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Quantifying daily methane production of beef cattle from multiple short-term measures using the GreenFeed system

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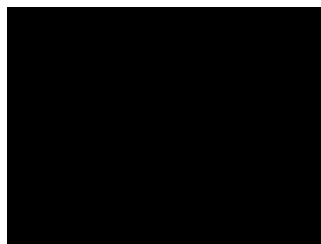
of the University of New England

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and it is not currently being submitted for any other degree.

I certify that, to the best of my knowledge, any help received in preparing this thesis and all sources used have been acknowledged in this thesis.

José Ignacio Velazco

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When everything seems to be going against you, remember that the airplane takes off against the wind, not with it.

Henry Ford (1863 – 1947)

Executive Summary

> On-farm CH_4 emissions have been identified as the largest contributors to the carbon footprint of livestock production systems. A requirement to quantify on-farm mitigation under commercial production conditions and a desire to establish the phenotype of thousands of ruminants for breeding programs, has fueled the development of techniques to estimate daily methane production (DMP) from short-term measures of methane concentration or methane flux.The accuracy, precision and applicability of these methods has been largely untested and forms the susbtance of this thesis.

> In assessing the accuracy of short-term emissions measures to estimate DMP, a high level of concordance was observed between DMP measured over 24h in a respiration chamber (RC) and estimated from multiple short-term measurement estimates using the GreenFeed Emission Monitoring system (GEM). Three independent experiments comparing DMP confirmed that estimates between methods differ by 5% to 8% (P>0.05). This implies that multiple short-term measures of emission rates are complementary to and consistent with respiration chamber-derived measures, providing capability to measure a greater number of animals, potentially in their production environment over extended periods of time.

> Methane yields (MY; g CH_4/kg DMI) were also derived based on multiple short-term emission measures, with results consistently within 10% of those calculated based on 24 h RC data. The overall MY of animals consuming roughages was 21.8 g CH_4/kg DMI using GEM data, in keeping with the 22.3 g CH_4/kg DMI average in the literature. That implies that GEM units can not only accurately estimate DMP of cattle but also support accurate MY estimates that can be used in quantifying livestock emissions for national greenhouse inventory calculations.

> Regarding the applicabilty of short-term emissions, the level of methane mitigation resulting from a reduction in the number of animals in a herd as quantified by GEM was comparable with that found by open path FTIR and RC (40%, 41% and 37% abatement detected by the 3 methods respectively). However, it was found caution is needed if the mitigation strategy affects the daily intake pattern of the animal. In a study of inclusion of nitrate salts in the total mixed ration, nitrate was found to increase the feeding frequency and reduce the meal weight (P<0.05) and it was considered this change could bias the estimated DMP as nitrate affected the interval between feed consumption and measurement by GEM.

>   In a further application of GEM to quantify mitigation, DMP and emissions intensity of grazing beef cattle genetically divergent for residual feed intake were studied using the GEM. It was concluded that faster daily growth by cattle was accompanied by lower methane intensity but only 39 of the 64 possible users regularly accessed the GEM, making the comparison weaker than expected from the original design.

>   Exhaustive analysis of feeding data (timing and weight of all meals in previous 72h) for each of 3048 short-term methane emission measures showed that, unlike the average emission rate over days, the short-term emission rate cannot be readily explained by feeding history, with meal size and timing explaining no more than 17% of the variance in spot emission rate. Consequently continuous monitoring of emitted gas over 24h may be required to verify mitigation from strategies that affect feeding behaviour such as nitrate.

>   As there is little literature around variance structure of short-term emission measures, power analysis was undertaken to determine the measurement requirements to detect a 10% difference in DMP between two treatment groups in a feedlot situation. A minimum of 10 animals per treatment measured twice a day over at least 30 days was required to detect 10% difference with 95% confidence based on the calcualted variance structure. By doubling the number of animals, the minimum duration of the test could be reduced to 20 days implying that duration and number of animals per treatment can readily be interchanged according to the budget and logistics without affecting power. Collection of more than 2 short-term measures/animal/d does little to improve the accuracy of the estimate as between-day variance is higher than within-day variance in short-term emission rate based on the calculated variance structure.

>   Further analysis identified that defining the methane emission phenotype of an animal (as may be required to develop estimated breeding values) to within 10% of the long term (64d) mean required from 60 to 70 consecutive short-term measures. This is compatible with recommended duration of feed efficiency tests, implying that quantifying methane traits during feed efficiency tests is feasible.

>   The proven accuracy of multiple short-term methane emission measurements in the estimation of DMP allows their use in verification of greenhouse gases inventories as well as statistical verification of mitigation claims, and offers capability to measure a greater number of animals in their production environment over extended periods of time than possible with other methods.

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Frequently Used Abbreviations

AA amino acid

Ac:Pr acetate:propionate ratio

ADF acid detergent fibre

ADG average daily gain

AEC animal ethics committee

AFIA Australian Fodder Industry Association

bLS backward Lagrangian Stochastic

BCM bromochloromethane

CH$_4$ methane

CI consumption index

CO$_2$ carbon dioxide

CO$_2$-eq carbon dioxide equivalent

CP crude protein

CSIRO Commonwealth Scientific and Industrial Research Organisation

CV coefficient of variation

DE digestible energy

DM dry matter

DMI dry matter intake

DMP daily methane production

DoE Department of the Environment, Australian government

XX

DOMD digestibility of the organic matter

DPI Department of Primary Industries, NSW Australia

EBV estimated breeding value

EE ether extract

Ei emission intensity

FAO Food and Agriculture Organization of the United Nations

FTIR fourier transform infrared

GE gross energy

GEI gross energy intake

GEM GreenFeed Emissions Monitor

GHG greenhouse gas (in this document, GHG refers primarily to CH_4 and N_2O)

GWP global warming potential

H$_2$ hydrogen

HFC hydrofluorocarbons

HRFI high EBV for residual feed intake

IPCC Intergovernmental Panel on Climate Change

LCA life cycle assessment

LI level of intake

LRFI low EBV for residual feed intake

LWG liveweight gain

ME metabolizable energy

MetHb methaemoglobin

MY methane yield (methane produced per kg of DMI)

N nitrogen

NDF neutral-detergent fibre

NH$_3$ ammonia

NIRS near-infrared reflectance spectroscopy

N$_2$O nitrous oxide

NO$_3$ nitrate

NPN non-protein N

OM organic matter

PFC perfluorocarbons

RC open circuit respiration chamber

REML residual maximum likelihood

RMP residual methane production

RFI residual feed intake

SD standard deviation

SE standard error

SEM standard error of the mean

SF$_6$ sulphur hexafluoride

THM Total Herd Methane emission

TMR total mixed ration

VFA volatile fatty acid

Ym CH_4 energy emitted as percent of gross energy ingested

Chapter One

GENERAL INTRODUCTION

1. General introduction

1.1. Anthropogenic greenhouse gas emissions

The accumulation of greenhouse gases (GHG) in the atmosphere is considered a major contributor to global warming (IPCC, 2013). Most recent atmospheric GHG changes can be attributed directly to human activity with the most dramatic increase after the industrial revolution of the late 1700s and early 1800s (Figure 1-1; Forster *et al.*, 2007). The industrial revolution was a period during which predominantly agrarian, rural societies in Europe and North America became industrial and urban and this phenomenon is happening currently in under-developed economies (Bruinsma, 2003).
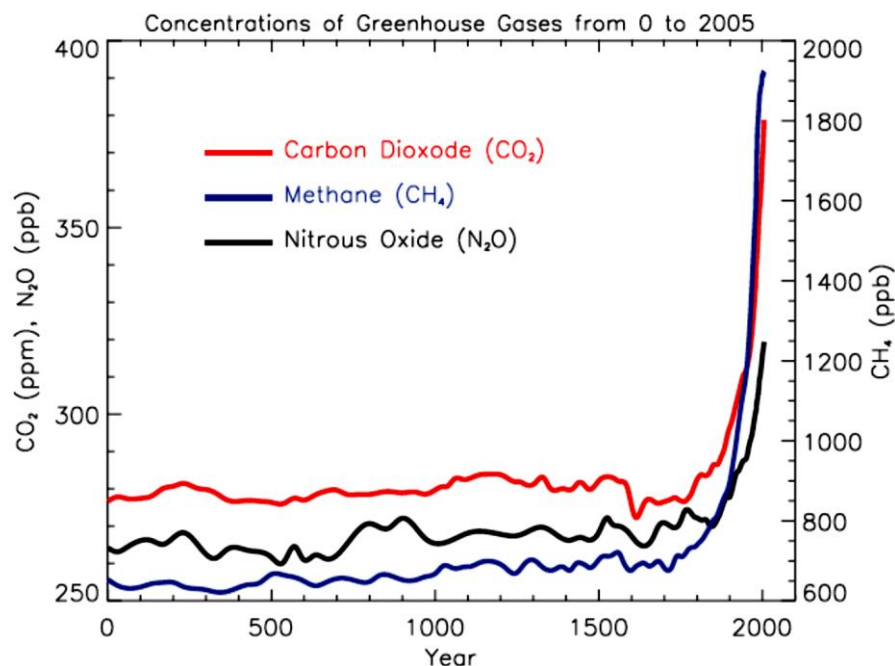
Figure 1-1 Evolution of atmospheric concentrations of the most relevant GHG (CO_2 in ppm and CH_4 and N_2O in ppb) over the last 2000 years. (Source: Forster *et al.*, 2007)

Non-CO_2 gases with the capability of absorbing and re-emitting radiation back to earth's surface include methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFC), perfluorocarbons (PFC) and sulphur hexafluoride (SF_6). The GWP (CO_2-equivalents) of each individual GHG has being determined by the IPCC (2013) and represents their radiative properties and atmospheric lifetime relative to an equal weight of CO_2. GWP of each of these gases based on a 100 year time horizon is presented in Table 1-1.

Table 1-1 Principal greenhouse gases, their atmospheric lifetimes and their global warming potential (GWP). Source IPCC (2013).

Gas name	Formula	Lifetime (yr) [#]	100-yr GWP [@]
Carbon dioxide	CO_2	50-200	1
Methane	CH_4	12	25
Nitrous oxide	N_2O	114	310
Perfluoromethane	CF_4	50,000	7,390
HFC-23	CHF_3	270	14,800
Sulphur hexafluoride	SF_6	3,200	22,800

[#] total atmospheric burden divided by the mean global sink of a gas in steady state
[@] global warming potential expressed on a weight basis relative to an equivalent weight of CO_2

Methane from natural and anthropogenic sources makes the second largest contribution to atmospheric GHG related global warming. The main natural methane sources include wetlands, termites and the oceans. Natural sources account for approximately one third of the total emissions. Important anthropogenic sources include landfills, livestock farming, as well as the production, transportation and use of fossil fuels. Human-related sources account for the majority (two thirds) of total methane emissions increasing by 2.2%/year over 2000 – 2010 (IPCC, 2013). Atmospheric concentration of CH_4 is determined

by the balance between surface emissions and photochemical destruction by the hydroxyl radical, the major atmospheric oxidant (Bousquet et $al.$, 2006). The global financial crisis in 2007/2008 only temporarily reduced emissions and the atmospheric CH_4 concentration (Forster et $al.$, 2007). According to Steinfeld et $al.$ (2006), agricultural activities account for 37% of the anthropogenic CH_4 emissions with CH_4 from ruminants' enteric fermentation the main single source.

Agriculture is also a major anthropogenic N_2O source (dramatically increased by industrial revolution as evident in Figure 1-1 due to usage of synthetic fertilizers, the combustion of fossil fuel for transportation, and nylon production (IPCC, 2006).

In summary, at 7-18% of total anthropogenic GHG emissions (depending on the accounting approach), ruminant livestock and cattle in particular are substantial contributors to global warming (Hristov et al., 2013). The short atmospheric lifetime of methane makes it a desirable target for mitigation as reduced methane production will rapidly result in diminishing of methane concentration and of its warming contribution.

1.1.1. The contribution of domesticated ruminants to global warming

Life cycle analysis shows the livestock supply chain accounts for approximately 18% (Steinfeld et $al.$, 2006) of total anthropogenic greenhouse gases (GHG), while direct on-farm emissions account for approximately half of that (8 - 10%, O'Mara, 2011). In all assessments, CH_4 represents the largest single source of livestock derived GHG and makes the second largest contribution to current global warming after CO_2. The global warming potential (GWP) or

relative radiative forcing of methane is 25 times that of an equal weight of CO_2 when considered over a 100 year time horizon. The relatively short atmospheric residence time of methane (12 years) means (a) modifying its emission will offer a more rapid change in global warming than modifying emissions of (the relatively long-lived) CO_2 and (b) its GWP increases when the time–horizon over which GWP is estimated is reduced.

Among domesticated ruminants', cattle are not only the most numerous (FAOSTAT, 2012) but also the largest and consequently make a greater contribution to global enteric CH_4 emissions (Table 1-2). When compared by region, almost 24% of the cattle are in South America and over 20% in Africa. Both regional distributions show a lower per-head emission from cattle in developing countries (Table 1-2), but due to their lower productivity a higher CH_4 emission intensity (Ei; emissions per unit of product) may also be expected (McCrabb *et al.,* 1999).

Table 1-2 Regional distribution of the global population (million head) and annual total (Mt CO_2-equivalents) and (derived) per head enteric CH_4 emissions t CO_2-equivalents/head/year (CO_2eq/hd) of cattle, buffalo, goat and sheep based on FAOSTAT 2012.

	Cattle			Buffalo			Goat			Sheep		
	n	CO_2eq	CO_2eq /hd	n	CO_2eq	CO_2eq /hd	n	CO_2eq	CO_2eq /hd	n	CO_2eq	CO_2eq /hd
Africa	298	215	0.72	4	4.8	1.14	346	36.3	0.1	322	33.8	0.1
South America	347	420	1.21	1	1.5	1.15	21	2.2	0.1	68	7.1	0.1
India#	213	150	0.7	114	132	1.16	160	16.8	0.11	75	7.9	0.11
China	114	118	1.04	23	27	1.17	183	19.2	0.1	183	19.2	0.1
North America	103	131	1.27	0	0	-	3	0.3	0.1	6	1.1	0.18
Aust/NZ	39	53	1.36	0	0	-	4	0.4	0.1	106	17.8	0.17
Europe	122	187	1.53	0.4	0.4	1	17	1.7	0.1	129	21.7	0.17
EU	88	133	1.51	0.4	0.4	1	13	1.3	0.1	98	16.5	0.17
World	1,479	1,518	1.03	198	229	1.16	993	104	0.1	1,167	138	0.12

FAO (2010) estimate.
World values are greater than the sum of rows as not all countries are included in this abbreviated table

Developed countries produced 46.3% of the ruminant milk and meat (calculated on an energy basis) but contributed only 25.5% of enteric CH_4 emissions in 2005 (O'Mara, 2011). A similar amount of milk and meat energy was produced in Latin America, Africa and Asia but with greater emission intensity (2.7 times higher Ei). This fact reinforces the need to target enteric CH_4 as part of a global strategy to mitigate GHG while recognising the major social and economic role of livestock beyond productivity. Mitigation from developing countries (while keeping production constant) accounts for about 70% of the global technical mitigation potential from agriculture (Hristov *et al.*, 2013).

