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**EVALUATION OF THE GREENFEED SYSTEM FOR
METHANE ESTIMATION FROM GRAZING DAIRY
COWS**

A thesis presented in partial fulfilment of the requirements for
the degree of

Master of Science

In

Animal Science

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ABSTRACT

Ruminant methane (CH₄) production contributes ~32% of New Zealand's total greenhouse gases (GHG) and a reduction in emissions is required under the Kyoto Protocol. Under this agreement, New Zealand has pledged to either reduce, or take responsibility for the GHG emissions above those in 1990. GHG emission factors used for inventory need to be accurate, reliable and applicable to animals grazing in New Zealand. Furthermore, identification and implementation of mitigation strategies also depends on accurate and reliable methods for measuring CH₄ emissions.

Current techniques to measure CH₄ have to compromise between accuracy and applicability. Respiration chambers are accurate, but do not represent a grazing environment, whilst the Sulphur Hexafluoride (SF₆) technique can be used at grazing, but has more variation than the chambers. Consequently, a new technique known as the Greenfeed system, has been developed to measure CH₄ from grazing animals. The evaluation of this technique applied to grazing animals, with minimal interference from humans, and its evaluation is reported here.

In the first experiment, CH₄ measurements were compared from the Greenfeed system with respiration chambers and the SF₆ technique using six dry dairy cows fed a silage-based diet. Intakes were measured and were approximately at maintenance levels and feeding regimens were similar throughout. The mean CH₄ production (g CH₄/d) ± standard deviation (Murray *et al.*, 2001) from the Greenfeed unit (150 ± 20.2) was higher and more variable than those from the respiration chambers (134 ± 9.8) and the SF₆ technique (128 ± 8.7). Similarly, the CH₄ yield (g CH₄/kg dry matter intake (DMI)) ± SD was higher from the Greenfeed unit (24.0 ± 3.2) than the respiration chambers (21.9 ± 1.6) or the SF₆ technique (20.5 ± 1.4). Correlations between CH₄ production by individual animals using the Greenfeed system and either respiration chambers or the SF₆ technique were weak ($r = -0.36$ and 0.13 , respectively).

The second experiment successfully implemented two Greenfeed units on-farm with 24 lactating cows, including four with rumen fistulae, and evaluated their behavioural interactions and estimated CH₄ production. Seventeen of the 24 cows (approximately 70%) visited the Greenfeed units, but this could be increased by training so most cows visit. An attractant/reward was used to encourage cows into the units and the cows preferred the Lucerne pellets to the Grain pellets. The mean CH₄ production measured using the Greenfeed units were 340.3 ± 61.8 g CH₄/d, suggesting about 21.3 g CH₄/kg DMI (based on calculated feed intakes). It appears that 14 days are needed for cows to get used to the Greenfeed units, and extending estimates (for five further weeks) did not reduce the variance in CH₄ emissions values within cows.

This research has shown that the Greenfeed system seems adequate for estimating CH₄ production of a whole herd. Further estimates are required before standard operating procedures can be determined and that confidence can be placed in the accuracy of CH₄ estimates.

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Dedicated in loving memory to my Mum

Christine Anne Garnett

3rd September 1950 to 24th February 2009

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LIST OF ABBREVIATIONS

% Percent

A

μM Micromole
ADF Acid detergent fibre
ATP Adenosine triphosphate
ADP Adenosine diphosphate

B

BCS Body condition score

C

C2 Acetate
C3 Propionate
C4 Butyrate
CH₄ Methane
CO₂ Carbon dioxide
CV Coefficient of variation

D

d Day
DM Dry matter
DMI Dry matter intake
DOMI Digestible organic matter intake
Dr Doctor

E

Ei Emissions intensity
ERUCT Emissions from Ruminants Using a Calibrated Tracer Technique

F

FACE Free air carbon dioxide enrichment

G

g Gram

GE	Gross energy
GEI	Gross energy intake
GHG	Greenhouse gas
GWP	Global warming potential
H	
H ₂	Hydrogen gas
H ⁺	Hydrogen ion
HFC	Hydrofluorocarbon
I	
Inc.	Incorporated
IPCC	Intergovernmental Panel on Climate Change
K	
Kg	Kilogram
Km	Michaelis constant
L	
LULUCF	Land-use, land-use change and forestry
M	
MAF	Ministry of Agriculture and Forestry
ME	Metabolisable energy
MJ	Megajoule
mV	Millivolt
N	
n	Number
N ₂ O	Nitrous oxide
NAD ⁺	Nicotinamide adenosine dinucleotide oxidised
NADH	Nicotinamide adenosine dinucleotide reduced
NIWA	National Institute of Water and Atmospheric Research
NDF	Neutral detergent fibre
O	
O ₂	Oxygen gas
OM	Organic matter
OMD	Organic matter digestibility
OMI	Organic matter intake

P

PFC Perfluorocarbon
pH Power of hydrogen

R

RRT Rumen retention time

S

SD Standard deviation
SSS Soluble sugars and starch
SF₆ Sulphur hexafluoride

T

TCA Tricarboxylic acid
™ Trademark

U

UNFCCC United Nations Framework Convention on Climate Change
US United States

V

VFA Volatile fatty acid

CHAPTER ONE

GENERAL INTRODUCTION



CHAPTER ONE: GENERAL INTRODUCTION

The last decade has seen an increasing international interest into global warming and climate change, which has been attributed to the production of greenhouse gases (GHG). These atmospheric gases absorb and re-emit radiation back to the Earth's surface, subsequently trapping energy and warming the environment; the 'greenhouse effect' (Le Treut *et al.*, 2007). The principal GHG are: methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), hydrofluorocarbons (HFC), perfluorocarbons (PFC), and sulphur hexafluoride (SF₆) (IPCC, 2007b). Methane has a global warming potential 25 times as great as CO₂ (kg/kg) but a short atmospheric half-life, so reduction in emissions could have an effect on global warming in the short term (Chynoweth, 1996). The likely contribution of GHG to increasing temperatures (IPCC, 2007a) has resulted in initiatives to reduce GHG emissions (Clark *et al.*, 2005).

Agriculture comprises over half of New Zealand's export products, and is facilitated by the favourable climate, abundance of agricultural land and grazing of perennial ryegrass-dominated pastures throughout the year (Clark *et al.*, 2011). However, this extensive livestock farming in combination with a relatively small human population means that approximately 47% of New Zealand's GHG are associated with agriculture (Ministry for the Environment, 2010). Ruminants are able to convert fibrous plant material to animal products for human consumption due to the microbial processes occurring in the rumen (Buddle *et al.*, 2011). The digestion results in nutrients leading to formation of animal products such as milk and meat. However, the digestion of feed in the ruminant is also associated with the creation of by-products, including hydrogen gas (H₂) and CO₂, which together form CH₄. The primary source of CH₄ is digestive fermentation in the rumen, which is released by eructation and exhalation (Murray *et al.*, 1976). Methane production during fermentation decreases the efficiency of animal production (Rossi *et al.*, 2001), with typical energy losses of 6-7% of the gross energy (GE) in feed, but with values ranging from 2-12% (Johnson & Johnson, 1995).

In 2005, New Zealand agreed to abide by the terms of the Kyoto Protocol, under which it is obliged to reduce GHG levels to 1990 levels between 2008 and 2012 or take

responsibility for the excess (Ministry for the Environment, 2010). To do this, accurate estimates of emissions are needed, including reliable CH₄ measurements from grazing animals under typical New Zealand farming systems. This will then provide a benchmark against which the effects of mitigation options can be measured (O'Hara *et al.*, 2003).

Techniques implemented in New Zealand to measure CH₄ emissions from ruminants include the respiration chambers and the SF₆ technique. The respiration chambers involve a cow being enclosed in a sealed box and the in- and out-going air is used to measure CH₄. This technique is currently the 'gold standard' as it measures rather than estimates CH₄ so is accurate. However, cows are placed in an artificial environment where movement, diet selection and grazing patterns are restricted so their applicability to grazing systems is questionable (O'Hara *et al.*, 2003).

The development of alternative CH₄ measurement techniques suitable for grazing animals has progressed, with the SF₆ technique being commonly used (Johnson *et al.*, 1994). The SF₆ technique requires a permeation tube releasing SF₆ gas at a known rate to be placed in the rumen and the ratio of SF₆ to CH₄ in respired breath collected near the nostrils is used to estimate the CH₄ production. This technique can be used in grazing or stall-fed animals, however, the CH₄ estimates are associated with greater variation than those derived from animals using the respiration chambers (Hammond *et al.*, 2009, Pinares-Patiño & Clark, 2008).

Limitations associated with the artificial environment of the respiration chambers and the high variation in CH₄ estimates derived from the SF₆ technique, has resulted in the development of the Greenfeed system (C-Lock Inc, South Dakota, United States, Patent 7966971), which could be ideal for estimating CH₄ from grazing animals. The Greenfeed system can be set up as a trailer-mounted unit that can be placed in a paddock to estimate CH₄ production from grazing cows and requires minimal human interference with the animals being measured. This system has potential advantages over the SF₆ technique because there is no equipment mounted on animals, so they can graze pasture without any interference from sampling equipment, which better

represents normal behaviour. There is no requirement to dose individuals with a permeation tube and the Greenfeed system does not need specialised (laboratory) equipment for sample analysis, so it may be less costly than the SF₆ technique.

Because of these features, the Greenfeed system has potential for use in the New Zealand farming situation. However, there is little known about either the accuracy of the CH₄ estimates from the Greenfeed unit compared to the respiration chambers and SF₆ technique, or the best way the units should be implemented on-farm.

CHAPTER TWO

REVIEW OF LITERATURE



CHAPTER TWO: REVIEW OF LITERATURE

2.1 GREENHOUSE GAS EMISSIONS

2.1.1 Principal greenhouse gases

Greenhouse gases (GHG) are atmospheric gases that absorb and re-emit radiation back to the Earth's surface, subsequently trapping energy and warming the Earth's surface. Each gas has a different global warming potential (GWP) due to differing radiative properties and atmospheric lifetimes (IPCC, 2007) (Table 2.1). The GWP are based on CO₂-equivalent (CO₂-e) emissions, attained by multiplying the GHG emissions by its GWP. For example; the GWP of carbon dioxide (CO₂) and methane (CH₄) are 1 and 25, respectively, meaning that 1 kg CH₄ has 25 times as much warming potential as 1 kg CO₂.

Table 2.1: Common greenhouse gases: their lifetimes and 100-year GWP.

Gas name and abbreviation	Lifetime (years)	100-year GWP
Carbon dioxide (CO ₂)	50-200	1
Methane (CH ₄)	12	25
Nitrous oxides (N ₂ O)	114	298
Sulphur hexafluoride (SF ₆)	3,200	22,800

GWP, global warming potential. From Ministry for the Environment (2010)

2.1.2 Global emissions

The contributions of CO₂, CH₄ and nitrous oxides (N₂O) to global GHG emissions are 75, 15 and 10% respectively (IPCC, 2007a). Between 1990 and 2009, global GHG were increasing at 0.9% per year (Ministry for the Environment, 2010). In 2005, global agriculture accounted for 10-12% of the total anthropogenic GHG emissions representing an increase of 17% from 1990 to 2005 (IPCC, 2007b). Agricultural GHG are those associated with animal and crop production. The principal contributors to global agricultural GHG are CH₄ and N₂O, each contributing equal amounts (7.5%) to global GHG emissions (Olivier *et al.*, 2005). The N₂O arise mainly from nitrification and denitrification in agricultural soils, and urine patches from ruminants. The CH₄ is produced from rumen fermentation and anaerobic rice production, accounting

for 29% and 12% of global agricultural CH₄, respectively (Ministry of Agriculture and Forestry, 2009).

Figure 2.1 shows the rate of change of atmospheric CH₄ concentrations between 1979 and 2012. The rate of atmospheric CH₄ concentrations is again increasing after plateauing during the

mid-1990's to mid-2000's, however the rate of increase is much less than during the 1980's. Global anthropogenic CH₄ emissions have been projected to increase

by 23% from 2005 to 2020 (Gunning, 2009).

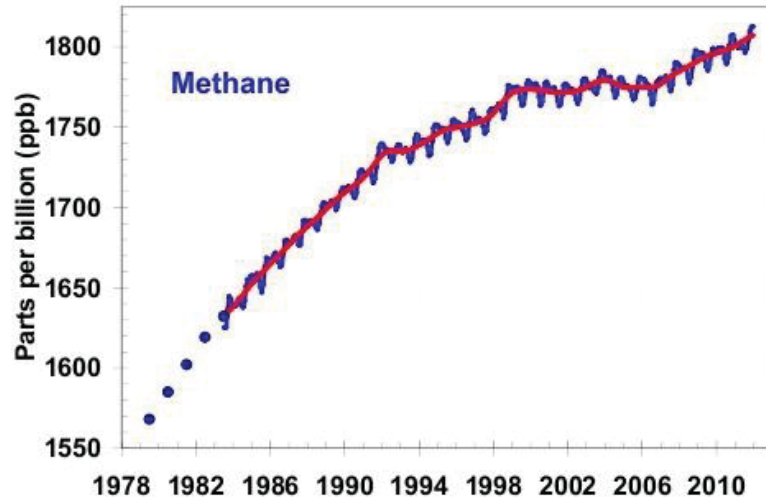


Figure 2.1: Change in atmospheric CH₄ concentrations. Actual emissions (blue line) and long-term trend (red line). From Butler (2012).

2.1.3 New Zealand agricultural emissions

New Zealand GHG emissions (Table 2.2) contribute only 0.2% of global emissions (Ministry of Agriculture and Forestry, 2010). In New Zealand, the agricultural industry is the largest source of GHG emissions, with 2009 emissions contributing 46.5% of national emissions (Ministry for the Environment, 2010), representing an 8.4% increase in emissions from 1990 to 2009 (Table 2.2). The emissions contributed by agriculture in New Zealand are much higher than other developed countries due to the high food production and a low population; with export of primary industry products a major component of New Zealand's economy, representing 66% of total merchandise exports (Ministry of Agriculture and Forestry, 2011).

Table 2.2: *New Zealand's emissions and removals by sector in 1990 and 2009.*

Sector	Gg CO ₂ equivalent 1990	Gg CO ₂ equivalent 2009	Change from 1990 (Gg CO ₂ equivalent)	Change from 1990 (%)
Energy	23,360	31,360	+8,000	+34.3
Industrial processes	3,380	4,350	+960	+28.5
Agriculture	30,280	32,810	+2,530	+8.4
Waste	2,050	2,020	-33	-1.6
Total (excluding LULUCF)	59070	70,540	+11,45 7	69.6
LULUCF	-23450	26680	-3230	-13.8
Net total (including LULUCF)	35660	43880	8220	+23.1

Adapted from Ministry for the Environment, (2010). Gg, Gigagram; CO₂, carbon dioxide; LULUCF, land use, land-use change and forestry. Columns may not total due to rounding

Figure 2.2 shows the change in contribution of the agricultural sector to GHG in New Zealand from 1990 to 2009, which indicates that both the agricultural and total GHG emissions increased gradually until 2006, after which there was a decline in emissions.

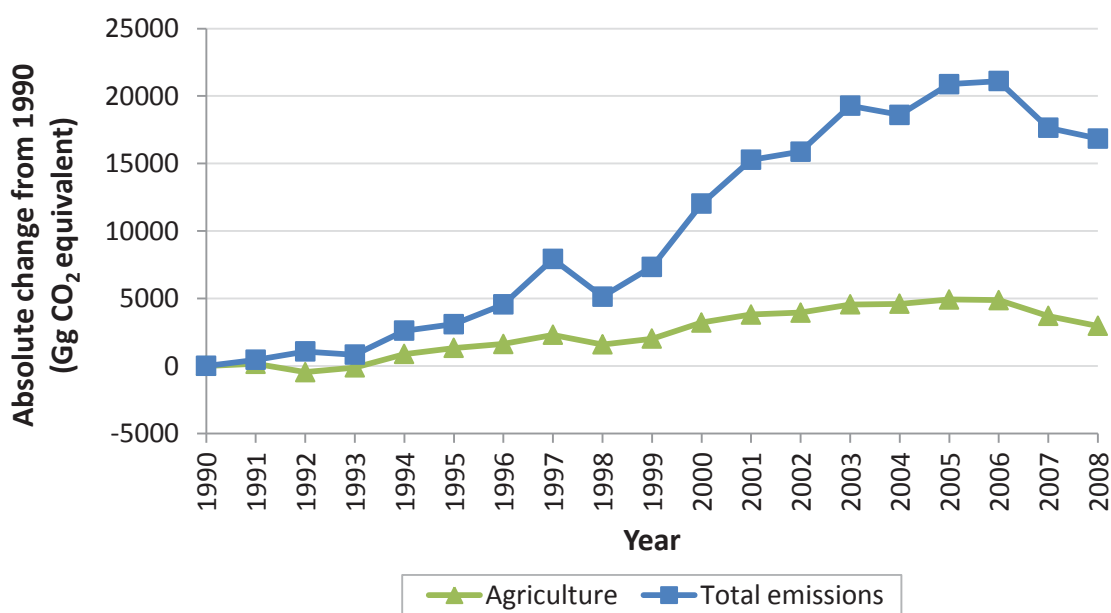


Figure 2.2: *Absolute change from 1990 to 2008 in New Zealand's total emissions and agricultural emissions. Adapted from Ministry for the Environment (2010). CO₂, carbon dioxide; LULUCF, land use, land-use change and forestry. Note: total emissions exclude emissions and removals from the LULUCF sector.*

Carbon dioxide is not included in the agricultural national inventory unless there is a change in land use (since 1990), such as the replacement of forests with grazing or cropping land. It is not included as it is recycled – CO₂ respired by ruminants is returned to the atmosphere for use by plants for photosynthesis, which are eaten by ruminants and again respired. However, a small portion of the ingested carbon is released as CH₄, from anaerobic fermentation (Figure 2.3).

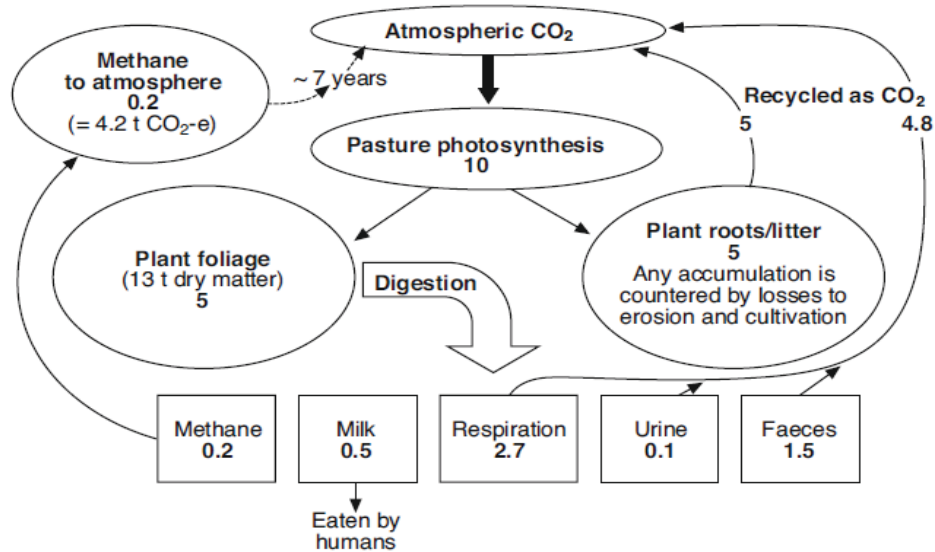


Figure 2.3: Carbon cycling in a typical one-hectare dairy pastoral system (all units are in tonnes of Carbon/annum). From Pinares-Patiño et al. (2009).

2.1.4 New Zealand ruminants

This thesis will primarily deal with agricultural CH₄ emissions from ruminant digestive fermentation, which in 2009 contributed 68.6% of agricultural emissions and 31.9% of New Zealand's total emissions (Ministry for the Environment, 2010). The large contribution of ruminant digestive fermentation to agricultural emissions means changes in livestock population and productivity may affect total CH₄ yields. Numbers of sheep have decreased, beef cattle have stayed reasonably constant and deer and dairy cattle have increased whereas the productivity of all livestock classes has increased and is likely to continue to increase (Ministry for the

Environment, 2010). Figure 2.4 shows changes in CH₄ yield per animal by livestock class from 1990 to 2008. The slight decrease in yields for dairy and beef cattle represent the dilution of maintenance whereby as feed intake and therefore productivity increase, there will be a decrease in CH₄ yield (Hammond, 2011). A reduction in overall emissions will only occur if animal numbers (and therefore overall productivity) increase at a slower rate than the decrease in CH₄ emitted per unit of product.

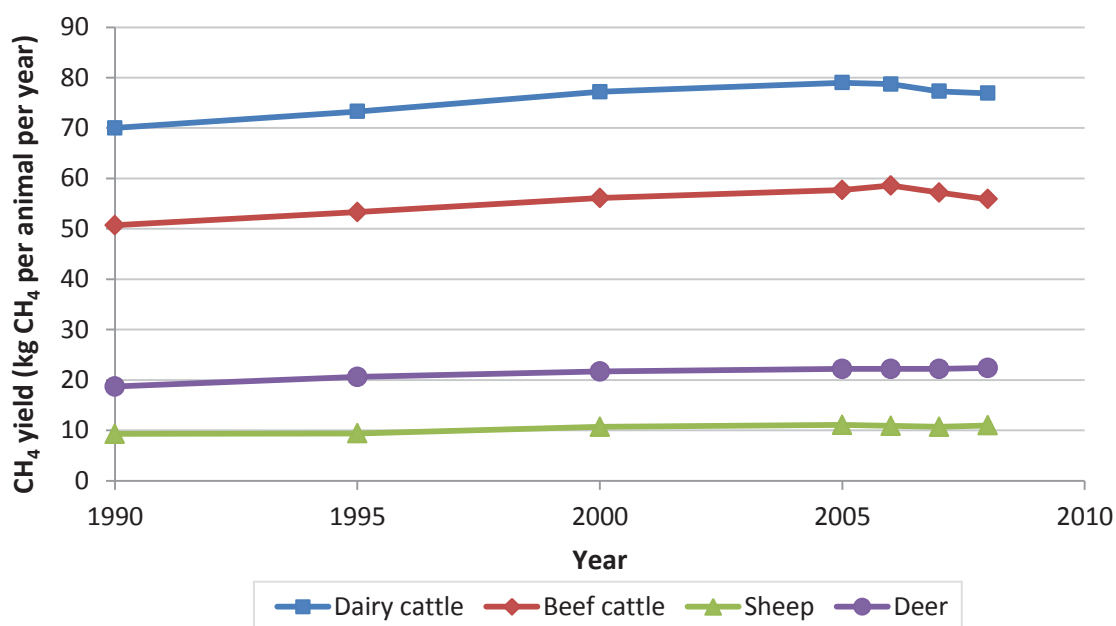


Figure 2.4: Annual CH₄ yields by livestock type used in inventory calculations (1990-2008). Adapted from Ministry for the Environment (2010).

2.1.5 Consequences for agriculture

The underlying cause of global warming is the accumulation of GHG in the atmosphere, which reduce the heat loss to space and cause the temperature of the atmosphere and ground to increase (Mitchell, 1989). There is no single factor responsible for global warming; it is instead due to the balance between the production (sources) and removal (sinks) of GHG.

The increasing ambient temperatures associated with global warming will also affect both pastoral and animal production. Increases in ambient temperatures may alter the plant species composition and decrease the feeding value of pasture,

which may decrease animal production (Hodgson *et al.*, 1992). This will affect our human populations as we rely on agricultural production as a food source (Pinares-Patiño *et al.*, 2009).

Changes in botanical composition can be inferred from results of a free air CO₂ enrichment (FACE) experiment conducted in New Zealand. This experiment investigated the effects of elevated CO₂ on herbage yield (in both C3 and C4 grasses and legumes). Results indicated that after the seven-year experimental period the total herbage yield was 8.4% higher (although not significant) in the elevated CO₂ treatment compared to the control (Newton *et al.*, 2006). Also legumes had significantly greater growth than the grass varieties (Newton *et al.*, 2006), which may affect the composition of typical New Zealand ryegrass-white clover pastures.

Global warming affects the pasture nutritive value and production (through decreased digestibility and decreased nitrogen uptake by the plant, respectively), which may affect animal performance. A higher ambient temperature decreases leaf/stem ratio and digestibility, associated with higher neutral detergent fibre (NDF) concentration and increased lignification (Buxton & Fales, 1994). In addition, studies by Newton *et al.* (2006) have shown that increased CO₂ levels reduce the nitrogen uptake of pasture plants which may lower the pasture production. As a result, the organic matter (OM) intake and metabolisable energy (ME) intake of typical grazing animals may be decreased along with animal production (Hodgson *et al.*, 1992).

Animal production is dictated by feed intake and pasture nutritive value which can be reduced when ambient temperatures are above 20°C (Payne, 1990). Lactating dairy cows have high intakes and high metabolic heat production, so heat loads may be increased in higher temperatures. The heat production and accumulation of radiant energy, combined with a decreased cooling ability due to the higher ambient temperatures, causes the body temperature of the cow to increase, further reducing food intake and consequently productivity (West, 2003). Hodgson

et al. (1992) predicted decreases in the production of lactating animals by 4.7% and 9.6% for 1.5°C and 3.0°C increases in ambient temperatures, respectively.

2.1.6 The National Greenhouse Gas Inventory

New Zealand was a signatory to the Kyoto Protocol in 2002 and in 2005 committed to maintaining GHG emissions at or below 1990 emissions or assume responsibility for emissions above that level. This requires New Zealand to compile an accurate inventory of all GHG emissions. The New Zealand Greenhouse Gas Inventory (Ministry for the Environment, 2010) is calculated using population and productivity data for livestock which enables dry matter intake (DMI) to be calculated. The DMI is then multiplied by an emission factor (g CH₄/kg DMI) to calculate New Zealand's CH₄ emissions for each ruminant species (sheep, dairy and beef cattle and deer).

If New Zealand is to meet the requirements of the Kyoto Protocol, considerable research and development in the area of agricultural GHGs will be required. Consequently, the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) was opened in March 2010, a partnership with several crown research institutes, universities and industry organisations within New Zealand. This centre co-ordinates and funds scientific research into agricultural GHGs, specifically the mitigation of CH₄ and N₂O and alternative sinks for soil carbon, and aims to demonstrate that farm businesses can be both low-emitting and profitable. The NZAGRC is 100% government funded, with approximately \$48.5 million being invested over the 2010 to 2020 period by the Primary Growth Partnership.

2.2 DIGESTION AND FERMENTATION

2.2.1 Ingestion and rumen fermentation

In New Zealand farming systems, ruminant diets are primarily fresh forages. When cows chew during eating it ruptures cells which increases the surface area, allowing the micro-organisms access to the cell contents for degradation. Saliva produced

during chewing facilitates bolus formation and swallowing and helps buffer rumen contents to reduce pH changes associated with volatile fatty acid (VFA) production.

Ingested feed is mixed with rumen fluid and subjected to microbial fermentation. The main components of the diet are carbohydrate (readily fermentable and cell wall polymers of cellulose and hemicelluloses and lignin) and protein. Fermentation of the carbohydrate fractions of the diet produce simple sugars which are degraded and contribute to VFA production. The cell wall components are degraded by extracellular (mainly bacterial and fungal) enzymes while the lignin component of the cell wall is indigestible.

About 70% (Waghorn *et al.*, 2007) of the protein in forage is broken down by micro-organisms and the ammonia derived from amino groups is absorbed and excreted as urea in the urine, or re-synthesised into microbial protein (Pacheco & Waghorn, 2008). The residual carbon skeletons are further degraded to produce VFA, while undigested protein passes to the intestine for endogenous digestion and amino acid absorption. Most carbohydrate and protein digestion takes place in the rumen, resulting in the production of VFA, hydrogen gas (H₂), CO₂ and microbial biomass (Janssen, 2010).

Microbial digestion involves the breakdown of substrates via glycolysis (and the tricarboxylic acid (TCA) cycle) to generate adenosine-tri-phosphate (ATP) for microbial growth and maintenance, with VFA, H₂ and CO₂ as by-products of fermentation (Russell & Wallace, 1997). As ruminants fed fresh forages absorb little or no glucose, they rely on VFA as their principal energy source, with acetate and butyrate being lipogenic and propionate being glucogenic (Waghorn *et al.*, 2007). The hydrogen must be removed from the system to maintain thermodynamic conditions favourable for sustained rumen fermentation (Janssen, 2010).

2.2.2 Methanogenesis

Methanogenesis is a form of anaerobic respiration, which enables ATP generation; the conversion of adenosine di-phosphate (ADP) to ATP is coupled to the reduction

of nicotinamide adenosine dinucleotide (NAD⁺) to NADH. For the anaerobic respiration to continue NADH must be oxidised back to NAD⁺, by the donation of electrons to hydrogen ions (H⁺) to form H₂. In the anaerobic rumen environment, the transfer of electrons and protons requires acceptors other than oxygen (Russell & Wallace, 1997), and in the presence of methanogens, H₂ provides electrons to CO₂ which is then reduced to CH₄ under strict anaerobiosis (McAllister et al., 1996)(Equation 2.1).

Equation 2.1: Stoichiometry associated with CH₄ production.



From Janssen (2010)

Methanogens are micro-organisms distinct from bacteria (domain Archaea) which are strict rumen anaerobes, present at 10⁸-10⁹/ml of rumen fluid. Their growth, survival and activity is largely dictated by the conditions in the rumen (Janssen, 2010) and the main means for methanogens to obtain energy is through rumen CH₄ formation. Methanogenic activity rate is highest when H₂ concentrations are high. The concentration of dissolved H₂ in the rumen ranges between 0.1 to 50µM and the rate at which H₂ enters the dissolved pool dictates the rate of methanogenesis (Janssen, 2010). The build-up of H₂ in the rumen is limited by its consumption by methanogens, which results in the production of CH₄.

