yield across both diets was 55%.

Table 5.7 Between- and within-animal variance for both absolute daily CH_4 production and daily CH_4 yield for four cows fed lucerne and TMR diets and the combined data from both diets.

	Lucerne	TMR	Combined data
Variance in methane production (g CH_4/day)			
Between-animal	211.85	770.67	464.85
Within-animal	217.56	281.69	320.80
Total	429.41	1052.36	785.65
Repeatability	0.49	0.73	0.59
Variance in methane yield (g CH_4/kg DMI)			
Between-animal	6.23	27.79	14.83
Within-animal	6.91	10.09	12.17
Total	13.14	37.88	27.00
Repeatability	0.47	0.73	0.55

5.4.3 Rumen parameters

The rumen volume of all four cows was greater when they were fed TMR than when they were fed lucerne (see Table 5.8). Rumen dry matter content was 8-10% for all animals and during both dietary periods. The mean rumen pH of all four cows followed a similar trend over time, being highest prior to the morning feed, falling just after the morning feed, and then gradually rising while consuming diet 1 (Figure 5.1). There was a similar drop in pH at 1 hour after feeding for the TMR diet, but in contrast to feeding the lucerne diet, pH remained low with the nadir around 4 hours after feeding.

Table 5.8 Rumen volume, dry matter content and dry matter percentage for four cows as measured prior to the morning feed on the last day of lucerne and TMR feeding.

	Lucerne			TMR		
Cow	kg wet	kg DM	DM %	kg wet	kg DM	DM %
1	30.5	2.8	9.1	37.4	3.2	8.5
2	45.6	4.6	10.0	52.6	4.8	9.1
3	39.6	3.6	9.0	46.1	3.8	8.3
4	44.2	4.0	9.0	51.6	4.1	8.0
Mean	40.0	3.8	9.3	46.9	4.0	8.5

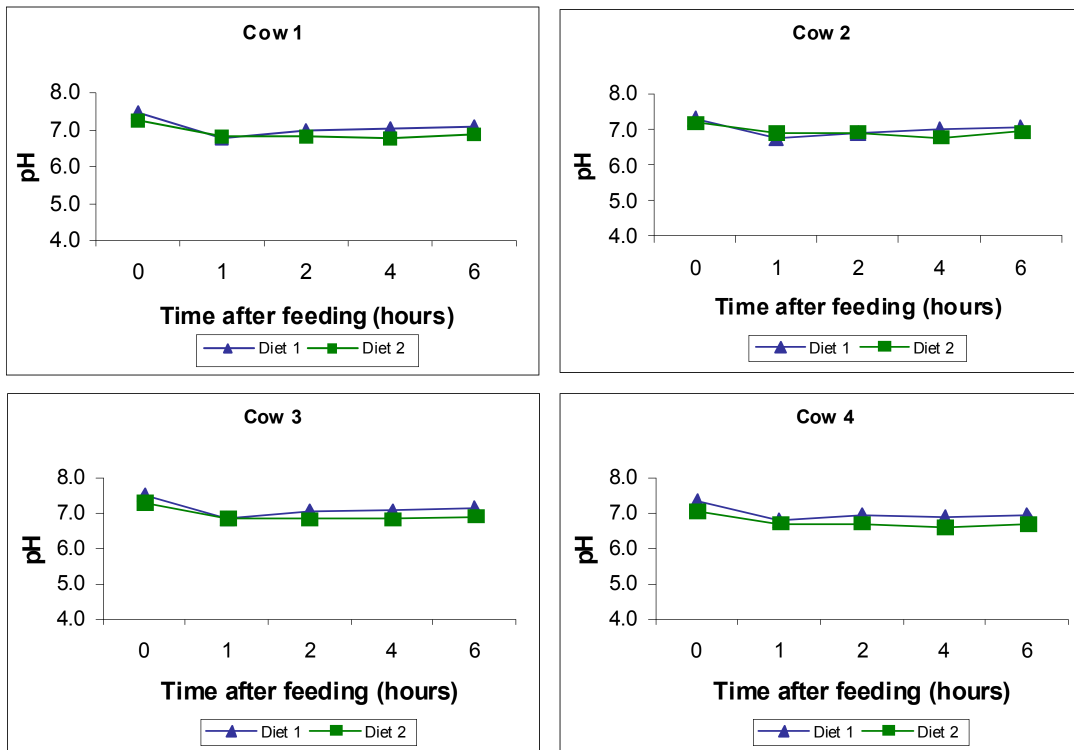

Figure 5.1 Mean pH from measurements on four consecutive Thursdays immediately prior to, and at 1, 2, 4, and 6 hours after the morning feed for four cows while fed either lucerne silage (diet 1) or TMR (diet 2).

Diet had a significant effect ($P < 0.0001$) on ruminal ammonia concentrations, with higher concentrations for lucerne (16.1 mmol/L) than TMR (8.2 mmol/L). Sampling time was significant ($P < 0.0001$). An interaction ($P < 0.0001$) occurred between sampling time and diet, indicating a different pattern of rumen fermentation for the lucerne diet than for the TMR diet, with concentrations remaining high at 4 hours post-feeding for the lucerne diet, but declining back to near pre-feeding concentrations for the TMR diet (Table 5.9).

Concentrations of acetate, butyrate, minor and total VFA in rumen fluid collected during lucerne feeding were significantly ($P < 0.01$) lower than when feeding the TMR diet. The ruminal acetate-to-propionate (A/P) ratio was also significantly ($P < 0.0001$) lower when feeding lucerne than the TMR diet. Concentrations of propionate tended to be higher on the lucerne diet than the TMR diet ($P = 0.059$), although pre-feeding concentrations were higher on the TMR diet than the lucerne diet ($P = 0.01$).

Table 5.9 Mean ± SD rumen fluid concentration of volatile fatty acids, ammonia and the acetate to propionate ratio (A/P) of four cows on two consecutive Thursdays prior to (0), and at 1 and 4 hours after morning feeding when cows were fed lucerne or TMR.

	Time	Acetic	Propionic	Butyric	Minor	Total	Ammonia	A/P
Lucerne	0	35.19 ± 3.13	5.56 ± 0.81	2.82 ± 0.33	1.98 ± 0.29	45.55 ± 4.21	5.37 ± 0.79	6.37 ± 0.53
	1	62.11 ± 9.33	20.13 ± 3.08	4.49 ± 0.90	3.54 ± 0.61	90.28 ± 12.97	21.60 ± 1.64	3.10 ± 0.41
	4	63.35 ± 8.24	19.03 ± 3.93	7.07 ± 1.33	6.93 ± 0.90	96.38 ± 14.00	21.29 ± 1.45	3.37 ± 0.39
TMR	0	48.39 ± 8.93	8.41 ± 1.51	6.18 ± 1.68	3.01 ± 0.29	65.99 ± 12.28	6.35 ± 0.88	5.75 ± 0.21
	1	65.72 ± 7.27	17.63 ± 1.57	8.94 ± 1.54	4.23 ± 0.45	96.53 ± 10.32	13.74 ± 1.30	3.73 ± 0.29
	4	72.97 ± 4.39	15.02 ± 0.74	10.72 ± 0.63	3.77 ± 0.16	102.47 ± 5.58	4.48 ± 1.18	4.86 ± 0.23

The A/P ratio was lower ($P < 0.0001$) at 1 and 4 hours post-feeding than prior to feeding (0 hour) for both diets. However, while there was no difference ($P = 0.21$) between 1 and 4 hours post-feeding for the lucerne diet, there was a higher ($P < 0.0001$) A/P ratio at 4 hours than 1 hour post-feeding for the TMR diet. This indicates that a different pattern of ruminal VFA production occurred between the diets.

5.5 Discussion

To allow selection of low CH_4-emitting animals as a mitigation strategy, animals would ideally have a low within-animal and a high between-animal variance in CH_4 yield. Results presented here indicate that the between-animal variance (CV = 12% for lucerne and 16% for TMR diets) is similar to the within-animal variance (CV = 8-18%). These results indicate that selecting for low-CH_4 emitting animals is a possible, albeit difficult, mitigation strategy to reduce enteric CH_4 emissions due to the within-animal variance being as large as the between-animal variance.

Unfortunately, there are few data on the variance in CH_4 yields in the literature, as variances tend to be reported for absolute daily CH_4 production. The distinction between absolute daily CH_4 production and daily CH_4 yield is very important since selecting animals with low absolute daily CH_4 production may simply result in selecting animals with lower daily feed intakes, due to the relationship between absolute daily CH_4 production and daily feed intake (see Blaxter and Clapperton, 1965).

Blaxter and Clapperton (1965) reported within-animal variation as 7-8% of the absolute daily CH_4 production from 210 sheep and 129 cattle experiments when individual animals were given a constant level of feeding, although different animals had differing levels of intake. These values are smaller than the within-animal variance (CV = 8-18%) in absolute daily CH_4 production reported in this experiment. Due to the constant daily intake for individual animals in this study, variation in CH_4 yield is the same as variation in absolute daily CH_4 production. Results from the current study suggest greater variation for estimated CH_4 yield

when using the SF_6 technique than for measurements of CH_4 yield using calorimetry.

The large within-animal variance reported in the current study may help partially explain why animals selected as high or low emitters using the SF_6 tracer technique have been found to change their ranking when measured at different points in time (Goopy and Hegarty, 2004; Pinares-Patiño et al., 2000, 2005). This variation may be caused by natural variation in daily CH_4 production from animals or other factors influencing the amount of variation. Other sources of variation include experimental error in the CH_4 measurement technique, expressing CH_4 relative to a predicted value that may not be fully accurate (Goopy and Hegarty, 2004), variation in intake estimation (Pinares-Patiño et al., 2000, 2005) or a combination of natural and technique variation. Any extra variation in CH_4 yield from using predictive equations to estimate absolute daily CH_4 production or estimating daily feed intake would lessen the ability to consistently identify low CH_4 emitters. However, this study indicates that differences between animals can occur, providing promise that selection for animals with low CH_4 yields may be possible.