As a direct consequence of the increasing expected demand for ruminant derived products and the consequent rise in GHG emissions (Hegarty, 2012), research is now focused on the development of mitigation strategies to reduce the Ei of animal products. Milk, beef meat and sheep meat production are expected to increase by 82%, 72%, and 110% respectively by 2050 compared with 2000 (O'Mara, 2011), likely increasing livestock GHG emissions. Methane intensity (Ei; gCH_4/kg ADG) may need to be decreased by corresponding proportions to avoid increasing ruminant contribution to global warming. Mitigation strategies will need to ideally deliver concurrent productivity increases (edible products per animal per day) where possible to reduce Ei. Improved forage quality, feeding management and the application of genetic selection may play key roles in the abatement of GHG emissions (see Section 4). In the absence of effective mitigation strategies, a large increase in livestock GHG emissions should be expected (IPCC, 2013).

1.1.2.　National inventories and targets

The United Nations Framework Convention on Climate Change (UNFCCC) was formed in 1992 to cooperatively address global warming and its potential impact. All participant countries recognized the complexity of global warming and almost 200 nations signed (1997) and ratified (2002) the Kyoto protocol which commits its members by internationally binding emission reduction targets (Oberthür and Ott, 1999).

Most industrialized countries accepted an obligation to reduce their GHG emissions to 5% below their 1990 levels by 2012. During the second commitment period (2013 – 2020), industrialized countries that are members of the OECD committed to reduce GHG emissions by at least 18 % below 1990 levels in the eight-year period (revised negotiation, 2009). Such targets require an ability to measure emissions and quantify mitigation.

The ability of the international community to prevent and reduce dangerous human-induced interference with the climate system is dependent on an accurate knowledge of GHG emissions trends, and the collective ability to alter these trends (UNFCCC, 2012).

Australia's emissions arising directly or indirectly from livestock production are classified in 'agriculture', and enteric methane is a major source quantified in that category (DoE, 2014).

1.2. **Methane production in the rumen**

The rumen supports a complex anaerobic ecosystem where organic materials are fermented to provide end-products to meet the animals' nutritional requirements. Ruminants depend on this microbial fermentation to catabolise complex carbohydrates in forages which cannot be broken down by mammalian digestive enzymes (McDonald *et al.*, 2011). The end-products of the ruminal fermentation are volatile fatty acids (VFA; principally acetic, butyric and propionic acids), CO_2, microbial cells and heat. During the formation of the total VFA, there is a net release of hydrogen with the residual being used by methanogenic archaea to reduce CO_2 (equation 1; Czerkawski, 1968). Understanding of the hydrogen yield of the component reactions (Figure 1-2 and equations 3,4,5) enables methane production to be calculated from residual hydrogen based on stoichiometry after direct measurement of individual VFA production rates (Leng, 1970), or stoichiometrically using VFA proportions and assumptions of feed material fermented and partitioning into cells or VFA.

Equation 1   Methane synthesis $CO_2 + 4H_2 \rightarrow CH_4 + 2 H_2O$

Equation 2   Propionate synthesis $C_6H_{12}O_6 + \mathbf{2H_2} \rightarrow 2C_3H_5O_2^- + 2H^+ + 2H_2O$

Equation 3   Butyrate synthesis $C_6H_{12}O_6 \rightarrow C_4H_7O_2^- + H^+ + \mathbf{2H_2} + 2CO_2$

Equation 4   Acetate synthesis $C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_3O_2^- + 2CO_2 + \mathbf{4H_2} + 2H^+$

0.5 Glucose

0.5 ATP
0.5 ATP

(A) NAD+
NADH+

ATP

PEP ·······PEP carboxykinase······· GDP
GTP

CO₂

NAD+ NADH
(g)

Lactate ← Pyruvate ATP → Oxaloacetate

Lactyl-CoA

CO₂ + H₂ (B#)

CO₂

H₂O 2 H₂O 4H₂
(J)

Acrylyl-CoA CH₄ Acetyl-CoA ×2

NADH
(e) NAD+

Malate

2H (H)
2H (I)

Acetyl-PO₄

H₂O

Fumarate

FADH ATP
(F) FAD+ ADP

Propionyl-CoA

ATP

Acetate CO₂ Succinate

Acetoacetyl-CoA

Propionyl-CoA CoA

NADH
(c)

NAD+

Propionate

Propionate

β Hydroxybutyrl-CoA

H₂O

Crotonyl + CoA

NADH

(d) NAD+

Butyrl-CoA

CoA
Butyrl-PO₄

ADP

ATP

Butyrate

Hydrogen balance/mole of product
Hydrogen (2H) produced : Hydrogen (2H) consumed
2A + P + 4B : 4M + 2B + 2P*
(AB) (A) 2(A)+2(B) : 4(J) + (C)+(D) + [(EF) OR (GHI)]
*: THERE ARE 2 PROPIONATE SYNTHESIS PATHWAYS BUT ONLY THE SUCCINATE PATH IS THOUGHT SIGNIFICANT
#: CO₂ AND H₂ ARE EITHER FORMED DIRECTLY OR BY RAPID DECOMPOSITION OF FORMATE

Figure 1-2 Microbial fermentation of organic matter (Glucose) in the rumen showing H_2 production and using reactions and net fermentation balance. (Source: Hegarty and Nolan 2007)

Consequently an inverse relationship between ruminal propionate proportion and methane production is often observed, with Moss *et al.* (2000) reporting a high correlation (r^2=0.78) between the ratio [(acetate + butyrate) / propionate] and CH_4 production, confirming that propionogenesis and methanogenesis are competing processes in the rumen. However, this stoichiometric approach to predicting CH_4 production is simplistic and methanogens using formate, ethanol, acetate and other methyl donors are known (McAllister *et al.*, 1996; Loughnan *et al.*, 2014). Further, a lower pH results in a higher proportion of propionate production due to changes in the more active species in the rumen and consequently less CH_4 is produced (Russell and Wilson, 1996).

Methane can also be produced in the lower digestive tract (hindgut). The proportion of CH_4 derived from the hindgut increases with feeding level but most of this CH_4 is excreted via the lungs after being absorbed into the blood (Murray *et al.*, 1978). Independent of where in the digestive tract the CH_4 is formed, almost all CH_4 is released via eructation and exhalation with 97.5% of CH_4 emission via the oesophagus and lungs and only 2.5% via flatus (Murray *et al.,* 1976)

While measures of rumen fermentation cannot be expected to perfectly predict daily methane production (DMP), biological associations exist that allow generic relationships between feed chemical composition and daily methane production to be established (e.g. Pelchen and Peters, 1998). For crude prediction, feed composition can be disregarded and DMP can be estimated from daily feed intake or feed energy intake. This is the approach used in IPCC Tier 2

methodology of estimating DMP as 6.5% of the gross energy intake of roughage-fed ruminants (IPCC 2006).

1.2.1.   Feed intake and methane production

Multi-factor regressions have been extensively used to relate DMP and DMI (as a proportion of energy intake or per unit feed intake). Initial analysis by Blaxter and Clapperton (1965) using 615 experiments in cattle and sheep, 55 diets, with 2500 determinations of DMP, concluded that the proportion of dietary energy lost as CH_4 increases as intake increases, but at a declining rate. Extensive investigations have followed to identify the ruminal and substrate factors responsible for this emission sensitivity (e.g. rumen pH, ADF:NDF, rumen retention time: Pelchen and Peters, 1998; Moss *et al.*, 2000; Barnett, 2013). However empirical relationships continue to be needed to estimate DMP (in g/d or MJ/d) from readily available data. The complexity of different approaches is evidenced in Table 1-3 (expanded from Storm *et al.*, 2012).

Table 1-3 A sample of predictive methane equations reported in the literature.

Reference	Equation
Kriss (1930)	CH_4 (MJ/d) = 75.42 + 94.28 × DMI (kg/d) × 0.05524 (MJ/g of CH_4)
Axelsson (1949)	CH_4 (MJ/d) = −2.07 + 2.636 × DMI (kg/d) − 0.105 × DMI (kg/d)^2
Blaxter and Clapperton (1965)	CH_4 (Kcal/100 Kcal feed) =1.30 + 0.112*D + L(2.37-0.050D)
Moe and Tyrrell (1979)	CH_4 (MJ/d) = 0.341 + 0.511 × NSC (kg/d) + 1.74 × HC (kg/d) + 2.652 × CEL (kg/d)
Kirchgessner et al. (1994)	CH_4 (g/d) = 63 + 79 × CF + 10 × NFE + 26 × CP - 212 × Cfat (kg/d)
Mills et al. (2003)	CH_4 (MJ/d) = 0.07 × ME (MJ/d) + 8.25
Mills et al. (2003)	CH_4 (MJ/d) = 0.92 × DMI (kg/d) + 5.93
Mills et al. (2003)	CH_4 (MJ/d) = 10.3 × forage (%) + 0.87 × DMI (kg/d) + 1.1
IPCC (2006)	CH_4 (kg/d) = GE (MJ/d) × Ym/55.65
Yan et al. (2006)	CH_4 (L/d) = 47.8 × DMI - 0.76 × DMI^2 - 41 (kg/d)
Yan et al. (2006)	CH_4 (L/d) = 0.34 × BW (kg) + 19.7 × DMI (kg/d) + 12
Jentsch et al. (2007)[#]	CH_4 (kJ/d) = 1.62 × d_CP - 0.38 × d_Cfat + 3.78 × d_CF + 1.49 × d_NFE +1142 (g/d)
Ellis et al. (2007)	CH_4 (MJ/d) = 0.14 × forage (%) + 8.6
Grainger et al. (2007)	CH_4 (g/d) = 18.5 × DMI (kg/d) - 9.5
Yan et al. (2009)	CH_4(L/d) = 1.959 x GEI 9MJ/d)+ 8.8
Kennedy and Charmley (2012)	CH_4 (g/d) = 19.6 x DMI (kg/d)

DMI=dry matter intake; D=digestibility of the diet at the maintenance level of feeding; L=level of feeding; NSC=non-structural carbohydrate; HC=hemicellulose; CEL=cellulose; CF=crude fibre; NFE=N-free extract; CP=crude protein; Cfat=crude fat; GE=gross energy; Ym=emission factor (MJ/100 MJ GE); BW=body weight; # d_ indicates the equation is based on digested amounts of each fraction

One of the limitations of predictive methane equations is the lack of agreement when models are applied to data beyond that used in their development, leading to large differences in their CH_4 estimates. For instance, Blaxter and Clapperton (1965) and Moe and Tyrrell (1979) show heavy bias (Ellis et al., 2010; Benchaar et al., 1998) and more recent assessments have required reporting of bias and standard error of prediction instead of coefficient of determination (eg. Yan et al., 2009; McPhee and Hegarty, 2008). However, all previously mentioned equations suffer from the limitation of requiring data that is not always available (i.e. digestibility of the feed, DMI, chemical composition of the diet).

1.2.2. Feed characteristics and methane production

Subsequent to Blaxter and Clapperton's (1965) review, nutritional effects on CH_4 production have been extensively investigated and the reader is referred to these reviews (e.g. Demeyer and Van Nevel, 1975; Kirchgessner *et al.*, 1995; Pelchen and Peters, 1998; Beauchemin and McGinn, 2006, Cottle *et al.,* 2011, Ramin and Huhtanen, 2013, also see table 1-3). In overview, there is a general positive association between fibre level and MY when considered across a wide spectrum of feedstuffs. This reflects in associated positive relationships between roughage inclusion level and MY, and between indigestibility and MY. Conversely, increasing grain inclusion will reduce MY (and often DMP) as a result of changes in digesta kinetics, lower rumen pH, a high starch substrate predominantly used by propionate producing organisms. On high grain diets where rumen fill does not limit intake and pH may drop, MY is suppressed (Ramin and Huhtanen, 2013) but this trend is not apparent in pasture fed animals (Kennedy and Charmley, 2012). Johnson and Johnson (1995) identified energy losses (in relation to the energy intake) in the range of 2 to 3% when cattle were fed high grain diets *ad libitum* and 6 to 7% when forage was fed at the maintenance level.

In summary, the crude effects of diet composition (concentrate v roughage) are made visible in the IPCC (2006) recommendations for % energy loss as CH_4 (3 ± 1% for grain-based diets and 6.5 ± 1% for roughage-based diets). These differences result from differences in the balance of H_2-generating and H_2-using reactions in the rumen, and this balance itself is a consequence of the differing form of the substrate (starch v cellulose) and the rate of digesta passage through the rumen.

1.2.3.   Individual animal variation in methane production

Large variations in DMP occur in the commercial environment over seasons (Munger and Kreuzer, 2008). Day to day variation in CH_4 was calculated when cattle and sheep were fed consistent quantities of the same feed by Blaxter and Clapperton (1965) in 989 24h determinations of CH_4 production using respiration chambers. Coefficient of variation (CV, % of the mean loss of diet energy as CH_4) of daily production was 7.2%, being the same for sheep and cattle. When feed on offer was assigned by liveweight, between animal variation was 8.1% of the mean. More recently and using similar measurement techniques, Garnsworthy *et al.* (2012a) reported the same CV (7.2%) on dairy cows and Robinson *et al.* (2011) reported a slightly lower CV (6.8%) in sheep. Grainger *et al.* (2007), measuring supplemented Holstein-Friesian grazing lactating cows, found a lower within-animal between-days variation (4.3%) when measuring CH_4 production using respiration chambers and 6.1% when the SF_6 technique was applied. The SF_6 technique was also used by Boadi and Wittenberg (2002) in Holstein dry heifers fed *at libitum* and they found a much higher variation in the between-day (26.9%) and between-animal (26.6%) DMP. When control of the consumed feed is not possible, a larger CV of DMP should be expected.