2.2.3 Site of methanogenesis and methane release

The rumen is the predominant site of CH₄ production (87-94% of total production), partly because most fermentation occurs in the rumen, with the remaining 6-13% derived from hindgut fermentation (Figure 2.5). Although the routes of CH₄ release from ruminants have not been studied extensively, Murray *et al.* (1976) reported the routes of total CH₄ release were eructation (83%), exhalation (16%) and flatulence (1%). Most CH₄ produced in the hindgut is absorbed into the blood stream and is expelled in exhalation (89%), with only 11% excreted in the flatus whereas 95% of the rumen CH₄ is expelled in eructation and 5% in the flatus (Murray et al., 1976).

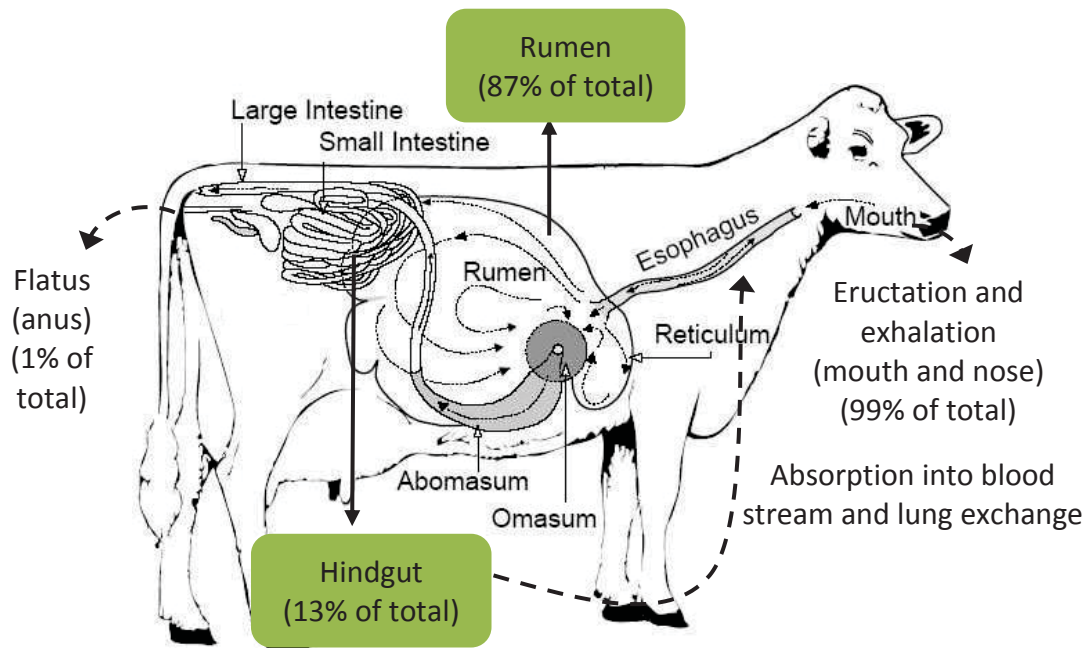


Figure 2.5: Sites of methanogenesis and release in a dairy cow. Adapted from Murray *et al.* (1976), Immig (1996) and Torrent and Johnson (1994). The solid line represents sites of CH_4 production whereas the dashed line represents sites of CH_4 release or absorption.

The low CH_4 release in the flatus is supported by studies using the sulphur hexafluoride (SF_6) technique to measure CH_4 production via eructation and exhalation (but not flatus), compared to total collection from animals in respiration chambers (Grainger *et al.*, 2007, McGinn *et al.*, 2006). In both studies the SF_6 and respiration chamber measurements of CH_4 production were similar, indicating that estimates of CH_4 production based on eructed and exhaled gases are only slightly less than total production, confirming the minor loss in flatus.

2.2.4 Expressing methane emissions

Methane emissions can be expressed in several ways depending on its end-use, whether it be inventory, feed utilisation efficiency or losses due to digestion (Hammond, 2011). It is important that these expressions are clearly defined to avoid misinterpretation (Waghorn & Clark, 2006). Examples of CH_4 expressions are:

- Absolute daily production ($\text{g CH}_4/\text{d}$); global mitigation requires total greenhouse gas emissions to be measured so the quantities can be reduced

- Yield (g CH₄/kg DMI, g CH₄/kg OMI); used to calculate the NZ inventory with standard values (population characterisation, performance and DMI required to achieve that performance) assumed for animals, without considering feed quality
- Methane emission is a general term that refers to either production (g CH₄/d) or yield (g CH₄/kg DMI)
- Relative to gross energy intake (% GEI); a similar expression to yield, relating energy losses in CH₄ to gross feed energy
- Per unit of digestible portion of feed (g CH₄/kg digestible dry matter intake (DDMI), g CH₄/kg digestible organic matter intake (DOMI)); relates CH₄ production to feed digested, so requires information on digestibility as well as CH₄ production and feed intake
- Emissions intensity (g CH₄/production); relates CH₄ to animal production (main reason ruminants are farmed), but this expression can be misrepresented for example by measurements at different stages of lactation or growth.

2.3 METHANE DETERMINATION TECHNIQUES

There are several techniques that are used to estimate CH₄ from individual ruminant animals. These include predicting CH₄ based on feed characteristics (Blaxter & Clapperton, 1965), feed composition (Moe & Tyrrell, 1979, Kirchgeßner *et al.*, 1995) or proportions of VFA produced (Whitelaw *et al.*, 1984) and the infusion of ethane (C₂H₆) (Machmüller & Hegarty, 2006). Individual measurements can be based on respiration chambers (Pinares-Patiño *et al.*, 2012b), ventilated hoods or masks (Kelly *et al.*, 1993), tracer technologies (Johnson *et al.*, 1994) or the Greenfeed system (C-Lock Inc, South Dakota, United States, Patent 7966971). However, the two most common measurement techniques used for CH₄ emissions from ruminants are (1) respiration chambers and (2) the SF₆ technique.

The aim of many respiration chamber studies up until around 1990 was to better understand the energetics of ruminant metabolism and feed conversion efficiency and this led to the realisation that CH₄ eructation represents a 2-12% loss in dietary energy. It has been postulated that this loss could otherwise be used for productive purposes (Johnson & Johnson, 1995). From the 1990s and continuing today, the main focus of ruminant CH₄ emissions has been driven by its impacts on global warming (Eckard *et al.*, 2010).

This section will focus on the open-circuit respiration chambers (and briefly closed circuit and ventilated hoods), the SF₆ tracer technique, and the Greenfeed system. This is because these techniques have been implemented in New Zealand.

2.3.1 Respiration chambers

A well-established technique for determining CH₄ emissions from individual animals is from respiration chambers; which can be either closed-circuit or open-circuit. Closed-circuit chambers were commonly used in earlier experiments by Blaxter & Wainman (1961) but also more recently (Robinson *et al.*, 2010). Closed-circuit chambers involve placing an animal in an airtight chamber, in some instances the air is then circulated within the system with adsorbents used to remove CO₂ and water, and oxygen gas (O₂) may be added (Blaxter, 1967). Other systems have no air circulation, and CO₂ concentrations increase over the short period (for example, 1 hour) of containment. Methane accumulates in the chambers during the measurement period and the production of CH₄ is calculated from the difference in the concentration of CH₄ in the samples taken before and after the measurement period and the volume of the chamber minus the estimated volume of the animal (Blaxter, 1967).

The open-circuit respiration chambers are considered the 'gold standard' for measuring CH₄ from individual animals (O'Hara et al., 2003). The open-circuit chamber system uses the compositions of the in-flowing and out-flowing air and outflow rate to calculate CH₄ emissions. Open-circuit respiration chambers (Figure 2.6, Photograph 2.1) have been widely used with various different species in order to measure CH₄ production. Typically, the CO₂ production and O₂ consumption are measured and sometimes H₂ production as well. The basic chamber is airtight, with an air conditioning system, a gas meter to measure outflow, and a system to analyse sub-samples of the air entering and leaving the chamber for CH₄, O₂ and CO₂ (Miller & Koes, 1988). Air is drawn past the animal inside the chamber and differences in inflow and outflow concentrations of gases in the air enable production or consumption to be measured (Blaxter, 1967). Details for chamber operation including calibration have been summarised by Pinares-Patiño *et al.* (2012b).

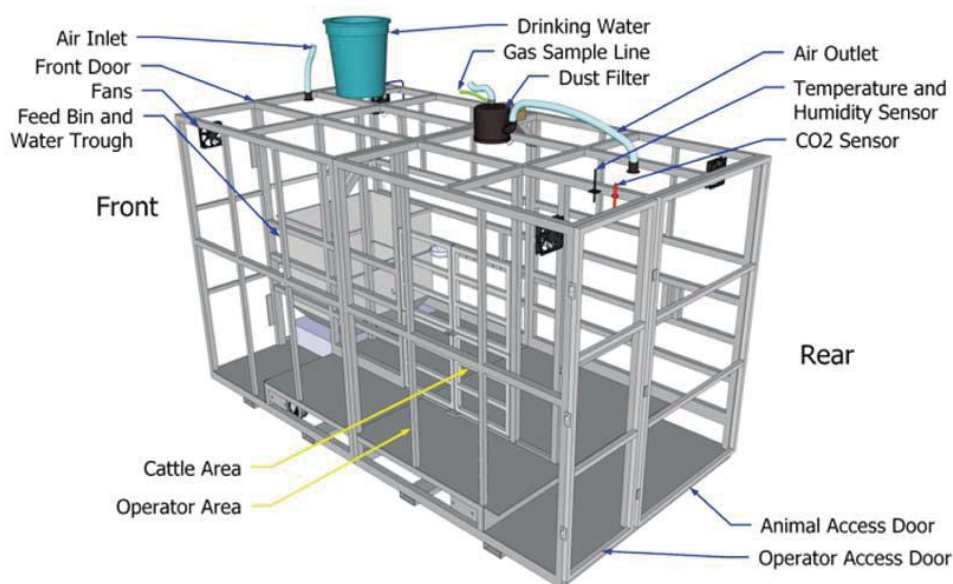


Figure 2.6: Design of the cattle respiration chamber showing its structural frame and associated equipment. The frame is covered with clear walls and roof. From Pinares-Patino *et al.* (2012b).



Photograph 2.1: A cow within a respiration chamber at the New Zealand Ruminant Methane Measurement Centre, AgResearch Grasslands, Palmerston North. From Hammond (pers. comm.).

Advantages of the respiration chambers

A major advantage of the chamber technique is that the CH₄ production can be measured directly from the animals, providing accurate and reliable CH₄ values. Another advantage is that individual animal feed intakes can be measured, allowing accurate CH₄ yield (g CH₄/kg DMI) to be calculated. This means that valid relationships between feed intake and CH₄ emission can be determined (Hammond, 2011). This is a major benefit as the amount of feed eaten is the most important determinant of CH₄ production (van Zijderveld *et al.*, 2011).

Disadvantages of the respiration chambers

The accuracy of measurements from respiration chambers must be balanced against some limitations. These include the costs of the facilities as well as operation, restrictions in number of animals that can be measured, and the artificial environment within the chamber. The expense of chambers includes the building to house them, set-up costs and the maintenance costs. Their operation is labour

intensive and includes requirements for animal training, which results in more consistent data collection (Williams *et al.*, 2007). Animals must be acclimatised by being placed in the chambers for six to eight hours on several occasions to become used to the environment. The time taken for training individuals will vary between animals and depends on previous handling. In addition, routine maintenance includes feeding according to the experimental protocol and filling of water containers as required, removal of faeces and urine and cleaning twice daily. This high labour input and overall expenses, reduces the number of animals that can be measured (Johnson *et al.*, 2007).

Differences in feeding regimes between controlled and grazing experiments may lead to differences in CH₄ production. Under controlled experiments such as the measurement of CH₄ production within respiration chambers, animals are placed in an artificial environment, removing the ability to choose their diet and graze at normal times of the day. Whereas, in a grazing situation the animal is free to eat throughout the day. The circadian pattern in CH₄ production reflects the fermentation activity in the rumen associated with the circadian pattern of feeding behaviour and DMI, being greatest after feeding (Clapperton & Czerkawski, 1969, Johnson *et al.*, 1994), when the substrate availability, fermentation and H₂ partial pressure in the rumen are highest (Smolenski & Robinson, 2006). Methane production then continues during rumination and declines as the rumen content falls (Lockyer & Champion, 2001).

Animals under controlled conditions, such as in the respiration chambers, are fed at set times of the day according to the experimental protocol, typically twice daily between 08:00 h and 09:00 h and again between 16:00 h and 17:00 h (Pinares-Patiño *et al.*, 2008, Murray *et al.*, 1999). This circadian variation in CH₄ production has been confirmed under controlled indoor conditions by Grainger *et al.* (2007) who reported that CH₄ production increased soon (0.5-1.0 hour) after feeding.

In contrast, animals under grazing conditions are free to choose their diet and eat throughout the day. The majority of grazing occurs during daylight hours, with

greater activity in the early morning and late afternoon, which may be associated with the provision of fresh feed or overnight fasting (Rook *et al.*, 1994). Larger meals follow afternoon milking, which may be a response to an accumulation of readily fermentable photosynthetic products in the pasture leaves during the day (Fulkerson & Donaghy, 2001), but overall patterns will be affected by feed availability and management. There are limited experiments which report on the circadian variation in CH₄ production from grazing dairy cattle; however this information is available for sheep. Experiments using sheep under grazing conditions, demonstrated that CH₄ production followed the circadian pattern of grazing behaviour (Murray *et al.*, 2001, Judd *et al.*, 1999, Lockyer & Champion, 2001).

The respiration chambers have the benefit over the SF₆ technique of being able to measure circadian variation in CH₄ production; however the degree to which the variation measured in respiration chambers can be applied to grazing animals is questionable.

Other techniques

Therefore, there have been some alternative systems developed using the principles of the respiration chambers in near-natural grazing conditions such as the tunnels methods (Lockyer & Jarvis, 1995). A polythene tunnel acts as a tent within which the animal can graze and a smaller wind tunnel at either end acts to blow air into and draw air out of, the large tunnel. The airflow and concentration of CH₄ within the tunnel are used to calculate CH₄ emissions through gas chromatography and flame ionisation detection (Lockyer & Champion, 2001). This technique has, however been reported to under-estimate CH₄ emissions compared to the respiration chambers, although a reason for this was not known (Murray *et al.*, 1999).

The ventilated hood or mask-type systems utilises a one-way stream of air passing across the face or head, which is measured at the outlet side of the head cage. The

system requires sub-samples of background air as well as expired and eructated air collected for gas analysis. Since the face mask covers the animal's face, they cannot eat or drink while measurements are being taken. The short duration of the measurement period (typically three one hour measurements per day (Kempton *et al.*, 1976)) means that quite variable results could be produced, due to the circadian variation in CH₄ production (Johnson & Johnson, 1995).

Although the respiration chambers allow for accurate CH₄ production and feed intake measurements, they confine animals and change their behaviour relative to their 'normal' production environment (McAllister *et al.*, 2011). Consequently, the relevance of the CH₄ measurements from respiration chambers to pasture-grazing animals has been questioned, and this has resulted in the development and implementation of techniques that are suitable for grazing animals (O'Kelly and Spiers 1992; Torrent and Johnson 1994; Harper *et al.* 1999; Leuning *et al.* 1999; C-Lock Inc (unpublished)).

2.3.2 The sulphur hexafluoride (SF₆) tracer technique

All tracer techniques operate on the principle that the tracer kinetics will be similar to those of CH₄, and knowing the amount of tracer administered, and its concentration (or specific activity for radioactive tracers) in expired gas will enable CH₄ production to be calculated. In order for tracer techniques to be effective in estimating CH₄ emissions they must meet several conditions; the tracer must (1) release at a constant and known rate (2) have no effect on ruminal fermentation; (3) be detectable in low concentrations and (4) be inert and non-toxic (Johnson *et al.*, 2007).

Pioneered at the Washington State University by Johnson *et al.* (1994), the Emissions from Ruminants Using a Calibrated Tracer Technique (ERUCT), is based on using SF₆ as a tracer and is the only standard method for estimating CH₄ in grazing animals. This method usually involves the release of SF₆ from a permeation tube at a constant rate in the rumen (Johnson *et al.*, 1994) as shown in Figure 2.7.

The implementation of the use of the permeation tube in New Zealand was led by Lassey *et al.* (1997).

The SF₆ technique relies on analysis of gases collected around the nose, typically for several 24 hour periods. Each animal is fitted with a halter which supports the sampling tube so its opening is close to the nose, and air is drawn into a capillary tube leading to an evacuated plastic collection yoke over the animal's neck (Photograph 2.2) or another part of the body. The typical yoke volume is about 2.5L for cattle. Gas chromatography is used to determine the CH₄ and SF₆ concentrations in collection and background samples calculated by using flame ionisation detection and electron capture detection, respectively (Lassey *et al.*, 1997).

The SF₆ technique is used to provide estimates of daily CH₄ emissions for individual animals, and estimates are usually made over 2-4 days. A longer measurement period provides a more accurate estimate of emissions than short periods. This method has been used with sheep, deer and cattle (Swainson *et al.*, 2008).

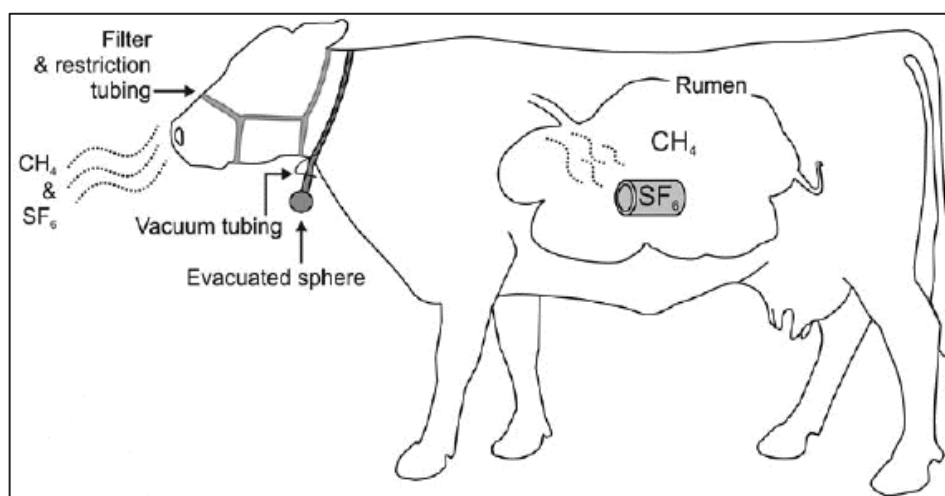
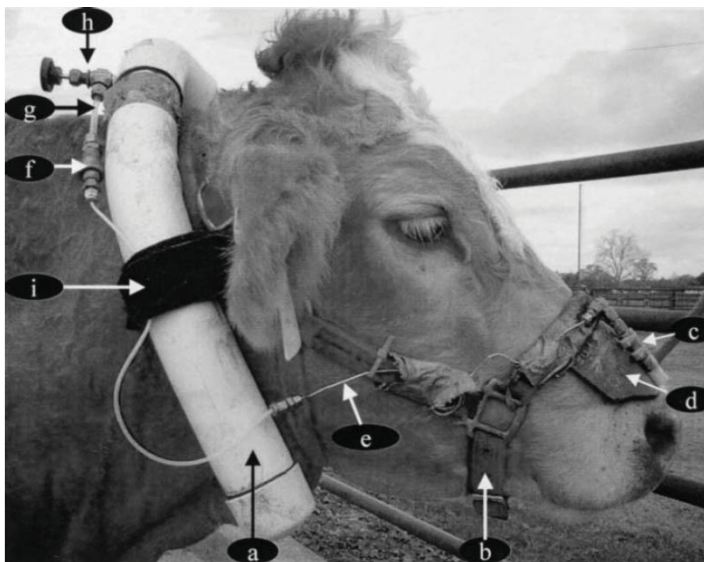


Figure 2.7: The tracer technique for individual ruminants showing the sampling of the target and tracer gas using an evacuated sampler at the head, and a permeation tube that releases sulphur dioxide (SF₆) at a known rate and is located in the rumen. From McGinn (2005).



Photograph 2.2: A cow fitted with the SF₆ apparatus; a; collection yoke, b; halter, c; filter inlet connected to capillary tubing, d; leather muzzle protector, e; stainless steel capillary tubing, f; quick-connect coupling of tubing to yoke, g; Teflon tubing shut-off valve and quick connect to capillary tubing, h; shut-off valve on yoke, i; Velcro strip to secure yoke to halter. From Johnson et al (2007).

Advantages of the SF₆ tracer technique

A significant advantage of this technique is its ability to estimate CH₄ from grazing animals and in New Zealand the estimates using the SF₆ technique have been used to compile the National Greenhouse Gas Inventory. The tracer methods can be applied at various locations and are lower cost although appropriate gas chromatography is needed to measure low concentrations of CH₄ and SF₆. Low costs enable measurement in more animals and estimates can be undertaken simultaneously, which allows for greater replication without time effects.

Disadvantages of the SF₆ technique

A notable feature of experiments measuring CH₄ from animals given the same amounts of feed are the differences in CH₄ emissions (Ulyatt *et al.*, 1999), regardless of the measurement technique used. Methane production from individual animals may vary over time even when animals are fed a constant amount of the same type and quality of feed and this is termed within-animal (day-to-day) variation (Table 2.3). Between-animal (animal-to-animal) variation (Table

2.4 and 2.5) has also been reported (Mbanzamihigo *et al.*, 2002, Lassey *et al.*, 1997, Boadi & Wittenberg, 2002). Both within and between-animal variation can be described in terms of coefficient of variation (CV).

Evaluations of the SF₆ technique have caused its accuracy for determining CH₄ emissions to be questioned by some (Vlaming *et al.*, 2005, McGinn *et al.*, 2006, Pinares-Patiño & Clark, 2008). The coefficient of variation for within-animal variation in CH₄ production was lower from respiration chambers (6.1%) than the SF₆ technique (4.3%)(Table 2.3). This indicates a need to repeat estimates over a greater number of days in order to obtain the same level of precision (Grainger *et al.*, 2007). This need is exacerbated for grazing experiments where conditions are more variable than under a controlled environment (Ulyatt *et al.*, 2002b). In addition, as the SF₆ technique collects an integrated sample it cannot determine the circadian variation in CH₄ emissions.

Between-animal variation in CH₄ emissions is a common feature of controlled and grazing experiments using the SF₆ technique (Ulyatt *et al.*, 2002b, Lassey *et al.*, 1997, Pinares-Patiño *et al.*, 2003), but the variation seems to be larger than those commonly reported for the respiration chambers (Johnson *et al.*, 1994, Grainger *et al.*, 2007, Boadi & Wittenberg, 2002). It would be expected that the CH₄ emissions under grazing conditions could be more variable than under controlled conditions because grazing animals have more opportunity to express natural grazing behaviour and select their diet than animals kept indoors (Arnold, 1981). Grainger *et al.* (2007) found a slightly higher between-animal variation in CH₄ production for the SF₆ technique compared to the respiration chambers (CV; 19.6 and 17.8%, respectively (Table 2.4)). Ulyatt *et al.* (2002a) suggested that in general between-animal differences account for most of the variation (70-80%).

There is evidence that the release rate of the permeation tube can affect CH₄ production (Vlaming *et al.*, 2007, Pinares-Patiño & Clark, 2008), which could further contribute to the variation in CH₄ emission estimates by as much as 6 to 13% (Pinares-Patiño & Clark, 2008). Vlaming *et al.* (2007) compared permeation tubes of low (2.88 mg SF₆/d) and high (7.34 mg SF₆/d) release rates and the estimates of CH₄

production were significantly different (134.2 g/d vs. 160.8 g/d, respectively) in cows fed the same intake of the same diet. There is also evidence that the change in permeation tube release rate over time (within rumen) can affect CH₄ estimates. This was confirmed by Lassey *et al.* (2001) who found that 150 d after tubes were filled there was a decline in SF₆ release rate, and because CH₄ production is calculated from the ratio of SF₆ to CH₄ in the breath, a decreased SF₆ release concentration would over-estimate CH₄ production (Lassey *et al.*, 2001).

A minor disadvantage of the SF₆ technique is the under-estimation of the CH₄ estimates due to losses in the flatus. This is because the SF₆ technique only captures exhalation and eructation (and not flatus) whereas the respiration chambers capture the total emissions. Studies have confirmed losses in the flatus to range from 1-3% (Murray *et al.*, 1976, Kempton *et al.*, 1976) of total emissions.

The SF₆ technique requires the animals to wear equipment to estimate CH₄ production, which from a practical perspective has two disadvantages. Firstly, this requires training of the animals to wear the equipment, which involves labour and time. Secondly, wearing the equipment has been known to interfere with an animal's ability to eat and drink (Pinares-Patiño *et al.*, 2008). This will affect feed intake, which as the most important determinant of CH₄ production (van Zijderveld *et al.*, 2011), is a significant disadvantage. If the equipment interferes with eating, then an under-estimation of feed intake will over-estimate CH₄ yield.

Table 2.3: Within-animal variation for daily CH₄ production (g/d) for cattle consuming forage-based diets.

Animal details	n	Diet type	DMI (kg/d) Mean ± SD [#]	Technique	CH ₄ production (g/d) Mean ± SD [#]	CV (%) ^δ	Reference
Not stated ¹	87*	Not stated but constant feed	Not stated	Chamber (closed)	103 ± 7.4	7.2	Blaxter and Clapperton (1965)
Dry heifers ²	6	Legume & grass hay (ad-libitum) (restricted)	8.2 ± 0.76 6.1 ± 0.40	SF ₆ SF ₆	172 ± 7.1 142 ± 7.8	26.9 27.1	Boadi & Wittenberg (2002)
Lactating cows ³	16	Ryegrass pasture & concentrate	18.2	SF ₆ Chamber	331 ± 74.6 322 ± 57.5	6.1 4.3	Grainger <i>et al.</i> (2007)

¹Unstated, ²Holstein, ³Holstein-Friesian, ⁴Beef breed, ⁵Friesian-Jersey, *A dataset.

DMI, dry matter intake; SF₆, sulphur hexafluoride; SD, Standard deviation; CV, coefficient of variation

[#]Not all experiments reported SD of the DMI or CH₄ production

^δSome CH₄ emissions values required a unit conversion (Appendix A)

Table 2.4: Between-animal variation for daily CH₄ production (g/d) for cattle consuming forage-based diets.

Animal details	n	Diet type	DMI (kg/d) (Mean ± SD) [#]	Technique	CH ₄ production (g/d) (Mean ± SD) [#]	CV (%) [§]	Reference
Lactating cows ³	221	Grass silage	17.0 ± 3.5	Chamber	377 ± 68.9	18.2 [†]	Yan <i>et al.</i> (1997)
Dry heifers ⁴	6	Barley (50%) & Lucerne (50%)	4.4 - 4.8	SF ₆	92 ± 2.7	11.7	Boadi <i>et al.</i> (2002)
Dry heifers ²	6	Legume & grass hay (<i>ad-libitum</i>) (restricted)	8.2 ± 0.76 6.1 ± 0.40	SF ₆ SF ₆	172 ± 7.1 142 ± 7.8	26.6 25.3	Boadi & Wittenberg (2002)
Lactating cows ¹	63* 20 13	Pasture (94% grass)	13.6 ± 1.7 11.8 ± 1.6 14.7 ± 3.3	Chamber	288 ± 27.0 234 ± 48.6 360 ± 36.0	9.4 [†] 20.8 [†] 10.0 [†]	Bruinenberg <i>et al.</i> (2002)
Lactating cows ³	16	Ryegrass pasture & grain	18.2	SF ₆	331 ± 74.6	19.6	Grainger <i>et al.</i> (2007)
Dry cows ⁵	4	Lucerne silage Cereal, lucerne silage & straw	5.3 - 6.0 5.2 - 5.4	Chamber SF ₆	322 ± 57.5 124 ± 22.2 170 ± 22.0	17.8 12.0 16.0	Vlaming <i>et al.</i> (2008)

¹ Unstated, ² Holstein, ³ Holstein-Friesian, ⁴ Beef breed, ⁵ Friesian-Jersey, *A dataset.

DMI, dry matter intake; SF₆, sulphur hexafluoride; SD, Standard deviation

[†] CV, coefficient of variation, has been calculated using = (SD x 100)/Mean.

[#] Not all experiments reported SD of the DMI or CH₄ production

[§] Some CH₄ emissions values required a unit conversion (Appendix A)

Table 2.5: Between-animal variation for daily CH₄ yield (g/kg DMI) for cattle consuming forage-based diets.

Animal details	n	Diet type	DMI (kg/d) (Mean ± SD) [#]	Technique	CH ₄ yield (g/kg DMI) (Mean ± SD) [#]	CV (%) ^δ	Reference
Dry cows ⁵	4	Lucerne silage Cereal, lucerne silage & straw	5.3 - 6.0 5.2 - 5.4	SF ₆ SF ₆	22.8 ± 4.0 32.0 ± 4.0	11 16	Vlaming <i>et al.</i> (2008)
Lactating cows ²	10	Ryegrass & white clover (<i>ad-libitum</i>)	16.2	Chamber	24.6	10.6	Munger and Kreuzer (2006)
Lactating cows ²	10	Ryegrass & white clover (<i>ad-libitum</i>)	12.3	Chamber	25.3	7.7	Munger and Kreuzer (2006)

¹ Unstated, ² Holstein, ³ Holstein-Friesian, ⁴ Beef breed, ⁵ Friesian-Jersey, *A dataset.

DMI, dry matter intake; SF₆, sulphur hexafluoride; SD, standard deviation, coefficient of variation

[#]Not all experiments reported SD of the DMI or CH₄ yield

^δSome CH₄ emissions values required a unit conversion (Appendix A)

Due to the greater variation in the CH₄ estimates and the consequent requirement for a longer measurement period from the SF₆ technique compared to the respiration chambers, there have been advances in the technology for determining CH₄ production accurately from grazing animals under a normal 'production' environment.