The current study suggests that between-animal variance in CH_4 yield can differ markedly on contrasting diets (see Table 5.6). If the between-animal variation is not constant across diets, this will create difficulties when trying to identify consistently high or low CH_4-emitting animals. Genetic selection relies on consistent differences in ranking between animals, so if this result is confirmed, animals will need to be selected for low CH_4 yield on the diet they typically consume.

It is not clear why the CH_4 yield increased when feeding the TMR diet, which had higher levels of starch and reduced NDF and ADF levels, especially as diets were formulated so the animals had the same level of metabolisable energy intake on both diets. This may perhaps be explained by the inclusion of straw in the diet. Straw is known to degrade slowly in the rumen, and may have reduced rumen outflow rates, thus increasing residence time in the rumen and CH_4 production (Okine et al., 1989). Reduced rumen outflow rate is linked to

higher rumen volume (Pinares-Patiño et al., 2003b), greater acetate production and, therefore, methane production per unit of feed eaten (McAllister et al., 1996). This is supported by the higher residual rumen volume for the TMR than the lucerne diet (4 versus 3.8 kg DM). The significantly higher acetate to propionate ratio for TMR (4.8) than lucerne (4.3) is consistent with higher CH_4 production, since higher acetate to propionate ratios have been shown to result in higher enteric CH_4 losses (Moss et al., 2000).

Estimated repeatability of CH_4 yield for cows was higher on TMR (0.73) than cows on lucerne (0.47). Repeatability is a measure of the likelihood of obtaining the same value on successive measurements and is calculated as the between-animal variance divided by the total variance. As between-animal variance increases as a proportion of the total variance, repeatability will increase. Therefore, if high within-animal variance exists, repeatability for CH_4 yield will be lower. In this experiment, the repeatability was higher on TMR than lucerne, despite the higher total variance on TMR. This can be explained by the fact that the within-animal variance was only slightly higher on TMR, while the between-animal variance was much higher. As the repeatability values are not consistent across diets (diet 1 = 0.47, diet 2 = 0.73), animals need to have their CH_4 yields measured on the type of diet they would typically consume to use selection for low CH_4 yields as a mitigation strategy. This supports research with both sheep and cattle by Pinares-Patiño et al. (2000; 2005) that showed changing the diet fed to animals resulted in changes in CH_4 yield ranking, while maintaining animals on the same diet resulted in consistent CH_4 yield rankings over a period of six months (Pinares-Patiño et al., 2003b).

Results from studies using the SF_6 technique are usually expressed as the mean of a 4-day measurement period, instead of the 23 days and 30 days for the lucerne and TMR diets, respectively. Using this standard protocol, the mean (± SD) of the rolling 4-day CH_4 yields for each animal on each diet are presented in Table 5.10.

Table 5.10 Mean ± SD of the 4-day rolling mean CH_4 yields (g CH_4/kg DMI) of four cows fed either lucerne silage or TMR.

	Cow 1	Cow 2	Cow 3	Cow 4
Lucerne	19.3 ± 0.6	23.4 ± 3.0	22.5 ± 3.0	25.4 ± 0.9
TMR	26.2 ± 2.0	29.3 ± 2.1	36.0 ± 2.4	36.6 ± 2.1

Using these data and some simplified assumptions detailed below, it is possible to provide approximate guidelines for selecting individual animals that differ significantly in their CH_4 yield. The first assumption is that the measured 4-day mean CH_4 yield for a given animal is the same as the mean of its overall CH_4 yield distribution. Second, the standard deviation in the CH_4 yield distribution for each individual animal is 3 g CH_4/kg DMI, based on the largest within-animal standard deviation from Table 5.10. Third, variability between animals is the same as the variation within-animal (as seen in the results for diet 1). Fourth, an animal's CH_4 yield is normally distributed. In this study, individual animal variances were not consistent, making it difficult to compare mean values. However, assuming the largest standard deviation (SD = 3.0 g CH_4/kg DMI), it is possible to estimate a conservative value for the differences in mean CH_4 yield required between individual animals to be confident that these differences are statistically significant. This difference is approximately two standard deviations or about 6 g CH_4/kg DMI.

Using the 6 g CH_4/kg DMI value to indicate a significant difference between animals in their mean CH_4 yield, on the lucerne diet cow 1 had a lower CH_4 yield than cow 4, but was not different to cows 2 and 3 (Table 5.10). Mean CH_4 yields from cows 2, 3 and 4 were not different to each other when feeding the lucerne diet. On the TMR diet, cows 1 and 2 were not different in their mean CH_4 yields, but both were significantly lower than cows 3 and 4, while cows 3 and 4 were not different in their CH_4 yields. This indicates that overall, cow 1 had a lower CH_4 yield than cow 4, but that this was the only consistent difference in CH_4 yields between cows on the two diets.

However, a value of 6 g CH_4/kg DMI only applies to animals measured indoors and fed a diet of constant quality and quantity and in a situation where

permeation tube release rates can be equalised for every animal (see Chapter 3). If animals are measured under less controlled circumstances, for example, in grazing animals where the quality of the diet is not strictly controlled for each animal, and intakes are estimated rather than measured, within-animal variation in CH_4 yield is likely to be greater than that found in this study. A simulation study, with ±10% for estimated DMI (Nicol and Brookes, 2007), was conducted using data from 681 cows measured during two grazing experiments (Pinares-Patiño et al., 2008) to assess the increased variability in CH_4 yield when estimating DMI. Each animal's DMI was randomly permuted to ±10% using an equal probability distribution, and the new CH_4 yield calculated. This procedure was repeated 10,000 times for each animal's data, with the mean and standard deviation for all 681 cows calculated each time. This simulation showed that estimating intake can increase the variation in mean CH_4 yield, compared with measuring intake, for all 681 cows by 6.9% with the standard deviation increasing from 2.9 to 3.1 g CH_4/kg DMI.

While further research is required to confirm the results presented here, the high within-animal variability in CH_4 yield measured using the SF_6 tracer technique may explain why there has been difficulty in obtaining consistent animal rankings in CH_4 yields when animals are measured on multiple occasions. Results from the current study also suggest that the SF_6 tracer technique may exaggerate apparent between-animal differences in CH_4 yields. If this is correct, the 40% difference between 'high' and 'low' CH_4-emitting animals reported by Ulyatt et al. (2002c) may be partially due to the technique. However, results from the present study do confirm the presence of large between-animal variation. Therefore, selection of animals for low CH_4 yields is still a possible mitigation strategy and further research is needed to quantify the achievable reductions with grazing livestock. However, these measurements may need to be conducted using calorimetry methods due to the greater variance associated with the SF_6 technique. If the SF_6 technique is to be used, it will be necessary to measure large numbers of animals on multiple occasions to confirm their CH_4 yield rankings.

Chapter 6

A comparison of methane emissions from lactating dairy cows using animal calorimetry and the sulphur hexafluoride technique using high and low SF_6 release rates

6.1 Abstract

Both animal calorimetry and the sulphur hexafluoride (SF_6) tracer technique are used to measure methane (CH_4) production from individual animals. Previous studies have shown reasonable agreement in the mean emissions between these two techniques, although greater variance has been reported for the SF_6 technique. These comparative measurements have been conducted using the SF_6 technique inside the calorimetry chamber, sometimes with high background SF_6 concentrations. Also, some studies using the SF_6 technique have shown an effect of SF_6 release rate from permeation tubes on estimated CH_4 production. An experiment was carried out to compare estimated CH_4 production from four Friesian dairy cattle in late lactation estimated using the SF_6 technique, both inside and outside calorimetry chambers, with measurements made using the calorimetry chambers. As part of this experiment, a repeat cross-over design was used to compare a low (2.77 mg SF_6/day) and high (6.53 mg SF_6/day) SF_6 release rate to ascertain which gave a more accurate estimate of CH_4 production when compared with calorimetry.

Contamination of the SF_6 gas samples by a refrigerant gas (F10) meant these results were unreliable and no comparison could be made between the two techniques. However, it appears from these few measurements that calorimetry measurements are less variable than estimates using the SF_6 technique with within-animal coefficients of variation (CVs) of 1-6% from this study compared with CVs of 7-10% reported for the SF_6 technique in Chapter 5. The between-animal CV from calorimetry measurements was 10%, which is lower than the 11-16% reported for SF_6 in Chapter 5. The smaller variance reported for calorimetry means fewer animals may be required to detect significant differences between treatments, and smaller differences required for animals to be significantly different in their CH_4 yields.

6.2 Introduction

With the advent of the United Nations Framework Convention on Climate Change (UNFCCC, 2008) has come the need for accurate national inventories of greenhouse gas emissions. This presents a challenge for countries with a relatively high proportion of their methane (CH_4) emissions from animal agriculture, due to the difficulty in obtaining accurate estimates of ruminant CH_4 production. In New Zealand, national ruminant emissions are calculated by estimating the dry matter intake of an average animal from that class, multiplying by the CH_4 yield (g CH_4/kg DMI), and then multiplying by the number of animals in each animal class (Clark et al., 2003). However, the large amount of uncertainty around the CH_4 yield values creates large uncertainties in the reported national emissions (Clark et al., 2003).

Two main methods are used to quantify CH_4 production from individual animals, animal calorimetry and the SF_6 tracer technique. Animal calorimetry provides a direct measure of CH_4 production by measuring gas concentrations entering and leaving an animal chamber (Blaxter, 1967), while the SF_6 technique provides an estimate using SF_6 as a tracer for CH_4 (Johnson et al., 1994a, and see Chapters 2, 3 and 4). While both these methods are widely used, only the SF_6 technique can be used with large groups of individual animals and with grazing animals. This characteristic has led to its application in many studies (Johnson et al., 1994a; Lassey et al., 1997; McCaughey et al., 1997; Boadi et al., 2002a; Pinares-Patiño et al., 2003b).