Between-animal variation has also been determined using portable accumulation chambers (PAC). Robinson *et al.* (2011) compared 24 h respiration chambers with 2 h PAC measures for sheep finding the between-animal CV was 6.2% in the respiration chambers and 16.7% in the PAC. This suggests more sources of variation are affecting the short-term PAC estimates, although this was not evident in the CV reported by the short-term emission measured by

Garnsworthy *et al.* (2012a) for dairy cows which also contains variation in intake and time post-eating.

Between-animal variation in CH_4 production has been recently used by geneticists as a tool for genetic selection. Selective breeding of low CH_4 emitting animals will result in a permanent reduction of emissions but caution should be exercised since CH_4 phenotype has low to moderate heritability (Crowley *et al.*, 2010, de Hass *et al.,* 2011) and low to moderate repeatability (Robinson *et al.,* 2010). It would only be used if selected animals did not compromise their production characteristics (Pickering *et al.*, 2013). The determination of genetic parameters of CH_4 production requires the CH_4 phenotypes of a large number of animals be known (Hayes and Goddard, 2010) that is impractical with the existing methods to measure CH_4 emissions.

Variability in DMP and emission related to feed intake has implications for those methods based on short-term measures of CH_4. Daily variation in feed intake deserves considered as well as daily variation in diet composition (both factors of key relevance in the enteric methane production (Hristov *et al.*, 2013). The optimum sampling schedule would need to account for within-day variation (length of individual measures to capture a number of eructation events and daily sampling frequency to account for diurnal variation) and between-day variation (duration of the test to account for longer term variation).

1.3. Measurements techniques to determine CH₄ emissions from beef cattle

Quantification of CH_4 emissions from ruminants is currently required to verify national inventories, to evaluate mitigation strategies and to underpin genetic selection programs (Storm *et al.*, 2012; Hegarty, 2013). In this section, more frequently used techniques to measure CH_4 emissions are evaluated with emphasis on the GreenFeed Emissions Monitoring system (GEM). A distinction needs to made between individual animal measurement methods versus group-based measurement techniques. Each method has different optimum conditions for application and expertise to be used properly. The advantages and disadvantages of the different techniques are discussed in this section.

1.3.1. Measurement of CH₄ emissions from individual animals

Agricultural emissions have to be accurately measured and this measurement challenge has spawned two reliable techniques for estimating CH_4 emission from individual animals: enclosure techniques and the SF_6 tracer technique.

For over 100 years, CH_4 emission measurement has been made from enclosure techniques (indirect calorimetry using open-circuit respiration chambers; RC) and attributes of modern systems have recently being reviewed (Berndt *et al.*, 2014). Most of our current understanding of animal CH_4 production and energy metabolism has been based on respiration chamber studies (e.g. Blaxter, 1962). For measuring housed animals, respiration chambers provide accurate, precise and reliable measurements of DMP but all aspects of the

feeding environment are regulated. Setting up and operational costs and expertise requirements, together with limited capacity, restricts the number of animals studied by RC.

In the 1990's use of SF_6 as a tracer gas for enteric CH_4 was developed (Johnson *et al.,* 1994) and even simpler approaches to quantifying DMP on-farm are now being assessed. Some of these novel measurement techniques are the breath spot sampling during milking for dairy cows (Garnsworthy *et al.*, 2012a and 2012b), PAC used for sheep (Goopy *et al.,* 2011) and the GEM unit (Zimmerman, 2013) used for dairy and beef cattle and currently tested in sheep (R. Hegarty, pers. comm.). The most commonly used methods to measure CH_4 emissions from individual ruminants are described below.

Whole animal chambers

Respiration chambers (RC) had been developed and used mainly to study the animals' energy metabolism (Blaxter, 1962). Using the composition of the in- and out- flowing air, open-circuit respiration chambers can monitor CH_4 losses throughout the confinement period (1 to 3 days) in an almost continuous manner. Advantages of RCs include high accuracy in measuring DMP, high repeatability over consecutive days (Pinares-Patino *et al.*, 2013) and knowing the exact quantity and composition of the diet consumed during confinement and usually on preceding days. An illustrative diagram of a RC setup is shown in Figure 1-3. The RC has not only been used to support national livestock GHG inventories (Charmley *et al.,* 2015) but also to quantify the nutritional responsiveness of

enteric CH_4 (Pelchen and Peters, 1998) and the efficacy of chemical and biological approaches to mitigation (Beauchemin *et al.,* 2006; Herd *et al.*, 2014).

Little thought has been given to the constraints to DMP determined in RCs until recently. The high cost of establishing and operating RCs and their low throughput means that very few studies have used RCs to quantify genetic variation in DMP and MY (Pinares-Patino *et al.,* 2013; Herd *et al.*, 2014). The observation of Pinares-Patino *et al.* (2013) that the repeatability of DMP was 0.94 on adjacent days but only 0.55 if measured 2 weeks apart and 0.53 if measured a year apart has also raised questions on their appropriateness when a long-term methane phenotype is required for an animal (Pickering *et al.,* 2014). The realization that the artificial confinement and feed environment of the RC prevents diet selection and may invoke genetic differences in feeding behaviour (H. Oddy, Pers. Comm.) means there is an increasing focus on emissions monitoring in the paddock over days or weeks.
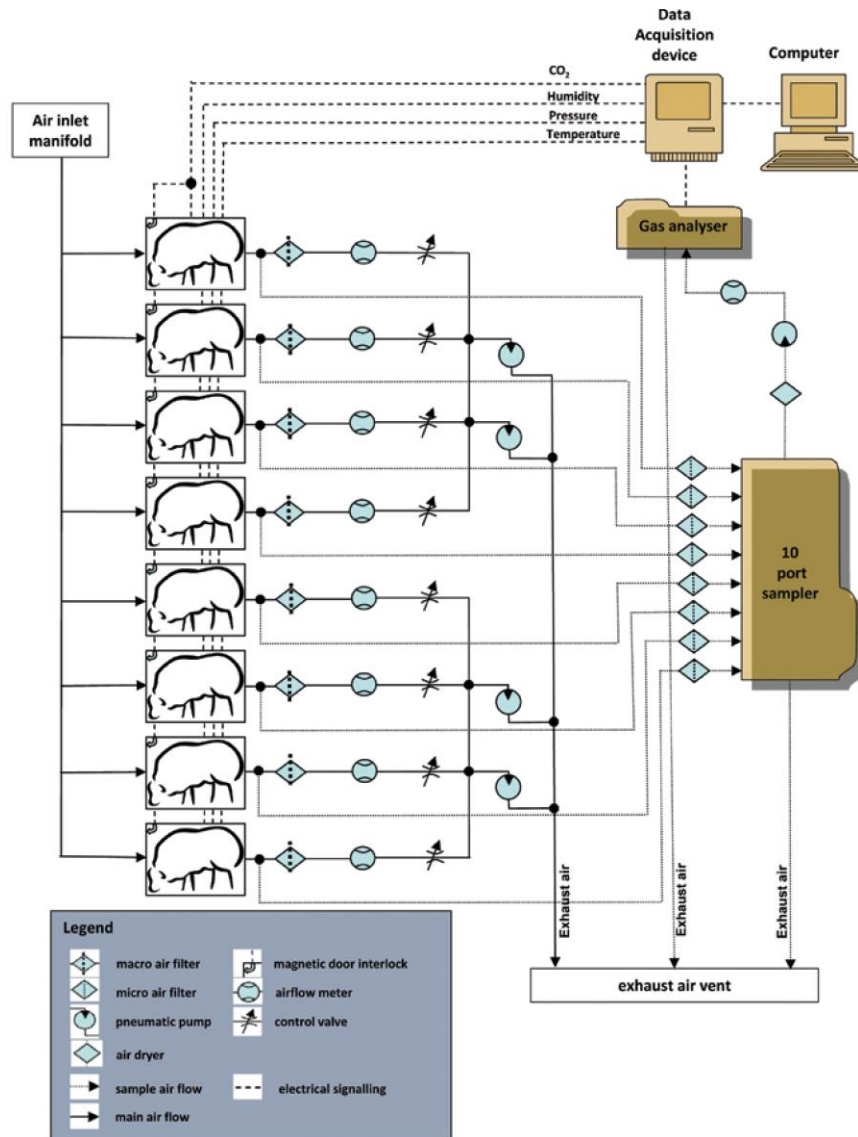
Figure 1-3 Diagram showing 8 open circuit respiration chamber. (Source: Pinares-Patino and Waghorn, 2014).

RC were used in the trials reported in this thesis in Chapters 2 and 3. A comprehensive description of the setting up, maintenance and operation can be consulted in the technical manual on RC design (Pinares-Patino and Waghorn, 2014).

Sulphur hexafluoride tracer technique

First described and patented in 1993, the sulphur hexafluoride (SF_6) tracer technique was developed to avoid the artificial environment of the RC and to allow investigation of energy usage in free-ranging cattle (Zimmerman, 1993). The method principle is that CH_4 emissions can be calculated if both the release rate of tracer and the tracer:CH_4 ratio are known (Johnson *et al.*, 1994; Figure 1-4). Several conditions must be met to ensure the accuracy and practicality of the tracer technique. The tracer gas must be non-toxic, physiologically inert, stable in rumen conditions and should mix with rumen gases as CH_4 does. The tracer must have a predictable and constant release rate and be detectable in low concentrations (Johnson *et al.,* 2007; Storm *et al.,* 2012). SF_6 was chosen as fulfilling those requirements, having a vapour pressure suitable for permeation tube release, having high molecular weight suitable for gravimetric release rate measurements and being detectable at parts per trillion in concentration (Machmuller and Hegarty, 2006).

The SF_6 is released in the rumen from a permeation tube at a constant rate. The breath gas collection system consists of a collection canister, a halter and capillary tubing placed on the nose of the animal and connected to the collection canister (Johnson *et al.*, 1994). The most common sampling time for this technique is 24-hour breath collection repeated over 3 to 5 consecutive days. Gas chromatography is used to determine the CH_4 and SF_6 concentrations in breath and background samples. Details can be consulted in Berndt *et al.*, 2014. A key component of the method is the permeation tube. The constant release of the tracer gas has been investigated and differences in permeation rate pre and

post experiment had been detected (Vlaming, 2008). Recently, Moate *et al.* (2013) found that Michaelis-Menten kinetics can be used to accurately describe the release rate of SF_6 from permeation tubes over extended time periods. Based on initial charge and pressure of SF_6 and initial release rate, they accurately predicted SF_6 permeation rates out to 600 days with the concomitant benefits of reducing costs, handling of the permeation tubes and all associated logistics. The permeation tubes are tested in the laboratory in dry air and it is assumed that the release rate remains the same when in the rumen. However, Vlaming (2008) reported a reduction of 6 to 11% in the release rate when comparing tubes placed in rumen fluid and in dry air.

Between and within animal variation in the estimates of CH_4 with the SF_6 technique are higher than those from respiration chambers (Pinares-Patino *et al.*, 2011). The higher variability in the results directly impacts on experimental design as more animals need to be measured for longer periods of time to detect differences between treatments and phenotypes. The most important constraints associated with the variability of the SF_6 technique are the poor implementation of the technique and/or the difficulty of accurately measuring SF_6 (Moate *et al.*, 2014).

With the objective of identifying and correcting substantial errors with the SF_6 technique, Deighton *et al.* (2014) demonstrated that when capillary-tube flow restrictors are used, the rate of sample collection declined and caused a bias of up to 15.6% in calculated methane emissions. A modification to the original SF_6 technique (using orifice plate flow restrictors to maintain a constant sample collection rate) minimised the decline in sample collection rate. When testing the

accuracy of the modified SF_6 technique, no differences in MY compared to RC (P=0.135) were found and methods gave a similar between-animal CV (6.5 and 7.5% respectively. The authors concluded that the modified SF_6 technique (using orifice plate flow restrictors) reduces the error associated with SF_6 release, sample collection and analysis, recommending the modified SF_6 technique for determination of enteric CH_4 emissions from ruminants
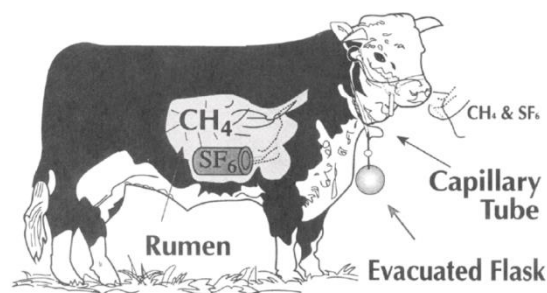
Figure 1-4 SF_6 tracer technique for individual ruminants showing the principal features. (Source: Johnson *et al.*, 1994)

Comparisons of measurement methods have been carried out with contrasting results. Usually the SF_6 technique underestimates CH_4 production relative to the RC, partly due to the proportion of gas lost via the rectum (Johnson *et al.*, 1994). Grainger *et al.* (2007) reported less than 3% difference in CH_4 emissions measured using SF_6 and RC with lactating dairy cows. These authors also reported higher between-day (6.1 vs 4.3%) and among-cows (19.6 vs 17.8%) coefficients of variance for the SF_6 technique than for RC. Muñoz *et al.* (2012) concluded that SF_6 measurements of CH_4 emissions for grazing livestock were accurate when compared with RC. They suggested an adjustment factor should

be applied with SF_6 measurements to account for CH_4 emissions from the rectum (3% in their experiment). In contrast, Wright *et al.* (2004) reported emission estimates were consistently higher with SF_6 than the RC estimates. They also reported no significant correlation between SF_6 and RC CH_4 emission estimates (n=30; r=0.33; p>0.05). The ability of the SF_6 technique to estimate CH_4 emissions from grazing animals has been highlighted by several authors (Lassey *et al.*, 1997, McCaughey *et al.*, 1997, O'Hara *et al.*, 2003; Johnson *et al.*, 2007).

Short-term measures of CH_4 emissions

In a recent review, Hegarty (2013) described and evaluated emerging measurement techniques reliant on short-term sampling such as the PAC (Goopy *et al.*, 2011), breath spot sampling during supplementation (using a laser technique reported by Chagunda and Yan, 2011) or during milking (using CH_4 analysers mounted in the milking robots reported by Garnsworthy *et al.*, 2012b) and breath spot sampling during supplementation (using the GEM system described by Zimmerman, 2011; Figure 1-5). Hegarty (2013) noted an increasing demand for non-expensive and high throughput CH_4 measurement techniques. Multiple short-term measurements of CH_4 emissions over 3-6 minutes may meet this demand to measure emissions from livestock in their normal production environment. Short-term measurements can be complementary to the RC-derived measures, offering capability to measure larger numbers of animals on-farm over extended periods of time.