2.3.3 The Greenfeed system

In an attempt to address both the artificial environment of the respiration chambers and the high variation in the CH₄ estimates from the SF₆ technique, there has been development of new techniques for overcoming these limitations. An example of such a development is the Greenfeed system (C-Lock Inc, South Dakota, United States, Patent 7966971), shown in Figure 2.8 and Photograph 2.3, which is designed for estimating CH₄ from grazing animals, specifically cows.

Full operational details are available in the Greenfeed Trailer Mounted Feeder Instruction Manual (S. Zimmerman, pers. comm., [www. C-Lockinc.com](http://www.C-Lockinc.com)) and brief details are given in Appendix B. Briefly, the Greenfeed unit is powered by batteries which are charged using solar panels, generators or mains power for indoor use. The Greenfeed unit can be placed in a paddock so cows are able to enter it voluntarily. When the cow's head is near the feeding station its electronic ear tag will be detected causing a pelleted feed (which is used as an attractant) to be dispensed. While the cow's head is in the feeding station, consuming the feed, air is then drawn past the animal's face and into a collection pipe, which allows CH₄ concentrations to be read. By measuring the change in CH₄ concentration relative to ambient levels, the air flow rate and applying ideal gas laws, the CH₄ emissions can be determined. However, since the feeding station is not completely enclosed, a known amount of an external tracer is released to estimate the recovery of gaseous emissions. Cows must use the Greenfeed several times a day for approximately five minutes at a time to obtain an estimate of emissions, and usage should be spread evenly over the 24 hour-period, to estimate daily emissions. Once the Greenfeed unit collects the data, it is automatically processed and CH₄ estimates are calculated

for each cow, which are then compiled into a daily report and emailed to the user by C-Lock Inc.



Figure 2.8: Components of the Greenfeed system design. From C-Lock Inc. (unpublished)



Photograph 2.3: A Greenfeed unit for use by pasture-grazing cows.

Advantages of the Greenfeed system

The Greenfeed system allows CH₄ estimates to be made while animals naturally graze in a pasture-based situation without any human-interference and allow circadian patterns in CH₄ production to be determined. This should provide a better representation of the diet, behaviour and environment compared to the artificial environment of the respiration chambers. Furthermore, the user-friendly web-based controls allow the user to adjust the settings such that the amount and type of attractant to be dispensed can be altered, and both the timing and meal interval can be manipulated. There are no requirements for laboratory equipment using this technique.

Disadvantages of the Greenfeed system

The disadvantages of the Greenfeed system lie in the large number of unknown variables that need to be considered when using the Greenfeed system in a pasture-based system. To date, the Greenfeed system has only been used once in an indoor experiment for lactating dairy cows consuming pasture carried out in the United States by Utsami (unpublished). This study reported a mean CH₄ production of 418 g/cow/day over a 17-day period.

Little is known about the on-farm implementation of the Greenfeed unit, especially under New Zealand conditions. Further work involving the use of the Greenfeed unit is required to determine how its CH₄ estimates compare to those from respiration chambers and SF₆ technique. In addition, the cow behavioural interactions and any training requirements with the Greenfeed unit need to be understood to ensure that reliable and representative CH₄ estimates can be obtained. Before the Greenfeed system can be considered for use, it needs to be tested in local conditions under New Zealand farming systems.

2.4 OBJECTIVES OF THIS THESIS

This study comprises two experiments, one to compare the Greenfeed CH₄ estimates with respiration chambers and the SF₆ technique, and the other to evaluate its on-farm implementation. This will be the first study with the Greenfeed units and will consequently involve the only two Greenfeed units in New Zealand. The objectives of this study are to;

- Compare CH₄ measurements from dry cows fed the same diet at similar (measured) intakes indoors using the respiration chambers, SF₆ technique and the Greenfeed unit (Chapter Three)
- Assess the interactions of outdoor grazing lactating dairy cows with the Greenfeed unit, including an assessment of their visiting behaviour (Chapter Four)
- Evaluate the CH₄ estimates obtained from the Greenfeed unit and investigate the on-farm implementation for its use in a New Zealand pasture-grazing system (Chapter Four)

2.5 HYPOTHESES OF THIS THESIS

Based on these objectives, the following hypotheses are given;

- In Chapter Three; that the CH₄ obtained from animals using the Greenfeed system would be similar to those from the respiration chambers and the SF₆ technique, with a similar degree of variation to respiration chambers and less variation than the SF₆ technique
- In Chapter Four; that the majority of cows would use the Greenfeed units at their own free will, that experienced users would 'train' new cows and that variance in the estimates of CH₄ production decrease over time as more estimates were taken

CHAPTER THREE

COMPARISON OF THE METHANE MEASUREMENTS MADE WITH THE RESPIRATION CHAMBERS, SF₆ TECHNIQUE AND GREENFEED UNIT



CHAPTER THREE: COMPARISON OF THE METHANE MEASUREMENTS MADE WITH THE RESPIRATION CHAMBERS, SF₆ TECHNIQUE AND GREENFEED UNIT

3.1 INTRODUCTION

The respiration chambers and sulphur hexafluoride (SF₆) tracer technique are common techniques used to measure methane (CH₄) from ruminants, and the Greenfeed system is a recent development. The respiration chambers are considered the 'gold standard' (O'Hara et al., 2003) as they measure, rather than estimate, CH₄ emissions directly from animals. A cow is placed in a chamber and the CH₄ concentrations in the in- and out-flowing air concentrations are measured every 3-5 minutes, and together with the measured airflow are used to calculate CH₄ emissions (Pinares-Patiño et al., 2012b). The advantages of the respiration chambers are that they are accurate and feed intakes can be measured, however, they are limited in their applicability to grazing systems (O'Hara et al., 2003) because of the artificial environment in which animals are placed, typically for 48 hours. Within the chamber, animals are often fed forage twice daily that has been cut and carried, which removes opportunities for diet selection and for natural grazing patterns to develop. Both the construction and operation of the respiration chambers is expensive and their operation is labour intensive, with animal training, daily feeding, cleaning and frequent exchange of animals required.

The SF₆ technique is based on the SF₆ marker gas released at a known rate from a permeation tube in the rumen and a halter with a collection tube near the cow's nose to sample the breath constantly. The CH₄ emissions are estimated based on the CH₄:SF₆ ratio in the breath sample, with a correction for background gas concentrations (Johnson et al., 1994). The SF₆ technique offers advantages over the respiration chambers because it enables CH₄ to be estimated from grazing cows, better representing the normal production environment (McAllister et al., 2011). However,

intakes must be estimated, and relative to respiration chambers, the SF₆ technique gives greater within-animal variation (Hammond *et al.*, 2011) so estimates need to be repeated over more days in order to obtain the same level of precision (Grainger *et al.*, 2007).

The Greenfeed system is a new method which has been developed to estimate the CH₄ production from cows in either a grazing situation or indoors, but without human interference. It estimates CH₄ emissions from short (3-7 minute) periods, usually up to six times per day. The raw data is sent electronically from the Greenfeed unit to C-Lock Inc. (South Dakota, United States, Patent 7966971) and they have algorithms to interpret the data and determine the CH₄ emissions, which are then reported back to the user (C-Lock, unpublished). The Greenfeed system has a potential advantage over the SF₆ technique because it allows CH₄ estimates to be made while cows graze pasture, better representing the behaviour, diet selection and environment that a grazing dairy cow would experience. However, as research on the Greenfeed system is still in its infancy, it is unknown how the CH₄ emissions from cows using the Greenfeed system compare to those from cows using the respiration chambers and the SF₆ technique.

Although the measurements of CH₄ derived from the respiration chambers, SF₆ technique and Greenfeed system should be similar, there may be differences in mean values and variation. This may be especially associated with the differing sampling frequencies for the animals; every 3-5 minutes for the respiration chambers, a daily integrated sample for the SF₆ technique and intermittent (up to six times a day) for the Greenfeed system. Therefore, the ability of the Greenfeed unit to detect circadian variation in CH₄ production may be limited due to the intermittent sampling compared to the respiration chambers and the SF₆ technique.

The experimental objectives will determine the need for a certain degree of accuracy and precision in the estimates obtained from the Greenfeed and SF₆ technique, relative to respiration chambers as well as other (for example, behavioural considerations). Accuracy refers to the agreement between measurements from each

system, relative to the value determined using respiration chambers, and precision refers to the variance around the mean. These evaluations can only be undertaken when intakes and feeding regimens are similar, in controlled (indoor) feeding experiments.

The objective of this experiment was to compare CH₄ measurements from cows fed the same diet at similar intakes using the respiration chambers, SF₆ technique and the Greenfeed system.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design

This experiment compared CH₄ emissions from cows using the Greenfeed unit, respiration chambers and the SF₆ technique. It was conducted over a period of 35 days as detailed in Table 3.1. From day 1 to 10 cows were given time to get used to the feed (lucerne silage), facilities, equipment and procedures (acclimatisation period) and from days 11 to 21 training was carried out to encourage the cows to use the Greenfeed unit (training period). Methane estimates using the Greenfeed unit were carried out from day 23 to 35 of the experiment. From day 22 to 29 all cows had CH₄ estimated using the SF₆ technique and from day 30 to 34 the respiration chambers were used. As there are only four respiration chambers at this facility, the first four cows were placed in the chambers from day 30 to 32, and the two remaining cows from day 32 to 34.

Table 3.1: Summary of activities carried out during the experiment.

Day	Activity (n = 6 for all periods)
1-10	Acclimatisation period: allowed cows to get used to the feed, facilities, equipment and procedures
11-21	Training period: cows given free access to the Greenfeed unit and cow training occurred
17	SF ₆ permeation tubes inserted into cows
23-35	Greenfeed unit estimates. During days 30-34 cows not in the respiration chambers had access to the units (ie. 2 cows from days 30-32 and 4 cows from 32-34).
22-29	SF ₆ technique estimates
30-34	Respiration chamber measurements (day 30-32 = 4 cows) (day 32-34 = 2 cows)

SF₆, sulphur hexafluoride

3.2.2 Animals and facilities

The experiment was carried out at the New Zealand Methane Measurement Centre at AgResearch, Grasslands in Palmerston North. All animal manipulations were approved by the Animal Ethics Committee of AgResearch Grasslands (Number 12301). Six dry Hereford x Friesian cows with a mean live weight of 365 kg ± 12 kg were used in this experiment. The cows were housed in a covered pen which comprised a roof with open sides allowing the circulation of air. It is unlikely that concentrations of CH₄ or SF₆ would have built up because of the air movement. The pen consisted of 12 feed stalls down one side and a Greenfeed unit was positioned at one end of the pen, with only the head-box of the Greenfeed unit extending into the pen (Figure 3.1). A chute (2.8m long x 0.6m wide) was positioned to allow only one cow to access the unit at any time. Metal gates were secured to either side of the chute to prevent cows getting close to the electronic identification (EID) reader to avoid possible interference with the EID of the cows using the unit. As estimates using the Greenfeed unit and SF₆ technique were carried out simultaneously the housing, feeds and feeding routine were the same (Table 3.1). All cows had access to water from a trough positioned at the opposite end to the Greenfeed unit as well as small individual troughs within each feed stall, which were on a reticulated system.

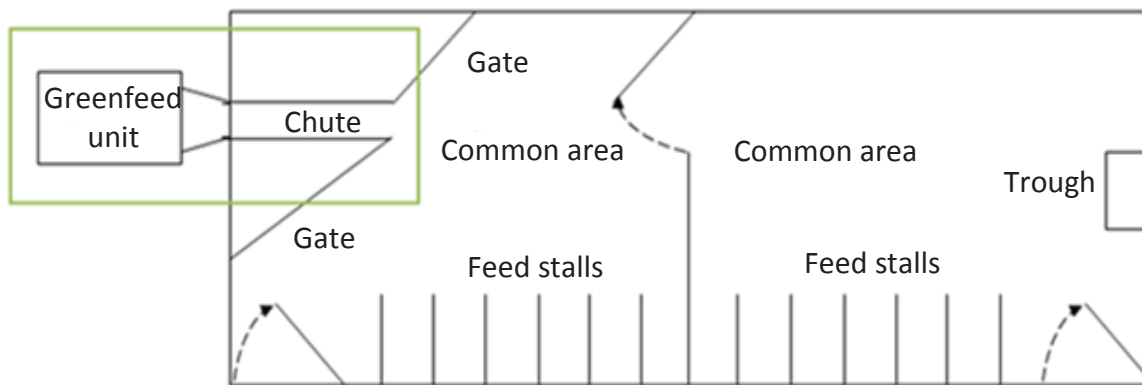


Figure 3.1: Pen used to house the cows during periods 1-4 of the experiment (top view) with Greenfeed unit setup (in green box).

When CH₄ was measured using the respiration chambers the cows (4 cows on day 30-32 and 2 cows on day 33-34) were removed from the covered pen and confined for a total of 48 hours in the respiration chambers, tethered to their feed bin. Each of the four open-circuit respiration chambers for cattle have a steel framework and the roof and walls are covered in 6mm thick UV stable polycarbonate sheets. Each chamber has a rear door for entry of the cows and a front door allowing access for feeding. A large feed bin was situated at the front of the chamber and a small water-trough to the right of the feed bin. This trough was supplied by a 50L water bucket situated on the roof of the chamber (Pinares-Patiño et al., 2012b).

3.2.3 Feeds and feeding

Cows were offered Lucerne silage (Fibre Fresh Fibre Pro, Reporoa, New Zealand) and pellets (Country Harvest Llama and Alpaca pellets, Hamilton, New Zealand) throughout the entire experiment at a maintenance level of intake. Daily 200 g samples of each the silage and pellets were used to estimate dry matter (DM) contents by oven-drying at 65°C for 48 hours. The wet chemistry analysis of the two feeds was conducted by the Institute of Food, Nutrition and Human Health Nutrition Laboratory, Massey University. The silage contained 45% dry matter while the pellets contained 90% DM. The laboratory used the following methodologies. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the Tecator Fibretec System

following the procedures of Robertson & Van Soest (1981). Crude protein content was determined using the Leco total combustion technique (AOAC, 1990). The ash content was determined by heating in a furnace at 500°C for 16 h (AOAC, 1990).

Greenfeed and SF₆ estimates

Cows were fed 2.7 kg DM of silage at 08:00 h and 15:45 h when tethered in the feed stalls during the acclimatisation, training and measurement periods with the Greenfeed unit and SF₆ technique periods. When the cows had finished eating the silage (generally 40 minutes after meal delivery) they were released into the common area and had free access to the Greenfeed unit. The mean pellet intake over the measurement period was 0.84 ± 0.05 kg DM/cow/day, which was dispensed by the Greenfeed unit over 4-6 meals per day, with a minimum meal interval of four hours. Therefore the total (silage and pellets) intake of cows was on average 6.24 kg DM/cow/day. Refusals were monitored after feeding but all cows ate all of the silage and pellets that was offered. Cleaning to remove faeces and urine was once daily.

Respiration chambers

When in the respiration chambers each cow was fed 2.7 kg DM of silage twice daily at 08:00 h and 15:45 h with 0.36 kg DM of pellets added to each meal to mimic the amount of pellets that was being dispensed from the Greenfeed unit. Cows during this period of the experiment consumed 6.12 kg DM/cow/day. Refusals were monitored an hour after meal delivery but all cows ate all feed (silage and pellets) offered. At all times each cow in the respiration chamber remained tethered to their feed bin. Cleaning of the respiration chambers to remove faeces and urine occurred twice daily and the water bucket on top of each chamber was used to fill the smaller trough within the chamber to ensure fresh water was always available *ad-libitum*.

3.2.4 Acclimatisation and training period

The six cows used in this experiment had been in the respiration chambers previously and were accustomed to the chamber facilities and associated experimental procedures. However, the cows had not had any previous experience with the SF₆ equipment or with the Greenfeed unit. Before any CH₄ estimates could start being taken, the cows needed to become acclimatised to the feeds, housing facilities and experimental equipment and procedures. This involved placing cows in the pen with free access to the Greenfeed unit without restrictions on the number of meals per day that the unit dispensed. There was varied interest in the Greenfeed unit by the cows, with some cows using it regularly and others not at all. Therefore, a 10-day training was undertaken to ensure all cows used the Greenfeed unit. Each day immediately prior to feeding the silage, all cows were placed and secured in their feed stalls and then one at a time, released and walked to, and encouraged into the Greenfeed unit. It was ensured that each cow remained in the unit for the required 3-5 minutes, which was the time required for a CH₄ estimate to be made. For encouragement a trail of silage was placed in the chute leading up to the Greenfeed unit (Photograph 3.1 A) and pellets were placed into the feeding station as an attractant (Photograph 3.1 B). At the end of this period the handlers were satisfied that all six cows would enter the Greenfeed unit at their own will once being released from their stall.



Photograph 3.1: The trail of silage (A) in the chute to attract cows into feeding station (B).

3.2.5 Methane measurements

Greenfeed unit

When CH₄ estimates started on day 23, the Greenfeed unit was set to operate continuously (day and night) during the experimental period. The units were adjusted to allow only six meals per cow per day to occur, at 4-hour intervals over a 24-hour period. However, cows were able to access the units at any time except when it was occupied by another cow, and their CH₄ would be estimated if she kept her head in for more than 3 minutes.

At each visit, the cow's EID ear tag was identified and if a meal was allowed, pellets would be dispensed over a 3-5 minute period, at 40-second intervals. The respired breath was sampled by the Greenfeed unit and concentrations of CH₄ (and carbon dioxide (CO₂)) were determined and sent to C-Lock Inc. where they applied algorithms to the concentrations and reported gas emissions to the users. This was standard procedure at the time of measurement. C-Lock Inc. used Equation 3.1 to determine CH₄ emissions;

Equation 3.1: *Components of the equation used for calculation of CH₄ production by the Greenfeed system.*

$$\text{CH}_4 \text{ production} = F_c \times C_R \times \sum_{tp} [\Delta t \times (\text{CH}_{4\text{eruct}} - \text{CH}_{4\text{bkd}}) \times Q_{\text{air}}]$$

Where:

C_R = proportion of emissions captured in airflow to sensors, determined using the tracer gas

Δ t = period over which emissions are measured

CH₄ eruct = average concentrations of CH₄ during the measurement period

CH₄ bkd = background concentrations of CH₄

Q_{air} = airflow during the measurement period

F_c = dimensional factor (undefined)

From C-Lock Inc. (unpublished)

Following the calculation of CH₄ volume, the number of CH₄ molecules per unit time was calculated and using ideal gas laws, then the mass of CH₄ emitted per time was calculated by multiplying by the molecular weight of CH₄.

SF₆ tracer technique

In preparation for CH₄ estimates by the SF₆ technique, each cow was orally dosed with a permeation tube containing the SF₆ tracer gas, five days before the start of the breath collection sampling. The permeation tubes released 3.066 ± 0.200 mg SF₆/d. Each cow was fitted with a halter with a collection tube positioned on top of a leather pad which was mounted about 5cm dorsal to the cow's nostrils. This collection tube was connected to a pre-evacuated yoke (approximately -100kPa) on the cow's neck by plastic tubing and a Quickconnect fitting (Johnson et al., 2007). Background yokes were placed at two fixed locations in the yards, in order to take into account the ambient concentrations of CH₄ and SF₆.

Changing the yokes for the SF₆ technique took place while the cows were tethered in individual feed stalls immediately prior to meal delivery each morning. The yokes were taken off the cows and a pressure gauge was used to ensure that the pressures were between -30kPa and -80kPa and those yokes that were in this range were deemed adequate samples and were stored for subsequent analysis. Inadequate samples were those with pressures outside this range and suggested either a leak (pressure close to zero) or block (pressure still close to -100kPa) in the sampling equipment (Pinares-Patiño *et al.*, 2012a), and the samples were discarded. Regardless of whether the yoke was adequate or inadequate, a new yoke was replaced on each cow each morning.

Those cows that provided inadequate samples had their equipment checked to ensure functionality, and these protocols also applied to the background samples. Breath samples from the cows were collected over 24-hour periods during the 8-day experimental period as well as background samples (Pinares-Patiño *et al.*, 2007). Those samples that had been deemed adequate were analysed using gas chromatography by the National Institute of Water and Atmospheric Research (NIWA) (Wellington, New Zealand), where flame ionisation and electron capture detectors were used for CH₄ and SF₆, respectively (Johnson et al., 2007). The SF₆ was used as a marker which accounted for the dilution of the gases by air as they left the cow's body. It was assumed that the SF₆ and CH₄ emissions were exactly matched, therefore the dilution

rates were identical. This allows the CH₄ emission rate to then be calculated from estimated CH₄ and SF₆ concentrations and correcting for the background concentrations using Equation 3.2;

Equation 3.2: Calculation of CH₄ production by SF₆ technique.

$$Q_{SF_6} = \frac{Q_{SF_6} \times ([CH_4 \text{ yoke} - CH_4 \text{ background}])}{([SF_6 \text{ yoke}] - [SF_6 \text{ background}])}$$

Where:

Q_{SF₆} = calibrated rate of permeation from SF₆ capsule

From Johnson et al. (2007)

Respiration chambers

Each cow was placed in a respiration chamber for 48 hours with an airflow of 1500 L/minute and gas concentrations measured at 3-minute intervals in the outlet airstream and hourly in the inlet airstream (Pinares-Patiño et al., 2012b). A multigas analyser (Servomex 4900) was used to determine concentrations of CH₄ and CO₂ in the samples. Gas recovery rates averaged 101% when measured by mass flow metering of a CH₄ calibration gas (Pinares-Patiño et al., 2012b). The cleaning of the chambers twice daily meant that the chamber doors had to be open for about 15 minutes, and gas production during this time was estimated by interpolation based on the 10 values immediately before the chamber doors were opened (Pinares-Patiño et al., 2012b).

The calculation of CH₄ production requires the wet ventilation rate, concentration of gas in dry sample (above background levels) and the gas recovery and involves the Equation 3.3 below;

Equation 3.3: *The basis of respiration chamber measurements for calculating CH₄ production.*

$$\text{CH}_4 \text{ production (L/min)} = (\text{Dry STP FR} \times ([\text{CH}_4 \text{ ppm}]/10^6))/\text{gas recovery}$$

Where:

STP FR = flow rate of dry air at standard temperature and pressure

ppm = parts per million

CH₄ concentrations have been corrected for CH₄ in incoming air

From Pinares-Patiño et al. (2012b)

3.2.6 Statistical analysis

Statistical analyses were preceded by an assessment of data to ensure only measurements that were accurate and useful were used in comparisons between techniques. This applied to a lesser extent for the Greenfeed estimates than it did for the SF₆ technique, and the calibration of the respiration chambers (Pinares-Patiño et al., 2012b) provided confidence in the data. Although both CH₄ and CO₂ data was collected using the Greenfeed unit and respiration chambers, only the CH₄ data was used in this analysis.

The raw data from the Greenfeed unit was assessed by C-Lock Inc. who provided data for analysis, and only CH₄ estimates in which the cow left her head in the Greenfeed unit for more than 3 minutes were included. Estimates provided by the SF₆ technique required several criteria to be met before acceptance of results. These included the need for a 24 hour collection (no blockages or leaks in the equipment) and that the concentrations of SF₆ and CH₄ in the sample was at least 6 times background values (Lassey et al., 2011). These assessments were made initially at sample collection after which they were sent to NIWA, where gas concentrations were determined. The screening procedure meant that all animals (except cow 2038) gave 5-7 adequate samples.

The time period for each of the three techniques differed slightly (Greenfeed; day 23-35, SF₆; day 22-29 and chambers; day 30-34; Table 3.1). However, in order to carry out the most direct comparison, the days of estimates needed to be close as possible. For

this reason, the Greenfeed data used for the analysis included a mean of the days prior to and after the chamber measurement period (Table 3.2), rather than day 23-35. The Greenfeed data therefore included 5-8 days of estimation for each cow distributed either side of the respiration chamber measurements. The difference in the number of measurement days is because at least 15 visits to the Greenfeed unit were required before the cows entered the respiration chambers, while all measurement days after the respiration chambers were included (S. Zimmerman, pers. comm.).

Table 3.2: Days included in analysis for the CH₄ estimates from the Greenfeed unit.

Cow	Before chambers	After chambers	Number of CH ₄ estimates
2010	27-30	32-35	29
2037	29-31	34-35	26
2038	26-29	32-35	20
2368	28-30	32-35	41
3656	29-30	34-35	23
2696	27-30	32-35	37

Days are inclusive

Statistical analyses used SAS version 9.2 (SAS, 2002-2008). A one-way analysis of variance (ANOVA) procedure compared the mean CH₄ production (g/d) for all 6 cows for each of the three techniques over the measurement periods (described above). The Tukey adjustment was used to correct for errors when multiple comparisons were made. Correlation coefficients (and associated P-values) and concordance correlation coefficients were made for CH₄ production averaged for the six animals in each treatment, to compare measurements from respiration chambers with the Greenfeed unit, and the respiration chambers with SF₆ estimates. In each case, the respiration chambers were treated as a reference. The level of significance was $P \leq 0.05$ for all analyses.

3.3 RESULTS

The silage fed throughout this experiment comprised (as a percentage of DM); 18% crude protein, 38.1% NDF, 19% ADF and 8.4% ash. The pellets contained; 17.9% crude protein, 36.6% NDF, 27.4% ADF and 8.1% ash.

Methane production (g CH₄/d) for individual cows measured using respiration chambers, the Greenfeed unit and the SF₆ technique are presented in Table 3.3. There was no significant difference between mean CH₄ production measured using the Greenfeed unit and respiration chambers (150 ± 20.2 vs. 134 ± 9.8 g CH₄/day), nor was there a difference between mean CH₄ production measured from the respiration chambers and the SF₆ technique (134 ± 9.8 vs. 128 ± 8.7 g CH₄/day). Mean CH₄ production estimated using the Greenfeed unit was significantly higher (150 ± 20.2 vs. 128 ± 8.7 g CH₄/day; P < 0.05) than those from the SF₆ technique. The mean CH₄ production estimated by the Greenfeed unit was more variable (higher standard deviation) than that measured from the respiration chambers and the SF₆ technique. The CH₄ yields (g CH₄/kg dry matter intake (DMI)) ± SD were higher from the Greenfeed unit (24.0 ± 3.2) than the respiration chambers (21.9 ± 1.6) or the SF₆ technique (20.5 ± 1.4).

Table 3.3: Methane production (g CH₄/d; means ± SD) from the respiration chambers, SF₆ technique and Greenfeed unit.

Cow	Respiration chambers ¹	SF ₆ technique ²	Greenfeed unit ³
2010	135 ± 7.4	118 ± 15.3	156 ± 28.5
2037	121 ± 2.1	133 ± 8.1	164 ± 34.3
2038	140 ± 4.7	118 ± 9.7	171 ± 46.7
2368	129 ± 7.0	126 ± 9.9	158 ± 74.8
3656	128 ± 2.1	131 ± 23.8	128 ± 21.8
3696	149 ± 5.2	140 ± 9.6	122 ± 35.2
Mean	134 ± 9.8 ^{ab}	128 ± 8.7 ^a	150 ± 20.2 ^b

^{a,b} Means within a row with different superscripts are significantly different (P ≤ 0.05) Methane measurements were based on a period of: ¹ 2 days, ² 8 days and ³ 5-8 days SF₆, sulphur hexafluoride

The strength and direction (as defined by the correlation coefficients and concordance correlation coefficients) of the relationships between the respiration chambers and the SF₆ technique, and the respiration chambers and the Greenfeed unit, are illustrated in Figure 3.2. Both relationships were weak but in opposite directions to each other. The correlation coefficient for the relationship between the respiration chambers and the SF₆ technique was 0.13 (P = 0.80) and between the CH₄ measurements for individual cows from the respiration chambers and the Greenfeed unit was -0.36 (P = 0.45).

Concordance correlation coefficients also demonstrated weak relationships between the measurements taken using the three techniques. Between the respiration chambers and SF₆ technique it was 0.11 and between the respiration chambers and the Greenfeed unit it was -0.19.

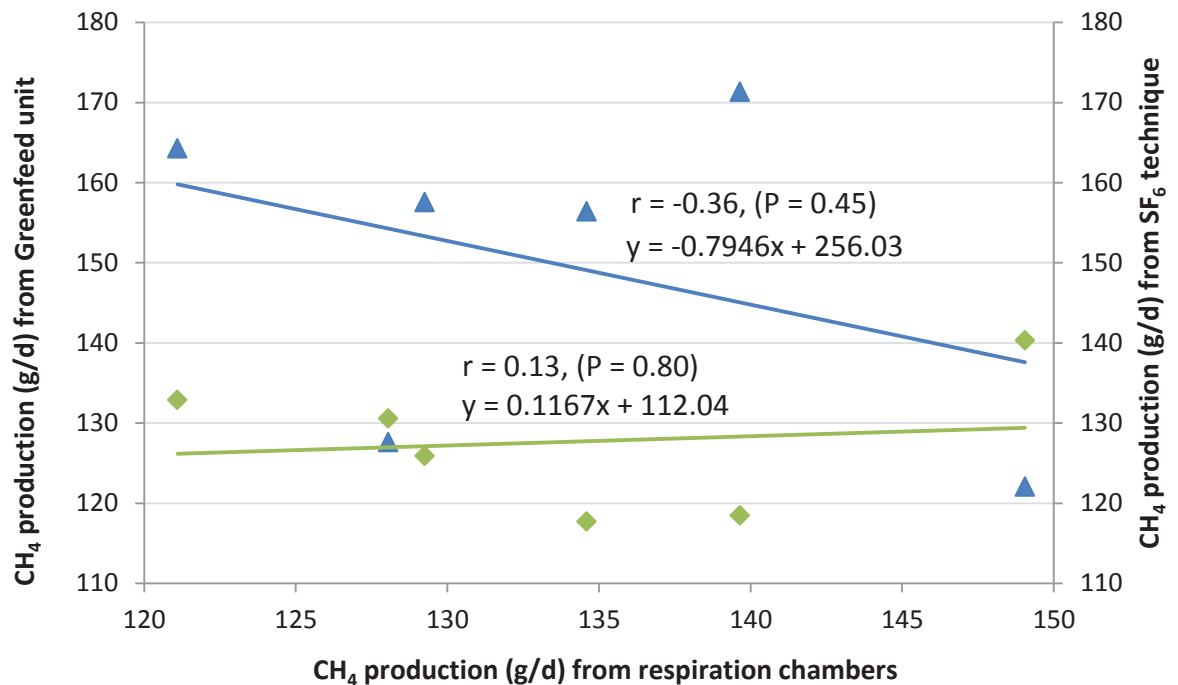


Figure 3.2; Methane production (g CH₄/d) from individual cows measured in respiration chambers and the SF₆ technique (◆) and the respiration chambers and Greenfeed unit (▲). The correlation coefficients (*r*), associated probability values (*P*) and relationships are indicated.