The CH_4 yields in the New Zealand national inventory are based on measurements made using the SF_6 tracer technique. The larger variability in CH_4 yield estimates (see below) using this technique results in larger variation in the national inventory. One aspect of this variation may be due to the SF_6 technique not accounting for CH_4 lost in flatus. Little research has been conducted to assess CH_4 loss in flatus, with just two studies indicating a loss of 1-2% (Murray et al., 1976; Kempton et al., 1976) of absolute daily CH_4 production. However, there is no indication of whether all animals excrete the

same proportion of CH_4 in flatus, or if feeding different diets has an effect on CH_4 loss via flatus.

A number of studies have been undertaken to validate the ability of the SF_6 technique to reliably estimate CH_4 production (Johnson et al., 1994a; Boadi et al., 2002a; McGinn et al., 2006; Grainger et al., 2007; Pinares-Patiño et al., 2007a). These studies indicate that group mean values obtained from the SF_6 technique are not significantly different to those obtained from calorimetry studies, although values using the SF_6 technique were 96 to 105% of those for daily CH_4 production using calorimetry.

Estimates from individual animals are more variable than group means. Johnson et al. (1994a) reported results from one animal where the SF_6 technique estimate was 10% higher than the calorimetry measurement, but average values from 55 SF_6 estimates and 25 calorimetry measurements were less than 1% different. Another study reported daily CH_4 production estimates ranged from 90 to 167 L/day for the SF_6 technique, but only from 108 to 145 L/day for a calorimetric hood (Boadi et al., 2002a). In a study with dairy cattle, lower variability was measured with open-circuit calorimetry (mean ± sd, 322 ± 58) than estimates from the SF_6 technique (mean ± sd, 331 ± 75) (Grainger et al., 2007). These studies indicate that while the two methods are not different when comparing group means, there does appear to be more variation in individual animal estimates when using the SF_6 tracer technique than when using calorimetry measurements. This is important as the uncertainty in CH_4 production is carried through to uncertainty in the New Zealand national greenhouse gas inventory

Comparisons of calorimetry with the SF_6 technique have not all been made in the same way. For example, the Grainger et al. (2007) measurements included CH_4 released from the rectum, while the McGinn et al. (2006) and Boadi et al. (2002a) study did not. It may be that some of the differences between calorimetry and the SF_6 technique are due to CH_4 released in the flatus. Murray et al. (1976) indicated that approximately 1% of the CH_4 was released in the flatus. However, this study was conducted with just three sheep fed lucerne

chaff. There is a need to confirm the results of this study from sheep and to measure the partitioning of CH_4 release from cattle.

Another issue to be considered when comparing SF_6 and calorimetry values is how the SF_6-based CH_4 estimates are being obtained. Johnson et al. (1994a) compared measurements using SF_6 for animals outside the calorimetry chamber with measurements from animals placed inside the calorimetry chamber. In the study of McGinn et al. (2006), the calorimetry chambers had high airflow rates and low gas concentrations inside the chamber, with gas samples for the SF_6 estimates of CH_4 production collected by the animal's nose and the background samples taken inside the chamber but away from the animal's face. This is different to the study of Grainger et al. (2007), where the chamber itself was treated as a giant 'rumen', with SF_6 samples collected inside the chamber, but background samples collected from the air inlet (outside air) of the chamber. The SF_6 technique relies on the assumption of low background concentrations relative to sample concentrations. The main issue is with the possible effect of the animal re-breathing high concentrations of SF_6, as this would tend to result in artificially high concentrations of SF_6 in collected breath samples. This methodology needs to be carefully assessed as it would appear to contradict the assumption of low background gas concentrations when using a conservative tracer. Further, taking background samples at a distant unrelated place to the animal measurements may not reflect the true backgrounds for the animal. Results from both studies appear to relate well to calorimetry measurements, but the issue of tracer gas sampling inside a calorimetry chamber needs to be addressed.

The absolute release rate of SF_6 from permeation tubes may be one area that could explain the additional variance that seems to be a characteristic of SF_6 estimates of CH_4 production (Chapter 2 and 3). There are three ways absolute release rate could affect the variation. First, differences in release rates between permeation tubes (each permeation tube has a slightly different release rate, ~ 2-5 mg SF_6/day) occur from the time of filling the tubes with SF_6 (Chapter 2). Second, the release rate for an individual permeation tube (within-tube) can gradually change over time (Lassey et al., 2001). A third possible

explanation is that the release rate from the permeation tube is not the same in the rumen as when the tube is calibrated in dry air.

It has been shown that the SF_6 release rate from permeation tubes is not constant over time and it has been recommended that corrections for SF_6 release rate be made if the permeation tube has been inside the rumen for more than 4 weeks (Lassey et al., 2001). This lack of linearity in SF_6 release may be partly due to the Teflon™ membrane used to restrict the release of SF_6, as SF_6 can chemically interact with the fluorine in the Teflon™ (Lassey et al., 2001). Recent studies have also indicated that there may be an effect of SF_6 permeation tube release rate on estimated CH_4 emissions when using the SF_6 technique (McNaughton et al., 2005; Chapters 2 and 3). McNaughton et al. (2005) found a significant relationship between SF_6 release rate and estimated CH_4 yield (g CH_4/kg DMI) in a study with grazing dairy cattle, with CH_4 yield increasing with increasing SF_6 release rate, while Chapter 3 reported an increase of 16% in estimated CH_4 yield when using an SF_6 release rate of 7 mg SF_6/day compared with a release rate of 3 mg SF_6/day. Results from these studies indicate that comparisons between SF_6 estimates of CH_4 production and calorimetry measurements will not be independent of SF_6 release rate. It is important, therefore, to state the release rates used when comparing the two techniques.

This study had multiple aims. The first was to make a 3-way comparison of daily CH_4 emissions from cattle. Measurements were made using animal calorimetry chambers as well as the SF_6 technique. Measurements with the SF_6 technique were made both in metabolism stalls and simultaneously with calorimetry measurements inside the calorimetry chamber. A comparison could then be made between calorimetry and the SF_6 technique to assess if the SF_6 technique gave the same value for daily CH_4 production as calorimetry. It would also give a preliminary measure of the variance from open-circuit calorimetry measurements.

Making estimates with the SF_6 technique inside and outside the chamber would allow a comparison of the two techniques, as well as ascertaining the validity of

using the SF_6 tracer technique inside a calorimetry chamber. This would help to resolve the issue of 're-breathing' SF_6. Further, the SF_6 estimate from inside the chamber would include flatus gas, due to re-circulation of air inside the chamber. This estimate from inside the chamber should be higher than the estimate taken outside the chamber, with the difference due to flatus CH_4.

A further aim was to compare the SF_6 CH_4 estimates using either a high or low SF_6 release rate with calorimetry. This was carried out to assess whether the 'high' or 'low' SF_6 release rate gave the most accurate estimate of CH_4 production when compared with open-circuit calorimetry. This was important due to the apparent relationship between SF_6 release rate and estimated CH_4 production reported in Chapter 3 of this thesis.

6.3 Materials and methods

6.3.1 Experimental design

An experiment was conducted at the Department of Primary Industries, Ellinbank, Australia to compare two techniques for measuring CH_4 production during March and April, 2006. Methane production from four rumen-fistulated Friesian dairy cows was measured using both the SF_6 tracer technique and animal calorimetry chambers in a repeat cross-over design experiment. Cows were adapted to both the chambers and SF_6 technique sampling equipment prior to the experiment. Simultaneous measurements were made from two cows housed in metabolism stalls (SF_6 outside chamber) and two cows using animal calorimetry chambers (calorimetry and SF_6 inside chamber) (Table 6.1). Both chamber and SF_6 technique measurements were conducted for three consecutive days with a four-day rest period on a sawdust loafing pad between measurements.

Table 6.1 Experimental design of the CH_4 measurement regime for four cows measured in both animal calorimetry chambers (Calorimetry plus SF_6 technique inside the chamber) and metabolism stalls (SF_6 technique outside the chamber).

	Cow			
	1	2	3	4
Period 1	Chamber	Chamber	Stall	Stall
Period 2	Stall	Stall	Chamber	Chamber
Period 3	Chamber	Chamber	Stall	Stall
Period 4	Stall	Stall	Chamber	Chamber

Either one (low SF_6) or two (high SF_6) permeation tubes were administered to each cow at the beginning of the experiment. Each cow was measured with both calorimetry and the SF_6 technique with either a high or low SF_6 release rate. After the first set of measurements with both calorimetry and the SF_6 technique the high and low permeation tubes were retrieved from the cows and re-administered such that cows that received low SF_6 release rates during the first set of measurements received the high SF_6 treatment for the second set of measurements and *vice versa*. After swapping the tubes, each animal was re-measured with both techniques. Cows were given a four-day rest period after the tubes were re-administered. The mean SF_6 release rates (mean ± SEM) were 2.77 ± 0.12 and 6.53 ± 0.33 for the low and high SF_6 release rate groups respectively.

During the chamber measurements of period 2 high air temperatures inside the chambers resulted in the automatic opening of the chamber doors. Measurements were taken for an extra three days to make up for the lost days during this measurement period. This resulted in the chamber measurements (calorimetry plus SF_6 inside the chamber) being taken three days after the SF_6 measurements in the metabolism stalls.

After the first cross-over (periods 1 and 2) was completed it was discovered that two cows had an existing permeation tube from a previous experiment. These permeation tubes were retrieved and taken to the laboratory and underwent serial weighing to determine their SF_6 release rates. The measured release

rates from these tubes were added to the release rates from the tubes administered for this experiment to correct the SF_6 release rates for estimating CH_4 production. One of the cows which had an extra tube was removed as it was due to calve soon after the end of the modified experimental design, so was replaced with another cow. This left only a correction to be made for one cow, with the release rate from the extra tube being 0.365 mg SF_6/day and this was added to the release from the permeation tube administered at the beginning of the experiment. At this time another cow which had pneumonia during the adaptation period and had not fully recovered was also removed from the experiment and replaced with another cow.