GreenFeed emission monitoring system

The GEM system for measuring DMP of cattle is not widely understood and is as yet poorly published. An overview of the principles and knowledge of the operation of the unit is provided below, prior to reviewing its accuracy. The GEM system was described by its inventor (C-Lock Inc, US) as *a "baiting station, where each animal visits periodically throughout the day to receive a small food reward. Animals can be encouraged voluntarily to visit three to five times each day by programming the food reward schedule for each animal. While the animals are visiting the GEM, a Radio Frequency IDentification (RFID) system identifies each animal, as a fan draws air over the animal's head and past its nose and mouth into a specially designed manifold and air handling system. At the same time, high resolution measurements of airflow rates, gas concentrations, and other environmental parameters are recorded. Using the sensor information, a volumetric flux (L/min) of gases emitted by the animal is directly calculated. Once the volumetric flux is known, the mass flux in (g/min) can be calculated using the ideal gas law"* (extracted from http://c-lockinc.com)*.*
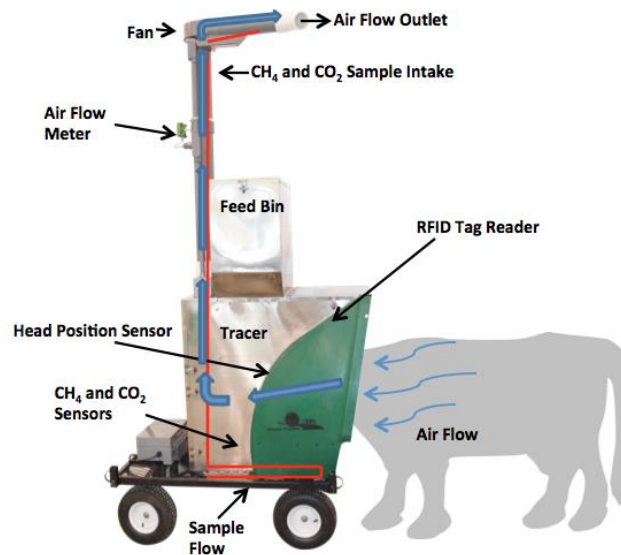
Figure 1-5 Basic instrumental layout of the GreenFeed emission monitor system. (Source: C-Lock inc., 2014)

A pelleted supplement is provided to cattle in a controlled manner (quantity/feed event and number of feed events/d) based on their identity detected by RFID ear-tag. To access the supplement, cattle place their head in an open shroud where the pellets are provided. Air is continuously drawn through the shroud and past the neck of the feeding animal at a precisely measured rate, and the concentration of gases (CH_4 and CO_2) and of propane (periodic release as a reference gas) are measured in the exhaust gas. Regular measures of background gas concentrations are also taken when no cattle are present. The background gas concentrations to be deducted from gas concentrations measured during an animal's visit are calculated before and after the visit. For each visit to the GEM a linear relationship is automatically fitted between the ambient pre-visit gas concentrations (averaged over 30 seconds prior to animal entry) and the gas concentrations post-visit (also averaged over 30 seconds after the animal has exited the GEM) to estimate background concentrations for every

second of measurement. CH_4 and CO_2 calibrations and CO_2 recovery tests are performed regularly during the experiments. The purpose of the calibrations is to define sensor responses to known concentrations of methane and CO_2, while recovery of released CO_2 (determined gravimetrically) verifies all gases released in the shroud are recovered by the flow system.

A feeding period of 3 to 6 minutes typically detects several eructation events. To avoid data that occur when animals step away from the shroud during CH_4 measurement, a proximity sensor in the shroud and data filtering system developed by C-Lock monitor the head-position of the animal throughout each feeding event. The system requires a 2m x 0.7m alleyway to be constructed in front of the shroud to allow only one animal access at a time. Adjustable width of the alleyway is recommended to avoid more than one small animal accessing the GEM and/or avoid physical limitation to access for big animals. Uniformity in size and weight of the animals is desirable if competition for usage of the system is detected.

Data filtering ensures only data from periods when the animal's head is consistently in the shroud are used in the calculations (termed useful feeding events). The emission rates over all useful feeding events (at least 3 minutes with head in position) are averaged to provide an estimate of DMP based on those few minutes' data. The pellets delivery can be programmed so that each animal is able to access up to a limited number of drops (of pellets) per feeding event, with a set interval between each drop and a set minimum delay between supplementation events. Changes to the supplement delivery program can be made on a herd basis, or on an individual basis. This flexibility allows differential

management of the animals to increase the recruitment rate, to ensure the dispersion and length of the measurements over the day and for training proposes.

The accuracy and practicality of the GEM has been investigated (Hammond *et al.*, 2013, Huhtanen *et al.*, 2013, Velazco *et al.,* 2013, Waghorn *et al.,* 2013). Agreement between estimates of DMP measured by RC and GEM techniques is high (Chapter 2) and demonstrates short-term measurement estimates of DMP are sufficiently accurate to be used for emission quantification. In indirect comparisons, Huhtanen *et al.* (2013) found a high level of agreement between GEM and predicted emissions (based on GEI). They also reported high repeatability (R=0.81) of DMP when measurements were made in dairy cows fed total mixed rations over two weeks. Another recent comparison (Hammond *et al.*, 2013) investigated the emissions from growing dairy heifers assessed using the SF_6 technique, the RC and the GEM. When comparing RC and GEM, they reported similar (*P*>0.1) mean emissions. It should be noticed that GEM was deployed indoors in this comparison. When comparing GEM with SF_6 technique under grazing conditions (outdoors), SF_6 estimates of DMP were higher (*P*<0.001) than GEM estimates. The authors attribute this difference to a lower number of effective measures/day (in comparison with the indoor experiment) at the GEM and conclude that timing and replication of the measures must be considered.

Under grazing conditions, Waghorn *et al.* (2013) reported a positive relationship (R^2=0.72, P=0.004) between DMP measured with GEM and DMP expected from consuming the quantity of feed required to meet the metabolisable energy requirements of the Holstein/Friesian cows. In terms of recruitment, more

than half of the cows in the experiment (17/24) adapted rapidly to GEM usage. The possible contribution of the attractant to the animals in a pastoral grazing environment was highlighted as a concern. In terms of practicality, they emphasised the need for preventing damage to pastures under wet conditions. A huge difference between animals with or without permanent rumen fistula was evident (339 vs 111 gCH_4/d) suggesting CH_4 leakage from the fistula.

Areas requiring further study that emerge from the reviewed literature include: a) establishing the required frequency and distribution of 3-6 minute measures of CH_4 flux to accurately quantify emissions (timing and replication of the measures), b) optimizing the sampling regime to define the phenotype of an individual animal, c) defining the best practices to maximize the recruitment rate in all production environments, and d) assessing the real contribution of the attractant (pellets) to the animals' diet (and therefore their CH_4 emissions).

Measurement-time relative to feeding and quantity and quality of the feed intake before measurements are additional sources of variation when short-term measurements are compared with RC and/or SF_6. This was investigated in Chapter 4 where a feed component (Nitrate) was proven to change feeding behaviour and therefore measurement time relative to feeding. An attempt to relate short-term measures of DMP and the feeding history of an animal is investigated in Chapter 6. The challenge of determining optimum sample size to estimate the methane production phenotype of an animal is discussed in Chapters 6 and 7 following two approaches: a) sample size for a trial based on power analysis combining number of animals, days and replicates to meet a given targeted precision of the estimated mean and b) a method that optimize the

sampling regime by calculating the minimum number of consecutive short-term measures needed to estimate the MP phenotype of an animal for a given level of confidence associated with the measured long-term estimate.

1.3.2. Measurement of CH₄ emissions from paddocks, herds and whole farms

There has been an increasing interest in the measurement of livestock CH_4 emissions while they are grazing or otherwise undisturbed in their production environment. Early studies used SF_6 (Ulyatt *et al.*, 1999) or mass balance approaches (Judd *et al.*, 1999) but micrometeorological approaches are increasingly used. The measurement of gas concentrations, air turbulence and wind direction in the atmosphere are the fundamental components of the micrometeorological methods (reviewed by McGinn, 2013). These systems account not only for the enteric CH_4 but for the manure and all possible sources of the target gas within the measurement footprint. These methods are non-intrusive so minimize the impact of the measurement procedure on the calculated CH_4 flux. Measurement of wind statistics and the need for high resolution instruments to measure concentrations is a common requirement of most micrometeorological methods. For example the eddy covariance technique (which is based on monitoring the vertical flux of a gas at a point above the source area) requires fast response sensors to measure vertical wind speed and CH_4 concentration (10 measures/s).

These methods are valuable tools for farm-system measurements and assist in the development of national inventories and mitigation claims.

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Considerable expertise is needed for the successfully measurement of CH_4 emissions from livestock using micrometeorological techniques. They have the advantage of being suitable for estimating feedlot emissions, paddock emissions and whole-farm emissions without modifying the production environment.

Galle *et al.* (2001) described one of the micrometeorological methods as used in Chapter 3. The method, called time correlation tracer, combines a controlled tracer gas release from the source with time-resolved concentration measurements downwind of the emitting source using fourier transform infrared (FTIR) absorption spectroscopy. Assuming the tracer gas release simulates the target gas well, an accurate estimate of the emissions is to be expected. Flesch *et al.* (1995) described and discuss the backward Lagrangian Stochastic (bLS) technique (also used in Chapter 3). In this inverse dispersion technique the emission rate is calculated from the rise in CH_4 concentration downwind of the emitting source and the software "WindTrax" (www.thunderbeachscientific.com) is used for the calculation in Chapter 3. In the bLS model thousands of trajectories are calculated upwind of the laser lines to mimic the transport of methane from the pen to the laser paths for the given wind conditions. The pen is assumed to be an area source in the model calculations so it is impossible to identify individual animal emissions. This method is reliant on a well delimited emission source and an accurately measured background concentration. Inadequate atmospheric conditions (for example low wind) compromise the effectiveness of the method to estimate emissions. The criteria to be met in order to fulfill the atmospheric requirements were discussed by Flesch *et al.* (2009) and are presented in Chapter 3.

Micrometeorological flux estimates are based on a series of assumptions related to the uniformity of strength of the emitting source. Emissions are typically assumed to arise from a uniform source over the measurement footprint but this does not reflect the discrete point emission sources that ruminants truly provide. In an attempt to account for the sparse and roving nature of the point emitting sources, a non-intrusive dispersion technique accounting for the location of each animal (using global positioning system, GPS) and treating each animal as a point-source emission was developed and tested by McGinn *et al.* (2011). They concluded that the technique resulted in more realistic and consistent CH_4 emission estimates when used during meteorological unstable periods. Frequent nocturnal deviations were caused by the weaker source strength and stability in the atmospheric parameters resulting in less convincing emission estimates. When averaged over longer periods of time, flux estimates based on this technique were similar to those reported in the literature for grazing animals (Laubach *et al.*, 2008).

1.3.3.   Repeatability and diurnal variation in methane production

From the preceding description of measurement methods it is apparent there is a practical trade-off between accuracy/duration of each measure of DMP and the number of days of measurement required, with between-day variation increasing with sampling interval, presumably due to rumen instability.

The RC is often viewed as a 'gold standard' in the measurement of emissions, having the highest between-measures repeatability when the comparison occurs on consecutive days (Pinare-Patino *et al.*, 2013). Relative to

RC, short-term measurement systems such as PAC have lower between measures repeatability so more measures will be required to accurately assess an animals' DMP. By making multiple measurements across days, short-term measurement systems are able to add more information to the emission phenotype. Bickell *et al.* (2011) conclude that 3 x 1h measurements in a PAC can define DMP comparably to 24h measure in the RC. McEwan *et al.* (2012) also conclude that little difference is expected when comparing 2 rounds of 2 x 24h in the RC 14 days apart and 4 x1h in the PAC when the intake is known and included as a covariate. The development of common procedures to estimate CH_4 will support efforts to underpin genetic selection for lower GHG emitting ruminants (Pickering *et al.*, 2013).

Crompton *et al.* (2011) investigated the effect of different feeding frequencies on the emission pattern of CH_4 in lactating dairy cows fed a total mixed ration. The response function describes an asymmetrical shape exhibiting a continuous rise to a peak followed by a period of linear decline, similar to a lactation curve (Wood, 1967; Dijkstra *et al.*, 1997). Fluctuation in CH_4 emissions in response to feeding events is evident in Figure 1-6. More recently, Jonker *et al.* (2014) confirmed this response when Hereford x Friesian heifers were fed lucerne silage at 4 different feeding levels and frequencies.
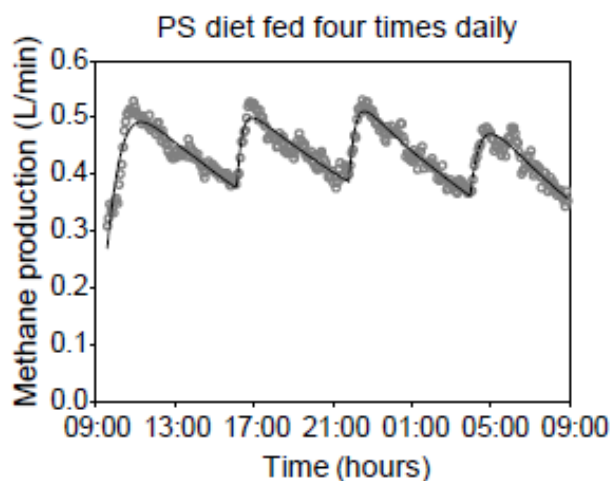
Figure 1-6 Effect of feeding frequency on diurnal pattern of CH_4 emissions in lactating dairy cows fed a TMR plus protein supplement (PS) four times a day. (Source: Crompton *et al.,* 2011)

From the above, the timing of measurement around feeding events should be considered if short-term measurement systems are to be applied in DMP estimation. All data must be assessed with the awareness that emission rates change over momentary, diurnal and longer seasonal patterns, requiring representative sampling. If the protocol does not incorporate sampling of emissions at least over the diurnal feeding and activity cycle, a scaling-up coefficient factor (as used by Garnsworthy *et al.*, 2012b) or adjustment factors (such as for animal activity and time spent in each activity as used by Chagunda *et al.*, 2009) may be required to avoid bias in estimating DMP. In addition to that, *ad libitum* feeding has been shown to reduce daily variation (Jonker *et al.*, 2014) but individual feeding behaviour under grazing conditions is difficult to know in commercial environments. As a general rule, ruminants consistently graze the greatest proportion of their daily intake early in the morning and late in the afternoon (Albright and Arave, 1997). When this grazing pattern was simulated in

the RC, an asymmetrical continuous rise to a peak followed by a period of linear decline was observed after each feeding session (Robinson *et al.*, 2011).