3.4 DISCUSSION

This experiment is the first to estimate CH₄ production using the Greenfeed unit, and compare values with those from the respiration chambers and the SF₆ technique. In all instances, the CH₄ measurements from the respiration chambers were treated as a standard to compare the Greenfeed unit and SF₆ technique against. Based on the CH₄ yield of 21.6 g CH₄/kg DMI (Ministry for the Environment, 2010) and a feed intake of 6.18 kg, a CH₄ production of 133.5 g/d would be expected, and this is within the range of mean values reported in the current experiment of 128 to 150 g/d (Table 3.3). The CH₄ estimates from the Greenfeed unit were higher (although not significant) than those from the respiration chambers, and significantly higher and more variable than

those from the SF₆ technique. It is important to note that only six cows were used in these evaluations. The decision to use only six cows in this experiment was due to logistical considerations given that this was an exploratory study to give an indication of the accuracy of the CH₄ estimates provided by the Greenfeed unit, and the need for similar intakes during all measurements.

The CH₄ yield measured by the respiration chambers and the SF₆ technique in the current experiment was comparable to reports by Vlaming *et al.* (2008) in which dry cows were also fed a maintenance diet of Lucerne silage (5.5 kg DM/d) and a yield of 22.8 g CH₄/kg DMI was reported using the SF₆ technique. There are limited reports of the CH₄ yield of dry cows measured by the respiration chambers. This is similar to those reported in the current experiment, with a yield of 20.5 g CH₄/kg DMI based on the SF₆ estimates. No comparable data are available for CH₄ production estimates obtained from Greenfeed unit in the literature. However, one study reported as an abstract (Utsami, unpublished), involved 61 lactating dairy cows in the United States fed pasture and concentrate (intakes unknown), and the Greenfeed estimates suggested CH₄ production was 418 g/d.

The CH₄ estimated from the Greenfeed unit may have been higher than those from the SF₆ technique and respiration chambers because of the sampling frequency used by each of the three techniques. The effect of sampling frequency becomes important when the circadian pattern of CH₄ production is considered. Typically, under controlled conditions such as those in this experiment, dairy cows spend approximately 3 hours feeding, 7 hours ruminating and 12 hours resting within a day (Krawczel & Grant, 2009). The CH₄ production and eructation reflects the fermentation activity in the rumen associated with the pattern of these behaviours and subsequent DMI. Therefore, the CH₄ production increases 0.5-1.0 h after feeding commences (Grainger *et al.*, 2007) and then continues during rumination and declines as the rumen content falls and is lowest during resting, matching the circadian feeding pattern (Lockyer & Champion, 2001).

The cows in this experiment were fed at maintenance twice daily at 08.00 h and 15.45 h and meals were generally consumed within 40 minutes, resulting in short periods of high CH₄ production after feeding and prolonged times with low CH₄ production throughout the night when cows were resting. The CH₄ production from the respiration chambers and the SF₆ technique were expected to be similar, as the measurements were almost continuous and were estimated during feeding, ruminating and resting for both techniques. However, the Greenfeed unit estimated CH₄ production approximately five times a day, only when cows visited the unit, and were actively feeding, so more variable CH₄ estimates could be expected. This intermittent sampling frequency of the Greenfeed unit means its ability to detect circadian changes in CH₄ production caused by feeding, depends largely on the time of day in which they visit the Greenfeed unit. The CH₄ production as estimated by the Greenfeed unit may have been higher due to the cows only visiting the Greenfeed unit when their CH₄ production was high, particularly soon after the cessation of feeding (Appendix C), so it may not be representative of the whole day. It is important to note that the cows were tethered for 40 minutes during feeding and it is 0.5-1.0 h after feeding that CH₄ production increases (Grainger et al., 2007). Despite this, it was unlikely that the cows would have left their feed and since only one cow could access the Greenfeed unit at a time, these periods of tethering are unlikely to affect the results.

The CH₄ production obtained from the Greenfeed unit may need to be adjusted to take into account the effect of sampling frequency and patterns of when animals visit the Greenfeed unit, in relation to feeding. It is important to note though that the Greenfeed unit was able to detect markedly different values of CH₄ over the course of the day. Further work may be required to evaluate the units in a large farm-scale, controlled experiment to determine what the best sampling pattern is.

Johnson *et al.* (1994) indicated that although the CH₄ measurements from the respiration chambers and the SF₆ technique were not significantly different, the CH₄ production measured by the SF₆ technique was lower. Similarly, in the current experiment, the CH₄ production from the SF₆ technique was 4.7% lower than from the respiration chambers, but this difference was not statistically significant. This

difference was similar to the 5-7% range suggested by Ulyatt *et al.* (1999) and Johnson *et al.* (1994) and part of the difference may be due to losses in the flatus, of about 1-3% (Murray *et al.*, 1976, Kempton *et al.*, 1976) of total emissions.

Literature reports of correlations between CH₄ measurements from respiration chambers and the SF₆ technique are not consistent, ranging from 0.14 (Pinares-Patiño *et al.*, 2011) to 0.83 (Muñoz *et al.*, 2012), 0.87 (McCourt *et al.*, 2008) and 0.93 (Pinares-Patiño *et al.*, 2008) in studies with sheep and cows. The correlation coefficient for the CH₄ measurements obtained from the respiration chambers and the SF₆ technique in the current experiment was 0.13, which is similar to that reported for sheep by Pinares-Patiño *et al.* (2011) and while still indicating a positive relationship between the two techniques, is considerably lower than the majority of reports. However, it is important to note that the current experiment used only six cows, compared to the 10 and 24 sheep (Pinares-Patiño *et al.*, 2008, Pinares-Patiño *et al.*, 2011), 18 and 20 dairy cattle (McCourt *et al.*, 2008, Muñoz *et al.*, 2012). Relationships determined in the current experiment may have been limited by the number of animals.

The concordance correlation coefficient between the respiration chambers and the SF₆ technique was 0.11, which although positive is still considerably lower than 0.79 which was reported by McGinn *et al.* (2006) for a similar comparison using eight beef heifers. Both the correlation and concordance coefficients (based on six cows) emphasises the much weaker relationship between the CH₄ measurements by the respiration chambers and the SF₆ technique compared to some literature reports.

The negative relationship between the CH₄ measurements from the respiration chambers and the Greenfeed unit is difficult to explain and contrary to expectations. In a more detailed evaluation of the Greenfeed system, Pinares-Patiño and Waghorn (unpublished) compared CH₄ production from the Greenfeed unit and respiration chambers (evaluated in the current experiment) in a report to the New Zealand Agricultural Greenhouse Gas Research Centre. Although some of the content of this report is beyond the scope of the requirements for this thesis, it is certainly of interest to mention that when the CH₄ production estimates from the Greenfeed unit and

respiration chambers were compared on an hourly basis, a very strong, positive relationship was obtained ($r = 0.91$). In the analyses of cow means, a similar relationship was expected, and the results presented here (Figure 3.3) may be due to the circadian variation in CH_4 production rates, due in part to the low intakes and short intense eating periods.

It is important to note how the emissions data may have been biased by cows visiting the unit, particularly as CH_4 production rates were so variable. There was a 5-fold range in CH_4 production rates within a day (Pinares-Patiño and Waghorn (unpublished)). The strong, positive relationship ($r = 0.91$) between techniques when estimates were compared on an hourly basis indicates the need for regular sampling in order to obtain accurate CH_4 estimates. The time period, number of measurement days and number of estimates included in the analysis differed for each of the techniques, but it is unlikely that time period would have contributed to differences in the CH_4 measurements between the techniques, because feed intakes were maintained at similar levels across the entire experimental period. However, the number of CH_4 estimates differed 2-fold among the cows (Table 3.2, Appendix C) and measurements over more days may have improved the accuracy of the Greenfeed estimates.

3.5 CONCLUSIONS

In summary, this experiment showed that the CH_4 estimates from the Greenfeed unit were higher and more variable than those from the respiration chambers and the SF_6 technique. However, the CH_4 production estimated from the Greenfeed unit was within the expected range considering the animal and dietary factors used in this experiment. The CH_4 yield of 24 g CH_4 /kg DMI provides confidence in the ability of the Greenfeed unit to determine the CH_4 production from a group of cows.

The weak relationship between the respiration chamber measurements compared to the Greenfeed unit and the SF_6 technique was disappointing, and the negative relationship between the Greenfeed unit and the respiratory chambers was

unexpected. However, only about five estimates per day were made from the Greenfeed unit, compared to continuous sampling from the other techniques. The strong relationship between the hourly CH₄ estimates from the Greenfeed unit and the respiration chambers reported by Pinares-Patiño and Waghorn (unpublished) indicates that it may be adequate for determining circadian changes in CH₄ production but is sensitive to the timing of visiting. In addition, there were only six cows used in this experiment, which may have affected the reliability of the CH₄ estimates obtained from the Greenfeed unit.

The use of the Greenfeed unit for estimating CH₄ from ruminant animals warrants further investigation using more animals, in a grazing environment, with differing feed levels representative of a typical farm system. Such studies will allow more CH₄ estimates to be obtained, providing more comprehensive evaluations of the Greenfeed system, and this could be undertaken simultaneously with the SF₆ technique.

CHAPTER FOUR

PRACTICAL APPLICATION OF THE GREENFEED UNIT ONTO PASTURE



CHAPTER FOUR: PRACTICAL APPLICATION OF THE GREENFEED UNIT ONTO PASTURE

4.1 INTRODUCTION

Measurement of methane (CH_4) emissions from animals, whether for inventory or mitigation, is best carried out in their “normal” environment. This is typically pastoral grazing in New Zealand, but in some Northern Hemisphere countries cows spend most of their time indoors. The CH_4 measurements should relate to emissions during normal animal management, because emissions may be changed by alterations to feeds, circadian patterns of feeding and effects of a new environment (for example, when in a respiration chamber), especially if intakes are changed.

The sulphur hexafluoride (SF_6) technique has been used to estimate CH_4 emissions from sheep, cattle and deer in New Zealand in both grazing and indoor environments (Swainson et al., 2008). These estimates have been used to determine emission factors ($\text{g CH}_4/\text{kg dry matter intake (DMI)}$) which are multiplied with the population and DMI to determine the enteric fermentation component of the National Greenhouse Gas Inventory (Ministry for the Environment, 2010). However, accurate measurements of CH_4 emissions are difficult unless animals are confined to respiration chambers, and CH_4 estimates obtained from animals using the SF_6 technique have been associated with greater variation between animals (and days) compared to respiration chambers (Grainger et al., 2007, Pinares-Patiño et al., 2008), although mean values can be similar (Hammond *et al.*, 2009).

Other disadvantages of the SF_6 technique include the additional human interference associated with changing the collection yokes daily, the costs of manufacture and calibration of permeation tubes that release the SF_6 gas and the expensive equipment required for measuring gases (gas chromatograph). Additional problems with the SF_6 tracer gas include an interaction between gas release rates and the estimate of CH_4

production (Vlaming et al., 2007) and under-estimation of CH₄ production due to losses in the flatus.

The Greenfeed system has recently been developed by C-Lock Inc. (South Dakota, United States, Patent 7966971) and can be mounted on a trailer and placed in a paddock to estimate CH₄ production from grazing cows. The development of the Greenfeed system has provided an alternative for estimating CH₄ production from grazing dairy cows and requires minimal human interference when the animals are being measured. This technique has potential advantages over the SF₆ technique because there is no equipment mounted on animals, so they can graze pasture without any interference from gas sampling halters and collection yokes, which better represents normal behaviour. There is no need to dose individuals with a permeation tube and the Greenfeed system does not require specialised (laboratory) equipment, so it may be less costly than the SF₆ technique.

The main limitation of the Greenfeed system include a lack of information about implementation on farms, little information about requirements for animals to be trained to use the unit, and that the pellets used as an attractant can contribute to the animals' diet (i.e. the diet is altered). Another concern is that intermittent visits to the unit will affect the quality of estimates (because CH₄ production rates vary 2-3 fold during a day (Murray et al., 2001)) and estimates of CH₄ production rely on algorithms used by the manufacturer to calculate emissions. There are no comparative data to provide confidence in the accuracy of the data from the Greenfeed system. Finally, the most effective way to use the Greenfeed unit in a rotational grazing system needs to be evaluated.

There do not appear to be any published information on the Greenfeed system, although Utsami (unpublished) have presented results from an indoor experiment undertaken in the United States. Before implementation of the Greenfeed system can occur in New Zealand evaluation in a typical farming system is required. Unknowns which require investigation for the on-farm implementation of the Greenfeed unit include interactions of the grazing cows with the Greenfeed unit, willingness of cows

to access the unit, and an assessment of the quality of the CH₄ estimates obtained from the Greenfeed unit, as well as its practical application.

The objectives of this experiment were therefore to;

- Assess the interactions of grazing dairy cows with the Greenfeed unit, including an assessment of the time taken for cows to access it, their visiting behaviour, and effects of group size and pellet (attractant) type
- Evaluate the CH₄ estimates obtained from the Greenfeed unit and investigate the on-farm implementation for its use in a New Zealand pasture-grazing system.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design

A grazing experiment involving two Greenfeed units was conducted at DairyNZ Lye Farm, Hamilton, New Zealand, during September and October 2011. The 38-day experiment used up to 44 Holstein x Friesian cows in early lactation. The cows were aged 2-4 years and were managed according to normal farm practice, as a single herd, and given a new break of pasture twice daily after each milking.

The experimental plan is summarised in Table 4.1 and over most (31 days) of the trial, 24 cows were used to evaluate the units, including four cows which had permanent rumen fistulae. A further 20 cows were added in the final week of the trial to investigate whether Group 1 cows would train Group 2 cows to use the Greenfeed units. Briefly, from day 1 to 7 cows became accustomed to the two Greenfeed units in the paddock. Although many of the cows accessed the units, others did not, and on day 8, training was carried out to encourage the non-user cows to use the Greenfeed units (Training period). Methane estimates continued from day 9 to 23 of the experiment and a single (grain based; Grain) pellet type was used as the attractant over this period. From day 24-31 attractant preference was evaluated to determine if

cows preferred a particular pellet type (Grain or Lucerne based; Lucerne) that was dispensed from the Greenfeed unit. The feed hopper was filled up on an as-required basis but was generally daily.

Behavioural observations were made and are presented in an ethogram (Table 4.5) for one hour after the morning milking on days 9, 10 and 11 of the trial following the training period and again for one hour after the morning milking on days 32, 33 and 34 following the addition of the cows. All procedures were conducted under Ruakura Animal Ethics Approval (Number 12409).

Table 4.1; *The experimental plan, showing treatment periods, numbers of cows, pellet type and principal measurements undertaken with the Greenfeed units.*

Day	Activity	Cow number	Pellet type ^a	Measurements taken (Between day 1 and 38)
1-7	Acclimatisation period: cows become accustomed to Greenfeed equipment	24		
8	Training period: cows with low usage encouraged to use the Greenfeed unit	8	Grain	Methane production (multiple times a day) Milk weight (daily) Milk composition (weekly) Live weight and BCS (fortnightly)
9-23	Continued estimates	24		Behavioural observations (days 9, 10, 11, 32, 33, 34)
24-31	Attractant x Greenfeed chute preference	24	Grain vs. Lucerne x Greenfeed unit	
32-38	Inclusion of additional cows	44	Lucerne	

^a Pellets with either a Grain or Lucerne base; components are given in the text. BCS, body condition score

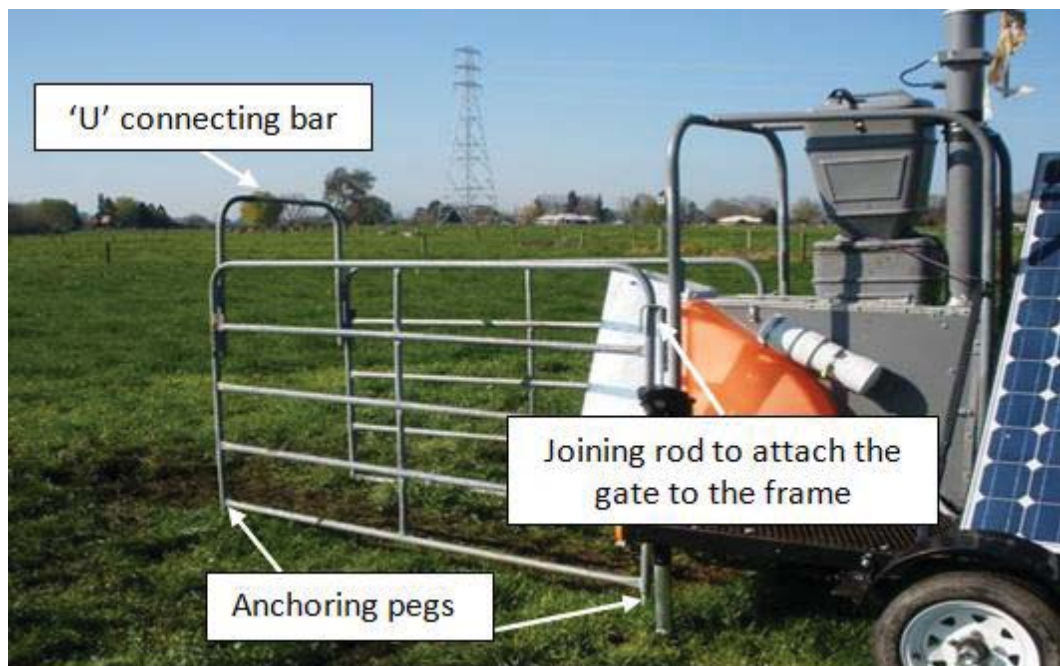
4.2.2 Greenfeed unit management

Each paddock was about 2 ha and provided sufficient feed for the 24 cows for two days managed according to standard commercial practice. Paddocks were divided into four using electric fences so that cows received a new break of feed after each milking. Breaks were allocated so the water trough was in the first break and was always available (breaks were not back-fenced). Two Greenfeed units were positioned approximately 15 m apart in the paddock, oriented to ensure the solar panels faced North to capture optimal sunlight for maintaining battery power of the units. The Greenfeed units were generally situated near the centre of the paddock (in the first allocated break) so that each time new pasture was allocated, the units were easily accessible.

The Greenfeed units themselves were identical but the entrance chute to each differed. To ensure only one cow could access the unit at a time, the operation of the units required chutes to be placed against the rear of the unit adjacent to the feeding station. Two types of chutes were constructed; a Sled chute (Photograph 4.1) that was towed behind the Greenfeed unit when it was moved and a Gate chute (Photograph 4.2) that was disassembled for moving. The Gate chute was lighter than the Sled chute and each gate had pegs that were forced into the ground to prevent movement, as well as a connecting bar to hold the rear of the gates in position. The Sled chute was also fitted with small gates near the feeding station to ensure there was no interference in the electronic identification (EID) from cows grazing outside the Greenfeed units but near the reader (Photograph 4.1).



Photograph 4.1: The Sled chute attached to Greenfeed unit with two gates placed near the feeding station and reader to avoid the ear tags of cows outside the unit being identified by the electronic identification reader.



Photograph 4.2: The Gate chute attached to Greenfeed unit.

When the cows were moved to a new paddock for grazing, the Greenfeed units were also moved. A four-wheel drive station wagon was used to tow the Greenfeed units and this was done while the cows were at the morning milking to avoid interference by the cows. Moving the Greenfeed units took approximately 30 minutes, and involved less time and work for the Sled chute compared to the Gate chute. On both of the Greenfeed units, the solar panels were lowered before transport to avoid damage, and the Greenfeed unit was put into 'sleep mode' when moved. 'Sleep mode' allows power consumption by the Greenfeed unit to be minimised if it is not being used for a short period of time but then immediately started back up when required.

Gas calibrations (using both nitrogen and a CH₄ and CO₂ gas mix) were carried out on the Greenfeed units when requested by C-Lock Inc. in order to counteract the "drifting" of sensors away from the baseline concentrations over time. This was carried out twice for each Greenfeed unit during the experiment.

4.2.3 Management during experimental periods

The cows were removed from the paddock at about 08:00 h and 15:00 h for milking and new pasture was offered after each milking. Greenfeed unit operation and estimates did not affect herd management, although the presence of the two units in the paddock did attract significant attention from some cows. No animals showed any apprehension toward the units, although some ignored them completely. The acclimatisation period (days 1-7) was no different to the operation throughout the trial with the 24 Group 1 cows allowed free access to the units. By monitoring the visiting behaviour of each cow via the web-based interface, those cows with low or limited usage could be determined, as well as those with high usage. During the acclimatisation period, some mechanical problems with the Greenfeed units became apparent and were remedied. These were mainly associated with cows chewing electrical wiring and disabling the powered legs, and water leakage into the feed hoppers. Cloudy weather sometimes reduced charging, so the fan (Photograph 2.3) was turned off (remotely) and only started when a cow entered a unit.

On day 8, the eight cows that had not yet visited the units or had very few (< 5) visits (up until day 8) were drafted into a small paddock to undergo training with one unit. The cows had little available pasture to encourage them to use the unit. Training involved placing a trail of Grain pellets leading towards the entrance of the unit and some pellets in the feeding station and then gently persuading the cows into the Greenfeed unit, without any physical contact. Following about three hours of training, all eight cows and the Greenfeed unit were returned to the herd grazing in the paddock.

From days 9 to 23, CH₄ estimates continued using Grain pellets as an attractant. An 8 day crossover trial was undertaken from days 24-31, where the two types of pellet were evaluated by dispensing Lucerne from the one unit and Grain from the other (days 24-27), and then the pellet types were dispensed from the other units from days 28-31. The Lucerne pellets were evaluated because it was considered undesirable to feed Grain to cattle being assessed for CH₄ emissions when grazing pasture.

On day 21, four fistulated cows had rumen sensing boluses (Kahne Limited, Auckland, New Zealand) inserted to measure pH and temperature within the rumen. Two versions of the same bolus were inserted into each cow at the manufacturer's request. The aim of using these boluses was to monitor and relate the rumen conditions to the CH₄ estimates and these results are presented in Appendix D.

On day 32, an additional 20 cows (Group 2) were added to the original 24 animals to determine if cows already familiar with the units would encourage the new group to visit the Greenfeed through social facilitation. During this time, both Greenfeed units dispensed the Lucerne pellets.

4.2.4 Feeds and feeding

During the experiment the cows grazed perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture, under normal commercial dairy farming practice in Spring. New pasture breaks were provided after morning (09:00 h) and afternoon (16:00 h) milkings, with pasture allowances that would allow intakes of 15-20 kg

DM/cow/day. Each week, immediately prior to morning grazing, 50 representative pasture 'grab' samples were taken. Their dry matter (DM) content was determined by drying at 65°C for 48 h, and composition was estimated by near-infrared reflectance spectroscopy (NIRS) analysis at the FeedTECH Laboratory, AgResearch, Grasslands Palmerston North.

As this was a grazing experiment, the intakes of the animals could not be measured accurately and this limited the potential for evaluation of Greenfeed prediction of CH₄ yields (g CH₄/kg DMI) by comparison with published values. However daily measurement of milk production, with weekly milk composition and fortnightly live weight measurements enabled intakes to be calculated on the basis of metabolisable energy (ME) requirements and the calculated ME content of the pasture. Intake calculations were based on the Australian Feeding Standards (CSIRO, 1990).

The Grain or Lucerne pellets dispensed by the Greenfeed units (Table 4.1) were compounded from a range of components, provided by the manufacturers (Cambridge Grains and Country Harvest) on an as-fed basis. The Grain pellets comprised distiller's grain (35%), palm kernel (25%), maize grain (15%), broil (15%), zeolite (5%) and lime (2.5%), and the Lucerne pellets comprised Lucerne (40%), broil (30%), premix (11.5%), copra (10%), barley (6%), hay (2.5%).

Following the acclimatisation and training periods, all cows that visited the units more than six times in a day had their feeding period interval restricted to 4-hour intervals so the maximum number of feeding periods per day was six. This was done electronically via the C-Lock Inc. website, so selected cows did not receive pellets if they visited the units within 4 hours after the last pellet drop. Five cows were restricted on day 10 and a further 5 on day 18 with another 4 restricted on day 14 and 2 on day 29. The two Greenfeed units were linked electronically so the restriction applied to both units. At each visit, a total of approximately 0.27 kg DM was dispensed by the Greenfeed unit, divided into three drops dispensed at about 45 second intervals. Therefore, the maximum intake of pellets was approximately 1.62 kg DM/cow/d, and there was no wastage.

4.2.5 Animal production measurements

Milk weights were recorded twice daily (at each milking) and milk composition was measured using a FT120 milk analyser from samples collected (pm/am) on days 7/8, 14/15 and 21/22. Live weights were measured after the morning milking fortnightly on days 3, 17, 31 of the trial, using manual weigh scales (TruTest, Hamilton). Data for milk yield and milk composition were recorded for the Group 2 cows that were added to the experiment, with live weight and body condition score (BCS) immediately before they entered the trial on day 32.

Based on the change in CH₄ concentrations (relative to ambient levels) due to eructation and exhalation, air flow rates and the application of ideal gas laws, the CH₄ production (g/d) for each cow was determined (refer to Equation 3.1) (C-Lock Inc, unpublished). Only CH₄ estimates from cows that kept their head in the Greenfeed unit for at least three minutes were included in the calculations. Methane estimates that lasted less than three minutes, or which were made immediately after the Greenfeed unit was re-started. These data were sent electronically to C-Lock Inc. (South Dakota, United States) where it was processed, and results were then provided to the user for analysis.

4.2.6 Analyses

Data presented in the Results (Section 4.3) are usually means with standard deviation. All analyses of CH₄ data were based on values provided by C-Lock Inc. under standard protocols and we had no control over the algorithms used for interpretation of CH₄ concentrations in respired breath. The majority of measurements undertaken in the trial were of a descriptive nature and not subjected to statistical analysis, with the exception of the pellet type and Greenfeed unit preference comparisons. These data were analysed using mixed models fitted by Restricted Maximum Likelihood (REML), with period (day 24-27 and 28-31), pellet type and Greenfeed unit as fixed effects and cow and period within cow as random effects. GenStat software, version 14.1 (Payne

et al., 2011) was used for statistical analysis. The level of significance was ≤ 0.05 for all analyses.

All data analysis was preceded by scrutiny of values obtained from the Greenfeed units. Not all cows accessed the units, even after training, and this limited potentially useful data to 14 of the 20 intact cows in Group 1 and three of the four fistulated animals. The Group 2 cows were added to examine the effectiveness of the Group 1 cows for training Group 2 cows that were new to the system, but there was limited opportunity to analyse these data because of low usage of the units by Group 2 cows.

Two criteria were used to accept or reject values used to calculate means for CH₄ production and other calculations:

1. Visits by the cows
2. The values obtained for CH₄ production during visits

The number of visits was an important measure in the trial, and only the data from cows that visited on their own free will (not during training) were used. Data from Group 1 cows averaging less than two visits per day giving useful data or from Group 2 cows with less than 10 visits in seven days were not used in evaluations of CH₄ production, because these were considered insufficient to provide useful values. The choice of number of estimates considered 'sufficient' was based on a need for 'routine' access to the unit, but allowance was made for cows that took time to begin using the Greenfeed unit, in Group 2. The typical pattern of usage was that once a cow accessed the unit and consumed pellets it either returned frequently or not at all for several days. In other words, cows were either "users" or "not users". These behaviours simplified selection of data, because once cows accessed the units they continued to do so.

The second criterion became important when data from the rumen fistulated animals were examined. These were not included in the evaluation of data from intact cows, but are covered separately in Appendix D. Only three of the four fistulates accessed the units and analyses are confined to those animals.

4.3 RESULTS

4.3.1 General animal and feed measurements

The estimates with the Greenfeed units were carried out with cows aged 2, 3 and 4-years in early lactation. Animals in Group 1 weighed 432 ± 45 kg, with a BCS of 4.0 and production was 21.6 kg of milk per cow per day during the 38-day experiment (Table 4.2). Group 2 cows weighed 427 ± 45 kg, with a BCS of 3.7 and produced 21.2 kg of milk per cow per day during the seven days that they were in this experiment.

Table 4.2: Mean milk yield, live weight and BCS of the Group 1 cows throughout the 38-day experiment, and for Group 2 cows during the final 7 days of the trial.

Age (years)	Group 1 cows			Group 2 cows		
	2	3	4	2	3	4
N	6	9	9	10	5	5
Milk yield (kg/d)	20.0	22.2	22.0	20.5	20.3	22.8
Live weight (kg)	385	438	458	398	441	471
BCS	4.2	4.1	3.9	4.0	3.5	3.5

N, number of cows; BCS, body condition score

The grazed pasture contained on average 24.2% crude protein while the neutral detergent fibre (NDF) content increased from 37.5 to 42.7% of the DM over the duration of the experiment (Table 4.3).

Table 4.3: Dry matter (DM) concentration and chemical composition (g/100g DM) of weekly pasture plucks taken approximately one day prior to grazing, during the measurement period.

Day	DM	SSS	Crude protein	NDF	ADF	Lipid	Ash
8	15.1	10.2	26.9	37.5	20.9	4.0	11.2
16	14.2	12.7	25.5	34.6	19.8	4.1	10.6
22	15.6	13.0	22.2	41.0	21.9	4.0	10.3
28	17.4	15.3	22.2	40.4	22.6	3.8	10.4
38	15.6	13.2	24.4	42.7	22.8	3.5	10.4
Mean	15.6	12.9	24.2	39.2	21.6	3.9	10.6

DM, dry matter; SSS, soluble sugars and starch; NDF, neutral detergent fibre; ADF, acid detergent fibre

The assessment of the Greenfeed unit was based on two main aspects; (1) the cow visiting behaviour and (2) the estimates of CH₄ production. Other assessments included the likely accuracy of the CH₄ estimates, value of data from cows with rumen fistulae and the suitability of the Greenfeed unit for on-farm use.