The forced replacement of two cows resulted in a new measurement regime as outlined in Table 6.2. The two original cows which continued on in the experiment are labelled cows 1 and 2, while the two replacement cows are labelled cows 3 and 4, with this labelling used throughout the remainder of this chapter.

Table 6.2 Modified experimental design of the CH_4 measurement regime for four cows measured in both animal calorimetry chambers (Calorimetry plus SF_6 technique inside the chamber) and metabolism stalls (SF_6 technique outside the chamber).

	Cow			
	1	2	3	4
Period 1	Chamber	Stall		
Period 2	Stall	Chamber		
Period 3	Chamber	Chamber	Stall	Stall
Period 4	Stall	Stall	Chamber	Chamber
Period 5			Stall	Stall
Period 6			Chamber	Chamber

6.3.2 Animals and animal measurements

The cows were all mature Friesian dairy cows in late lactation, with mean ± SD weight of 583 ± 63 kg and condition score 4.8 ± 0.3. Each of the cows was

rumen-fistulated to allow entry into the rumen for retrieval of the SF_6 permeation tubes. The four cows which began the experiment were milked once-daily in the morning throughout the experiment. The two replacement cows had higher live weight (640 ± 30 kg) but similar body condition score (4.9 ± 0.5). These two replacement cows were milked twice daily during the experiment due to their greater milk production.

Live weight was recorded once per week before the morning feed. Cow body condition score was recorded twice by the same experienced technician using a scale from 1 to 8 (as described by Earle, 1976). Milk production was measured daily, and a sample taken for compositional analysis. Milk samples were added to bronopol preservative (0.5% wt/wt) and composition (fat, protein, and lactose) were determined with an infrared milk analyser (model 2000, Bentley Instruments, Chaska, MN). Rumen fill was measured once at the end of the experiment by emptying and weighing the rumen contents prior to the morning feed.

6.3.3 Diet

The diet, on a wet basis, comprised lucerne hay (46%) and maize silage (32%), with lesser amounts of barley (14%) and canola (8%). Urea (40 g/cow) and a mineral supplement (250 g/cow) were also included in the diet. Feed was prepared once daily and weighed out for both the morning and afternoon feedings. The feed was stored in a chilled room until offered to the cows. Representative samples of the daily diet offered were oven dried, bulked by measurement period, and sub-sampled for analysis. These samples were ground through a 0.5 mm sieve, then analysed by near-infrared spectroscopy by a commercial laboratory (FeedTest®, Hamilton, Victoria, Australia). Chemical composition of the diet offered is shown in Table 6.3. Refusals were collected prior to each feed, weighed and sub-sampled for dry matter (DM) determination to calculate daily DM intakes for each cow.

Table 6.3 Chemical composition, including estimated metabolisable energy content (ME), of the diet offered to the cows throughout the experiment, as estimated by near-infrared spectrometry.

	Chemical component			
	Crude protein (g/100 g)	NDF[1] (g/100 g)	DOMD[1] (g/100 g)	ME[1] (MJ/kg DM)
Mean ± SD	16.3 ± 1.1	39.1 ± 2.4	68.0 ± 2.5	10.1 ± 0.4

[1]NDF, neutral detergent fibre; DOMD, digestible organic matter in dry matter; ME, metabolisable energy; SD, standard deviation

A constant quantity of the diet (13 kg fresh weight) was offered twice daily at 8:30 h and 16:00 h and cows had *ad libitum* access to fresh drinking water throughout the experiment. The two replacement cows were offered 15 kg fresh weight twice daily due to their greater milk production.

6.3.4 Methane by the SF_6 technique

Sulphur hexafluoride permeation tubes were administered to the animals 7 days prior to the start of measurements, with cows receiving either one (low SF_6) or two (high SF_6) permeation tubes. Release rates were measured by gravimetric weighing over a two month period prior to insertion into the animals. Methane production was measured using the method described in Chapters 2 and 3. Two background air samples were collected during each day from the barn close to the metabolism stalls where the cows were housed.

6.3.5 Methane by Calorimetry

Methane measurements were made using the chambers and procedures described by Grainger et al. (2007). Each chamber was designed to house one cow. Humidity was maintained at 55% by a dehumidifying unit and temperature at 20ºC by a temperature control unit. Air was re-circulated inside each chamber (7.2m^3/min) and chamber air was replaced with background air (1 m^3/min). Exhaust air was ducted outside the barn through a polyvinyl chloride exhaust duct. Air was sampled for oxygen, carbon dioxide and CH_4 at both the intake and exhaust of the chamber at 10 second intervals and recorded on a

data logger (DT800, Datataker Pty Ltd., Rowville, Victoria, Australia). Exhaust air flow rates, CH_4 concentrations, relative humidity and temperature were recorded at 10 second intervals over a 4-minute period. Each chamber was measured for a 4 minute period out of every 12 minutes.

When the concentration of CH_4 was at steady state, the CH_4 gas flux was measured as the difference between the exhaust and inlet CH_4 concentrations. Twice daily, the doors of the chambers were opened to allow feeding, cleaning and milking of the animals. While the doors were open, the CH_4 flux was assumed to be the same as just before the doors were opened. Once the doors were closed, it took 90 minutes for the chambers to reach steady state. During this time, the change in CH_4 concentration in the chamber was calculated and used to account for storage of CH_4 inside the chamber (Grainger et al., 2007). The quantity of stored CH_4 was added to the total CH_4 measurement from the steady state period to give daily CH_4 production. The gas analyser for the chambers was calibrated using ultra-pure nitrogen as a zero standard and a standard gas mixture (CSIRO Atmospheric Research Division, Victoria, Australia) manufactured to have a similar gas concentration to that measured in the exhaust air from the calorimetry chambers. The ultra-pure nitrogen was flushed through the analyser to remove any impurities before the standard gas was analysed. The standard was analysed once every 4 hours throughout measurement periods.

6.4 Results

During the analysis of breath samples collected when using the SF_6 technique, it was discovered that these samples were contaminated. This caused very large peak areas on the gas chromatogram which were entirely unrealistic. After analysing several samples, the gas analysis system became contaminated. Results were, therefore, completely unreliable and all CH_4 measurements made using the SF_6 technique were discarded. Contamination of the gas chromatographs meant SF_6 samples collected inside the chambers were also unreliable. Due to this contamination, only data related to the chamber measurements are presented.

Intake for the two cows milked once-daily (cows 1 and 2) was lower than for the cows milked twice-daily (cows 3 and 4), as was milk production (Table 6.4). Residual rumen fill was measured on the morning after the last CH_4 measurement for each cow to see if there was a relationship between residual rumen fill and daily CH_4 yield.

Table 6.4 Three-day means ± SD of DM intake and milk production from four cows during two chamber measurement periods and a single rumen fill measurement from before the morning feed of the last day of the experiment.

	Period	DM Intake (kg/d)	Milk Yield (kg/d)	Rumen fill (kg DM)
Cow 1	1	14.61 ± 0.36	9.99 ± 0.98	
	3	14.11 ± 0.48	6.56 ± 0.89	4.95
Cow 2	2	15.22 ± 0.16	14.17 ± 3.09	
	3	14.11 ± 0.48	11.94 ± 1.29	7.33
Cow 3	4	16.34 ± 0.24	11.57 ± 0.66	
	6	15.22 ± 0.23	11.51 ± 0.87	10.84
Cow 4	4	15.48 ± 0.61	17.49 ± 0.55	
	6	15.22 ± 0.23	17.48 ± 1.57	7.19

Mean daily CH_4 production from individual animals was in the range from 300 to 400 g CH_4/day (Table 6.5). Coefficients of variation in absolute daily CH_4 production for individual animals ranged from 0 to 4%. Methane yield (g CH_4/kg DMI) was consistent within period of measurement, with coefficients of variation from 1 to 6% (Table 6.6). However, there were small differences between periods, even though intake was constant in both quantity and quality. No data are available for the first calorimetry measurement for cow 2 as no two consecutive days of CH_4 data were recorded due to problems with the temperature sensors in the chambers at the time of measurement.

Table 6.5 Mean ± SD daily CH_4 production (g CH_4/day) from two 3-day measurement periods for individual dairy cows using animal calorimetry.

		Cow 1	Cow 2	Cow 3	Cow 4
Measure 1	Mean ± SD	303.2 ± 6.5	NA[1]	403.2 ± 3.2	363.1 ± 2.7
	CV (%)	2.1	NA[1]	0.8	0.8
Measure 2	Mean ± SD	326.9 ± 11.3	301.1 ± 1.3	350.0 ± 6.5	331.6 ± 2.4
	CV (%)	3.5	0.4	1.9	0.7

[1]Not available

Table 6.6 Mean ± SD and coefficient of variation (CV) of daily CH_4 yield (g CH_4/kg DMI) from two 3-day measurement periods for individual dairy cows using animal calorimetry

		Cow 1	Cow 2	Cow 3	Cow 4
Measure 1	Mean ± SD	20.8 ± 0.2	NA[1]	24.7 ± 0.3	23.5 ± 0.9
	CV (%)	1.0	NA[1]	1.2	3.6
Measure 2	Mean ± SD	23.2 ± 1.5	21.4 ± 0.8	23.0 ± 0.8	21.8 ± 0.5
	CV (%)	6.3	3.8	3.3	2.1

[1]Not available

6.5 Discussion

Due to sample contamination the main aim of this experiment, a three way comparison of CH_4 production using calorimetry measurements and SF_6 estimates both inside and outside the calorimetry chamber was not possible. Also precluded was any comparison between the high and low SF_6 release rates to ascertain which estimate was in closer agreement with the calorimetry measurements. The main reason this contamination resulted in unreliable samples was because of an overlap between the contaminant peak and the SF_6 peak during analyses on the gas chromatograph. During later experiments at the same site it was discovered that this contamination was still occurring (C. Grainger, pers. comm.). Further testing showed that it was a refrigerant gas, F10, leaking from one of the air-conditioning units located inside the animal house (C. Grainger, pers. comm.).