1.4. **Strategies to reduce enteric methane production**

Many reviews on enteric CH_4 mitigation technologies have been published in the last decade (Smith *et al.,* 2008, Martin *et al.,* 2010, Cottle *et al.,* 2011, Patra, 2012, Hristov *et al.*, 2013, Doreau *et al.*, 2014). Consequently the current review focuses on two of the most promising strategies for on-farm mitigation; being nitrate in the diet and selective breeding of animals for improved residual feed intake.

1.4.1. **Competitive inhibition of methane production by dietary nitrate**

Dietary inclusion of compounds with high affinity for hydrogen such as sulphates, nitrates and fumerate has been shown to provide an alternative hydrogen sink to methanogenesis (Van Zijderveld *et al.*, 2011a and 2011b). Nitrates compete successfully for hydrogen in the rumen and routinely achieve 80% of the theoretical methane mitigation expected, reflecting some loss of nitrate or nitrite across the rumen or down the tract.

The ruminal metabolism of nitrate and the opportunity to use nitrate as a fermentable nitrogen source in the rumen for microbial protein synthesis have been reviewed by Leng and Preston (2010) and Cottle *et al.* (2011) respectively. The inclusion of nitrate in a sugar cane-based diet (low-protein) diets was tested in young goats (Anh *et al.*, 2010) without evident differences in growth rate (when

compared with urea treated goats). Nolan *et al.* (2010), investigating the effect of the addition of 4% potassium nitrate to sheep fed a chopped hay-basis diet found a 23% reduction in CH_4 emissions. In a recent study, Guyader *et al.* (2014) investigated the combined effect of linseed oil and nitrate fed individually and associated to dairy cattle. They reported CH_4 emissions reductions of 17, 22 and 32% respectively when compared with the control treatment (grassland hay and concentrate 50:50). Daily pattern of CH_4 emissions showed most of the reduction attributed to the inclusion of nitrate occurs during the first 3 hours post-feeding.

While stoichiometry suggests complete reduction of NO_3 should directly decrease rumen methanogenesis by approximately 10% per 1% NO_3 ingested, nitrate can also indirectly reduce emissions by reducing feed intake (Pereira *et al.*, 2013, Hulshof *et al.*, 2012, Lichtenwalner *et al.*, 1973). In most cases NO_3 reduces feed intake but when feed is available *ad libitum* DMI is sometimes not affected. The persistency of the CH_4 emission mitigation effect caused when nitrate is added to a corn silage based diet in dairy cows was investigated by van Zijderveld *et al.* (2011a) over 107 days. They found the mitigation stable and concluded that CH_4 'persistently decrease by 16% without negatively affecting diet digestibility and milk production'. Ascensão (2010) reported a 41.6% reduction in CH_4 emissions when nitrate feed Nelore bulls were compared with urea counterparts without affecting DMI. In this experiment feed on offer was restricted to 2.15% of the body weight.

An additive effect in CH_4 abatement was reported by van Zijderveld *et al.* (2010) when combining nitrate and sulphates in a corn silage-based diet for Texel lambs. Lamb CH_4 emissions decreased with nitrate inclusion by 32%, with

sulphate inclusion by 16% and with combination of both by 47% relative to the control. The effect of supplemental sulphate on animal health is unclear but, acting as electron donors in the reduction of nitrite, may reduce the nitrite accumulation in the rumen decreasing the methaemoglobinemia risk.

Diet management to allow a ruminant to express it´s genetic capacity for growth or other production will substantially reduce the Ei of products from the cattle industries (Verge *et al.*, 2007 and 2008; Pinares-Patino *et al.*, 2009) by reducing the proportion of ingested energy expended on maintenance. One genetic trait expressing efficiency is the residual feed intake (RFI), being the difference between the expected intake of an animal and its' actual intake. A lower RFI indicates the animal has a better feed efficiency as it consumes less DM than expected for a given performance.

RFI in British cattle has a moderate heritability (Herd *et al.*, 2003; h^2 =0.39) as also found by de Haas *et al.* (2011; h^2 = 0.4) for dairy cows. Selection by efficiency over ten years would result in a theoretical CH_4 reduction by 11 to 26%. Modelling the long-term effectiveness of selecting beef cattle for superior RFI by Alford *et al.* (2006) identified an individual adopting herd may reduce its emissions by almost 16% in 25 years while at the national beef herd level, the expected abatement was 3.1% after 25 years. They conclude that selection for reduced RFI will lead to substantial and lasting methane abatement. Selecting for RFI could be seen as a long-term mitigation option but its effect on CH_4 production under grazing conditions deserves more investigation as data is sparse (Jones *et al.*, 2011, Herd *et al.*, 2011).

The between-animal variability in DMP can be attributed to diversity of methanogenic community in animals differing in RFI (Hernandez-Sanabria *et al.*, 2012) and in MY (Goopy *et al.*, 2014; Shi *et al.,* 2014). When DMP was analysed using the correlation with DMP was highly positive (Grainger *et al.*, 2007). Selection for RFI, which implies lower DMI at equal body size and production, may also mean decrease methane emissions, as DMP is proportional to DMI (Grainger *et al.*, 2007). Generally, the highest DMI, the higher DMP as more substrate is available for rumen fermentation and more hydrogen is available for methanogenesis. However, increased DMI is also associated with reduced retention time in the rumen, a lower acetate:propionate ratio and lower MY. Despite these counter balancing biological processes, low RFI cattle are still reported to emit less methane compared with high RFI cattle.

Selection for RFI to reduce CH_4 emissions is being investigated by the Animal Selection Genetics and Genomics Network of the Global Research Alliance for reducing greenhouse gases from agriculture. In their white paper (Pickering *et al.*, 2013) it was suggested that RFI could be used as an associated trait to reduce CH_4 but these and other authors (Hayes and Goddard, 2010) emphasized the need for a large number of true CH_4 measurements to be related with DMI and RFI levels. If breeding for lower emitting livestock is to be adopted as a global mitigation strategy, accurate, low cost, relevant measurements need to be available for its rapid implementation.

1.5. **Conclusions and hypothesis**

From the reviewed material it can be concluded that enteric CH_4 emissions from the dairy and beef supply chain represent a significant GHG source contributing to anthropogenic climate change. On-farm CH_4 emissions have been identified as the largest contributors to the carbon footprint in livestock production systems and therefore several GHG mitigation research programs are currently targeting ruminant gaseous emissions (Hristov *et al.,* 2013). The proportional contribution of livestock emissions to national GHG inventories is greatest in agriculturally based countries such as New Zealand, Ireland, Australia, Argentina and Uruguay, where beef and dairy production systems are pasture-based.

On-farm CH_4 emissions and their contribution to global warming are a concern because Steinfeld *et al.* (2006) estimated human consumption of ruminant meat is already approximately one third of the total meat consumption and ruminant milk is by far the predominant source of milk for human nutrition. Accounting for the anticipated increase in human population and the changes of habits in the emerging economies, ruminant meat and milk demand will increase by 50% in the next two decades (Steinfeld *et al.*, 2006) so an increase in livestock enteric emissions can be anticipated.

In consequence, the focus on CH_4 mitigation must be coupled with animal performance to effectively reduce emissions without compromising the food supply. Selective breeding for reduced emissions without compromising productivity may result in a long-lasting mitigation strategy, while sustained mitigation by nutrition will require continuous on-going application. A requirement for evidence of effective mitigation on-farm and a desire to establish the

phenotype of thousands of ruminants for breeding programs has fueled the development of new techniques to measure methane emissions on-farm. Many of these approaches have not yet been properly tested to give confidence in the accuracy of the estimation of DMP they provide or to understand sampling regimes appropriate for their use.

Spot measures of methane concentration or methane flux are increasingly being used to estimate DMP so this thesis sought to investigate the applicability of short-term measures of methane flux in estimating DMP using the GEM as the measurement device. Specifically the objective of this thesis was to validate the accuracy of DMP estimates derived from multiple short-term emission measures and verify the usefulness (or otherwise) of this approach to quantify the efficacy of mitigation strategies on-farm. The objective was met by addressing the following three specific research questions:

- *Are the estimates of DMP based on multiple measures of CH_4 flux over 3-6 minutes different from RC measures of DMP from the same cattle consuming the same quantity of the same ration?*
- *Can multiple 3-6 minutes measures of CH_4 flux be used to detect a reduction in the emissions expected when a mitigation strategy is implemented?*
- *What would be the required number and timing of 3-6 minute measures of CH_4 flux to detect 10% mitigation at $P<0.05$ and what would be the optimal sampling regime to define the phenotype of an individual animal?*

In Chapter 2, the first research question is addressed with two experiments comparing the DMP of 4 (experiment 1) and 10 (experiment 2) beef cattle. Chapter 2 represents the validation stage of the GEM system against open circuit RC. Chapter 3 is a further cross-validation of individual animal and group-based micrometeorological emission measurement systems as tools to detect methane mitigation for beef cattle on-farm. The group-based techniques are micrometeorological including N_2O-tracer and bLS techniques applied to open path laser and FTIR data, while individual animal emissions were monitored by GEM and RC. The emission measurement systems were compared at 2 stocking rates to simulate implementation of a mitigation practice. Application of multiple 3-6 minute flux measures by GEM to detect the effect of a dietary intervention in the diet of feedlot beef cattle and its effect in methane production was investigated in Chapter 4. In Chapter 5, GEM was further used to assess differences in DMP among beef cattle divergently selected for Residual Feed Intake under grazing conditions. Data from cattle using GEM in a feedlot situation where the time and weight of each meal by each animal was known, was then used to assess the relationship between methane production rate over 3-6 minutes and the animals' recent feeding history. Additionally, the variance structure of this data (collected over 70d) was used to develop recommendations on the sampling program required in using multiple 3-6 minute measures of methane production to accurately estimate DMP and MY of cattle.

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STATEMENT OF ORIGINALITY

General introduction

We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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27/2/2015

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Chapter Two

Use of short-term breath measures to estimate daily methane production by cattle.

2. Use of short-term breath measures to estimate daily methane production by cattle

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2.1. **Abstract.**

Methods to measure enteric methane (CH_4) emissions from individual ruminants in their production environment are required to validate emission inventories and verify mitigation claims. Estimates of daily methane production (DMP) based on consolidated short-term emission measurements are developing, but method verification is required. Two cattle experiments were undertaken to test the hypothesis that DMP estimated by averaging multiple short-term breath measures of methane emission rate did not differ from DMP measured in respiration chambers (RC). Short-term emission rates were obtained from a GreenFeed Emissions Monitoring (GEM) unit which measured emission rate while cattle consumed a dispensed supplement. In experiment 1, (Expt. 1) 4 non-lactating cattle (LW=518kg) were adapted for 18d then measured for 6 consecutive periods. Each period consisted of 2d of ad-libitum intake and GEM

emission measurement followed by 1d in the RC. A prototype GEM unit releasing water as an attractant (GEM-water) was also evaluated in Expt.1. Experiment 2 (Expt. 2) was a larger study based on similar design with 10 cattle (LW=365kg), adapted for 21d and GEM measurement was extended to 3d in each of the 6 periods. In Expt. 1 there was no difference in DMP estimated by the GEM unit relative to the RC (209.7 v 215.1 g CH_4/d) and no difference between these methods in methane yield (MY, 22.7 vs 23.7 g CH4/kg of Dry Matter Intake, DMI). In Expt. 2, the correlation between GEM and RC measures of DMP and MY were assessed using 95% confidence intervals, with no difference in DMP or MY between methods and high correlations between GEM and RC measures for DMP (r=0.85; 215 v 198 g CH4/d s.e.m.=3.0) and for MY (r=0.60; 23.8 v 22.1 g CH4/kgDMI s.e.m.=0.42). When data from both experiments was combined neither DMP nor MY differed between GEM and RC based measures (P>0.05). GEM-water based estimates of DMP and MY were lower than RC and GEM (P<0.05). Cattle accessed the GEM-water unit with similar frequency to the GEM unit (2.8 v 3.5 times/d respectively) but eructation frequency was reduced from 1.31 times/min (GEM) to once every 2.6 min (GEM-water). These studies confirm the hypothesis that DMP estimated by averaging multiple short-term breath measures of methane emission rate using GEM does not differ from measures of DMP obtained from RCs. Further, combining many short-term measures of methane production rate during supplement consumption provides an estimate of DMP which can be usefully applied in estimating MY.

Keywords: methane; cattle; measurement; greenhouse gases

2.2. **Implications**

Estimates of daily enteric methane production by individual cattle derived by averaging multiple short-term measures of emission rate are complementary to and consistent with respiration chamber-derived measures, offering capability to measure many animals in their production environment over extended periods of time. Repeated short-term measures made using a GreenFeed Emission Monitoring unit thus provide a valid means of quantifying livestock methane emissions. This may have application in verifying on-farm mitigation claims for carbon trading schemes and in generating high volumes of individual animal data to enable genomic selection of cattle based on enteric emission rates.

2.3. **Introduction**

Accurate methods of measuring of daily methane production (**DMP**; g CH_4/d) and methane yield (**MY**; g CH_4/kg of dry matter intake, **DMI**) from ruminants are essential for discovery of methane mitigation strategies and development of national inventories. There is a strong need to develop fast, simple and low-cost methods to measure enteric methane emissions on-farm (Pickering *et al.,* 2015). For many years, most DMP measurements have been made over one or more 22-24 h period in respiration chambers (**RC**), or in the field using the sulphur hexafluoride (SF_6) tracer method (Johnson *et al.,* 1994; Deighton *et al.,* 2014). Recently, multiple short-term enteric emission measurements have being used for regularly measuring larger numbers of animals within production systems (Garnsworthy *et al.,* 2012a). Estimates of DMP based on short-term flux (3-120 min) including by confinement (Goopy *et al.,*

2011; Robinson *et al.,* 2010) or imputed from the methane concentration in expired air (Chagunda and Yan, 2011; Garnsworthy *et al*., 2012b) are being evaluated and compared (Huhtanen *et al.,* 2014; Dorich *et al.,* 2015) The GreenFeed emission monitoring system (**GEM**; C-Lock Inc., USA) is a commercial system developed to estimate DMP of cattle from repeated short-term measures of methane emission over a period of days, weeks or months. The objective of this study was to compare DMP estimated by GEM systems with DMP measured by RC for cattle fed roughage-based diets. It was hypothesized that multiple emission measurements made over short (3-5 min) periods could be averaged to provide an estimate of DMP that does not differ from that obtained by complete collection of expired gases in a RC.