4.3.2 Visiting behaviour

One of the criteria for evaluating the Greenfeed units was the incidence of cow visits, based on the number of cows visiting the units, mean number of visits per cow per day and the willingness of non-users to become users. Assessment was made in terms of total visits and visits when useful CH₄ data were obtained. The data are presented for individual cows over the entire measurement period (Table 4.4) and total number of visits for the 24 cows in Group 1 over the 38-day experiment and for the 20 cows in Group 2 over seven days (Figure 4.1). Of the 20 intact (non-fistulated) cows in Group 1, only 14 provided defensible estimates of CH₄ emissions (visiting and providing useful data at least twice per day) and only three of the four fistulated cows provided CH₄ data, although the values were not sensible. Within Group 2, only four of the 20 cows visited adequately (more than 9 visits) to provide CH₄ estimates. The difference in criteria used for the two groups was because the second group of cows were introduced to evaluate learning to access the units, rather than estimates of CH₄ emissions. Methane data from Group 2 cows could not be considered a reliable estimate of emissions.

Table 4.4: Number of visits to the Greenfeed units by all cows, and number and percentage of visits that provided useful CH₄ estimates and those that did not

	Number of animals	Number of total visits	Number of useful visits giving CH ₄ data	Percentage of visits giving useful CH ₄ data	Number of animals that did not provide useful CH ₄ data
Group 1					
2 years	6	737	595	81	1
3 years	9	767	560	73	4
4 years	9	1149	885	77	2
Group 2					
2 years	10	73	40	55	7
3 years	5	12	0	0	5
4 years	5	42	26	62	4

Table 4.5 illustrates the frequency of different behaviours that were displayed during the two observation periods (following the ‘humans training cows’ and ‘cows training cows’) regimes. The most commonly observed behaviours were the movement of cows into the Greenfeed unit with no interaction between the cows. The least frequent observed behaviour was that in which two cows interacted and some form of aggression was used to physically displace the other cow.

Table 4.5: Ethogram of the frequency of behaviours displayed during observation periods following the two training regimes.

Situation	Humans training cows				Cows training cows			
	9	10	11	32	33	34		
Trial day	4	4	4	10	2	11		
Both units unoccupied, cow enters A								
Both units unoccupied, cow enters B		6	6	4	7	5		
A occupied, cow enters B		5	3	7	6	4		
A occupied, cow enters A		5	4	4	2	3		
A occupied, another cow enters, bunts her out & then uses, B unoccupied whole time				1		1		
B occupied, another cow enters, bunts her out & then uses, A unoccupied whole time		1	1	2				
A occupied, another cow enters, bunts her out & then uses, B occupied whole time								
B occupied, another cow enters, bunts her out & then uses, A occupied whole time		2		1	1			
Cow enters A then B (these cows are likely to be restricted, but are still seeking pellets)		1			2			
Cow enters B then A (these cows are likely to be restricted, but are still seeking pellets)	1		2					
Cow enters A, another cow follows and is frustrated waiting so returns to pasture	1	1	2	2	1	1		
Cow enters B, another cow follows and is frustrated waiting so returns to pasture	1							
Cow is using A, another cow observes this and moves to B								
Cow is using B, another cow observes this and moves to A								
A occupied, another cow approaches and waits for its turn in A	1	1	1	3		1		
B occupied, another cow approaches and waits for its turn in B		1	1					

A = Greenfeed unit with the Sled chute attached, B = Greenfeed unit with the Gate chute attached

Number of cows visiting and cow training

Cow visits/day is summarised in Figure 4.1 for each group. Up until day 14, there was a large variation in the number of cows visiting, ranging from 11 to 18 per day, with the latter occurring on the training day. From day 14 through until day 38 the number of cows that visited appears to stabilise at around 18 cows per day, but some visits did not provide useful CH₄ data. The addition of the Group 2 cows on day 32 caused the number of Group 1 cows that visited the units to increase to 21 per day and from day 32-38 the mean number of cows visiting per day was 18.7. The number of Group 2 cows that visited was extremely variable over the 7-day period, ranging from 3 to 11, while the number of visits per day ranged from 12 to 29 for this group.

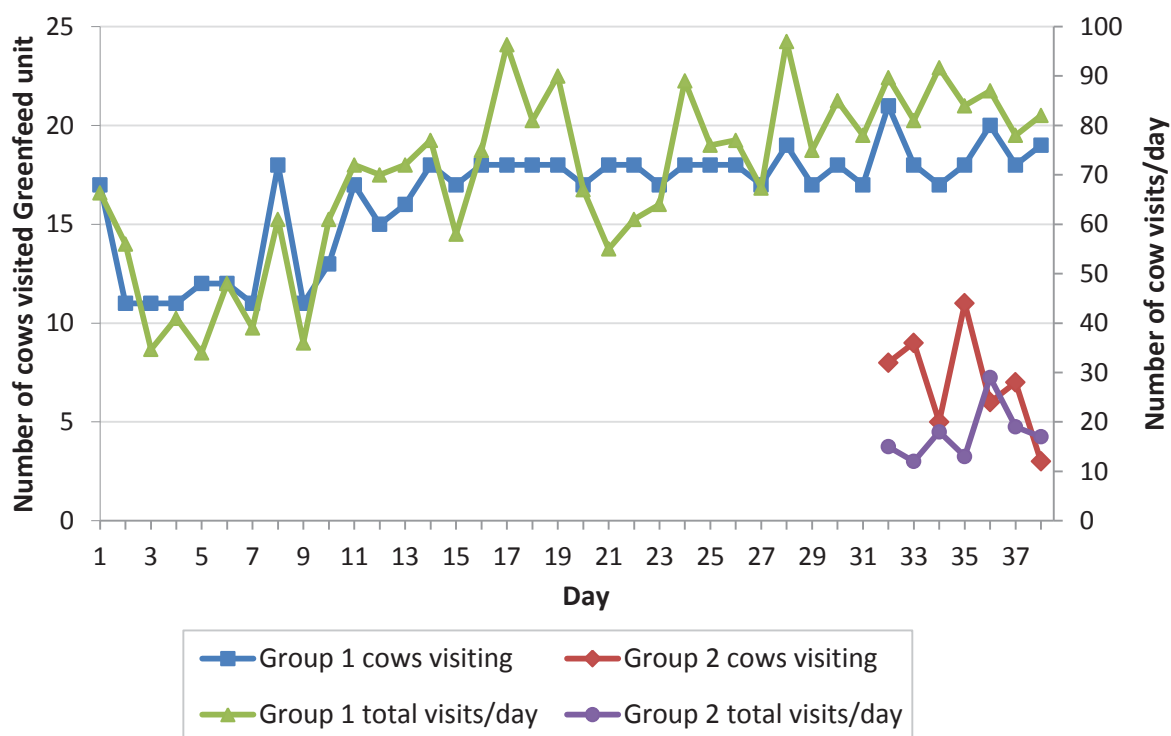


Figure 4.1: Number of cows using the Greenfeed units per day, and the number of cow visits per day. Data includes the Group 1 cows (from day 1-38) and Group 2 cows (from day 32-38).

Allocation of cows to the experiment resulted in three age groups; 2-year old (heifers), 3-year old and 4-year old (mature) animals. This allocation was due in part to suggestions from farm staff that young cows may be more inquisitive than mature cows, so number of visits per cow between age groups and are presented in Table 4.6. There was a lower incidence of visiting throughout the experiment by 3-year old cows compared to either heifers or mature individuals. However, these data must be interpreted with care as individual cows with high usage (>6 visits/day) had their access to pellets restricted on days 10, 14, 18 and 29 to prevent excessive intake of pellets. The restrictions have constrained mean visits per day to 6 or less (Table 4.6).

Table 4.6: Number (mean \pm SD) of visits per cow per day providing useful CH₄ data for the 2, 3 and 4-year old cows from Group 1. There were 6, 9 and 9 cows in the respective age groups. Visits applied to both Greenfeed units.

Age (years)	2		3		4	
	Mean \pm SD		Mean \pm SD		Mean \pm SD	
1-10	1.4	0.4	1.7	0.4	2.6	0.9
11-20	3.9	0.6	2.3	0.6	3.6	0.6
21-30	3.8	0.8	2.3	0.4	3.5	0.7
31-38	4.0	0.5	2.8	0.2	3.9	0.4
All days	3.2	1.2	2.2	0.6	3.4	0.8

Before the training on day 8, two cows never visited the Greenfeed unit and 10 cows visited less than five times in total over the 7-day period, despite frequent use by other cows. Of the eight cows that were trained, only five willingly entered the chutes to the Greenfeed unit and three of these maintained their usage in subsequent days (Table 4.7), providing useful CH₄ data. Of those three cows that were reluctant to use the Greenfeed unit during the training period, two did not use it for the rest of the experiment.

There was an increase in the mean number of visits per cow per day from day 9 to 38 (Table 4.7), even with restrictions imposed on some cows. For those five cows that visited on the training day, CH₄ estimates were obtained from three cows from day 9 to 38, averaging 2.4 visits/day (Table 4.7).

Table 4.7: *Visits by the eight cows introduced to the Greenfeed unit on the training day.*

	N	Mean visits/cow/day		Cows with useful CH ₄ data	
		Before training	After training	Before training [#]	After training
Entered the Greenfeed chute during training day	5	0.2	2.4	0	3
Did not enter Greenfeed chute during training day	3	0.4	1.4	0	1

[#]*Occasional visits before training were insufficient to provide useful data*

Table 4.8 summarises cow use over time, and illustrates the effect of ‘cows training cows’ on the mean number of visits per cow per day, and the number of cows with useful CH₄ data. There was an increase in the mean number of visits per cow per day up until day 32 when the Group 2 cows were added. The addition of the Group 2 cows had little effect on the mean visits per cow per day or the number of cows with useful CH₄ data from Group 1. However, a total of four the 20 Group 2 cows provided useful CH₄ data.

Table 4.8: *Mean number of visits per cow per day, number of cows visiting and number of cows with useful CH₄ data during the experimental periods. Day 1 to 31 includes only Group 1 cows whereas day 32 to 38 include both Group 1 and 2 cows.*

Day	Group	n	Mean visits/cow/day	Mean number of cows visiting	Mean number of cows with useful CH ₄ data
1-7	1	24	1.9	12.1	5.6
9-23	1	24	2.9	16.6	12.3
24-31	1	24	3.4	17.8	13.9
32-38	1	24	3.5	18.7	14.0
32-38	2	20	2.6	7	3.0

Visits per cow

The number of visits per day (Figure 4.1) tended to increase over time, and this was due both to an increasing number of cows using the Greenfeed unit and to a high frequency of use by some cows. Excessive use resulted in the pellets forming a substantial proportion of the animals' diets, so access to the Greenfeed units was restricted (Section 4.2.4). Table 4.9 shows cows that provided useful CH₄ estimates, and the days from when a restriction to the Greenfeed units was imposed. In Group 1, all except three cows that gave useful CH₄ estimates were restricted by day 18 of the experiment, but there were seven cows that had very few visits (17 or less over the 38 day measurement period; Table 4.4). No Group 2 cows had their access restricted.

Table 4.9: The cows, grouped by age, that were used for evaluation of the Greenfeed units, with animals that provided useful CH₄ estimates in bold, and the day from which access to the pellets was restricted to 6 visits/day indicated by the subscript.

Group 1 cows			Group 2 cows		
Age (years)					
2	3	4	2	3	4
2013 ₁₄	2085	2033	2112	2124	2048
2080	2122	2043 ₁₀	2142	2130	2131
2152 ₁₈	2195 ₁₀	2176 ₁₀	2246	2160	2156
2395 ₁₈	2328 ₁₀	2193 ₁₈	2341	2375	2264
2473 ₂₉	3716 ₁₄	2247 ₂₉	2342	3713	3661
4749	3719 ₁₈	2330 ₁₀	2357		
	3812 ₁₈	2424 ₁₄	3786		
	3694	3720 ₁₄	4703		
	4657	3730	4731		

The mean number of visits per cow per day to the Greenfeed units is illustrated in Figure 4.2, for the restricted, unrestricted and all cows. The data are combined for both units, and the values for all cows up to day 8 were fairly stable, averaging 2-3 visits per cow per day, after which there was an increase in visits, stabilising at 4-5 visits per cow per day. The first group of five cows were restricted on day 10, after which there was a gradual increase in numbers of visits, so a further group of four cows were restricted on day 14, then another group of five on day 18, and a further two on day 29. All but one of the frequent users were restricted by day 29, after which use stabilised at about five visits per cow per day.

It was apparent that some cows did not access the Greenfeed units, and others infrequently. Figure 4.3 illustrates the number of visits per cow per day to the Greenfeed units, averaged for groups restricted at either 10, 14 or 18 days. The mean visits after restriction was about 5 per day, even though 6 visits were theoretically possible.

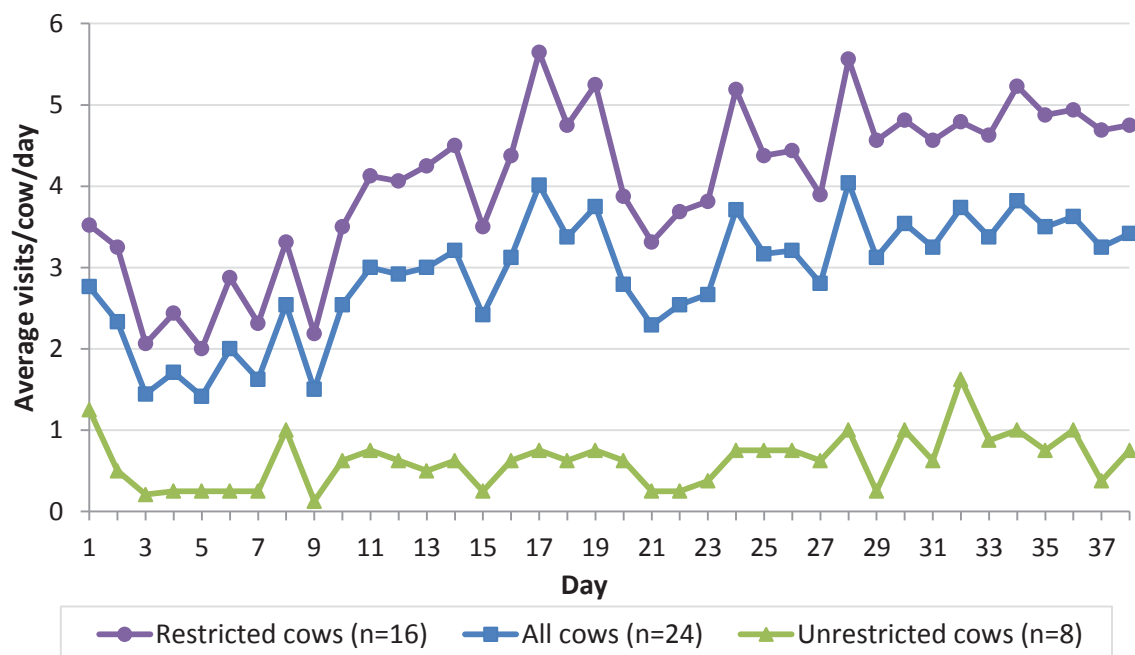


Figure 4.2: The mean number of visits per day to the Greenfeed units for Group 1 cows over the 38-day experiment. Data are means for all cows and those either restricted or unrestricted.

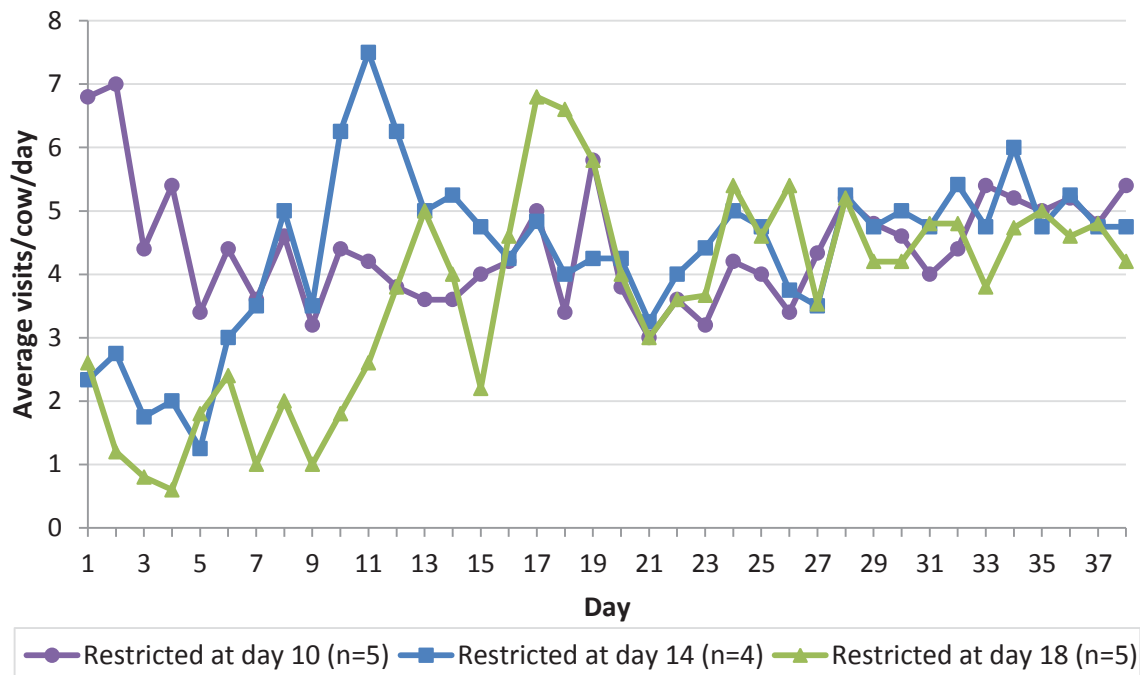


Figure 4.3: The mean number of visits per day to the Greenfeed units for Group 1 cows over the 38-day experimental period for animals that were restricted from day 10, 14 and 18, respectively.

An important aspect of these estimates that could affect the quality or robustness of the data was the circadian pattern of visits. The rate of CH₄ production from fermentation varied by up to 5 times a day (Pinares-Patiño and Waghorn, unpublished) when cows had low intakes, and measured using respiration chambers over a 24 hour period. Circadian variation in CH₄ production, in association with intermittent access to the Greenfeed units, may affect estimates of CH₄ per day, and the pattern of visits per cow is presented in Figure 4.4. When averaged over the whole experiment for Group 1 cows a similar number of visits per hour were evident over 24 h at the commencement of the trial (days 1-8), but frequency of visits in the early hours of the morning (03:00-04:00 h), around noon and at 20:00 h declined toward the end of the trial.

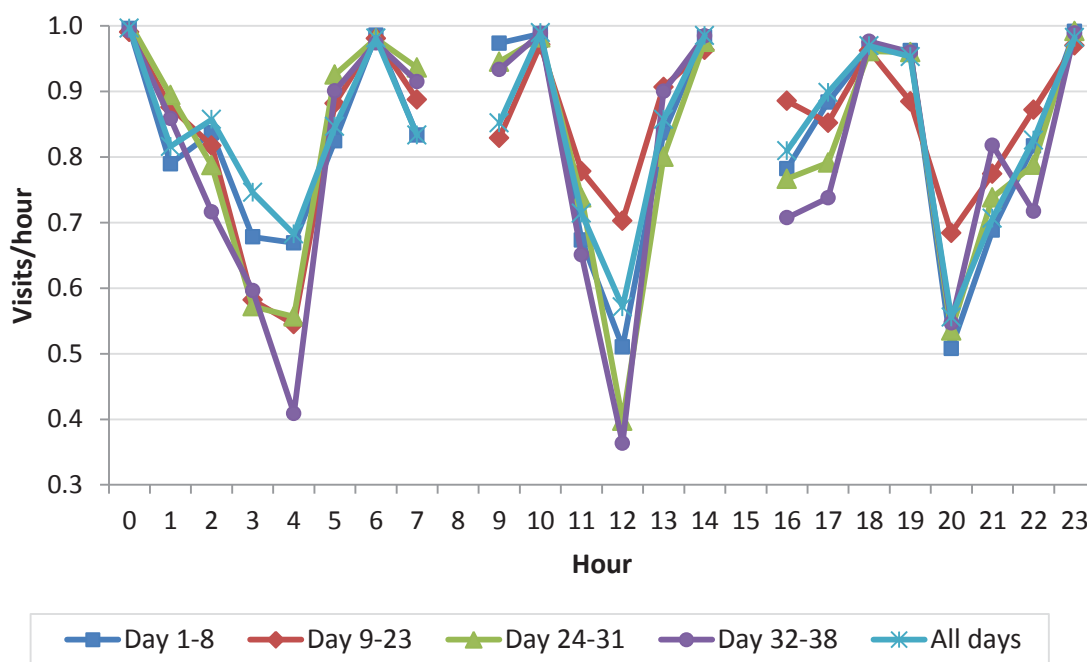


Figure 4.4: Number of visits/hour for Group 1 cows averaged over 38 days and for four periods during the experiment. Data are omitted for 08:00 h and 15:00 h when cows were milked. Zero on the x-axis represents midnight, while 12 represents midday.

Attractant preference

The number of visits to each Greenfeed unit, with the two pellet types during the 8-day crossover period is presented in Figure 4.5. There were more visits per cow in the second period (day 28-31) than the first period (day 24-27) (4.91 and 4.08 respectively; $P=0.032$) and there was a significant preference for the Greenfeed unit with the Sled chute over the unit with the Gate chute (2.64 vs. 1.85 visits per cow per day, $P<0.001$; Figure 4.5) during the 8-day crossover period at a 6:4 ratio. Cows had a significant preference for the Lucerne pellets over Grain (2.60 vs. 1.89 visits per cow per day, $P<0.05$; Figure 4.6) during the 8-day crossover period at a 6:4 ratio.

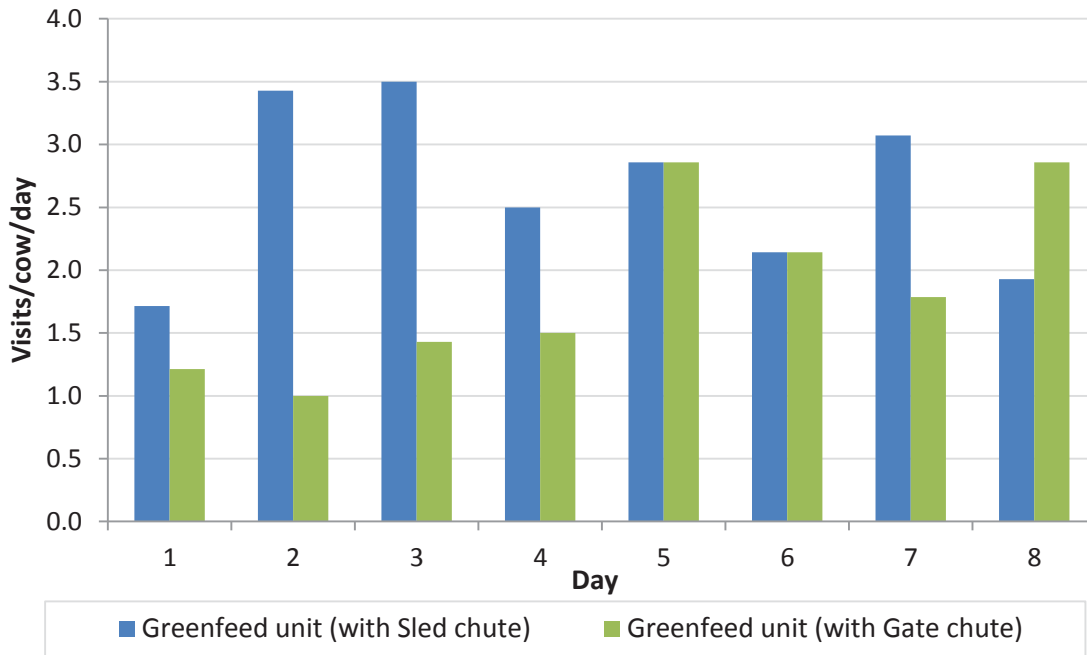


Figure 4.5: Number of visits/cow/day to both Greenfeed units over days 24-31.

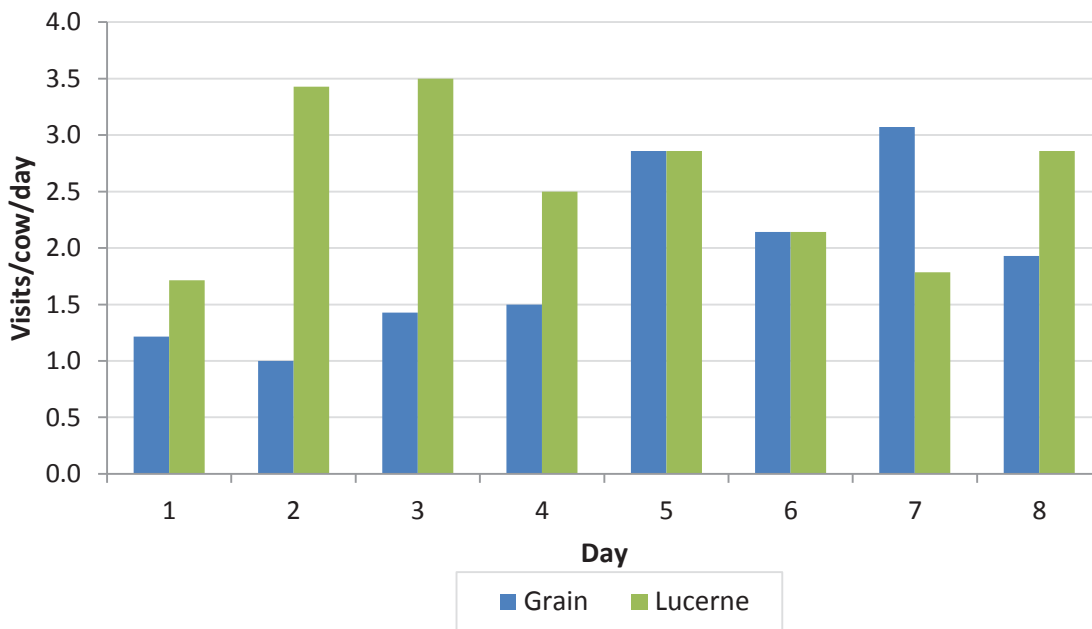


Figure 4.6: Number of visits/cow/day for both Grain and Lucerne pellets over days 24-31.

Estimates of CH₄ production were higher (P=0.043) in the second period (356.1 g/; day 24-27) compared to the first period (330.0 g/d; day 28-31) and there was a tendency (P=0.070) for higher CH₄ production to be recorded from the Greenfeed unit with the Sled than with the Gate (353.6 vs. 335.6 g/cow/day; P = 0.057). Methane production from cows accessing units with Lucerne pellets was similar to those assessing Grain pellets (352.3 vs. 336.8 g/cow/day; P=0.098)

4.3.3 Methane production

Methane production can be expressed as daily values averaged for all cows in the herd over the duration of the trial (Table 4.10) and as values for individual cows during defined measurement periods (Appendix E). The CH₄ estimates from the Greenfeed system may be affected by the number of cows visiting (Figure 4.1), frequency of visits per cow (Figure 4.2 and 4.3), the circadian pattern of visits (Figure 4.4) and the value derived from visits where useful estimates were obtained (Table 4.12 and 4.13). All of these variables will affect the accuracy of CH₄ estimates, but it was not possible to validate the CH₄ data obtained in this experiment because no other CH₄ estimates were made. The need for both accuracy and minimising the time required to obtain reliable estimates (ie. days of access to the Greenfeed units) has resulted in data presentation for individual cows (with standard deviations and coefficient of variation) in 10-day increments and cumulatively over the measurement period (Appendix E). These tables indicate if the accuracy of the CH₄ data improves as the estimates progress, or over the duration of the experiment.

Mean CH₄ production (Table 4.10) was preceded by an assessment of data from fistulated cows, and their emissions were very low (Table 4.11), so their data were excluded from the analyses to obtain means (Tables 4.10). Mean CH₄ production from intact cows ranged from 278.5 to 438.0 g/d (Table 4.10; Figure 4.7).

Table 4.10: Total number of cows visiting, number of cows providing useful data, mean number of visits/cow/day and calculated CH₄ production \pm SD for Group 1 cows over the 38-day experimental period. Total number of cows visiting, number of cows providing useful data and mean visits/cow/day (excluding fistulates) is based on those cows with 2 or more visits/day whereas CH₄ production \pm SD is based on up to 14 cows depending on those which provided useful CH₄ data (fistulates and those cows without useful data are excluded).

Day	Total cows visiting	Cows providing useful data	Mean visits/cow/day	Mean CH ₄ production (g/d)	SD
1	17	2	4.5	311.5	8.9
2	11	5	6.5	342.0	100.8
3	11	4	4.0	438.5	80.0
4	11	5	4.3	372.3	80.8
5	12	8	4.1	306.9	93.2
6	12	8	4.6	304.0	70.0
7	11	7	4.1	340.1	70.0
8	18	6	4.9	338.1	47.7
9	11	8	3.8	322.9	54.6
10	13	9	5.4	345.6	87.3
11	17	13	5.2	316.1	61.0
12	15	11	4.9	315.7	62.8
13	16	12	5.0	312.2	60.2
14	18	12	4.9	295.3	49.8
15	17	12	4.2	340.6	78.3
16	18	13	4.6	307.6	57.0
17	18	14	5.4	347.2	73.3
18	18	14	4.7	309.0	76.9
19	18	14	5.2	374.4	57.6
20	17	13	4.1	341.6	58.0
21	18	13	3.3	278.5	59.4
22	18	12	3.7	338.0	64.2
23	17	14	3.8	317.4	51.8
24	18	14	5.2	324.5	37.8
25	18	14	4.4	336.7	73.1
26	18	14	4.5	359.8	45.3
27	17	14	4.0	356.6	59.4
28	19	14	5.6	385.8	53.7
29	17	14	4.4	338.7	69.8
30	18	14	4.9	343.8	37.8
31	17	13	4.8	348.8	37.8
32	21	14	4.6	362.0	65.2
33	18	14	4.7	367.8	57.3
34	17	14	5.4	362.2	53.5
35	18	14	4.9	370.0	58.5
36	20	14	4.7	358.3	57.6
37	18	14	4.5	353.8	70.9
38	19	14	4.7	346.1	66.7
Mean	16.4	11.5	4.6	340.3	61.8

Table 4.11: Mean daily CH₄ production (g/d), SD and CV over the 38-day experiment. Data are based on the 17 cows (including 3 fistulates (in grey shading)) with two or more visits/day that provided CH₄ production data. Table includes Group 1 cows only.