Within-animal variation (CV) was between 0% and 4% of absolute daily CH_4 production from calorimetry measurements when cows were fed a constant diet. This is smaller, but similar to the 7-8% of the absolute daily CH_4 production previously reported for sheep and cattle with constant intake and measured over about 4 days with calorimetry (Blaxter and Clapperton, 1965). Grainger et al. (2007) reported a CV of 4.3% for within-animal variance from 3-day calorimetry measurements with sixteen lactating dairy cows fed *ad libitum*. These values are lower than the within-animal CV (8-18%) reported for SF_6 estimates of absolute daily CH_4 production reported in Chapter 5. This would indicate that there is less variation in day-to-day measurements using calorimetry than estimates using the SF_6 technique.

Between-animal variance (CV) calculated from the mean absolute daily CH_4 production of each animal was 10%. This is lower than the 18% reported from calorimetry measurements made with *ad libitum* fed dairy cattle at the same facility on a previous occasion (Grainger et al., 2007). Lower variation reported in this study may be at least partially due to the lower intakes than in the Grainger et al. (2007) study, as Pinares-Patiño et al. (2003b) suggested that differences in CH_4 production between animals may be greatest at high levels of feed intake. Results from this study are also lower than the 12% and 16% reported in Chapter 5 for cattle fed a constant daily quantity of feed on two contrasting diets when using the SF_6 technique. However, they are close to the CV (7-8%) for cattle with constant intakes reported in the study by Blaxter and Clapperton (1965). This appears to confirm previous reports (Johnson et al., 1994a; McGinn et al., 2006; Grainger et al., 2007) of greater variation in estimated daily CH_4 production when using the SF_6 tracer technique compared to calorimetry measurements.

Within-animal variation in daily CH_4 yield (CV = 1-6%) for these calorimetry measurements was lower than that reported for SF_6 (CV = 8-18%) in Chapter 5. This result would suggest that CH_4 yields from individual animals are reasonable consistent over a period of 3 days when feed intake is relatively constant. Another implication is that the higher day-to-day variance found with the SF_6 technique is due to the technique itself, and not to natural animal

variation. This would support the work from Chapter 5 suggesting that the lack of consistency of animals in their estimated CH_4 yield ranking over time may be at least partially due to the SF_6 technique.

The between-animal variance (CV) in daily CH_4 yield from the four cows measured in this study was 6%. This is lower than the 11% and 16% reported for the lucerne and TMR diets in Chapter 5. While only four cows were used in both studies, these values would suggest that even under highly controlled conditions, estimates using the SF_6 tracer technique tend to be more variable than measurements made using calorimetry, and that between-animal differences may be exaggerated by the SF_6 technique.

Using the assumptions of Chapter 5 (95% confidence interval and a *t*-statistic for a large sample) to estimate a significant difference, means will differ if they are approximately two standard deviations apart. The largest standard deviation from CH_4 yield measurements in this study is 1.5 g CH_4/kg DMI. Therefore, a conservative estimate of the difference required between two animals for them to be likely to differ significantly in their CH_4 yields would be 3 g CH_4/kg DMI. This is half the value reported for the SF_6 tracer technique under highly controlled conditions. If this is correct, far fewer animals would need to be measured using calorimetry to identify high and low emitters.

While the main comparison for this experiment could not be made due to sample contamination, calorimetry results provide a preliminary value for within- and between-animal variation. When these values are compared with the long-term measurements using the SF_6 technique reported in Chapter 5, estimates of CH_4 yields using the SF_6 technique appear to be more variable than calorimetry measurements. This smaller variation means fewer animals may be required to detect significant differences between treatments, and smaller differences required for animals to be likely to be significantly different in their CH_4 yields.

Chapter 7

General Discussion

7.1 Introduction

The initial aim of this research work was to identify high and low CH_4 emitting cattle in an attempt to discover why some animals produce less CH_4 per kg DMI. This would then lead to the possibility of breeding livestock for low CH_4 yields (g CH_4/kg DMI) by selecting animals with identified characteristics related to low CH_4 yield. Breeding animals with low CH_4 yields is one option that could provide an inexpensive and long-term reduction in CH_4 emissions from ruminant livestock. An early experiment measuring CH_4 yield using the SF_6 technique (Pinares-Patiño et al., 2005) failed to identify any consistency in the rankings of animals for CH_4 yield. This result, in combination with similar results from other studies estimating CH_4 yield using the SF_6 technique (Pinares-Patiño et al., 2000; Goopy and Hegarty, 2004) caused a change in the focus of this study.

The observed lack of repeatability in rankings among animals may be due to natural variation in individual emissions, variation in the measurement technique, or a combination of both. The aim of this study changed to try to understand the basis for the lack of consistency in estimated CH_4 yields. One aspect of this was the quantification of animal variance. A further aspect was to understand why estimates of CH_4 yield using the SF_6 tracer technique appear to be more variable than calorimetry measurements.

7.2 Discussion

7.2.1 Animal variance

The SF_6 tracer technique was developed as a simpler, cheaper alternative to animal calorimetry (Johnson et al., 1994a). This technique has the advantage that it can be used to measure CH_4 production from large numbers of individual animals, and also from grazing animals. Comparisons with animal calorimetry to date have shown that mean values for absolute daily CH_4 production estimated using the SF_6 technique are not different to measurements made using animal calorimetry (Johnson et al., 1994a; Boadi et al., 2002a; McGinn et al., 2006; Grainger et al., 2007; Pinares-Patiño et al., 2007a). While the group mean value

from SF_6 estimates of absolute daily CH_4 production may not be different to calorimetry measurements, there does appear to be greater variation in the SF_6 estimates (Johnson et al., 1994a; Boadi et al., 2002a; McGinn et al., 2006; Grainger et al., 2007; Pinares-Patiño et al., 2007a).

Selection of low CH_4-emitting animals would need to be done on the basis of CH_4 yield (g CH_4/kg DMI). At present, calorimetry is considered the 'gold' standard for measuring CH_4 production (O'Hara et al., 2003). Variation (CV) in CH_4 yields from calorimetry measurements reported in Chapter 6 was 1-6% for within-animal and 6% for between-animal variance. These values are less than half the reported within- (CV = 8-18%) and between-animal (CV = 11-16%) variance in estimates of CH_4 yield for housed stall-fed animals when using the SF_6 technique (Chapter 5). The diet eaten by animals in the experiment where the SF_6 technique was employed was consistent from day to day in both quality and quantity, so any extra variation in the SF_6 estimates compared with calorimetry measurements is likely due to technique variation and not true animal variation. These results confirm the larger variation from estimates using the SF_6 technique than measurements made using calorimetry.

Results from the experiments described in this thesis confirm the existence of measured between-animal variance in daily CH_4 yield from both calorimetry (Chapter 6) and the SF_6 technique (Chapter 5). However, between-animal variation appears to be much larger from SF_6 estimates of CH_4 yield than calorimetry measurements. The implication is that the SF_6 technique greatly exaggerates the differences between individual animals, so the 40% difference in CH_4 per unit intake between high and low CH_4 emitters as reported by Ulyatt et al (2002c) seems to be partly due to technique variance and not simply to true differences between animals. This extra variance due to the SF_6 technique itself will be discussed further in section 7.2.2.

Chapter 5 reported that the variance in CH_4 yield for cows fed a lucerne silage diet (within = 6.9; between = 6.2) was lower than when the same cows were fed a TMR diet (within = 10.1; between = 27.8). The within-animal variance was almost double and the between-animal variation was almost five times as high

on the TMR diet compared with the lucerne diet. This implies that diet can have a strong influence on variation in daily CH_4 yields, and this may affect the consistency of animal rankings. Pinares-Patiño et al., (2003b) showed that when animals were maintained on a pasture diet, they maintained their rankings in CH_4 yield over a period of six months. However, Pinares-Patiño et al. (2000; 2005) showed that when the diet fed to either housed sheep or cattle was changed, their CH_4 yield rankings also changed. These data imply that selection of animals for high or low CH_4 yield would, therefore, need to be undertaken when animals are consuming the diet they would typically consume. This has implications for experiments selecting for low CH_4 yields when animals are housed and fed a conserved forage diet or TMR diet instead of grazing fresh pasture. However, while it is best to select animals at grazing to avoid dietary differences compared with housed animals, it is not possible to accurately measure or estimate daily dry matter intakes for grazing livestock and thus to accurately calculate CH_4 yield.

Studies assessing CH_4 mitigation options rely on detecting differences in group means to quantify any possible reduction in CH_4. Chapter 6 showed that using the within-animal variance data and some simplified assumptions, a difference of 3 g CH_4/kg DMI was required between individual animals for them to be significantly different when using calorimetry, while the same calculation for data from Chapter 5 showed a difference of 6 g CH_4/kg DMI was required when using the SF_6 tracer technique for housed stall-fed animals. A simulation study, with ±10% for estimated DMI (Nicol and Brookes, 2007), using data from 681 cows measured during two grazing experiments (Pinares-Patiño et al., 2008) showed that estimating intake resulted in a 7% increase in the standard deviation. Using the larger standard deviation of 3.2 instead of 3.0 g CH_4/kg DMI, two animals would need to be approximately 6.4 g CH_4/kg DMI apart in their CH_4 yields for them to be likely to be significantly different from each other when their CH_4 yield is estimated using the SF_6 technique.