2.4. **Material and methods**

Two experiments were conducted 10 months apart with measurements of methane emission being determined from cattle using GEM and RC techniques within each of six experimental periods in each experiment. The six measurement periods were consecutive and animals left the RC from one period to commence the first day of GEM measurement in the next period. The GEM system delivering pelleted supplement as an attractant was evaluated in both experiments while a GEM system delivering water as an attractant (GEM-water) was only evaluated in Expt.1. These studies were approved by the University of New England Animal Ethics Committee (AEC 11-126 for Expt. 1 and 12-077 for Expt. 2).

Animals and feeding

In Expt. 1, five female Shorthorn cattle varying in age and in live weight (LW) from 392 to 680 kg (518 ± 132 kg LW s.d.) were group-housed in a pen (8 m x 12 m) in an open barn with access to an outside exercise pen (10 m x 12 m). The range in animal LW was chosen to induce variable voluntary feed intakes so the emissions would span a range of DMP. Cattle were not lactating with one heifer in the first trimester of pregnancy. Cattle had 18 d to adapt to the diet, environment and GEMs prior to the measurements commencing but had no RC experience prior to the study. One animal exhibited high feed refusals in the RC so all data from this animal was excluded from analysis. . The main ration was a lucerne/oaten chaff blend (Manuka Stockfeeds NSW; 8.8 MJ ME/kgDM; 11.2% CP; 55% NDF, 33% ADF in DM). The chaff was provided *ad-libitum* to all animals through a "Ruddweigh" (Ruddweigh, Guyra NSW Australia) feed dispenser with cattle identified by radio frequency ear-tag (**RFID**), so that each meal of each individual animal was recorded (Bindon, 2001). The weight of all meals consumed in a day was summed and compared to the known weight of chaff added to the feeder daily to confirm the accuracy of the scales in the feed dispenser.

In addition to the chaff, cattle were also provided with a measured quantity of pelleted supplement each time they accessed the GEM unit (13.2 MJ ME/kg DM, 12.7 %CP, 24% NDF, 11% ADF, 3.1% fat; 70% barley, 20% lucerne). When in the RC each animal was offered a quantity of mixed chaff and pellets equal to its voluntary consumption of these feeds (from Ruddweigh and GEM units) over the 2 days preceding RC entry. As it is known that methane production is affected by intake not only on the day of measurement, but at least on the preceding 2

days (Robinson *et al.*, 2011), the DMI used in calculation of MY by all techniques was the mean intake (chaff + pellets) on the day of measurement and of the 2 preceding days (i.e. for RC = chamber day + 2 preceding days in pen; for GEM = intake in previous RC day + intake during two days in pen).

The conduct of Expt. 2 was as for Expt.1 except that ten Aberdeen Angus steers (365.2 ± 50 kg LW s.d.) were used, commencing after steers had been adapted to the diet, GEM unit and facilities for 21 days including multiple training periods in RCs. There were no animals removed in Expt. 2. In further difference to Expt. 1, there were slight diet differences with the chaff (ME = 9.4 MJ ME/kg DM; 13.0% CP) and supplement (ME=9.5 MJ ME/kg DM, 13.4%CP, 25% NDF, 7% ADF, 2.7% fat in DM) and feed allowance in the RC was based on intake averaged over 3 d prior to RC entry.

GreenFeed Emission Monitoring units

The GEM unit was manufactured by C-Lock Inc. (U.S. Patent 7966971) and the principle of the unit is explained by Zimmerman (2013). For both experiments, a pelleted supplement was provided to cattle in a controlled manner (quantity of supplement/event and number of supplement events/d) based on animal identity as detected by RFID ear-tag. To access the supplement, cattle placed their head in an open shroud where the pellets were provided (Figure 2-1a) in a scheduled manner. An extraction fan in the airflow system continuously drew air through the shroud and past the neck and head of the feeding animal at a precisely measured rate. This air was filtered and the concentrations of methane, CO_2, and propane (released periodically as a reference gas) were determined in the exhaust. Air filters were not changed during either experiment as airflow did

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not fall below the 27 L/s minimum criteria. The background gas concentrations to be deducted from gas concentrations measured during an animal's visit were calculated for each second of the visit. For each visit to the GEM a linear relationship was automatically fitted between the ambient pre-visit gas concentrations (averaged over 30s prior to animal entry) and the gas concentrations post-visit (again averaged over 30s after the animal has exited the GEM) to estimate background concentrations for every second of measurement.

Methane and CO_2 calibrations and CO_2 recovery tests were performed weekly during both experiments. The purpose of the calibrations was to define sensor responses to known concentrations of methane and CO_2. Recovery of a gravimetrically determined quantity of CO_2 released over 20 min into the shroud was calculated from the CO_2 concentration and air flow rate through the GEM to verify gases released in the shroud were completely drawn into the exhaust stream.

A feeding period of 3 to 5 min typically detected several eructation events. To identify occasions when animals stepped away from the shroud during methane measurement and methane could have been lost, a proximity sensor in the shroud monitored the head-position of the animal throughout each feeding event. A measure of methane production rate (expressed as g CH_4/d or DMP) was only generated when an animal's head was continuously in the shroud for 3 min (described as a useful GEM visit). Data from GEM visits when animals did not keep their head in the shroud for the full 3 min were not utilized. To estimate the DMP rate of a given animal on a given day, the arithmetic mean of emission rates of all useful GEM visits by that animal on that day was calculated. No data was

removed because of being unexpectedly high or low, so any outlier emission measurements contributed to the DMP of that day.

In Expt. 1 pellets delivered by the GEM unit were made at the University of New England using a pellet press with a 6 mm dia. Pellet delivery in the GEM was programmed so that each animal was able to access up to 5 drops of pellets per supplement session (54.9 ± 0.8 g pellet/drop), with 40s between each drop and a minimum of 3h between supplement sessions. In Expt. 2 the supplement (Pryde Easy Ride pellets; 6mm dia) were again delivered to provide 5 drops of pellet (30.5 ± 0.9 g/drop) with 50 s between drops and a 4 h delay between supplement sessions. The smaller drop size, longer between-drop and between-session delays were implemented to increase duration of each visit while keeping supplement as a minor proportion of the total feed intake. The pellet hopper was checked daily and kept filled throughout the study.

The GEM was located at the end of a 2 m x 0.7 m alleyway which restricted access to one animal at a time. The extraction fan in the GEM was turned off when cattle were first introduced to the unit to reduce noise and maximize visitation. All GEM units were powered by 240V mains power and were operated within a portion of a large shed (36m x 25m).

The GEM-water unit (used in Expt. 1) was designed and built by C-Lock Inc. and was used concurrently with the supplement delivering GEM device. Water was used as the attractant in place of pellets (Figure 2-1b). Water was supplied from a high-pressure source into a shallow stainless steel bowl in the shroud. Water was replenished automatically as long as an animal remained at

the unit but there was no rationing or quantification of water as there was for pellets.
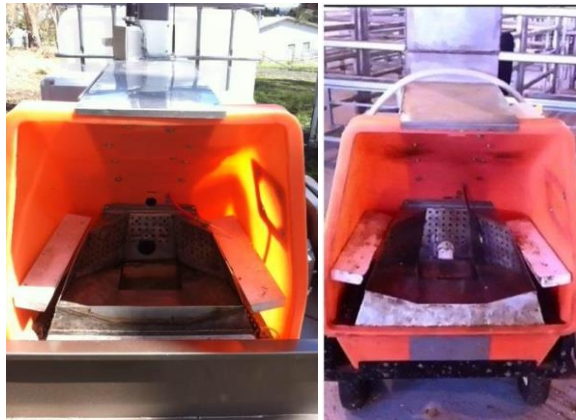
Figure 2-1 (a)GreenFeed emission monitoring units showing shroud where cattle enter to receive water (GEM-water; LHS) or (b) a pelleted supplement (GEM-supplement; RHS) based on animal RFID identification

Respiration chambers

Five open circuit RC were used in Expt. 1 and ten RC used in Expt. 2. Chambers (3.6m x 2.4m x 2.4m) were constructed of polycarbonate sheet (4mm thickness) fixed to a hot-dipped galvanized frame (Hegarty *et al.,* 2012). The RC did not have a floor but were able to seal into a water-filled rebate in the concrete floor and then be lifted by pneumatic rams to allow cleaning. Within the polycarbonate box, a pen made of steel cattle panels (3m x 1.8m x 1.8m) was bolted to the floor to confine the cattle. Cattle entered and exited the RC by a polycarbonate door fitted into the RC frame and a steel gate on the internal pen.

The daily feed allocation (chaff + supplement) was provided as a single meal immediately prior to sealing the RC. An air flow system (outside shed – RC – flow meter – high pressure fan) drew fresh air through each RC (approximately 1400 L/min), with the rate of flow though the exhaust line from each RC measured

by an SCI mass flow meter (Model ST75V, Fluid Components International, San Marcos, California USA). A subsample of air from each RC (2L/min) was continuously drawn from the exhaust line adjacent to the site of the flow meter, dried using a refrigerated drier (4°C) and passed through a multiplexer. The CO_2 and methane concentrations in these dried samples of exhaust air from each RC and a dried sample of concurrent ambient air were determined consecutively throughout the 24 h measurement period, with the dried sample pumped into a Servomex 4100 analyser fitted with GFx infrared sensors. Each sample took 60 s for purge and analysis so gases leaving each RC were measured every 12 min.

Sample drying, analytical and data processing software were configured by AZCO Holdings (Auckland NZ). The gas analyzer was calibrated each morning using a standard mixed gas and recovery of methane through RC was checked by introducing a standard pulse of methane (99% purity) before and after each experiment, with all emission data corrected to 100% methane recovery. Animals were randomly rotated through RC, with each animal measured in a different RC in each period.

Predicted methane yield

Methane yield and DMP were also predicted from the gross energy of the feed and the Intergovernmental Panel on Climate Change (**IPCC**) emission factor (IPCC, 2006) for comparison to values determined in experiments 1 and 2. Gross energy intake (**GEI**) was calculated from the chemical composition of the chaff and pellets in each experiment assuming a 19% loss of the apparently digestible energy (excreted in the urine and as methane; McDonald *et al.,* 2011). Metabolisable energy (**ME**, MJ/kg DM) was calculated using the prediction

equations recommended by the Australian Fodder Industry Association (2011) laboratory methods manual for roughages other than silages (ME = 0.203 * DOMD (%) – 3.001). Digestibility of the organic matter in the dry matter (**DOMD**) was estimated using the Pepsin-Cellulase method (Australian Fodder Industry Association 2011).

Statistical analysis

Mixed model analyses were conducted for MY and DMP (covariate-adjusted for DMI) in Genstat (Payne *et al.*, 2011), using the residual maximum likelihood (REML) procedure. Animal was fitted as a random effect, and measurement technique as a fixed effect (RC, GEM, GEM-water when present). This simplified model was adopted after first testing for day and period effects. Including 'day' (of measurement) as a covariate showed no effect for DMP or MY (P=0.4–0.8), justifying the assumption (of no time-trend) which is necessary for the analysis of this systematic random design. Residual plots were used to check the validity of the underlying statistical assumptions of homogeneity of variances and normality. The estimated means for the measurement techniques were subjected to protected least significance difference testing (at the 5% level) in Expt. 1. With the prolonged multi-period design, there was the possibility of animals adapting differentially to each measurement technique over time (e.g. as they became more familiar with the GEM unit and with confinement in RC units). This was investigated in both experiments by fitting the period x technique interaction (for DMP and MY). 95% confidence intervals (CI) were calculated for the measurement techniques in each experiment, and used in the combined analysis of both experiments. RC and GEM-supplement means from both

experiments were pooled and a statistical hypothesis test was performed. All 95% CI were compared against the IPCC predicted emissions. Steers in Expt. 2 were ranked from the lowest and highest according to their averaged DMP and correlation between methods was calculated (using the Pearson coefficient). In Expt. 2 diurnal variation in GEM derived DMP estimates was investigated using a spline model which included animal as a fixed effect.

2.5. **Results**

Feed intake

In Expt. 1 there was no difference ($P>0.05$) between DMI during the RC and GEM monitoring periods, based on intake averaged over these 3 days (Table 2-1). In Expt. 2, daily intake of the steers did not differ ($R^2=0.88$, $P>0.05$) between GEM and RC methods (9.3 and 9.3 kg/d, respectively), with refusals in the RC <5% of the feed offered.

Table 0-1 Dry Matter Intake (DMI), daily methane production (DMP) and methane yield (MY) measured by open circuit respiration chambers (RC), by GreenFeed Emission Monitoring units delivering supplement (GEM-s) or delivering water (GEM-w) as attractants.

Experiment	Technique	DMI (kg/d)	s.e.m.	DMP (gCH$_4$/d)	s.e.m.	MY (gCH$_4$/kgDMI)	s.e.m.
	RC	9.30$_a$		215.8$_a$		23.71$_a$	
1	GEM-s	9.27$_a$	0.92	208.6$_a$	9.2	22.71$_a$	1.01
	GEM-w	9.27$_a$		105.7$_b$		11.40$_b$	
2	RC	8.98	0.25	198.3	3	22.14	0.42
	GEM-s	9.3		214.6		23.83	
Combined analysis	RC	9.02	3.5	206.7		22.9	0.45
	GEM-s	9.13		212.1		23.24	

[a,b] Values within a column with different superscripts differ significantly at *P*<0.05.

Animal visitation and emission profile of GEM units

Experiment 1. Of the 40 available feed drops/day (in up to 8 supplement sessions), the cattle averaged 22.4 drops/d providing a minimum monitoring time of 14.9 min/animal/d and a mean daily pellet consumption of 1,230 g/d. Either because cattle were interrupted during a feeding period or because they did not stay with head fully inside the shroud for a constant 3 min, an average of 3.1 useful GEM visits/animal/d were made with an average visit duration 4.4 min/visit.