Cow	CH ₄ production (g/d)	SD	CV	Number of visits/cow/day
2043	121.6	35.0	28.7	4.4
2176	102.2	32.0	31.3	4.7
2424	111.8	21.2	19.0	4.6
2013	298.0	37.5	12.6	5.4
2152	289.9	42.2	14.5	3.2
2193	361.4	60.2	16.7	5.3
2195	359.3	44.1	12.3	4.4
2247	330.2	58.9	17.8	2.9
2328	312.3	47.6	15.2	4.8
2330	443.7	48.8	11.0	3.8
2395	318.8	36.9	11.6	3.4
2473	332.6	48.5	14.6	3.8
3716	324.5	49.9	15.4	3.9
3719	396.2	58.6	14.8	3.1
3720	387.6	50.2	12.9	3.6
3812	248.7	37.5	15.1	3.5
4749	338.5	48.6	14.4	3.5

NA; insufficient data was obtained from Group 2 cows to determine feed intake and calculated CH₄ production; CV, coefficient of variation

The CH₄ production has been plotted, along with milk production (Figure 4.7), over the measurement period. There was a gradual increase in CH₄ production over time, but milk production declined from approximately 25 to 21 kg/d over the 38-day measurement period. On day 34, pasture allowance was less than adequate, and although this was not reflected in the CH₄ production, milk yield was markedly reduced. The sharp increase in CH₄ emissions on day 3 was due to unusually high emissions from Cow 2330 (524.7 g) and estimates being obtained from only 4 cows.

Figure 4.8 depicts the circadian variation in CH₄, based on data from the 38-day measurement period. The CH₄ production was lowest between midnight and 07:00 h, then increased until 11:00 h, and remained stable until 20:00 h after which it gradually decreased.

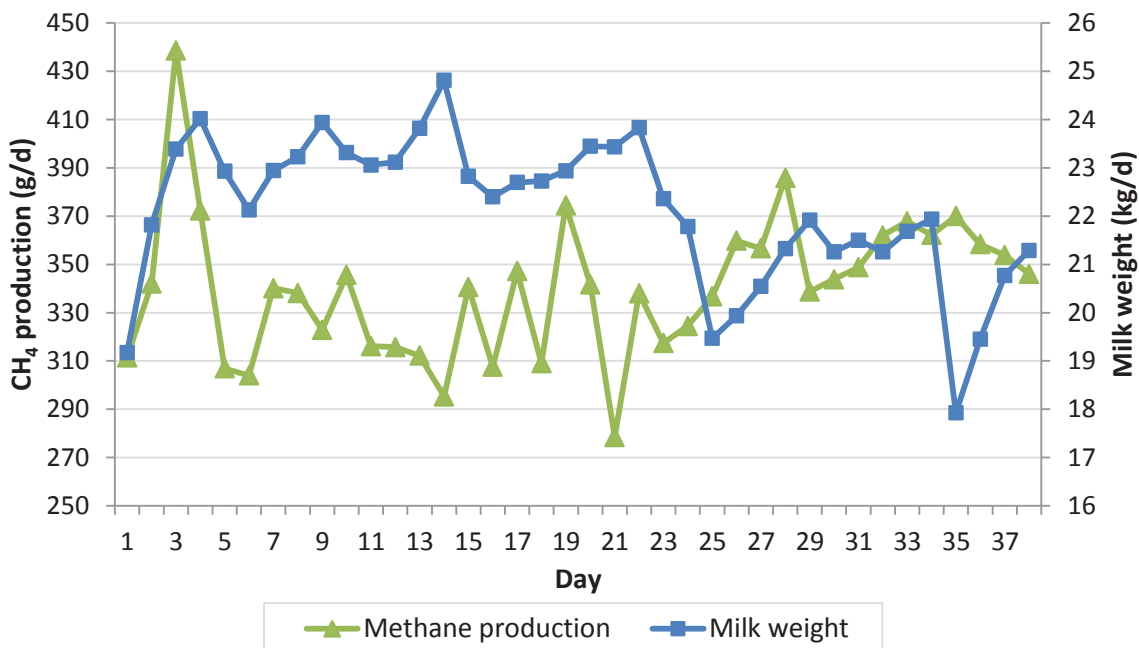


Figure 4.7: Methane production ($g\ CH_4/d$) and milk weight (kg/d) of the Group 1 cows (excluding the 3 fistulates) over the 38-day experiment. On day 34 there was an unplanned shortage of pasture.

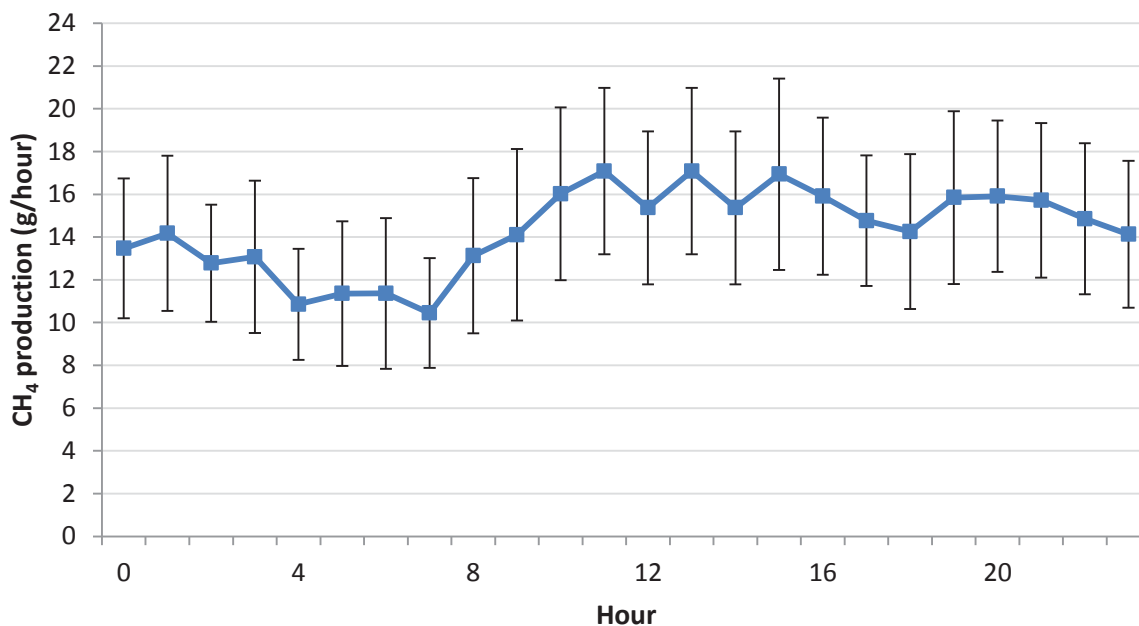


Figure 4.8: Methane production ($g\ CH_4/hour$; means \pm SD) for all of the Group 1 cows that provided useful data over the 38-day experiment (excluding the 3 fistulates). Zero on the x-axis represents midnight, while 12 represents midday.

Calculated and estimated methane production

Although it was not possible to validate absolute CH₄ emissions (g/d), calculations of expected emissions were made on the basis of estimated cow intakes. This was done by estimating feed ME intake (CSIRO, 1990) from cow live weight, live weight change and milk production (Table 4.11) and pasture ME content to determine the quantity of pasture needed to meet the animal requirements.

Predicted values by NIRS for OMD of pasture grazed over the 38-day trial were 88.0-92.5%, and ME 13.3 MJ/kg DM, which were out of the expected range and these were not used in the feed intake calculation. Instead, the ME was derived from actual estimates of digestibility by lactating cows fed pasture with a similar composition to that used here; Kolver and Aspin (2006) and Rius *et al.* (2012). The pasture grazed in the current experiment contained 24.2% crude protein and 39.2% NDF; comparative values for Kolver and Aspin (2006) were 19.8% CP, 41.0% NDF and DM digestibility was 78.8%, whilst Rius *et al.* (2012) reported a DM digestibility of 78.0% from cows fed fresh pasture containing 23.1% CP and 36.4% NDF in the DM. So, if the OMD was assumed to be 80%, then the ME will be $18.4 \text{ (gross energy of pasture)} \times 0.80 \text{ (OMD)} \times 0.82 \text{ (proportion of ME in digestible energy for forages; CSIRO, 1990)} = 12.1 \text{ MJME/kg DM}$.

The quantity of pasture DM required to meet cow ME requirements was calculated, assuming a ME content of 12.1 MJ/kg DM (Table 4.11) and this was used to estimate likely CH₄ emissions (Table 4.12) by assuming a yield of 21.6 g CH₄/kg DMI (Ministry for the Environment, 2010).

Table 4.12: Cow live weights, live weight change, milk production and composition data over the 38-day experiment, used to calculate feed DMI of Group 1 cows. Shaded values represent cows with rumen fistulae, and cows with sufficient visits to the Greenfeed system to provide useful CH₄ data production are above the dashed line did while those that did not are below

Cow	Mean live weight (kg)	Mean BCS	Age (year)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	Live weight change (kg/d)	DMI (kg/d)
2033	470.2	3.7	4	20.8	4.0	3.6	-0.24	14.3
2043	448.2	3.7	4	20.0	3.8	3.9	-0.05	14.1
2176	428.6	3.8	4	21.9	3.6	3.3	0.13	14.7
2424	477.4	3.7	4	19.4	4.3	3.9	-0.31	14.0
2013	330.4	3.7	2	19.7	4.1	3.5	0.4	14.7
2152	361.6	4.4	2	20.9	3.8	3.4	0.1	13.9
2193	440.8	3.7	4	22.7	5.2	3.9	-0.4	16.3
2195	482.0	3.8	3	22.6	4.6	3.6	0.5	18.4
2247	466.8	3.9	4	25.6	3.9	3.7	-0.2	16.7
2328	367.8	4.1	3	21.7	4.6	3.8	-0.3	14.6
2330	488.2	3.7	4	24.8	4.6	3.4	0.1	18.2
2395	360.0	3.8	2	20.8	4.4	3.5	0.4	15.7
2473	405.2	3.9	2	19.9	3.9	3.5	0.3	15.1
3716	430.6	3.3	3	22.3	3.8	3.5	0.4	16.3
3719	438.6	4.1	3	20.5	4.3	3.7	0.3	16.1
3720	481.8	4.5	4	20.9	4.5	3.8	0.5	17.5
3812	429.2	4.4	3	24.3	4.6	3.5	-0.7	15.3
4749	435.8	4.4	2	20.1	4.4	3.6	-0.1	14.9
2080	412.6	4.2	2	18.3	4.1	3.4	0.1	13.8
2085	463.0	3.9	3	24.4	4.1	3.3	-0.1	16.4
2122	428.8	3.6	3	20.3	4.4	3.8	0.1	15.4
3694	442.2	4.1	3	19.9	4.7	3.6	0.1	15.5
3730	376.8	3.6	4	21.8	4.4	3.6	0.0	15.4
4657	441.8	4.2	3	23.6	4.9	3.6	-0.8	15.1

Dry matter intakes based on the Australian Feeding Standards (CSIRO, 1990)

Table 4.13: The mean number of visits to the Greenfeed units, estimated and calculated[#] (and the difference) in daily CH₄ production from individual cows over the 38-day experiment. Shaded values represent cows with rumen fistula. Group 1 cows are listed above the dashed line while Group 2 cows are below

Cow	Mean (visits/cow/d)	Estimated CH ₄ production (g/d)	SD	CV (%)	Calculated CH ₄ production (g/d)	Difference (estimated – calculated) (g/d)
2043	2.4	121.6	35.0	28.7	305.4	-183.8
2176	3.3	102.2	32.0	31.3	318.4	-216.2
2424	4.6	111.8	21.2	19.0	301.8	-190.0
2013	4.9	298.0	37.5	12.6	318.4	-20.4
2152	3.9	289.9	42.2	14.5	300.9	-11.0
2193	4.7	361.4	60.2	16.7	352.5	8.9
2195	3.2	359.3	44.1	12.3	397.0	-37.7
2247	3.4	330.2	58.9	17.8	359.6	-29.4
2328	4.2	312.3	47.6	15.2	315.8	-3.5
2330	3.8	443.7	48.8	11.0	393.3	50.4
2395	3.0	318.8	36.9	11.6	338.3	-19.5
2473	4.0	332.6	48.5	14.6	325.3	7.3
3716	2.9	324.5	49.9	15.4	352.5	-28.0
3719	3.6	396.2	58.6	14.8	348.2	48.0
3720	3.9	387.6	50.2	12.9	377.1	10.5
3812	3.0	248.7	37.5	15.1	330.1	-81.4
4749	2.8	338.5	48.6	14.4	321.2	17.3
2048	3.3	289.9	50.8	14.6	NA	
2246	2.2	359.3	23.8	8.7	NA	
2342	1.5	361.4	66.7	17.6	NA	
4703	3.1	298.0	28.8	7.7	NA	

NA; insufficient data was obtained from Group 2 cows to determine feed intake and calculated CH₄ production; CV, coefficient of variation

[#]Based on a CH₄ yield of 21.6 g CH₄/kg DMI (Ministry for the Environment, 2010)

There was a strong significant relationship between the estimated and calculated CH₄ production (Table 4.12) for individual cows ($r = 0.72$, $P = 0.0036$), averaged over 38 days, as indicated in Figure 4.9. Only one cow (3812) seemed to be an outlier, with a CH₄ estimate of 248.7 g/d which was substantially lower than the calculated value (330.1 g/d).

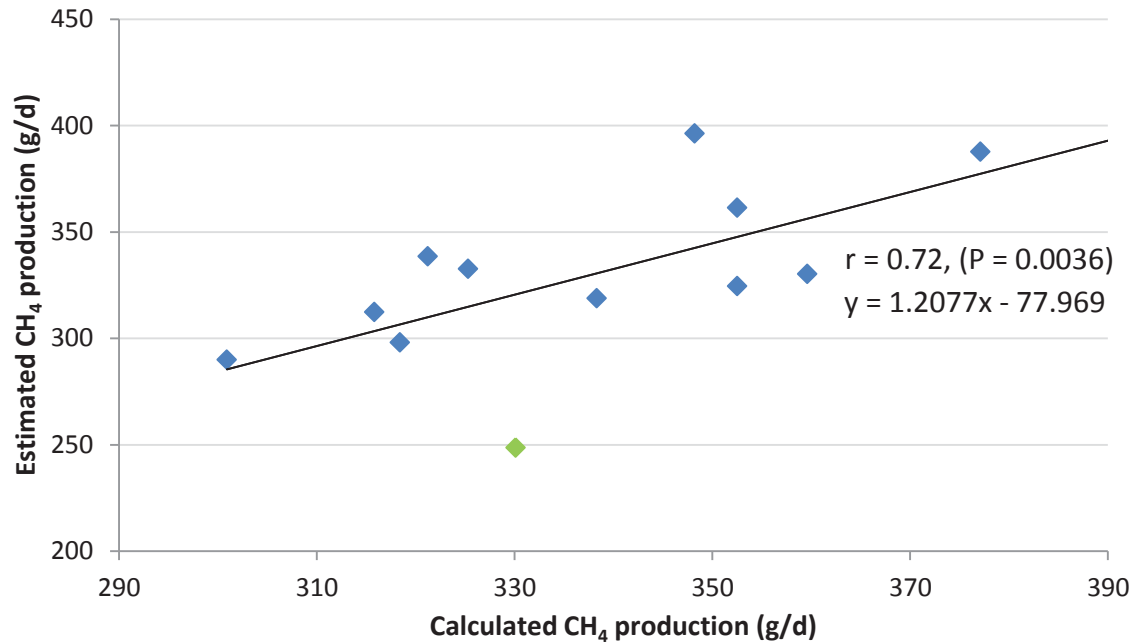


Figure 4.9: Relationship between estimated and calculated CH₄ production (g/d) for 14 Group 1 cows (fistulates and those cows with insufficient data are excluded) over the 38-day experiment. The outlier is depicted in green. The correlation coefficient (r), associated probability value (P) and relationship are indicated.

Fistulated cows

Figure 4.10 illustrates the mean CH_4 production from the three fistulates (from which CH_4 data were obtained) as well as the mean number of visits per cow per day over the duration of the 38-day experiment. This figure shows variation in daily emissions from less than 80 to about 160 g/d, with substantial variation between days, despite a high incidence of visits from three of the four cows. Caution needs to be made when interpreting CH_4 estimates from the fistulated cows because values were about a third of that from intact cows, and the data appear unrealistic.

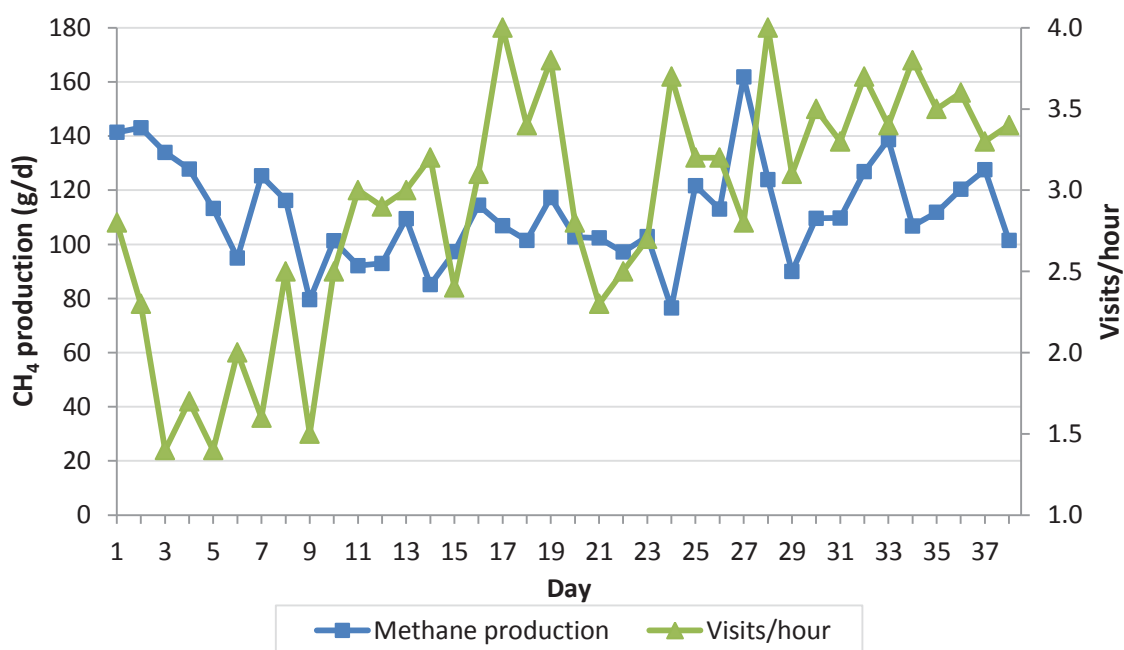


Figure 4.10: CH_4 production (g/d) and number of visits/cow/day for the fistulated cows. Methane data is based on 3 cows and number of visits/cow/day is based on 3 cows. Cow 2033 visited insufficiently and therefore provided no adequate CH_4 data.

4.3.4 On-farm application

As well as the evaluation of behavioural interactions between cows and the Greenfeed units and the validity of the CH₄ estimates, there was a need for implementation of the units on-farm. These units were the first trailer mounted systems evaluated by clients, and the need to move them frequently, and assess their operation on pasture had not been assessed previously. Two types of chutes were designed; the Sled chute and the Gate chute, and both regulated cow access to the Greenfeed units satisfactorily. However, unit design affected their ease of movement. As described in 4.2.2, the Sled chute was easier to move between paddocks than the Gate chute, and there did not appear to be any interference from recording of EID tags from cows adjacent to the unit (but not in the chute). The small gates on the Sled chute were not necessary.

The Greenfeed units were typically left in the paddocks for two days before being moved. An unexpected problem arose from the considerable use of the Greenfeed units, which resulted in significant pugging of the paddocks around the entry to the chute, especially within the chute and also surrounding the Greenfeed units (Photograph 4.3).



Photograph 4.3: Pugging damage following two days of Greenfeed unit use.

This pasture damage due to pugging is presented in Photographs 4.3 and 4.4. More important were the large depressions (ruts) that were left in the ground within the

chute (Photograph 4.4). These were sometimes 150-200 mm deep, and once the pasture has re-established around these areas, the depression may not be visible to farm staff travelling on farm bikes, and will pose a safety hazard.

Photograph 4.4: *Pugging damage after two days of Greenfeed unit use (including depression (in inset)).*



4.4 DISCUSSION

4.4.1 General animal and feed measurements

Methane estimates undertaken with the Greenfeed units were based on normal farm practice, so the animals were treated as a small herd, given new breaks of pasture after each milking, and the cows were similar in live weight and condition to those on a typical New Zealand dairy farm (DairyNZ, 2010). Pasture allowance was also similar to normal farm practice, as was the chemical composition of the pasture on offer (DairyNZ, 2010).

4.4.2 Visiting behaviour

There has been only one unpublished study concerning the Greenfeed system for calculating CH₄ production from dairy cows (Utsami, unpublished). Their work was with dairy cows held in confinement, and because there are no comparable reports, this discussion relates mainly to cow behavioural interactions with the Greenfeed unit, and some consideration of CH₄ production estimates. However, there is some literature relating cow visits to concentrate dispensers used indoors (Wierenga & Hopster, 1991b, Collis *et al.*, 1978, Wierenga & Hopster, 1991a). Even when Greenfeed units are positioned in an indoor controlled environment, such as confinement systems, or in association with robotic milkers (Ketelaar-de-Lauwere *et al.*, 1999), the principles are common to the Greenfeed unit and concentrate feeders, because the cow is required to voluntarily access the unit. An ear tag is then electronically read allowing feed to be dispensed, while the cow then has either her CH₄ production estimated (Greenfeed unit) or her milk harvested (robotic milking system).

Adaptation to Greenfeed unit

An important point of difference of the Greenfeed unit to the respiration chambers and the SF₆ technique is that it requires the animal to voluntarily enter the unit for a CH₄ estimate to be made. Consequently, an important criterion for assessing the Greenfeed units in a grazing situation is the incidence of cow visits, which may be

based on the number of cows visiting the units or the number of visits per cow per day. For Group 1 cows, the results indicated that both the number of cows visiting the Greenfeed units and the number of cow visits per day was low for the first seven days of the experiment, despite a high frequency of visiting on the first day of the experiment. There was a gradual increase until about day 14, after which visiting seemed to plateau. However, when the visiting behaviour is expressed as mean number of visits per cow per day, the pattern is more variable and the visits do not appear to stabilise until day 29, when access to all but one of the cows was constrained to a maximum of 6 visits/day. From this experiment it appears that it takes cows around two weeks to get used to the unit.

Cattle are able to learn quickly while adjusting to new equipment and environmental changes (Albright, 1981). Collis et al. (1978) found that cows rapidly adjusted to an automatic concentrate feeder, and after an initial learning period the cows used the feeders regularly, and with almost the same daily frequency. Similarly, the cows in the current experiment began using the feeders regularly, although the time taken for this adaption varied considerably between individuals; a consistent frequency was achieved once access was restricted.

Cows may respond to signals such as the presence of another cow visiting the feeder (social facilitation (Curtis & Houpt, 1983)), and may associate this with the opportunity to eat, although cows do not always consistently respond to these signals (Wierenga & Hopster, 1991b). The gradual increase in visiting over the experiment may have been a response to several signals, for example, other cows visiting or learning there would be a feed reward. Often cows would note the presence of a cow in the Greenfeed unit, and would attempt to access either the occupied, or the other Greenfeed unit (Table 4.5).

Excessive use and training

On a herd basis, the number of visits increased over time but behaviour of individual cows was highly variable, with some cow's having their use restricted and other cows requiring training. A high incidence of visits may provide a more representative

estimate of CH₄ emissions than intermittent use, but the restriction to visiting frequency was necessary to prevent some cows from dominating the units and to prevent pellets becoming an excessive proportion of the animal's diet.

Cows that had visited infrequently within the first seven days of the experiment were subjected to a training period using 'humans to train cows' in an attempt to stimulate Greenfeed use. Towards the end of the experiment, the effect of 'cows training cows' was investigated by the addition of 20 cows (Group 2) to the original herd of 24 cows (Group 1). The outcomes of both forms of training will be discussed.

Humans training cows

On day 8 of the experiment, five of the eight cows that were encouraged to enter the Greenfeed unit did so. Of the five cows that entered the unit on the training day, three became regular users (2 or more visits per day) after 2, 3 and 4 days following training. Training did increase usage of the Greenfeed units, although there were three cows that even with gentle persuasion could not be made to use the unit during training. There was an increase in the mean number of visits per cow per day from day 11 onwards (Figure 4.2) and a small increase in numbers of cows visiting (Table 4.10) which provided useful CH₄ estimates. The increased visitation may have been due to the cows adapting to the Greenfeed unit without human interference, but some of this increase was attributable to the training period.

Cows training cows

The effect of 'cows training cows' was evaluated throughout the last week of the experiment, when 20 cows were added to the herd on day 32. The mean number of visits per cow per day for the Group 2 cows was lower than that of the Group 1 cows during their first week (days 1-7). There was no benefit in adding the cows to those already familiar with the Greenfeed unit, with only four Group 2 cows providing useful CH₄ estimates, compared to the 14 Group 1 cows (excluding fistulates). It was hypothesised that the addition of (Group 2) cows to those already familiar with its operation would facilitate visiting the Greenfeed unit, but the opposite effect was

observed; cows did not train cows. Social dominance plays a pivotal part in any existing, or newly formed group of dairy cows (Grant & Albright, 2001) and may have contributed to the lower number of Group 2 cows visiting and visits per day) in the last seven days. A low incidence of visits may be a consequence of increased competition associated with an increase in the number of cows per Greenfeed unit, as described by Olofsson and Wiktorsson (2001) with cows using feeding stations, with dominant cows having priority to feeding (Jenkins & Leymaster, 1987). Olofsson and Wiktorsson (2001) investigated the effect of the number of animals per feeding station on the competition for a total mixed ration diet fed at maintenance levels, but their work involved one and four cows per feeding station. They found that, as expected, dominant cows were often involved in highly competitive situations such as the displacement of cows from feeding stations or them gaining access to the feeding stations immediately following feeding, but this was rarely evidenced in the experiment with the Greenfeed units (Table 4.5).

By the time the Group 2 cows were introduced, all but one of the frequent users from Group 1 had their access restricted. Hence mean visits per cow per day was about four for 17 cows (Table 4.11) and if each visit lasted six minutes, then total time in Greenfeed units was about 400 minutes. This is approximately 3.5 hours per Greenfeed unit, so there was ample time for the introduced Group 2 cows to access the Greenfeed unit, ruling out lack of access to the units as the cause for low visitations in that group. Increased competition associated with the addition of Group 2 cows seems unlikely to be a cause of their low usage, even if dominant cows attempted to displace submissive cows in an attempt to gain her allotment of pellets. Alternatively, the seven days that Group 2 cows had access to the Greenfeed units may have been insufficient for them to take an interest in the pellets, especially if hierarchies were still being established.

Grouping of unfamiliar animals has been found to increase aggression (Collis *et al.*, 1979), social stress (Mench *et al.*, 1990, Hasegawa *et al.*, 1997) and locomotion behaviour (Hasegawa *et al.*, 1997, Nakanishi *et al.*, 1993). Grant and Albright (2001) explained that when cows are introduced to a socially stable group, the social

structure of the entire group may be disturbed until the new cows have established their new social status within the original group. This may take about a week for the dominance hierarchy to become established and stable (Schein & Fohrman, 1955), although 5-15 days has been reported for changes in social behaviour and locomotion activity to return to a basic level after the introduction of unfamiliar animals into the group (Kondo *et al.*, 1984, Hasegawa *et al.*, 1997). Therefore, those dominant Group 1 cows within an already stable social group may have displaced Group 2 cows from the Greenfeed units because they were in the process of establishing their social status in the newly formed group. One week may not have been enough time for the dominance hierarchy to form and stabilise, and this may have taken precedence over Greenfeed visits for most Group 2 cows.

Not only is a cow required to visit the Greenfeed unit, but she needs to keep her head in the feeding station for at least three minutes for adequate eructation and respiration events to occur and samples to be made and CH₄ estimate to be determined. Therefore, the duration of the visit to the Greenfeed unit is an important determinant of whether a cow provided useful CH₄ estimates. Of the cows providing useful data from Group 1 (2 or more useful visits/day), those visits represented 52-98% of total visits for individual cows. This is similar to the percentage of useful visits from Group 2 cows with more than nine visits (64-87%) during the week they were being estimated, so there did not seem to be any effect of competition on duration of visits, as reported by Olofsson and Wiktorsson (2001) in relation to visits to feeding stations dispensing a total mixed ration diet.

Cow age

Effects of cow age were evaluated in Group 1 animals where the number of visits per cow per day appeared lower for the 3-year old cows compared to either the 2-year old heifers or 4-year old (mature) animals. It was suggested that 2-year old heifers might be more inquisitive and visit the Greenfeed units more than older animals, but the only difference was the lower use by 3-year-olds. However, care needs to be taken when

interpreting these data as cows with high usage had their access to the Greenfeed unit restricted, and cow numbers were very low.