While the 6.4 g CH_4/kg DMI gives a rough guide to the difference required between animals for them to be likely to be significantly different, they may not remain different in their CH_4 yield when re-measured at a later date due to

within-animal variance. Pinares-Patiño et al. (2005) showed that two groups of three cows were, on average, 9.5 g CH_4/kg DMI (high = 24.8 ± 3.7, low = 15.3 ± 1.1) different in their CH_4 yields, with the smallest difference between a high and low CH_4-emitting cow being 5.4 g CH_4/kg DMI. When these cows were re-measured at a later date, the largest difference was 5.4 and on average was 2.1 g CH_4/kg DMI and during a third measurement of these animals the average difference was only 0.6 g CH_4/kg DMI. These data illustrate that although animals may be measured as different at one point in time, this does not necessarily mean they are truly different and repeat measures are needed when using the SF_6 technique to confirm whether animals are truly different in their CH_4 yields.

Due to the high within-animal variance in CH_4 yield measured in current study and the exaggerated between-animal variance it is suggested the SF_6 technique may not be suitable for detecting individual animals with high or low CH_4 yields.

7.2.2 Variance due to the SF_6 Technique

The first large grazing experiment aiming to identify high and low emitters from a cohort of 300 lactating dairy cows identified a small, but significant, effect of SF_6 release rate on estimated CH_4 yield (McNaughton et al., 2005). This effect was not seen in a subsequent 400-cow experiment conducted the following year at the same location (Cavanagh et al., 2008). The main difference in the second year was a much smaller range in SF_6 release rates. An examination of data from twenty-one CH_4 experiments with either housed or grazing animals (sheep or dairy cattle) conducted in New Zealand assessed the effect of SF_6 release rate on estimated CH_4 production and yield (for detail see Chapter 2). This work showed that a significant relationship did sometimes occur in estimated absolute daily CH_4 production for grazing dairy cattle and housed sheep experiments, but not in CH_4 yield.

The lack of any apparent effect of SF_6 release rate on CH_4 yield may be related to the inaccuracies of estimated intake in grazing studies. Any error in

estimating intake is directly related to CH_4 yield, which is calculated as absolute CH_4 production (g CH_4/day) divided by feed intake (kg DMI/day). A range of methods were used to estimate intakes in the experiments examined, and errors in CH_4 yield due to the inability to accurately measure the feed intake from grazing animals could easily mask any small effect of SF_6 release rate on estimated CH_4 yield.

In the sheep dataset there was a mix of immature and mature animals. Two recent studies have shown that immature sheep have lower CH_4 yields than mature sheep (Clark et al., 2003; Ulyatt et al., 2005). This is in contrast to Pelchen and Peters (1998), who reported that absolute daily CH_4 production was higher for growing animals, although CH_4 yield (% GE) was not different between growing and mature animals. Any extra variability in CH_4 yield due to age differences could mask any possible effect of SF_6 release rate. The data appear to support this, with greater variability in CH_4 yield for the sheep datasets than for the dairy cattle datasets even though they contain many more measurements.

Results from the examination of previous CH_4 experiments indicate that permeation tube release rate may be related to estimated CH_4 production, with higher SF_6 release rates resulting in higher estimated CH_4 production. Therefore, having a range in SF_6 release rates for a group of animals could introduce extra variance into estimates of CH_4 production.

In order to confirm the findings for the examination of previous CH_4 experiments, a tailored experiment was conducted under controlled conditions with low (2.9 mg SF_6/d) and high (7.3 mg SF_6/d) release rates (Chapter 3). Results from this experiment showed a significant effect of release rate on estimated CH_4 yield. The difference in estimated CH_4 yield from release rates at the low (3 mg/d) and high end (5 mg/d) of the normal range of release rates used in cattle experiments was approximately 8.5%, assuming the relationship between SF_6 release rate and estimated CH_4 yield was linear. A subsequent experiment with a larger range in SF_6 release rates showed a similar pattern in increasing estimated CH_4 yield with increasing SF_6 release rate (Pinares-Patiño

et al., 2008). This implies that a lower proportion of SF_6 is recovered in breath samples as the SF_6 release rate increases. The SF_6 technique relies on the assumptions that SF_6 is inert, well-mixed with rumen gases, and eructated and breathed out in the same way as CH_4. Results from these three studies suggest that this may not be the case (Chapter 2; Chapter 3; Pinares-Patiño et al., 2008).

Having a range in permeation tube release rates in any given experiment will increase the between-animal variation in CH_4 yield estimates only slightly, as there is approximately a 4% increase in estimated CH_4 yield for every 1 mg/day increase in SF_6 release rate over the range from 2 to 7 mg SF_6/day. While this is important for comparing individual animals, it should not have a significant effect on group means due to the random allocation of permeation tubes to animals.

As results from Chapters 2 and 3 indicated the occurrence of a positive relationship between SF_6 release rate and estimated CH_4 yield, it was considered that permeation tubes may not behave the same in the rumen as during calibration in the laboratory. In Chapter 4 it was reported that immersing permeation tubes in fluids could result in a reduced daily release of SF_6. Release rates were lower from a permeation tube submerged in water (6%) and strained rumen fluid (11%) than in dry air when measured over ten to twelve one-hour periods (Chapter 4). These results contradict the current assumption of the SF_6 technique that the release rate from permeation tubes measured by gravimetric weighing is the same as the release rate in the rumen. Further research is required to confirm and quantify the inhibition of SF_6 release when permeation tubes are submerged in rumen fluid.

A lower than expected SF_6 release rate would result in a systematic bias in CH_4 estimates using the SF_6 technique, as the technique would overestimate the CH_4 yield. If release rates are 11% lower in the rumen, then estimated CH_4 yields would be 11% too high. While more research needs to be carried out to confirm the results of this preliminary study and to quantify any effect of fluids on permeation tube release rate, it indicates that calibration in dry air may not

give an accurate value for release rate in the rumen and the method of calibration may need to be revised if this proves to be the case. However, this result is in contrast to comparative studies showing that mean values of absolute CH_4 production estimated using the SF_6 technique are not different to calorimetry measurements. It does suggest that any comparison of CH_4 production between the SF_6 technique and calorimetry may not be independent of the SF_6 release rate used.

Variation from day to day in the release rate of an individual permeation tube could account for some of the extra within-animal variance measured with the SF_6 technique. It appears from the study reported in Chapter 4, that the SF_6 release rate is more variable when permeation tubes are immersed in fluids than in air. It is plausible that the cause of variation could be the Teflon® membrane (Lassey et al., 2001). Lassey et al. (2001) discussed the possibility of SF_6 'sticking' to the Teflon® membrane, as these two compounds can chemically interact. It is also known that there is variation in the Teflon® sheeting used in the manufacture of permeation tubes (K. Lassey pers. comm.). Any variation in the Teflon® membrane between tubes has the potential to result in different release behaviour between tubes, and this may be implicated in the variation in SF_6 release.

One of the primary assumptions of the SF_6 technique is that all the SF_6 released from the permeation tube is released via the mouth and nose. Preliminary data looking at the release of SF_6 from sites other than the mouth and nose showed that that very little SF_6 is excreted in either urine (~0.00007 mg SF_6/day) or gas trapped in faecal material (8.2×10^{-6} mg/day) (Chapter 4). It is possible, however, that some SF_6 is excreted in faecal material due to its adherence to organic material (Wilson and Mackay, 1996). Studies have reported diffusion of SF_6 into the lungs from venous blood (Schrikker et al., 1989; Schimmel et al., 2004), so it may also be possible for SF_6 to be absorbed from the digestive system into the blood and lungs followed by excretion in the breath. As long as the SF_6 is excreted via the breath and not stored in the body, this route of excretion should not affect the efficacy of the SF_6 tracer technique for estimating CH_4 production.

While a number of studies have confirmed that estimates of mean absolute daily CH_4 production from the SF_6 technique are not different to calorimetry, they do report greater variation for SF_6 estimates than for calorimetry measurements (Boadi et al., 2002a; McGinn et al., 2006; Grainger et al., 2007; Pinares-Patiño et al., 2007a). Research presented here indicates that the release rate of SF_6 from the permeation tube may contribute to this greater variance. As most experiments are conducted using a small range in release rates, the SF_6 release rate effect on CH_4 yield will have very little impact on estimated CH_4 yields, as indicated by an experiment with 388 grazing dairy cows (Pinares-Patiño et al., 2008) using a range of SF_6 release rates from 2.2 to 3.6 mg SF_6/day, where there was no significant effect of release rate on estimated CH_4 yields. The analysis of previous CH_4 experiments undertaken in New Zealand also showed only a very small effect, or no effect. So while the SF_6 release rate may contribute to the extra variance in estimated CH_4 yields, there should be very little systematic bias when the SF_6 release rates used in any given experiment are within a small range.

7.3 Animal numbers for treatment comparisons

The original aim of this study was to identify individual animals that had high and low CH_4 yields. As has been discussed in section 7.2, this was not possible due to the higher than expected within-animal variance, exaggerated between-animal variance, and due to extra variance associated with the SF_6 technique estimates compared to calorimetry measurements. However, the estimates of repeatability and within- and between-animal variances in daily CH_4 yield from Chapter 5 were used to develop a power analysis for repeated measures to estimate the number of animals and number of measurements per animal required to detect a significant difference at the $P < 0.05$ level between two groups of animals. Development of the power analysis was conducted by Dr. N. Lopez-Villalobos. Repeated measures of CH_4 yield from the same animal were sampled from a multivariate normal distribution using a Cholesky decomposition. The residual matrix was assumed to have an autoregressive structure with correlation of 0.7 (repeatability value). The model was designed so the experiment had two treatments, a high CH_4-emitting group and a low

CH_4-emitting group. The difference between these groups could be altered, with a difference of 1.0, 0.5, and 0.25 standard deviations. Each experiment had varying numbers of animals per treatment, with each animal being measured from 2 to 10 times. A Bootstrapping algorithm (Efron, 1979) was implemented to compute the likelihood of detecting a significant difference between CH_4 groups. For each combination of group differences, number of animals and number of measures per animal, 1000 bootstrapping samples were performed and power was defined as the number of occasions in 1000 that a significant difference would be detected between the two groups (Table 7.1).