The amount of water consumed by cattle using the GEM-water unit was not recorded. Differences in eructation pattern were apparent between cattle using a GEM-water unit and a standard GEM unit (Figure 2-2). Animals visited the GEM-water unit with similar frequency to the GEM unit (2.80 v 3.46 visits/d respectively), but the eructations were much less frequent, with 0.38 v 1.31 (sd = 0.08) eructations per min, being one eructation per 2.6 min instead of one eructation per 46 s as for the GEM unit. GEM-water measures included a number of visits with no eructations which were discarded from the analyses as were periods with < 3 min of continuous monitoring data, lowering the number of useful GEM visits (1.52 visits per day sd = 0.52) that contributed to the average DMP.
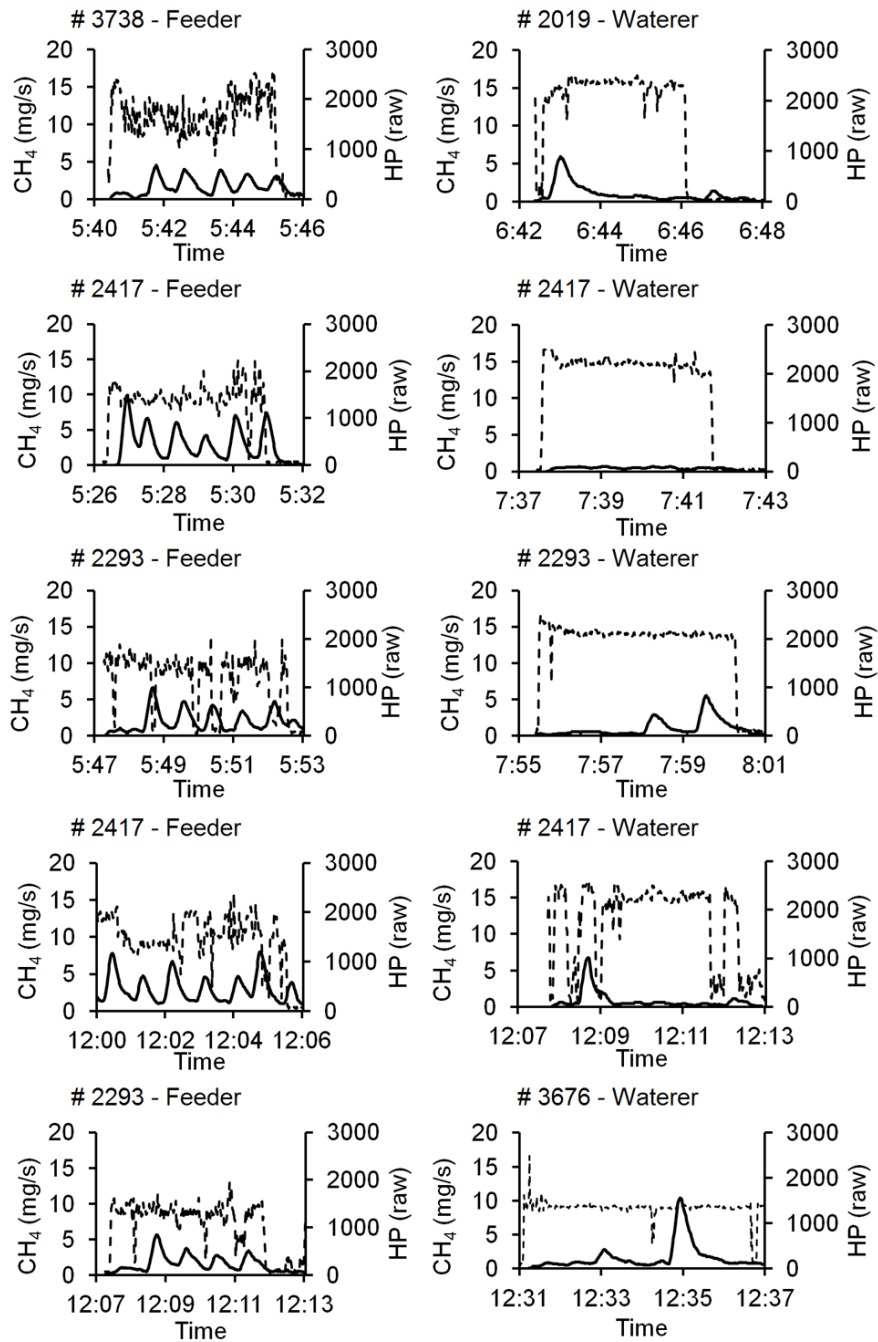
Figure 0-1 Short-term emissions profile of methane concentrations (solid lines) in exhaust air of cattle over 4-6 minutes monitored using the GreenFeed Emission Monitors with pelleted supplement as an attractant (LHS: "Feeder" = GEM-supplement) or water as an attractant (RHS: "Waterer" = GEM-water). Head position (HP, dashed line) units are arbitrary, with a value over 1000 indicating the head is in a position suitable for data collection. Eructation events appear as a sharp peak in solid line, being less frequent and less regular from cattle when they attend the GEM–water unit

Experiment 2. An average of 4.6 useful GEM visits were made from the 6 scheduled potential visits/animal/d. Each steer consumed an average of 27.5 drops of pellets/d, giving a mean daily pellet consumption of 840g/animal/d. Average GEM visit duration was 5 min (± 0.2 min) totaling 230 min of data collected/d for the group.

The spline model of short-term emission rates showed significant cyclic diurnal patterns for DMP estimated by the GEM (P<0.01) with a 14.9% difference between daily maximum and minimum emission rates. Visits to the GEM showed a uniform pattern over the daily period for all the 10 steers over the 6 periods (Figure 2-3). An analysis of the rate of methane production showed the model explained only 2.5% of the variation in DMP among all the individual useful GEM visits (Figure 2-3) but if all emission values within each 12 min period were averaged the spline through averaged values explained 89% of the variation.
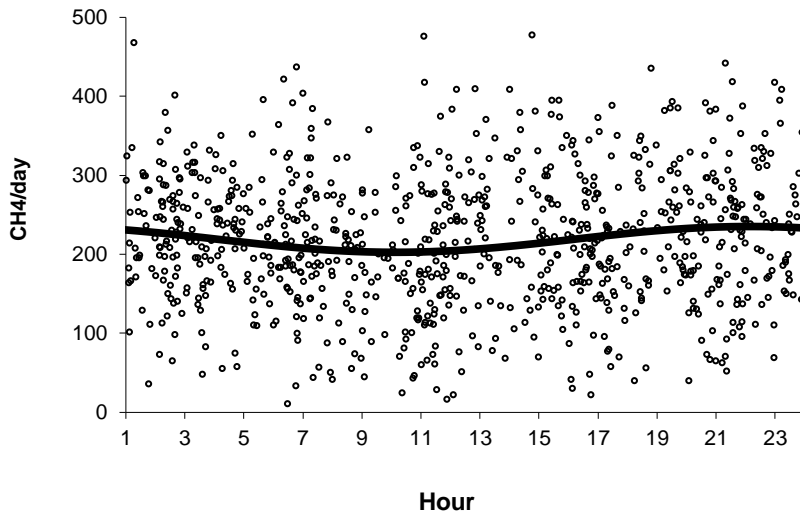
Figure 0-2 Compilation of all individual methane production rate (gCH$_4$/d) estimates from 10 steers collected from the GEM unit during experiment 2. Each useful supplementation event (>3 min continuous data collection) is shown as a spot, with a spline curved fitted to indicate diurnal variation in mean emission rate. Number of visits in each consecutive 4h period from midnight to midnight are 152, 152, 138, 106, 143 and 137.

Methane production and yield

Experiment 1. There was no difference in DMP or MY determined by the GEM and RC techniques ($P>0.05$; Table 2-1). Emissions measured from the GEM-water unit were significantly lower than emissions measured by GEM and RC ($P<0.05$). Because of the small number of animals and the high variation between animals in Expt. 1, an alternative analysis of DMP was utilized based on the CI of the data. The 95% CI was calculated to compare measured MY versus MY predicted by IPCC (2006). Predicted MY (21.3 g CH_4/kg DMI) calculated as 6.5% of the GEI was more than 1 s.e.m. lower than MY measured by RC.

Experiment 2. Agreement between methods was assessed using 95% CI and Pearson's correlation coefficients between DMP and MY rankings of individuals. Correlation coefficients were calculated for DMP and MY using individual rankings (periods were averaged to get one mean per individual per method). The strength of the relationship between methods was high for DMP ($r=0.85$) and moderate for MY ($r=0.58$) with no difference between measurement techniques (Figure 2-4) for MY. IPCC predicted MY (21.3 gCH_4/kg DMI), however, was lower than that measured using RC (22.1 g CH_4/kg DMI) and GEM-supplement (23.8 g CH_4/kg DMI).
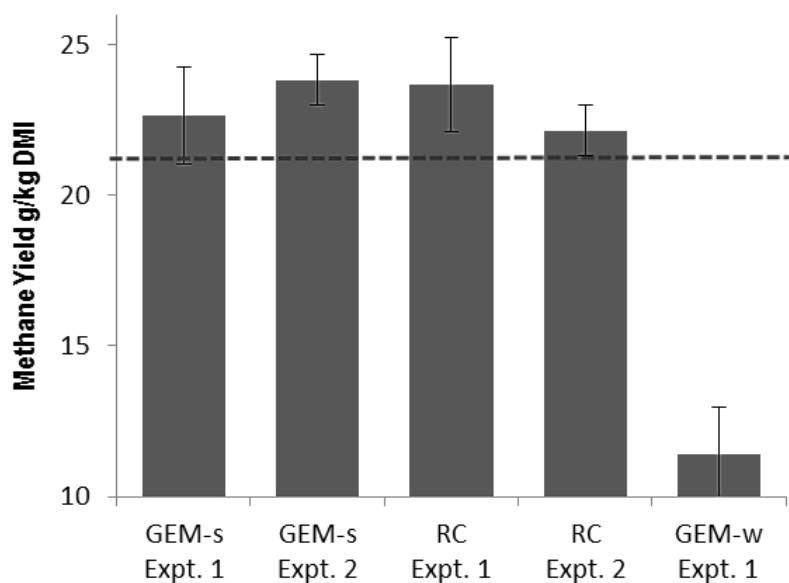
Figure 0-3 Methane Yield results (g/kg DMI) by method (GEM dispensing supplement [GEM-s] or water [GEM-w] or respiration chamber [RC]) and by experiment with 95% confidence interval. Dotted line corresponds to the predicted Methane Yield based on IPCC 2006.

Combined analysis of DMP and MY from Expt.1 and Expt. 2

Combined data from Expts. 1 and 2 was used in a post-hoc comparison of GEM and RC techniques for determining DMP and MY. There were no differences in DMP or MY (P=0.282 and 0.596 respectively) within data pooled across experiments for these two measurement techniques. Average MYs over Expt. 1 and Expt. 2 were 23.24 and 22.9 g CH_4/kg DMI respectively, while the corresponding DMP was 212.2 and 206.7 g CH_4/d respectively (Table 2-1).

2.6. **Discussion**

Increasing demand to measure DMP and MY of large numbers of ruminants for genetic or mitigation studies, necessitates development of methods

to measure animal emissions in their production environment. This has been attempted using the SF_6 tracer technique (Woodward *et al.,* 2004; Deighton *et al.,* 2014) and by short-term confinement in portable accumulation chambers for sheep (Goopy *et al.,* 2011), sampling methane concentration during milking for dairy cows (Garnsworthy *et al.,* 2012b), during feeding (Velazco *et al.,* 2013, Huhtanen *et al.,* 2013), or when drinking water (McGinn *et al.,* 2010). The CV of DMP estimated from short-term emission measures (2 min-2 h) is often higher than for RCs (Hegarty 2013), but emission estimates based on shorter measurement periods are highly correlated with DMP (Robinson *et al.,* 2011). Consequently there is scope to obtain measures of DMP from 2 min-2 h emission measurements if multiple measurements can be obtained.

Diurnal patterns in methane emissions of grazing ruminants are known (Lockyer and Jarvis, 1995) and match the bimodal diurnal grazing pattern (Goopy *et al.,* 2009) due to the rapid rise then decline in emissions post-feeding (Nolan *et al.,* 2010). Consequently it can be anticipated that sampling emissions in a schedule that will account for diurnal variation (as achieved in Expt. 2, see Figure 2-3), would be important to accurately quantify DMP by multiple short-term emission measures. What has not been clearly defined is the optimum duration of measurement required; how the measurement may be compiled from subsets of data; and how these samplings must be spread within days, over days, weeks or seasons. This is now being addressed by the Animal Selection, Genetics and Genomics Network (Pickering *et al.,* 2014) as they seek to standardize protocols to determine the methane phenotype of individual animals.

The agreement in DMP determined by GEM and RC techniques together with the low SE of fitted values for DMP and MY in this study, demonstrates that the GEM unit is sufficiently accurate to be used for emission quantification. This is consistent with other recent comparisons (Hammond *et al.*, 2013, Huhtanen *et al.*, 2013, Hegarty, 2013) where a high level of agreement between GEM and other methods were observed (differences less than 8%, P>0.10). Huhtanen *et al.*, (2013) also found high repeatability (R=0.81) of DMP when RC measurements were made of dairy cows fed total mixed rations over two weeks. Under grazing conditions, Waghorn *et al.* (2013) reported a positive correlation (R^2=0.72) between DMP as measured by GEM, and the DMP estimated to arise from feed consumed to provide the ME requirements of the Holstein Friesian cows.

Emissions are potentially variable over seasons due to feed quality and availability as documented in Australia's Tier 2 greenhouse gas inventory (DoE 2014). It is possible that by sampling an animal for a short period of time, but repeating the sampling many times, short-term measures of enteric methane eructation could provide a more accurate estimate of the emission rate over weeks, months or season than would a single intensive 24 h emission measure in a RC.

Prediction equations developed using RC data show over 70% of the variation in DMP can be explained by DMI or DOMI (Kennedy and Charmley 2012) but an inability to measure intake of large numbers of individuals (Cottle 2013) is still the limiting factor to predicting DMP from DMI under commercial grazing conditions. The IPCC estimations for MY compared with GEM and RC (or any equation-based on GEI and/or DMI) cannot predict the mitigation effect of

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dietary compounds (sulphates, nitrates, tannins), diet selection of grazing animals or genetic merit of the animals. The RC is able to identify some such mitigation effects but is expensive, time consuming and subjects animals to an artificial environment in which feed intake is controlled (Pickering *et al.*, 2013).