Dominance order has been reported by Collis *et al.* (1979), to increase with age so older cows would be expected to be more dominant than younger cows, or heifers (Grant & Albright, 2001). Many authors (Friend & Polan, 1974, McPhee *et al.*, 1964, Kabuga, 1992) have noted that dominant animals in a herd have priority whenever there is a competitive situation, such as at a feeding station. Collis *et al.* (1979) reported that younger, sub-ordinate, animals visited a feeding station more frequently than older cows, and this has been supported by Katainen *et al.* (2005). This was evident with young cows in Group 1, but there was no clear relationship between Greenfeed use and cow age in this study.

Greenfeed unit and pellet-type preference

The cows in the current experiment demonstrated a preference for the Greenfeed unit A (Sled) over unit B (Gate) (at approximately a 6:4 ratio) during the 8-day crossover period, although care needs to be taken when interpreting this result as the chutes were not switched over between the two units. Information in the literature in relation to preference experiments with differing feeders and the attractants they dispense is limited. Of the information available, both Hyde *et al.* (1974) and Forbes (2007) observed that when cows were grouped with access to multiple feeders they generally move between the feeders, indicating no preference, which does not agree with the observations made in the current experiment. Hyde *et al.* (1974) further explained that if a cow received no concentrate from one feeder she would often move to the other and if she received none in the second, she would return to the first. This switching of position may be repeated, particularly if a cow in the adjacent feeder is receiving concentrate. This movement between feeders agrees with the observations made in the current experiment, as indicated in Table 4.5 (and in Photograph 4.1).

The current experiment indicated that there was a preference for the Lucerne pellets over the Grain pellets dispensed from the Greenfeed units (at approximately a 6:4 ratio). Unless animals are restricted (as with the Greenfeed units), more visits to an

automatic concentrate feeder will result in an increased concentrate intake. While many visits may acquire more CH₄ estimates, high intakes of the supplement feed, particular high starch grains, could affect appetite, feeding behaviour, and also the digestion (Miron *et al.*, 2004a), possibly over-estimating CH₄ production. Therefore, it was desirable to choose a type of pellet that attracted the cows to the Greenfeed units, was low-cost and had a similar composition to the feed offered (Miron *et al.*, 2004a). There is limited information on the attractiveness of pellets to motivate cows to visit an automatic concentrate feeder and Miron *et al.* (2004a) found the mean number of rewarded visits, total visits per day and pellet intake were similar for pellets based on either barley grain or soy hulls. Other work by these authors (Miron *et al.*, 2004b) showed a high number of visits to an automated milking system when a soy hull/corn gluten feed mix was offered, compared to the high-starch pellets. Explanations proposed by Miron *et al.* (2004b) for this preference were based on NDF digestion rate versus higher digestibility of the respective attractants, but these will be affected by the basal diet. In the experiment reported here, the starch content of the Grain pellet would have been small, probably less than 2% of dietary DM and any improvement in diet quality would have been minimal. It is difficult to explain cow preference for the Lucerne based pellets. Further evaluations may be warranted as pellet attraction was shown to affect visits, otherwise the preferred attractant would be one with a composition that closely resembles the basal diet.

The mean number of visits to a Greenfeed unit may have been under-estimated by cows unable to access the unit with a particular pellet type if it was already occupied, so the cow returned to pasture. This was observed in both the current experiment (Table 4.5) and by Winter and Hillerton (1995).

Circadian variation in visiting behaviour

The expectation was that cows would first have to learn to use the Greenfeed system, and those who had their intakes restricted (to 6 visits/day) to prevent over-use, would adopt a regimen where they would visit the units every 4-5 hours. As a consequence, visiting would become regular and evenly spread over the day, so that any circadian

variation in CH₄ production could be detected. However, this was only partially correct, with visits appearing to be more evenly distributed throughout the day at the beginning of the measurement period compared with the end (Figure 4.4). In general, the visits were distributed throughout the day, so the circadian pattern of variation in CH₄ production would have been represented, but the visits were more even during the beginning of the experiment, compared to at the end (Figure 4.4). There was no reduction in either standard deviation or coefficient of variation (CV) of emissions as the experiment progressed (Appendix E).

Visiting the Greenfeed unit is voluntary, and therefore the time in which the CH₄ production estimates were made was dependent on the cows' decision to visit, and the restrictions imposed to prevent excessive visits. The less even spread of visits towards the end of the experiment was unexpected and may indicate either a lack of learning or that the reward was insufficient to warrant visits in the early hours of the morning (Figure 4.4). Wierenga and Hopster (1991b) suggested cows may fit their visits into their other daily activities, particularly while they were already active, and this was confirmed by Winter and Hillerton (1995) who noted that the voluntary visiting to an automatic milking system occurred as a function of normal forage feeding behaviour. To some degree, this pattern was evident in the current experiment where there were higher hourly visiting frequencies following each of the milkings, possibly indicating that the cows would visit the Greenfeed unit on their way back to pasture following milking and this was also observed by Miron *et al.* (2004b) in an indoor experiment.

The higher visiting frequency to the Greenfeed unit during the daylight hours compared to the night hours provides further evidence that the cows were fitting their visiting into their normal daily routine. Dairy cows usually feed during the day and rest at night (Winter & Hillerton, 1995), but the frequencies of visitation around 23:00 h may be associated with a perception of an increased chance of a reward by standing up and visiting, even during a resting period (Wierenga & Hopster, 1991b). Birke and Archer (1983) suggested that the amount of time or energy spent on exploring the environment will depend on the effort required and the benefit to the animal.

Therefore, the costs for a night visit may have been less than the reward (pellets), after which there was little activity until dawn.

The uneven pattern of visits to the Greenfeed unit over 24 h will have less effect on the estimates of CH₄ from animals with high intakes (and a small circadian variation in emissions) compared to low intakes and a high variation as shown in Appendix C.

4.4.3 Methane production

The CH₄ production (g/d) (Mean ± SD) obtained from the 14 intact Group 1 cows (excluding fistulates) that provided useful CH₄ data was 340.3 ± 29.4. The CH₄ production and standard deviation is similar to those reported in the literature for lactating dairy cows grazing forage-based feeds at similar intakes to those reported in the current experiment; McNaughton *et al.* (2005) reported 332 ± 42.1 g/d (Mean ± SD) for lactating cows (estimated with the SF₆ technique) with a daily intake of 17.4 kg DM (estimated with alkane markers and prediction equations), while Bruinenberg *et al.* (2002) reported a CH₄ production of 360 ± 36 g/d (Mean ± SD) for lactating dairy cows with an intake of 14.7 kg DM/d of grass using the respiration chambers.

The mean CH₄ production as estimated from the fistulated cows was unexpectedly low (111.5 g/d); approximately a third of those CH₄ production estimates from the intact cows (338.7 g/d). The initial objective of including the fistulated cows into the experiment was to use rumen sensing boluses to relate CH₄ production to rumen temperature and pH. However, the lower CH₄ production from the fistulated cows suggested an escape of gas (and rumen fluid) from around the fistula and that this objective could not be achieved. Liquid leakage is common but gas escaping from the fistula is measured from animals in respiration chambers and the SF₆ technique uses the ratio of CH₄: SF₆ in the breath to determine CH₄ production (Pinares-Patiño *et al.*, 2012a) so the CH₄ escaping via the fistula cannot be determined. However the Greenfeed unit differs in this regard, as it captures only the CH₄ released via eructation and exhalation and the tracer is not mixed in the rumen, and therefore the loss of CH₄ via a leaky fistula can be determined. The CH₄ estimates from the fistulated cows were 70% lower than those from the intact cows which was unexpected. However, they

were comparable with findings from Utsami (pers. comm) who used fistulated cows to measure CH₄ production using a Greenfeed unit who reported 20-50% lower CH₄ emissions compared to intact animals.

As the current experiment is the first to be carried out using a Greenfeed unit to estimate CH₄ production from grazing lactating dairy cows, there is little comparable CH₄ production information. Although the mean emissions were similar to published data from lactating dairy cows fed fresh feeds (Grainger et al., 2007)(refer to Table 2.4 and 2.5), the lack of comparable information, or data from pasture fed cows where intakes were measured, resulted in an assessment of emissions by other means, including:

1. The extent to which the number of measurement days affects the accuracy of the CH₄ estimates
2. A comparison of estimated CH₄ data with calculated estimates based on predicted intakes
3. Examining the sensitivity of the Greenfeed estimates in relation to an unplanned pasture deficit, and daily variation in emissions.

When the CH₄ estimates and their respective standard deviation and coefficient of variation (CV) were presented for both 10-day increments as well as cumulatively as the experiment progressed, the trends in variance for individual animals seemed inconsistent. There were several cows whose CV's decreased and one cow whose CV's increased over the specified periods within the 38-day feeding period for Group 1 animals. Overall, the herd mean CV (%) declined from 14.3 to 13.5 and 9.8 g CH₄/day with increasing number of data (days 11-20, 21-30;31-38, Table 4.11) suggesting less variance in the latter part of the trial (Table 4.12) The low CV's obtained from the first 10 days were somewhat meaningless because these were based on fewer cows, with large numbers of estimates prior to restrictions being imposed (commencing on day 10 (Table 4.11 and 4.12)).

There is no comparable literature on either the length of the measurement period or on the accuracy of the CH₄ estimates obtained from the Greenfeed unit and benefits of protracted recording. However, a recent study by Pinares-Patiño *et al.* (2012a) used the SF₆ technique to evaluate the effects of extended sample collection periods (5-day and 10-day) compared to a standard 24-hour daily collection period, on CH₄ production from cows fed the same intakes, found similar estimates of CH₄ production from each of the three collection periods. In the current experiment, CH₄ production estimates in the first 10 days were high (obtained from fewer cows than for most of the experiment), after which production was relatively constant (Figure 4.7). However, a detailed interpretation of variation is not possible because intakes were neither measured nor controlled. When the calculated CH₄ production values (based on cow production) were compared to those estimated from the Greenfeed unit for individual cows over the whole 38-day period, there was a strong relationship ($r = 0.71$), with one possible outlier and the herd mean CH₄ production estimated by the Greenfeed unit was very similar to values calculated over the 38 days (338.7 and 345.0 g/d, respectively).

There were no obvious reasons why estimated (based on Greenfeed unit) values for Cow 3812 (Figure 4.9) differed from predicted values, and this suggests a limitation of the Greenfeed system for identifying CH₄ production for individuals, despite the whole herd CH₄ production estimate being comparable to the literature and calculated values. If the calculated intake of the cow 3812 is correct, it is possible that the CH₄ production value for cow 3812 did represent true divergence in CH₄ yield (g/kg DMI), but if the intake was 15.3 DM/d, the yield would be 16.3 g CH₄/kg dry matter, compared to a yield of 21.6 g CH₄/kg DM used in the calculations, which is unlikely. Another potential source of error is in the estimates of DM (based on metabolisable energy) requirements of individual cows, as described by Waghorn and Hegarty (2011), which shows that efficient animals would eat less feed than average, and may be expected to produce less CH₄ than the population average. Differences between predicted and actual intakes could explain some of the reasons associated with cow 3812, but again these are unlikely to account for the differences between predicted and estimated CH₄ in Figure 4.9.

Although not a planned part of the experiment, a misjudgement in the feed requirements for the cows on day 34 resulted in a significant deficit in the pasture allowance, so the cows were under-fed on this day. There was a 3-4 kg drop in milk production immediately after the pasture deficit (Figure 4.7), and the expectation was that CH₄ production would also decrease, as DMI is the main source of CH₄ production (van Zijderveld et al., 2011). However, the CH₄ estimates did not show a decrease in CH₄ production despite the decreased milk production. It is not known whether this was an artefact of the Greenfeed unit estimation, or simply that there was no decrease in CH₄ production.

Circadian variation in methane production

There were variations between days in Greenfeed estimates of CH₄ production (Figure 4.7), probably in association with variations in feed intake, but no information is available to indicate whether the variations are in response to feed intake or other factors (such as feed quality). Provided the cow keeps her head in the Greenfeed unit for at least three minutes, a CH₄ estimation can be obtained, so the timing of the visit in relation to feeding will affect the CH₄ estimation. Sampling frequency is important and could affect the circadian pattern of CH₄ estimates, depending on whether the estimates were made during a period of eating, ruminating or resting. Both CH₄ production and eructation is affected by the fermentation activity in the rumen, associated with the pattern of feeding behaviour and DMI. Therefore, the CH₄ production increases after feeding (Grainger et al., 2007) and is maintained during the day (Figure 4.8), but declines over night when chewing is dominated by rumination (Gregorini *et al.*, 2012) in association with the circadian feeding pattern (Lockyer & Champion, 2001).

The intermittent sampling frequency of the Greenfeed unit (approximately 5 times a day) may limit its ability to detect circadian variation in CH₄ production. There was an approximate 1.5-fold difference in the hourly CH₄ production over the day (Figure 4.8), associated with feeding, which indicates the importance of the timing of visiting

throughout a 24 h day in order to obtain a representative estimation of the CH₄ production.

Information on the circadian pattern of CH₄ production in a grazing dairy cow is scarce because the SF₆ technique is not appropriate for determining within-day changes in CH₄ production as it collects a daily integrated sample (Storm *et al.*, 2012). There is information on the circadian variation in CH₄ production for grazing sheep (using tunnel methods) which indicates that CH₄ production gradually increases over the day reaching a peak at 15:00 h to 16:00 h (Murray *et al.*, 1999). After this it gradually decreases during the night (Murray *et al.*, 2001) and reaches a minimum at around 09:00 h (Murray *et al.*, 1999, Lockyer & Champion, 2001) which is similar to that described in Figure 4.8. Minor differences, such as the minimum CH₄ production being at 07:00 h in the current experiment, compared to 09:00 h with grazing sheep (Murray *et al.* (1999), Lockyer and Champion (2001)) are probably a consequence of the feeding regimen.

4.4.4 On-farm application

The practical application of the Greenfeed technology was an important component of this evaluation, especially the potential for its integration into a New Zealand farming system. One aspect requiring consideration was the chute design (Sled and Gate) which were to ensure only one cow entered the unit at a time, and after making behavioural observations (Table 4.5), it was clear that they were essential to the operation of this technology. This was because cows were observed pushing and bunting each other to gain access to the Greenfeed unit (Table 4.5), and without the chutes, the cows may have caused considerable damage to the Greenfeed units, and the minimum of three minutes recording time may have been difficult to achieve. The Sled chute was the most practical, because not only did it allow only one animal to access the unit at a time, it required less labour to move locations.

An important observation was the significant pasture damage around the Greenfeed units and especially in the chute, where deep ruts sometimes formed. This damage was exacerbated in wet conditions leading to pugging which will reduce pasture

growth. This may have been relatively minor in dry (summer) conditions. Future use should evaluate rubber mats positioned inside the chutes to minimise the damage to pasture by preventing direct contact with cow hooves. However, mats may also affect the willingness of cows to access the feeders and will need to be evaluated in future.

4.5 CONCLUSIONS

This experiment is the first to successfully implement a Greenfeed unit on-farm with lactating dairy cows grazing pasture under New Zealand conditions. The results from this chapter were broadly grouped into those relating to visiting behaviour, CH₄ production, and the on-farm implementation of the Greenfeed unit. Results indicated that there was a gradual increase in visiting as the experiment progressed, and that visiting was affected by; the type of cow training undertaken, herd size, cow age, time of day, pellet type and the Greenfeed unit itself. This gives an indication of some of the factors that need to be taken into account in future experiments involving the Greenfeed unit. Of the 24 cows in Group 1, 17 cows visited adequately for CH₄ estimates to be obtained. With the exception of the fistulates, the mean herd CH₄ production from the Greenfeed unit was similar to those reported in the literature. When calculated and estimated individual animal CH₄ estimates were compared, a strong relationship was obtained. Further assessment led to questioning of the quality of the CH₄ estimates as there was inconsistent changes in the variation in the estimates as more estimates were obtained over time, and a lack of sensitivity in CH₄ estimates to a feed deficit, both of which were unexpected. The Greenfeed unit was implemented on-farm during a typical Waikato Spring despite some considerable pasture damage associated with excessive Greenfeed unit use. Following the evaluation of two chute types, the Sled seemed the better choice as it involved less labour during movement of the Greenfeed units.

In summary, this experiment illustrated the practical application of the Greenfeed unit in a farming system. It was found that a variety of animal and behavioural factors affected the visiting behaviour to the Greenfeed unit. Further experiments would be required to make recommendations on standard operating practices in relation to visiting behaviour to the Greenfeed unit and its on-farm implementation. Future

experiments should involve both controlled indoor and grazing stages, with animals fed at levels simulating farming systems, in order to better define the accuracy and precision of CH₄ estimates obtained from the Greenfeed unit.

CHAPTER FIVE

GENERAL CONCLUSIONS AND RECOMMENDATIONS



CHAPTER FIVE: GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 GENERAL CONCLUSIONS

Before it could be used on-farm for grazing animals, the Greenfeed unit (C-Lock Inc, South Dakota, United States, Patent 7966971), needed to be validated against the two existing techniques, using the 'gold-standard' respiration chambers as a reference under a controlled indoor environment with constant intakes (Chapter Three). The CH₄ estimates from the Greenfeed unit were higher and more variable compared to the respiration chambers and SF₆ technique. This is likely due to the infrequent sampling of the Greenfeed unit compared to the continuous sampling of the respiration chambers and the integrated daily sampling of the SF₆ technique. The strong relationship between the hourly CH₄ estimates (Pinares-Patiño and Waghorn, unpublished) from the Greenfeed unit and the respiration chambers indicates that the unit may be adequate for determining circadian changes in CH₄ production but is sensitive to the timing and frequency of the visit. When individual animal CH₄ measurements from the respiration chambers were compared to the Greenfeed unit and to the SF₆ technique, weak relationships were found in opposite directions. This was likely due to the experimental design and feeding protocol. This experiment was conducted under controlled indoor conditions and involved six cows fed at maintenance levels, which may have limited the ability for defensible relationships to be determined. It was concluded that although the Greenfeed unit CH₄ estimates were higher and more variable, they were within the expected range considering the animal and dietary factors. Therefore, this provided confidence in the ability of the Greenfeed unit to determine the CH₄ emissions at least at an animal group level and warranted further investigation using more animals, in a natural grazing environment, with differing feed levels representative of a typical farm system.

A second experiment was then conducted on-farm to assess the interactions of grazing dairy cows with the Greenfeed unit and evaluates the CH₄ estimates obtained from the

Greenfeed unit and provide practical recommendations for its use in a New Zealand pasture-grazing system (Chapter Four). The results were broadly grouped into those relating to visiting behaviour, CH₄ production and the on-farm implementation of the Greenfeed unit. Results indicated that there was a gradual increase in visiting as the experiment progressed, and that visiting was increased by 'humans training cows' whereas there was no benefit in cows training cows. Cows preferred Lucerne pellets and the Greenfeed unit with the Sled chute. With the exception of those from the fistulates, the mean herd CH₄ estimates from the Greenfeed unit were similar to those reported in the literature. At an individual animal level, comparison of calculated and estimated CH₄ values indicated a strong, significant relationship. Furthermore, it appeared that 14 days were needed for cows to get used to the Greenfeed units, and extending estimates (for a further five weeks) did not consistently reduce the variance in CH₄ estimates within cows. Other issues to consider was the pasture damage that occurred while the units were in use and that a chute was required to restrict visiting to the unit to only one cow at a time. In this case, the Sled chute was the best suited to a rotational grazing system.

This study was the first to use the Greenfeed units under New Zealand farming conditions. The units were successfully implemented into both a controlled indoor (Chapter Three) and an outdoor grazing experiment (Chapter Four). This thesis has gone some way to firstly demonstrate that the CH₄ estimates from the Greenfeed unit at the group level are comparable to those reported in the literature from respiration chambers and the SF₆ technique. The study also demonstrated that the Greenfeed unit can be used on-farm to determine CH₄ emissions from grazing animals in their 'normal' environment. This allowed the circadian variation in CH₄ production to be determined, which the SF₆ technique does not provide. This study evaluated how best the Greenfeed unit may be used in an experiment to measure CH₄ emissions from grazing animals and has hence established some of the factors that affect the visiting behaviour and CH₄ production obtained from the Greenfeed unit. Therefore, this information can be used to begin developing standard operating procedures for the Greenfeed unit, if it is to be implemented in New Zealand as a standard technique for estimating CH₄ production from cattle.

5.2 RECOMMENDATIONS FOR FUTURE RESEARCH

It is important to note that research into the recently developed Greenfeed unit is still in its infancy and therefore further evaluations are required. Recommendations are broadly classified into those related to the practical aspect of future experiments and those related to the determination of CH₄ emissions by the Greenfeed unit.

Practical recommendations for future research include; carrying out further experiments with both controlled indoor and grazing components and animals offered high and also low feeding levels, representative of a typical farming system. This would allow more robust and comprehensive evaluations of the accuracy and precision of the CH₄ estimates. Despite the lack of meaningful CH₄ estimates from the fistulated cows they did indicate that at least half of the CH₄ produced in the rumen is lost through the fistula. It is recommended that they are not included in further Greenfeed experiments. Indeed, if defensible relationships were required to compare CH₄ emissions to rumen conditions, it would be recommended that CH₄ measurements should be made using the respiration chambers. Future research should address the length of the experimental period in relation to the accuracy on the estimates, as the extent of the measurement period has implications on the logistics and cost of an experiment. With regard to the considerable pasture damage associated with the on-farm use, it is suggested that slatted rubber mats be placed immediately around the Greenfeed unit and within the chute. A suggestion for future research is that the Greenfeed unit be moved more regularly, perhaps daily, however this can be labour intensive. The Sled Chute was easiest to move as it could be towed behind the Greenfeed unit.

Future experiments assessing the behavioural interactions with the Greenfeed unit would be required to provide definitive conclusions in terms of standard operating procedures. An evaluation of cow age in relation to Greenfeed unit use would require more cows, preferably with similar numbers in each age group with a greater range of ages and some consideration of social hierarchy. More cows could be taught using the

'human training cow', but the timing, extent and details of training needs to be refined to achieve a high cow usage in a short period of time.

In these experiments, C-Lock Inc. determined the values of CH₄ emissions from the Greenfeed system and supplied this information to the users. This means that the users have limited knowledge of the process undertaken to determine the CH₄ estimates. In contrast, the determination of CH₄ production for the SF₆ technique and the respiration chambers are both documented in the literature (Johnson et al., 2007, Pinares-Patiño et al., 2012b) and are controlled by scientists undertaking the measurements. With the estimates reported here, C-Lock Inc. both manufacture the Greenfeed units and interpret the CH₄ data. In future it would be better if end-users undertook responsibility for analysing Greenfeed data and to do this they would need to complete a training course provided by C-Lock Inc.

Another consideration in relation to the data is the strong relationship that was obtained when the hourly measurements from the Greenfeed unit and the respiration chambers were compared ($r = 0.91$). This was considerably higher than the correlation between daily means for each cow (g CH₄/d) determined from the Greenfeed unit and respiration chambers ($r = -0.36$). This point indicates that the timing and frequency of visits to the Greenfeed can affect the accuracy of the estimates. Therefore, the feeding frequency and sampling pattern should be considered when planning future experiments, especially when intakes are low.

The findings from this study provide confidence in the ability of the Greenfeed unit to determine CH₄ emissions from animals in both controlled indoor and grazing environments. However, there are still plenty of questions that need answering with the Greenfeed unit and future research is required to fine tune the use of the units on farm in grazing pasture systems.

CHAPTER SIX

APPENDICES

CHAPTER SIX: APPENDICES

6.1 APPENDIX A: UNIT CONVERSIONS

The equations below were used to make conversions from CH₄ measured in L/d and MJ/d to g/d (in Table 2.4)

Conversion of CH₄ production (L/d) to CH₄ production (g/d)

$$\text{Methane production (L/d)} \times 10^{-3} = \text{Methane volume (m}^3\text{)}$$

$$\text{Methane volume (m}^3\text{)} \times \text{Methane density (kg/m}^3\text{)}^* = \text{Methane weight (kg)}$$

$$\text{Methane weight (kg)} \times 1000 = \text{Methane production (g/d)}$$

*Density of methane: 0.668 kg/m³

Conversion of CH₄ production (MJ/d) to CH₄ production (g/d)

$$\frac{\text{Specific energy (MJ/kg)}^*}{\text{Methane production (MJ/d)}} = \text{Methane production (kg/d)}$$

$$\frac{1000}{\text{Methane production (kg/d)}} = \text{Methane production (g/d)}$$

*Specific energy of methane: 55.6 MJ/kg

6.2 APPENDIX B: OPERATIONAL/TECHNICAL DETAILS FOR GREENFEED SYSTEM

Given below are some aspects of the operational and technical functioning of the Greenfeed system.

6.2.1 Feeder Controls

Turning the Greenfeed unit on

The 3-way switch was set in the 'feeder' position to turn on the Greenfeed unit. Both the 12 and 24 DC volt power cords were connected to the feeder and the Greenfeed unit was set to 'run' and the power switch on the main power box was switched on. Following this there was a 30 minute warm-up time for the CH₄ concentration sensor. To overcome this warm-up time, the Greenfeed unit generally remained on (in a powered state) for the duration of the experiment. However, while changing or cleaning air filters or when moving the unit between paddocks, the Greenfeed unit was placed in 'sleep mode', which reduces the power usage of the unit. Once the 'sleep mode' was activated, the Greenfeed unit stopped collecting any data and uploaded any saved data and disabled the fan, feeder, tracer release and RFID reader.

Turning the Greenfeed unit off

To turn off the Greenfeed unit it was placed in 'Sleep mode' which enabled any recent data to be uploaded. Once the data had uploaded, the fan stopped running and the 'sleep' light started blinking, which indicated that the Greenfeed unit could continue to be shut down. Finally, the 'power' switch was switched to the off position.

6.2.2 Maintenance of air filters

The airflow rate during the measurement period is one of the variables in the calculation of CH₄ emissions (Equation 3.1), so any changes in this variable would affect the CH₄ emissions estimated. It was therefore important for the air filter to be cleaned every two weeks or when the 'change filter' LED was on. Cleaning involved

switching the system to 'sleep mode', opening the air filter box lid and inserting a clean or new air filter, replacing the lid and returning the system to 'run' mode. Cleaning the air filter involved tapping the filter to remove debris and then rinsing with water and storing in a clean dry place until it was replaced.

6.2.3 Gas calibrations

As the sensors can 'drift' over time, it was recommended the sensors be calibrated using both a span gas (methane (CH_4) and carbon dioxide (CO_2)) and a zero gas (nitrogen). These calibrations were conducted when instructed by C-Lock Inc. The calibration procedure involved filling a standard bag with a standard gas mixture, connecting it to the calibration tube and pressing the calibration button. Turning the valve counter-clockwise two full turns allowed airflow to begin pulling air out of the bag. This continued until only about 10% of the capacity was remaining in the bag and the unit was then switched back to the 'run' setting. Finally the sample bag was closed and disconnected from the pipe and a rubber stopper was placed in the calibration tube.

6.2.4 Data flow

A computer situated inside the Greenfeed unit stored the real-time data which was then uploaded to the Greenfeed server daily. Once the data was completely transferred to the server, it was then erased from the local computer. Once daily, this data was then processed by C-Lock Inc. to determine CH_4 emissions (Equation 3.1).

6.3 APPENDIX C: CIRCADIAN VARIATION IN METHANE PRODUCTION BY THE GREENFEED SYSTEM

Figures C1 to C6 illustrate the circadian pattern of CH₄ production over the measurement period during the first experiment (Chapter Three), which ranged from 5 to 8 days, depending on the cow. The patterns were quite variable between the six cows. Despite this variation between cows, there appeared to be a general pattern amongst all cows (except 2037; Figure C2), whereby the visiting and CH₄ production declined from midnight until approximately 08:00 h when feeding occurred. This feeding caused a steep peak in the CH₄ production, followed by a trough and another peak in CH₄ production following the 15:45 h feeding, after which there was a gradual decrease as the night progressed until midnight. The pattern illustrated for all cows (except 2037; Figure C2) is in agreement with the literature under indoor, controlled conditions with both sheep (Murray et al., 1999) and dairy cattle (Grainger et al., 2007). The reasons for the differing circadian pattern in the CH₄ estimates made from Cow 2037 were unknown.

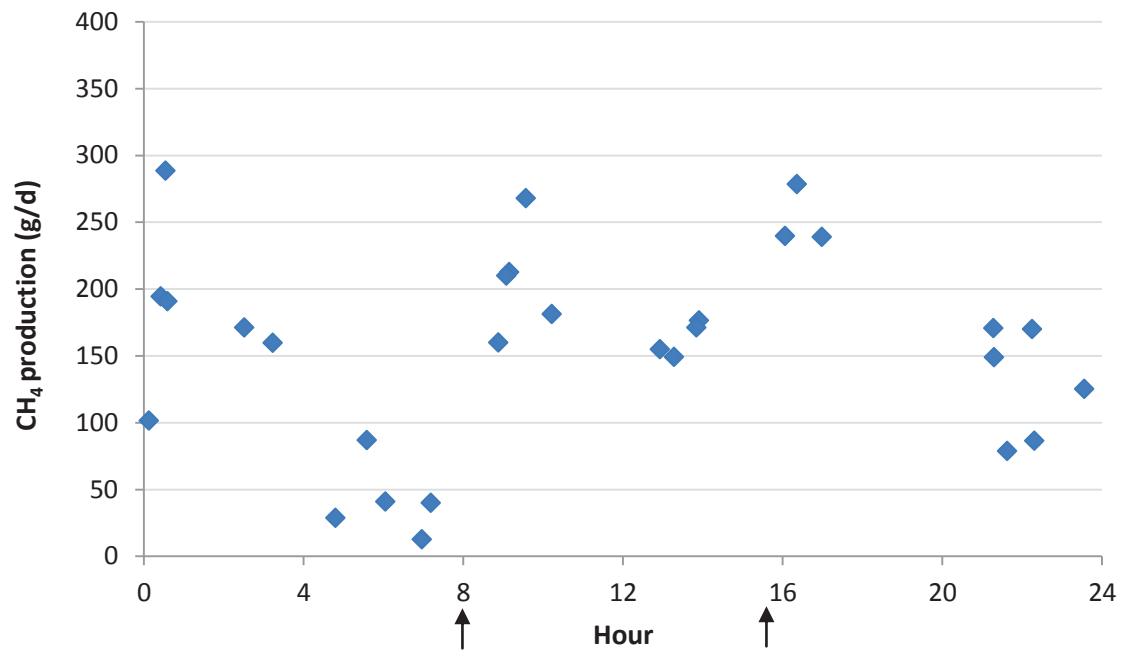


Figure C1: Circadian variation in CH₄ production estimates made over 8 days as estimated from the Greenfeed unit for Cow 2010. Feeding times are indicated by the arrows. Zero on the x-axis represents midnight, while 12 represents midday.