The power analyses show that when the difference between the mean of two groups of animals is small, a larger number of animals is required to detect a significant difference (Table 7.1). Increasing the number of measurements per animal increases the power of the experiment up to four to five measurements per animal. After this, little is gained by undertaking further measurements on individual animals and will not increase the likelihood of detecting significant differences between groups of animals. It is also possible to optimise experiments to take the minimum number of measurements. For example, using the standard 80% power requirement and 1 standard deviation between the mean of two groups, 10 animals would need to be measured eight times (80 samples) or 14 animals measured twice (28 samples).

Table 7.1. Likelihood, out of 100, of detecting a significant difference (at $P < 0.05$) in CH_4 yield estimated using the SF_6 technique between the means of two groups of animals either 1, 0.5 or 0.25 standard deviations (SD) apart, with a set number of animals per group and a set number of consecutive measurements.

Animals per group	Measurements per animal								
	2	3	4	5	6	7	8	9	10
1 SD									
10	64	72	71	76	77	77	81	79	79
12	72	80	80	81	83	84	86	87	88
14	80	83	84	89	87	89	90	92	92
16	87	88	90	91	92	93	94	93	95
0.5 SD									
40	67	72	73	75	76	81	79	81	80
45	74	77	79	79	80	81	83	84	86
50	78	84	83	84	88	87	89	87	88
60	85	88	91	93	91	92	93	93	94
0.25 SD									
100	49	55	54	55	57	58	60	60	61
125	58	62	66	65	68	69	70	71	71
150	67	70	73	77	76	75	77	78	80
200	77	81	82	87	85	86	87	88	88

Another approach is to use the expected difference between two group means and the variation to calculate the total number of experimental animals required. This was carried out using a power analysis based on a simple two-sample t-test comparison of means. The power analysis calculates the number of animals required to achieve a power of 80%, which is the accepted standard for power analyses. Reported variation in CH_4 yield is higher for estimates made using the SF_6 tracer technique than for calorimetry measurements (see Chapters 5 and 6). Calculations use a mean daily CH_4 yield of 20 g CH_4/kg DMI, with standard deviations calculated using the commonly reported coefficients of variation for each technique and estimation of each animal's CH_4 yield conducted once using the SF_6 technique. Results for the SF_6 tracer technique are shown in Table 7.2, while results for calorimetry measurements are shown in Table 7.3.

Table 7.2 Total number of experimental animals required to detect a significant difference in mean estimated CH_4 yield (g CH_4/kg DMI) between two groups of animals with a specified coefficient of variation (CV) and expected difference between treatment means when using the SF_6 tracer technique.

		Expected difference between treatment means				
		5%	10%	15%	20%	25%
CV (%)	10%	64	17	8	5	4
	15%	143	37	17	10	7
	20%	253	64	29	17	12
	25%	394	100	45	26	17

Table 7.3 Total number of experimental animals required to detect a significant difference in mean estimated CH_4 yield (g CH_4/kg DMI) between two groups of animals with a specified coefficient of variation (CV) and expected difference between treatment means when using animal calorimetry.

		Expected difference between treatment means				
		5%	10%	15%	20%	25%
CV (%)	2.5%	5	2	2	2	2
	5%	17	5	3	2	2
	10%	64	17	8	5	4
	15%	143	37	17	10	7

As the coefficients of variation for between-animal variance in estimated CH_4 yield is lower when using calorimetry than the SF_6 technique, fewer animals are required per treatment. For example, if a 10% difference is expected between the means, between 5 and 17 animals per treatment are required using calorimetry, but between 37 and 64 animals per treatment when using the SF_6 technique. Results show that more animals are required when undertaking experiments using the SF_6 technique than when using calorimetry. Another implication is that further research is required to reduce the variance associated with the SF_6 technique, so in future fewer animals would be required to conduct experiments.

These results provide insight into the combination of number of animals and number of measurements per animal required to be likely to detect a significant

difference. This information will help to ensure the most efficient use of resources in experiments, and minimise the number of animal measurements required, while still ensuring a high likelihood of detecting any reduction in CH_4 yield between groups due to a mitigation strategy.

7.4 Recommendations and future research

Research to date suggests that mean values obtained using the SF_6 technique are similar to those obtained using hood calorimetry (Boadi et al., 2002a) and open-circuit calorimetry (McGinn et al., 2006; Grainger et al., 2007). Results from the studies presented here confirm that there is larger variation in estimated CH_4 yields when using the SF_6 tracer technique than measurements made using calorimetry.

While confirmation of between-animal variance provides an opportunity to select animals for low CH_4 yield, these differences appear to be exaggerated when using the SF_6 technique compared with animal calorimetry. Consequently, it is recommended that the SF_6 tracer technique not be used to attempt to identify high and low CH_4 emitting animals, as very large numbers of animals would need to be measured multiply times in an attempt to identify animals that had significantly and consistently low CH_4 yields.

The effect of SF_6 release rate on estimated CH_4 yield identified here may contribute to the greater variability identified with this technique. Having a range in permeation tube release rates may result in small amounts of additional variation, but if tubes are randomly allocated to animals there should not be any systematic effect of release rate on the average estimated CH_4 yield from a group of animals. It is recommended that researchers using the SF_6 tracer technique ensure that permeation tubes used in experiments have a small range in release rates; for example, less than 1 mg SF_6 release for sheep and less than 2 mg SF_6 release for cattle and that SF_6 release rate is used as a covariate in statistical analyses. Ideally, future research will enable the production of permeation tubes with a pre-determined release rate, removing any possible variation due to SF_6 release rate. This may involve finding an

alternative membrane that can be used instead of the Teflon® sheeting that is currently used. An important factor in choosing an alternative material would be that SF_6 does not chemically interact with it, as it does with Teflon®.

If a large range in release rates is used in an experiment, such as in the McNaughton et al. (2005) study, it is recommended that permeation tubes are blocked by treatment. This will help avoid the possibility of treatment bias by having a higher average release rate in one treatment than another. Otherwise SF_6 release rate may need to be used as a covariate in statistical analyses to remove the effect of SF_6 release rate. Further, any comparison of the SF_6 technique with calorimetry will need to state what SF_6 release rate is used, as the comparison will not be independent of release rate over the range of 2 to 7 mg SF_6/day.

Further research is required to confirm that permeation tubes have lower SF_6 release rates when immersed in rumen fluid than when measured in air. If measured release rates are lower in rumen fluid, a new method for calibrating permeation tubes may need to be developed. Further, the larger variation in SF_6 release in fluid needs to be quantified to confirm the minimum sampling time required to obtain reliable measures of SF_6 release. As permeation rate is known to become non-linear over time, it is also recommended that future research be conducted into assessing the release rate from permeation tubes over periods up to several months inside the rumen.

Initial measurements indicate that SF_6 is not appreciably lost through either gas trapped in faecal material or in urine. However, measurement of SF_6 in flatus and in solution with faecal material still need to be carried out to confirm the robustness of the hypothesis that all SF_6 is released via the mouth and nose. Confirmation and quantification of the routes of SF_6 loss will provide confidence in the basic assumption that SF_6 behaves as a conservative tracer, and can provide reliable estimates of CH_4 production from livestock.

A power analysis has been developed based on the measured within- and between-animal variances when using the SF_6 technique (Chapter 5).

Information on the number of animals and CH_4 measurements per animal presented in Tables 7.1-7.3 will aid researchers to optimise the number of animal measurements taken during CH_4 experiments.

It is recommended that future research aiming to identify high and low CH_4-emitting livestock is conducted with calorimetry chambers and not the SF_6 tracer technique. The reason is that high within-animal variation and extra variation in SF_6 technique estimates of CH_4 yield make it difficult to obtain consistency in the CH_4 yield ranking of animals. This is supported by the guide developed using variance data from the current study of a 6.4 g CH_4/kg DMI difference required between two animals for them to be likely to be significantly different when estimating their CH_4 yield using the SF_6 tracer technique, and the data of Pinares-Patiño et al. (2005) that showed even differences of this magnitude did not result in animals that were consistently different in their CH_4 yield rankings. In addition, animals will need to be fed a forage diet typical of that eaten by grazing livestock in New Zealand due to apparent differences between diets.

Chapter 8

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Appendices

Appendix 1 Raw data of the daily intake, SF_6 release rate and CH_4 production of 12 steers during an experiment to assess the effect of SF_6 release rate on estimated CH_4 production from Chapter 3.

Steer	Time	Feed	Release rate	CH_4 (g/day)	Release rate (mg SF_6/day)	DMI (kg/day)	CH_4 (g/kg DMI)
52	1	high	high	204.53	7.54	8.98	22.76
57	1	high	high	219.94	7.36	9.65	22.79
58	1	high	high	186.05	7.36	9.16	20.32
48	1	low	high	139.03	7.19	5.81	23.93
51	1	low	high	128.45	7.36	6.41	20.04
55	1	low	high	131.57	7.22	5.81	22.65
49	1	high	low	153.80	2.90	8.56	17.97
50	1	high	low	154.55	2.81	8.87	17.42
53	1	high	low	140.31	2.96	8.05	17.42
47	1	low	low	111.06	2.78	5.91	18.79
54	1	low	low	97.68	3.07	6.11	15.99
56	1	low	low	99.70	2.75	5.41	18.43
52	2	high	low	152.99	2.90	8.67	17.64
57	2	high	low	175.56	2.81	10.22	17.17
58	2	high	low	168.32	2.96	9.09	18.52
48	2	low	low	135.87	2.78	5.69	23.89
51	2	low	low	109.64	3.07	6.41	17.10
55	2	low	low	110.73	2.75	5.81	19.06
49	2	high	high	184.01	7.54	9.01	20.41
50	2	high	high	189.82	7.36	8.80	21.58
53	2	high	high	170.44	7.36	8.75	19.48
47	2	low	high	123.15	7.19	5.27	23.35
54	2	low	high	133.82	7.36	6.11	21.90
56	2	low	high	118.64	7.22	5.41	21.93

Appendix 2 Raw data of the daily intake of four cows fed two diets (diet 1 = Lucerne silage, diet 2 = Lucerne, straw and grain mix) and their estimated CH_4 production from Chapter 5.

Cow	Diet	Day	CH_4 (g/day)	DMI (kg/day)	CH_4 (g/kg DMI)
714	1	1	115.43	5.3	21.92
714	1	2	121.38	5.3	23.05
714	1	3	103.42	5.3	19.64
714	1	4	106.22	5.3	20.17
714	1	5	90.19	5.3	17.12
714	1	6	107.83	5.3	20.47
714	1	7	96.00	5.3	18.23
714	1	8	94.47	5.3	17.94
714	1	9	115.00	5.3	21.84
714	1	10	100.05	5.3	19.00
714	1	11	98.45	5.3	18.69
714	1	12	93.65	5.3	17.78
714	1	13	102.66	5.3	19.49
714	1	14	94.22	5.3	17.89
714	1	15	102.48	5.3	19.46
714	1	16	103.86	5.3	19.72
714	1	17	102.41	5.3	19.45
714	1	18	101.29	5.3	19.23
714	1	19	103.38	5.3	19.63
714	1	20	94.92	5.3	18.02
714	1	21	113.26	5.3	21.50
714	1	22	103.13	5.3	19.58
714	1	23	83.15	5.3	15.79
714	2	1	118.53	5.4	22.07
714	2	2	130.51	5.4	24.30
714	2	3	125.75	5.4	23.41
714	2	4	128.46	5.4	23.92
714	2	5		5.4	
714	2	6	113.56	5.4	21.15
714	2	7	149.98	5.4	27.93
714	2	8	143.22	5.4	26.67
714	2	9	129.02	5.4	24.03
714	2	10	142.77	5.4	26.59
714	2	11	149.08	5.4	27.76
714	2	12	142.87	5.4	26.60
714	2	13	142.77	5.4	26.59
714	2	14	113.92	5.4	21.21
714	2	15	145.75	5.4	27.14
714	2	16	136.76	5.4	25.47
714	2	17	122.96	5.4	22.90
714	2	18	134.46	5.4	25.04
714	2	19	134.20	5.4	24.99
714	2	20	145.90	5.4	27.17
714	2	21	152.31	5.4	28.36
714	2	22	140.95	5.4	26.25
714	2	23	159.07	5.4	29.62
714	2	24	129.76	5.4	24.16
714	2	25	147.41	5.4	27.45
714	2	26	174.97	5.4	32.58
714	2	27		5.4	
714	2	28		5.4	
714	2	29	158.80	5.4	29.57
714	2	30	151.72	5.4	28.25
715	1	1	129.95	5.4	23.98

Cow	Diet	Day	CH_4 (g/day)	DMI (kg/day)	CH_4 (g/kg DMI)
715	1	2	149.80	5.4	27.64
715	1	3	126.67	5.4	23.37
715	1	4	118.74	5.4	21.91
715	1	5	125.52	5.4	23.16
715	1	6	122.05	5.4	22.52
715	1	7	114.11	5.4	21.05
715	1	8	116.23	5.4	21.44
715	1	9		5.4	
715	1	10	131.15	5.4	24.20
715	1	11	115.65	5.4	21.34
715	1	12	143.37	5.4	26.45
715	1	13	133.16	5.4	24.57
715	1	14	204.59	5.4	37.75
715	1	15	122.43	5.4	22.59
715	1	16		5.4	
715	1	17		5.4	
715	1	18	120.03	5.4	22.15
715	1	19	110.99	5.4	20.48
715	1	20	100.28	5.4	18.50
715	1	21	107.23	5.4	19.78
715	1	22	112.28	5.4	20.72
715	1	23	106.41	5.4	19.63
715	2	1	168.34	5.2	32.21
715	2	2	160.62	5.2	30.73
715	2	3	125.06	5.2	23.93
715	2	4	144.04	5.2	27.56
715	2	5	140.34	5.2	26.85
715	2	6	132.17	5.2	25.29
715	2	7	146.17	5.2	27.97
715	2	8	138.38	5.2	26.48
715	2	9	148.07	5.2	28.33
715	2	10	139.13	5.2	26.62
715	2	11	180.33	5.2	34.50
715	2	12	167.37	5.2	32.02
715	2	13	112.84	5.2	21.59
715	2	14	169.98	5.2	32.52
715	2	15	200.51	5.2	38.36
715	2	16	192.63	5.2	36.86
715	2	17	143.24	5.2	27.41
715	2	18	144.07	5.2	27.56
715	2	19	157.17	5.2	30.07
715	2	20	157.73	5.2	30.18
715	2	21	177.73	5.2	34.00
715	2	22		5.2	
715	2	23	150.81	5.2	28.86
715	2	24	120.16	5.2	22.99
715	2	25	145.26	5.2	27.79
715	2	26	151.35	5.2	28.96
715	2	27	149.51	5.2	28.61
715	2	28	188.17	5.2	36.00
715	2	29	158.42	5.2	30.31
715	2	30	136.37	5.2	26.09
718	1	1	143.24	6.1	23.61
718	1	2	135.05	6.1	22.26
718	1	3	130.42	6.1	21.50
718	1	4	118.86	6.1	19.59
718	1	5	132.41	6.1	21.83
718	1	6	137.71	6.1	22.70

Cow	Diet	Day	CH_4 (g/day)	DMI (kg/day)	CH_4 (g/kg DMI)
718	1	7		6.1	
718	1	8	117.71	6.1	19.40
718	1	9	127.69	6.1	21.05
718	1	10		6.1	
718	1	11		6.1	
718	1	12		6.1	
718	1	13		6.1	
718	1	14	205.73	6.1	33.91
718	1	15	89.27	6.1	14.71
718	1	16	128.84	6.1	21.24
718	1	17	140.15	6.1	23.10
718	1	18	147.04	6.1	24.24
718	1	19	135.56	6.1	22.35
718	1	20	134.49	6.1	22.17
718	1	21		6.1	
718	1	22		6.1	
718	1	23		6.1	
718	2	1	214.35	5.2	41.01
718	2	2		5.2	
718	2	3		5.2	
718	2	4		5.2	
718	2	5	167.48	5.2	32.04
718	2	6	181.11	5.2	34.65
718	2	7	167.08	5.2	31.97
718	2	8	166.39	5.2	31.84
718	2	9	167.61	5.2	32.07
718	2	10	163.83	5.2	31.35
718	2	11	227.75	5.2	43.58
718	2	12	233.69	5.2	44.71
718	2	13	173.23	5.2	33.14
718	2	14		5.2	
718	2	15	187.98	5.2	35.97
718	2	16	181.50	5.2	34.73
718	2	17	200.51	5.2	38.36
718	2	18	185.26	5.2	35.45
718	2	19	171.73	5.2	32.86
718	2	20		5.2	
718	2	21	196.59	5.2	37.61
718	2	22	198.63	5.2	38.00
718	2	23	206.80	5.2	39.57
718	2	24	191.86	5.2	36.71
718	2	25	200.78	5.2	38.42
718	2	26	182.97	5.2	35.01
718	2	27	171.70	5.2	32.85
718	2	28	201.40	5.2	38.53
718	2	29	186.86	5.2	35.75
718	2	30	228.84	5.2	43.78
720	1	1	141.00	5.3	26.77
720	1	2	141.22	5.3	26.81
720	1	3	138.90	5.3	26.37
720	1	4	140.66	5.3	26.71
720	1	5	135.91	5.3	25.81
720	1	6	138.34	5.3	26.27
720	1	7	129.08	5.3	24.51
720	1	8	115.00	5.3	21.83
720	1	9	142.90	5.3	27.13
720	1	10	113.32	5.3	21.52
720	1	11	132.91	5.3	25.24

Cow	Diet	Day	CH_4 (g/day)	DMI (kg/day)	CH_4 (g/kg DMI)
720	1	12	131.28	5.3	24.93
720	1	13	131.35	5.3	24.94
720	1	14	136.32	5.3	25.88
720	1	15	138.96	5.3	26.38
720	1	16	141.14	5.3	26.80
720	1	17	147.37	5.3	27.98
720	1	18	117.90	5.3	22.39
720	1	19	126.95	5.3	24.10
720	1	20	131.77	5.3	25.02
720	1	21	140.64	5.3	26.70
720	1	22	151.77	5.3	28.82
720	1	23	115.28	5.3	21.89
720	2	1	164.28	5.4	30.59
720	2	2	193.54	5.4	36.04
720	2	3	196.69	5.4	36.63
720	2	4	205.40	5.4	38.25
720	2	5	187.97	5.4	35.00
720	2	6	143.02	5.4	26.63
720	2	7	164.12	5.4	30.56
720	2	8	185.64	5.4	34.57
720	2	9	209.81	5.4	39.07
720	2	10	215.88	5.4	40.20
720	2	11	209.77	5.4	39.06
720	2	12	230.39	5.4	42.90
720	2	13	202.90	5.4	37.78
720	2	14	198.68	5.4	37.00
720	2	15	202.28	5.4	37.67
720	2	16	211.48	5.4	39.38
720	2	17		5.4	
720	2	18	200.38	5.4	37.31
720	2	19	188.75	5.4	35.15
720	2	20	198.42	5.4	36.95
720	2	21		5.4	
720	2	22	195.76	5.4	36.45
720	2	23	206.06	5.4	38.37
720	2	24	181.82	5.4	33.86
720	2	25	203.15	5.4	37.83
720	2	26	197.14	5.4	36.71
720	2	27	161.68	5.4	30.11
720	2	28	209.68	5.4	39.04
720	2	29	243.93	5.4	45.42
720	2	30	194.96	5.4	36.30