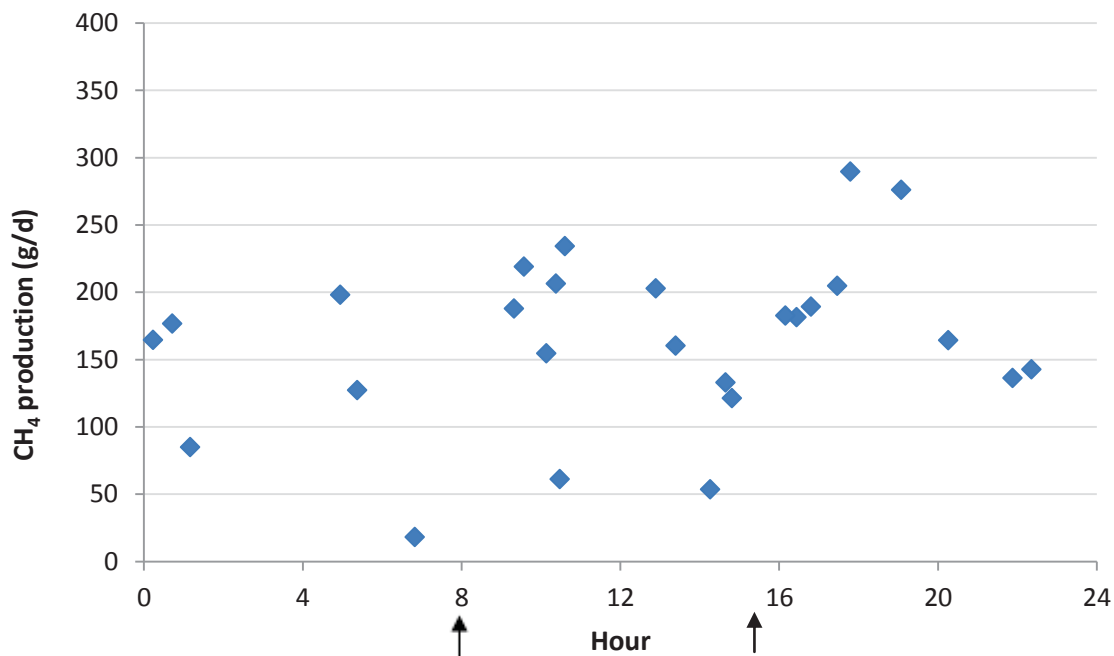


Figure C2: Circadian variation in CH₄ production estimates made over 5 days as estimated from the Greenfeed unit for Cow 2037. Feeding times are indicated by the arrows. Zero on the x-axis represents midnight, while 12 represents midday.

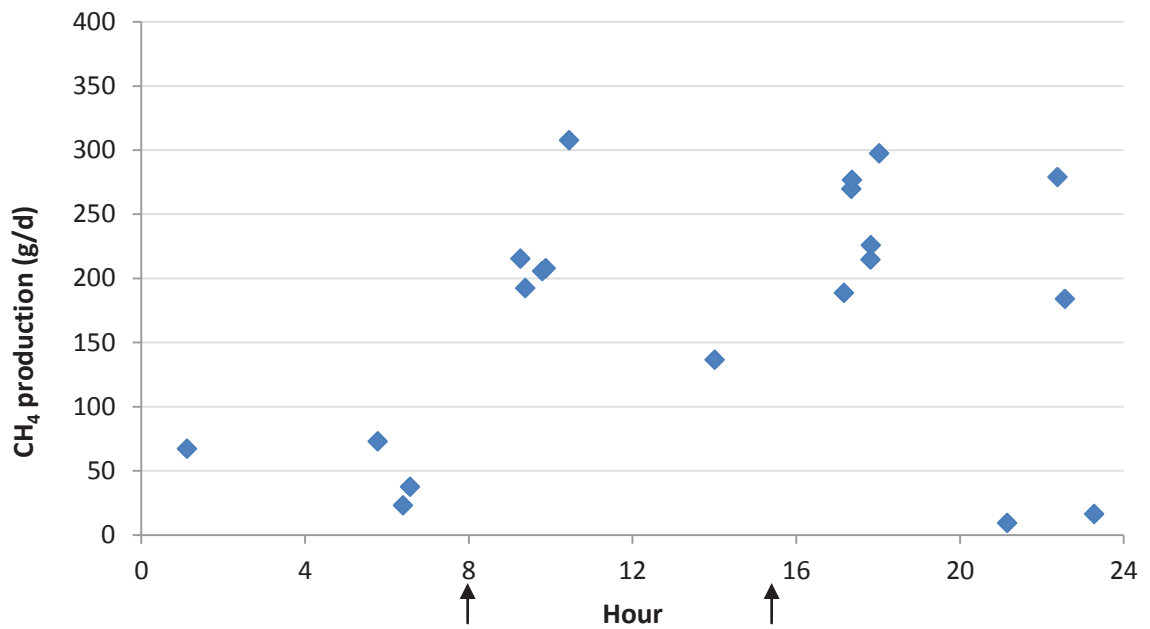


Figure C3: Circadian variation in CH₄ production estimates made over 8 days as estimated from the Greenfeed unit for Cow 2038. Feeding times are indicated by the arrows. Zero on the x-axis represents midnight, while 12 represents midday.

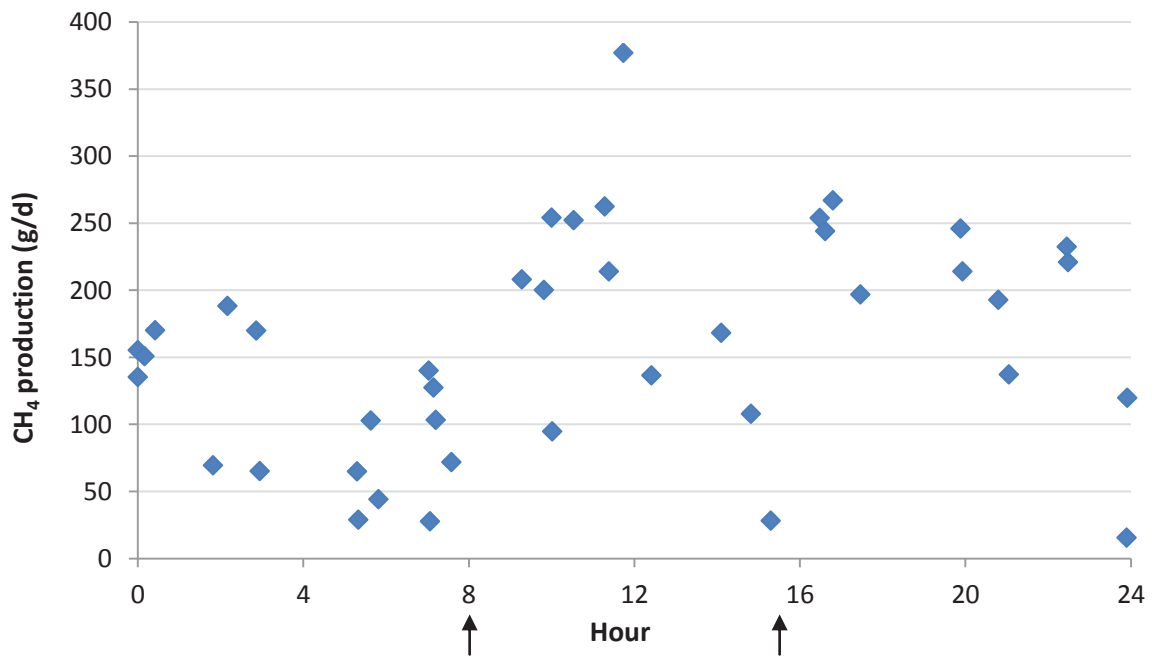


Figure C4: Circadian variation in CH₄ production estimates made over 7 days as estimated from the Greenfeed unit for Cow 2368. Feeding times are indicated by the arrows. Zero on the x-axis represents midnight, while 12 represents midday.

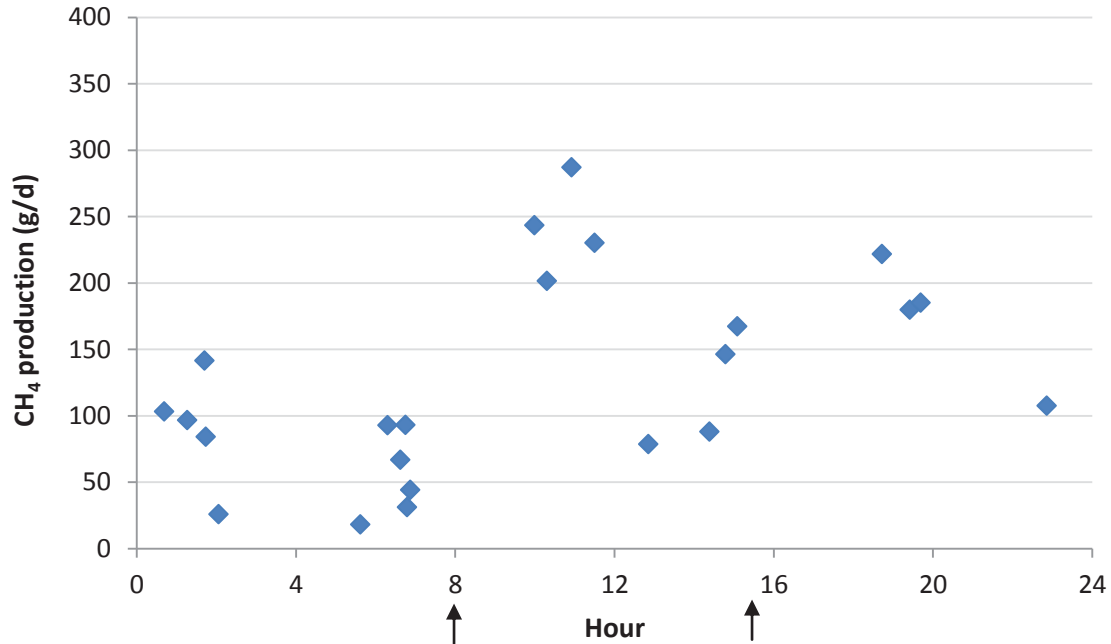


Figure C5: Circadian variation in CH₄ production estimates made over 6 days as estimated from the Greenfeed unit for Cow 3656. Feeding times are indicated by the arrows. Zero on the x-axis represents midnight, while 12 represents midday.

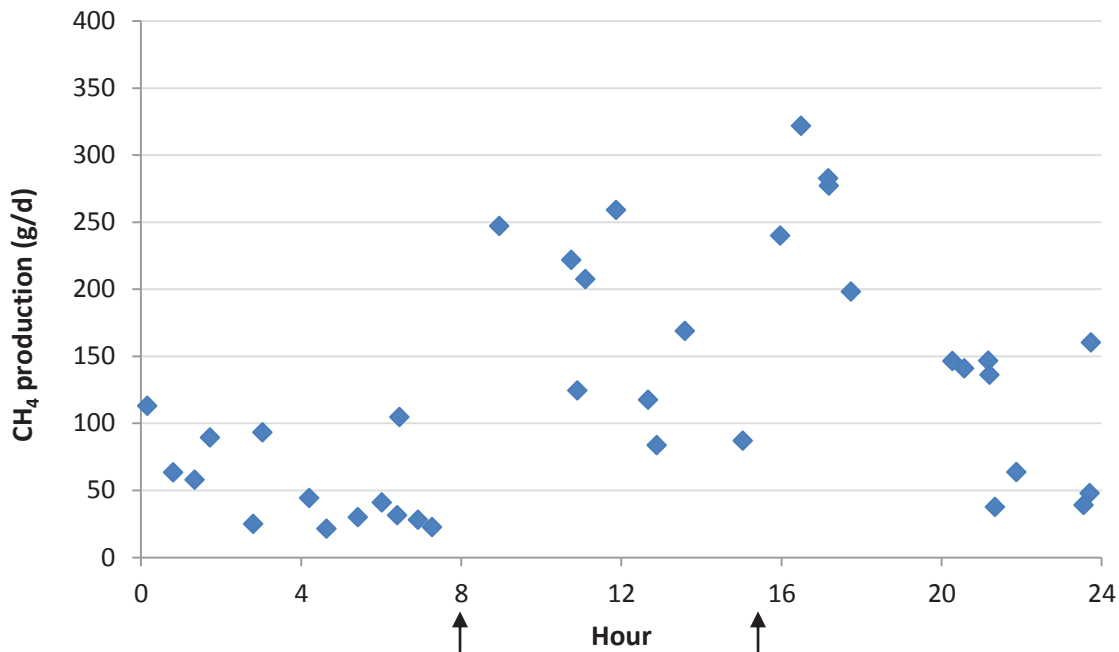


Figure C6: Circadian variation in CH₄ production estimates made over 8 days as estimated from the Greenfeed unit for Cow 3696. Feeding times are indicated by the arrows. Zero on the x-axis represents midnight, while 12 represents midday.

6.4 APPENDIX D: CIRCADIAN VARIATION IN RUMEN TEMPERATURE AND pH FROM FISTULATED COWS

Figures D1 to D4 show similar patterns for rumen temperature and pH for all four fistulated cows. The rumen temperature and pH occur in opposite directions which was also reported by AlZahal *et al.* (2009). This was to be expected because the end-products of fermentation are VFA, CH₄ and heat (Hungate, 1966), thus a low rumen pH (due to VFA production) complemented by a high rumen temperature would be expected. In general, there was a decrease in rumen temperature in the morning until midday followed by an increase through until midnight, interrupted by a trough in late afternoon. The pattern of rumen pH increased in the morning followed by a gradual decrease from midday until midnight.

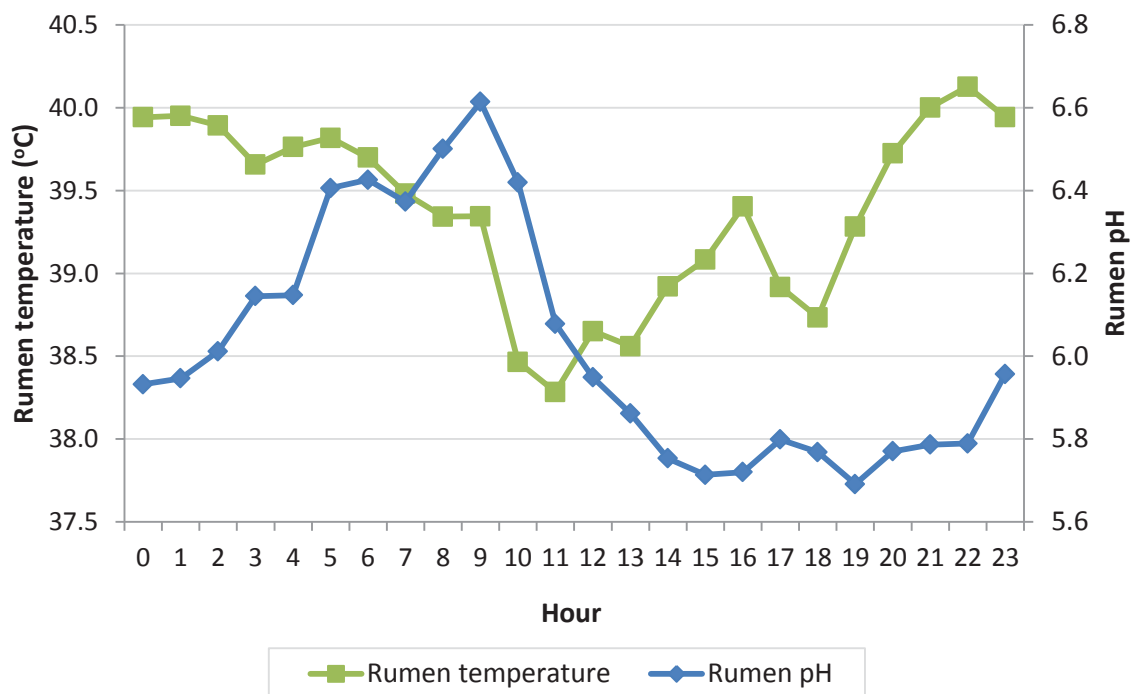


Figure D1: Circadian variation in rumen temperature and pH for Cow 2176 based on Kahne bolus data.

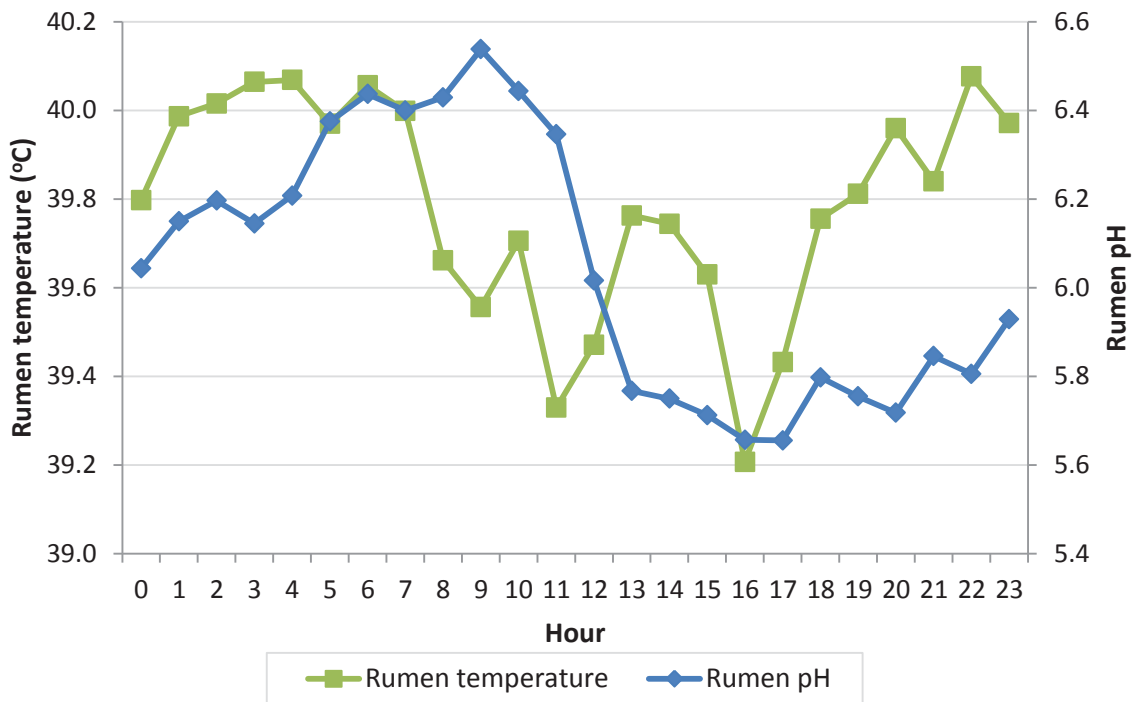


Figure D2: Circadian variation in rumen temperature and pH for Cow 2043 based on Kahne bolus data.

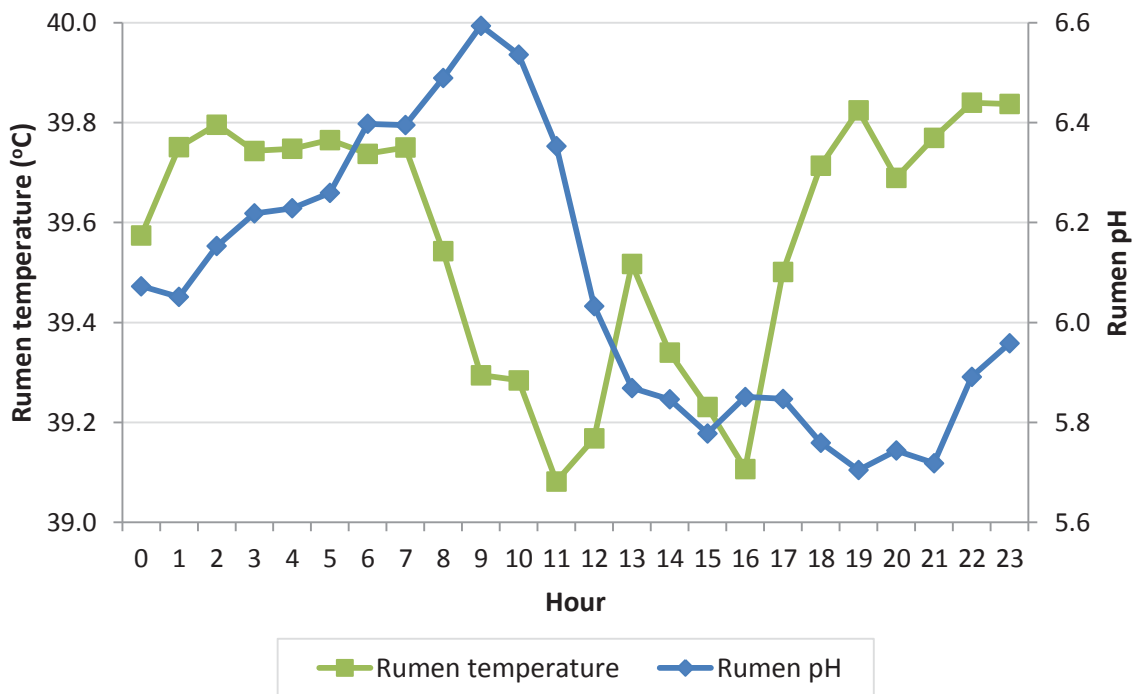


Figure D3: Circadian variation in rumen temperature and pH for Cow 2033 based on Kahne bolus data.

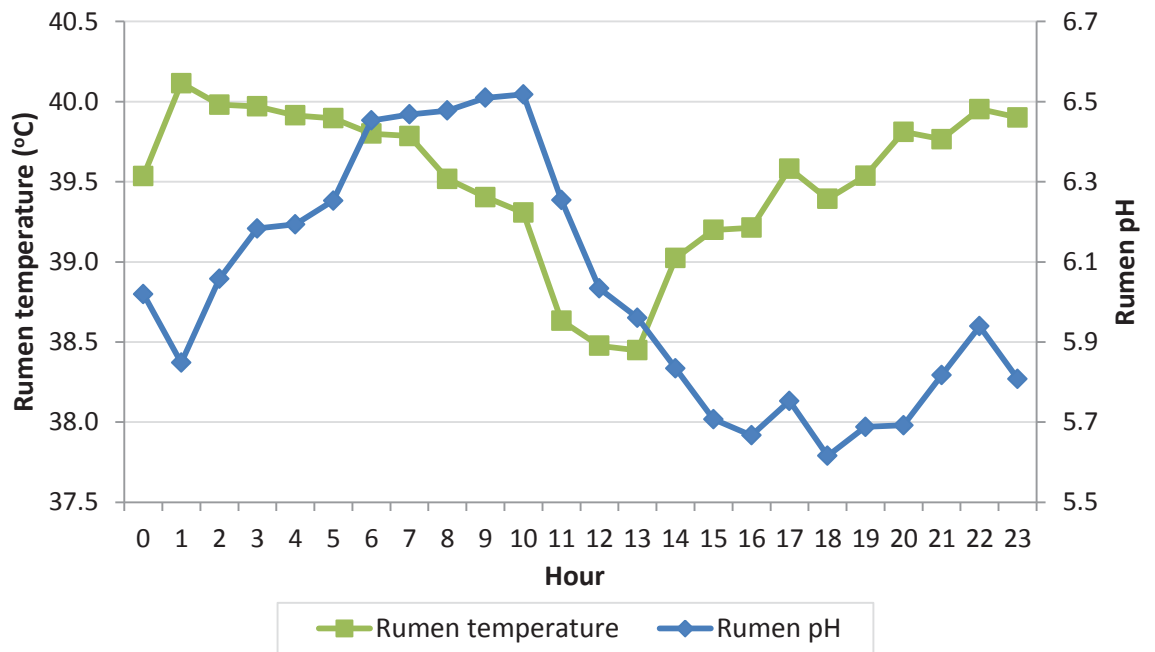


Figure D4: Circadian variation in rumen temperature and pH for Cow 2424 based on Kahne bolus data.

6.5: APPENDIX E: METHANE PRODUCTION AS THE EXPERIMENT PROGRESSED

Data for individual cows illustrate trends over the measurement period, with measures of variance. Similar data are presented in Table E2, but with progressively more days included in the analyses. These values may be used to define the optimal length of measurement period required to obtain defensible data.

Table E1: Mean CH₄ production (g/d) ± SD and CV for the 14 cows over 10-day increments (Data from fistulates and cows with less than 2 visits providing useful data/day were excluded). Table includes Group 1 cows only.

Day	COW														Mean
	2013	2152	2193	2195	2247	2328	2330	2395	2473	3716	3719	3720	3812	4749	
Mean															
1-10	288.1	NA	352.4	354.7	NA	297.2	458.1	341.4	309.2	333.7	NA	542.0	248.0	310.8	348.7
11-20	277.4	264.9	341.4	374.0	317.5	291.1	441.4	304.5	310.8	329.7	394.7	359.6	243.6	336.5	325.7
21-30	285.2	278.5	348.1	361.3	333.7	304.5	434.0	316.4	329.4	322.5	385.1	377.3	246.6	343.7	339.3
31-38	332.0	315.1	411.9	352.9	324.4	340.1	472.7	326.8	340.5	331.2	420.7	411.7	255.4	329.6	354.6
All days	298.0	289.9	361.4	359.3	330.2	312.3	443.7	318.8	332.6	324.5	396.2	387.6	248.7	338.5	338.7
SD															
1-10	37.9	NA	78.8	54.6	NA	32.6	50.7	3.6	NA	51.4	NA	NA	14.0	38.6	40.2
11-20	22.6	29.9	39.8	43.1	85.0	50.0	37.1	30.2	37.8	61.0	71.4	34.6	53.3	44.3	45.8
21-30	38.6	33.0	60.5	40.0	53.3	37.5	50.2	54.3	60.1	48.4	47.4	42.0	36.7	37.4	45.7
31-38	23.6	41.7	21.9	34.4	25.5	30.8	32.9	19.0	30.6	45.8	46.1	25.4	41.1	54.8	33.8
All days	37.5	42.2	60.2	44.1	58.9	47.6	48.8	36.9	48.5	49.9	58.6	50.2	37.5	48.6	47.8
CV															
1-10	13.1	NA	22.4	15.4	NA	11.0	11.1	1.1	NA	15.4	NA	NA	5.7	12.4	11.9
11-20	8.4	11.3	11.9	11.1	26.8	17.6	8.7	10.0	12.2	18.7	18.1	9.9	22.4	12.6	14.3
21-30	12.8	11.3	16.6	11.8	15.5	11.3	12.1	16.3	17.4	15.6	12.7	10.6	14.8	10.6	13.5
31-38	7.1	13.2	5.3	9.7	7.9	9.1	7.0	5.8	9.0	13.8	11.0	6.2	16.1	16.6	9.8
All days	12.6	14.5	16.7	12.3	17.8	15.2	11.0	11.6	14.6	15.4	14.8	12.9	15.1	14.4	14.2

SD; standard deviation, CV; coefficient of variation, NA; not available as cows had not used the Greenfeed unit sufficiently to obtain useful data

Table E2: Mean CH₄ production (g/d) ± SD and CV for the 14 cows over 10-day cumulative increments (Data from fistulates and cows with less than 2 visits providing useful data/day were excluded). Table includes Group 1 cows only.

Day	Cow														Mean		
	2013	2152	2193	2195	2247	2328	2330	2395	2473	3716	3719	3720	3812	4749			
Mean																	
1-10	288.1	NA	352.4	354.7	NA	297.2	458.1	341.4	309.2	333.7	NA	542.0	248.0	310.8			348.7
1-20	277.4	264.9	341.4	374.0	317.5	291.1	441.4	304.5	310.8	329.7	394.7	359.6	243.6	336.5			327.7
1-30	285.2	278.5	348.1	361.3	333.7	304.5	434	316.4	329.4	322.5	385.1	377.3	246.6	343.7			333.3
All days	298.0	289.9	361.4	359.3	330.2	312.3	443.7	318.8	332.6	324.5	396.2	387.6	248.7	338.5			338.7
SD																	
1-10	37.9	NA	78.8	54.6	NA	32.6	50.7	3.6	NA	51.4	NA	NA	14.0	38.6			40.2
1-20	32.1	29.9	58.3	49.3	85.0	85.0	44.9	30.2	35.9	55.3	71.4	65.1	41.8	49.8			52.4
1-30	35.1	37.1	58.7	47.0	69.9	47.4	47.5	41.7	54.0	51.9	58.3	55.5	37.3	46.6			49.1
All days	37.5	42.2	60.2	44.1	58.9	47.6	48.8	36.9	48.5	49.9	58.6	50.2	37.5	48.6			47.8
CV																	
1-10	13.1	NA	22.4	15.4	NA	11.0	11.1	1.1	NA	15.4	NA	NA	5.7	12.4			11.9
1-20	11.6	11.3	17.1	13.2	26.8	29.2	10.2	9.9	11.6	16.8	18.1	18.1	17.1	14.8			16.1
1-30	12.3	13.3	16.9	13.0	20.9	15.6	10.9	13.2	16.4	16.1	15.1	14.7	15.1	13.6			14.8
All days	12.6	14.5	16.7	12.3	17.8	15.2	11.0	11.6	14.6	15.4	14.8	12.9	15.1	14.4			14.2

NA; not available as cows had not used the Greenfeed unit sufficiently to obtain useful data

CHAPTER SEVEN

REFERENCES

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