Unlike a RC, a GEM unit can potentially monitor enteric emissions over multiple short periods from in excess of 20 cattle able to access it, providing the ability to measure this population over longer periods (e.g. months) in their production environment while expressing largely natural diet selection and consumption. The prolonged measurement period (daily data for weeks or months) should allow for the detection of small treatment differences in emissions using GEM. Making measurements in the grazing environment would also allow changes in emissions due to diet selection or diurnal grazing pattern based on animal choice to be detected; in difference to RC systems which constrain the ration available and the timing of feeding so diurnal variation observed. While the GEM can estimate DMP of animals in their production environment, accurate feed intake data is less likely to be available in such a situation than when DMP is being measured by RC, so GEM methane emission data is less likely to be used for MY calculation than is RC data. The possibility of the GEM-supplied supplement affecting grazing behavior, feed intake and DMP requires investigation.

The fact that data were collected for 4.95 min/visit on average, indicates that cattle stayed in position in the GEM until supplement delivery was complete (delivery of supplement took maximum of 3.33 min/visit in Expt. 1 and 5 min/session in Expt. 2). Uniform distribution of visits over the day would ideally

sample the diurnal feeding cycle of grazing animals (Albright and Arave, 1997) minimizing the risk of biasing the estimation of DMP. A very even distribution of sampling time over the 24h by the herd was apparent throughout this study (Figure 2-3) indicating that scheduling supplement supply to occur at intervals gave GEM the capacity to adequately sample the daily emission profile of the herd. The weak diurnal variation in emissions apparent in Figure 2-3 suggests such sampling across the 24h cycle would avoid biasing the DMP estimate.

Aside from the need to manage the monitoring schedule to avoid bias, the provision of supplement by the GEM unit risks affecting DMP by two means. Firstly the supplement itself could affect DMP by affecting the total fermentable energy entering the rumen. In Expt.1, the average ME consumed as supplement (14.9 MJ/d) was 17% of the average daily ME intake; potentially affecting DMP by not only augmenting the fermentable ME intake, but also by providing nutrients that could enhance the fermentability of the chaff diet. To minimize that risk without compromising the duration of the data collection, ME consumed as supplement was reduced to 9% of the daily ME intake in Expt. 2 (by reducing ME content in the pellets and reducing the weight delivered per drop) and further reductions may be possible. A second means by which a supplement could affect emissions is by potentially affecting intake of the basal ration or pasture. Repeated accessing of the GEM could affect the grazing habits of the cattle being measured (Bowman and Sowell, 1997), thereby biasing their DMP by increasing or decreasing the composition and/or quantity of basal pasture consumed. Data are required on these two possibilities to be sure that the supplement provided by GEM has minimum effect on the DMP of livestock.

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To alleviate the possible problem of the supplement affecting DMP, and also because animals may choose to not access supplement, a GEM system using drinking water as the attractant was tested in Expt. 1. The observation that eructation pattern was different when water was used to attract animals (Figure 2-2) identifies the need to use different data screening of emission profiles from a water unit than from a supplement unit. There is also a need to optimize the rate of water supply through the GEM–water unit to ensure that, with the different eructation pattern, cattle remain in the unit long enough to give a measurement period containing adequate eructation events. So while the GEM-water prototype showed some weaknesses, the fact it does not introduce exogenous energy to bias DMP and the fact that all animals must drink, means further development appears warranted.

It is uncertain what proportion of a herd would voluntarily visit a GEM unit, but this study found that familiarising animals for 3 weeks before commencing measurements minimised the risk of low recruitment. Based on observations in these and other GEM studies an introductory protocol for high recruitment is likely to include: scheduling supplement delivery to be liberal (multiple sessions of 6 or more drops/session); use of pellets flavoured with a taste or smell attractant (e.g. aniseed); turning off the GEM extraction fan to make the unit quieter and avoid frightening the animals; widening the alleyway to promote the visit of shy feeders; reducing the amount of supplement delivered to dominant animals based on their identity.

Since many researchers require MY measurement, there is also opportunity to deliver indigestible markers through the GEM and GEM–water

units, coupling this with faecal sampling to estimate faecal output and so DMI. Indigestible markers have previously been used to estimate intake in grazing sheep, giving rise to genetic parameters for DMI (Fogarty *et al.*, 2006). Recently provision of markers in supplement dispensers to estimate feed intake has been patented (Patent 13/391,116).

In summary, the arithmetic mean of many short-term measurements of methane production rate from the GEM unit provided an estimate of DMP that did not differ from that determined by open circuit calorimetery of roughage-fed cattle. On an individual animal basis, ranking in the GEM and RC systems correlated highly (Expt. 2; R^2 =0.71). This supports development of short-term emission rate monitoring as an approach to quantify emissions for both inventory and mitigation purposes. The ability of short-term emission measurement devices such as the GEM unit to estimate DMP in animals in their production environment creates opportunities for detecting emission changes arising from grazing behavior and diet selection, which are not possible in RCs. Validation of GEM-derived estimates of DMP in the grazing environment are still required. The possibility of coupling emission measures with digesta markers and faecal sampling may allow simultaneous estimation of DMI and so of MY during studies using GEM.

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Journal-Article Format for PhD Theses at the University of New England

STATEMENT OF AUTHORS' CONTRIBUTION

Use of short-term breath measures to estimate daily methane production by cattle

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

	Author's Name (please print clearly)	% of contribution
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27/2/2015

Candidate   Date

27/2/2015

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Journal-Article Format for PhD Theses at the University of New England

STATEMENT OF ORIGINALITY

Use of short-term breath measures to estimate daily methane production by cattle

We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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27/2/2015

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27/2/2015

Principal Supervisor   Date

Chapter Three

Atmospheric and GreenFeed measurement systems to quantify beef cattle methane emissions benchmarked using respiration chambers

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3. Atmospheric and GreenFeed measurement systems to quantify beef cattle methane emissions benchmarked using respiration chambers

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3.1. **Abstract**.

The development and validation of management practices to mitigate livestock greenhouse gas emissions demands accurate emission measurement techniques. The objectives of this study were to assess the accuracy of field methods to quantify daily methane production (gCH_4/d) from cattle and to establish their capacity to quantify the effectiveness of mitigation. Daily methane production of a cattle herd was estimated by two atmospheric methods; the tracer-ratio technique using an open-path FTIR sensor (Tracer-FTIR), and the bLS technique using either an open-path Laser or FTIR (bLS-Laser, bLS-FTIR). These were compared with an individual-animal based method (Greenfeed emission monitors [GEM]). Emissions were then benchmarked using open circuit respiration chambers (RC). Field measurement systems were compared at two stocking rates (85 cattle/ha, period 1 and 42 cattle/ha, period 2) to simulate the effect of mitigation. Five to 10 days after the field experiment, daily methane production was measured on the same animals in RCs (periods 3 and 4), using the same diet at the same feed intakes. There was a high level of consistency between DMP estimated from the atmospheric methods (321-329 gCH4/d, p>0.05) when meteorology conditions were suitable, but these measurements were higher than predicted (IPCC 252 gCH4/d), higher that measured by the GEM (225 g/d) and higher than RC measures (234 g/d). Significant mitigation was detected by Tracer-FTIR, bLS-FTIR, and GEM (p<0.05) when stocking rate was reduced, with the observed reduction approximately that expected to result from the decline in total feed intake. It was concluded that both individual-animal and group-based

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techniques for measuring methane can be useful in quantifying enteric emission mitigation from livestock.

3.2. Introduction

The combined pressures of global population increase, usage of fossil fuel and climate change are demanding the agricultural sector to manage its environmental impacts, especially its greenhouse gas (GHG) emissions that contribute to global warming (Howden et al., 2008; Thornton 2010; Hegarty 2013). An array of feed supplements, rumen modifiers and animal management and breeding opportunities are under investigation for their potential to mitigate emissions of enteric methane, the principal GHG originated from livestock (Beauchemin et al., 2009, Cottle et al., 2011). Increasingly there is a requirement for mitigation claims to be validated when these practices are applied on-farm (DoE 2014) and so methods for on-farm measurement of livestock methane emissions are required.

On-farm enteric emissions have been measured by portable respiration hoods for tethered and non-tethered animals (Garnsworthy et al., 2012, Zimmerman 2013); by use of an exogenous tracer source in or near the animal (Johnson and Johnson 1995; Phillips et al., 2013) and by micrometeorological means reliant on knowledge of air turbulence, wind speed and direction (Harper et al., 2011). While some comparisons of methods have been reported (Laubach et al., 2008, 2013, Pinares-Patiño et al., 2011, Tomkins et al., 2011) an assessment of emerging methods such as the Greenfeed emission monitor that may have application in the extensive production environments is needed. The objective of

this study was to examine the accuracy of group emission measures derived from atmospheric open-path tracer ratio (SF_6 Tracer-FTIR) and backward Lagrangian Stochastic (bLS-Laser, bLS-FTIR) modelling approaches, together with measures of individual-animals by a novel technique based on multiple short-term measures (Greenfeed Emission Monitor, GEM) benchmarked using the gold standard for measuring individual methane emissions (open circuit respiration chambers, RC) to determine total herd methane production (kg CH_4/d), and their capability to detect a reduction in the emissions when a mitigation strategy is implemented.

3.3. Method and Materials

3.3.1. Overview

The study was conducted at Chiswick pastoral research laboratory (CSIRO Chiswick, 30° 37' S, 151° 33' E) and the University of New England (Armidale, NSW, Australia) after approval by relevant animal ethics committees (UNE AEC 12/077; CSIRO ARA12/28). The study consisted of 4 measurement periods; being 2 consecutive 7 days outdoor periods in which 32 (period 1) and then 16 (period 2) yearling steers were held in group pens. Emissions were measured with micrometeorological techniques (using open-path Laser and open-path Fourier Transform Infrared Spectrometry, FTIR), by an atmospheric tracer technique (using N_2O as a tracer and FTIR) and by a Greenfeed automated emissions monitoring system (GEM). Immediately after the field measurements, the 16 steers were transferred and then housed in individual pens. During period 3, steers were offered daily rations equal to the mean daily intake corresponding to period 2 for at least 3 days and during period 4, steers were offered rations equal

to the mean daily intake corresponding to period 1 for at least 3 days. Daily methane emission was then measured by open circuit respiration chambers (RC) as described by Hegarty et al., 2012.

3.3.2. Field site layout

The field site was established in an open field in which 4 pens, each 30 m x 30 m in size were constructed in a square design with a 3m wide runway running roughly east-west between the two northern and two southern pens (Figure 3-1). The nearest trees were at least 300 m from the site with a height of approximately 40 m. The site had been sprayed twice with glyphosate to kill all vegetation and was heavily grazed by sheep before the study to ensure there was less than 500 kg of dry matter per ha at the start of the study, meaning the only feed available was that provided in feeders.

Each pen was fitted with an automated feeder recording the time and weight of each meal consumed by each animal based on radio frequency identification (Ruddweigh International Pty Ltd, South Guyra, NSW, Australia). Water was offered *ad libitum* in troughs in all pens. Of the 4 pens only one pen (north-east corner) had a GEM unit installed to which cattle in that pen (n=8) had continual access. Easterly winds were expected during the experiment, and open-path (OP) FTIR and Laser systems were positioned to monitor the background methane mixing ratio of air coming onto the site and the enhanced mixing ratio leaving the site (Figure 3-1). An automated weather station was set up in a nearby area to monitor turbulence and wind speed and direction as shown in Figure 3-1. In period 1 each 30 m x 30 m pen held 8 cattle. In period 2, the laneway and

internal dividing fences were removed, and 16 cattle were removed, leaving 16 cattle within the 60 m x 63 m pen.

3.3.3.   Preparation and feeding of animals

Angus steers (n=32; 373 kg ± 59 sd) used in the study included 16 steers that had little prior research management and 16 steers that had previous exposure to experimental procedures including consuming diet from automated feeders, accessing GEM units and confinement in respiration chambers. A single source of blended oaten and lucerne chaff was offered *ad libitum* from automated feed dispensers (Bindon 2001) during field studies (Table 3-1). Feeders were cleaned out daily and any remaining feed weighed as a means of checking that total feed consumed matched the sum of the individual-animal intakes in each pen as recorded by the automated feeders. At the field site cattle were adapted to the ration for 3 weeks prior to the first measurements being made. During the last week of adaptation cattle were fitted with 600 g backpacks (attached by contact adhesive) to become accustomed to carrying the weight of N_2O canisters used during measurement periods, as part of the tracer technique proposed by Jones et al. (2011).
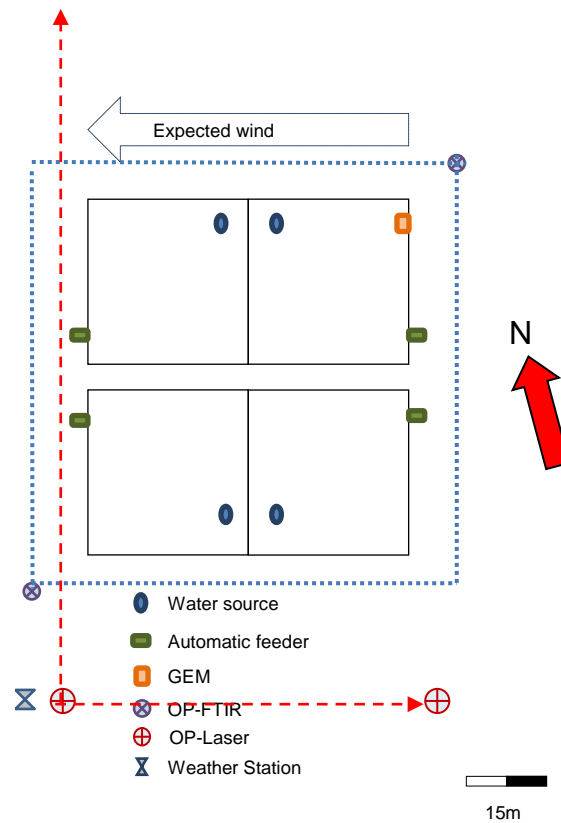
Figure 3-1 Schematic layout of the experimental site. OP-FTIR, open-path Fourier Transform Infrared Spectrometry sensors; OP-Laser, open-path Laser sensor; GEM, Greenfeed emission monitor.

3.3.4. Meteorological measurements

A portable weather station installed south-west of the site provided 3-dimensional wind speed and wind direction data at 10 Hz resolution averaged to 15 minutes (sonic anemometer, height 2.8 m, CSAT3, Campbell Scientific Inc, Logan Utah, USA). A wind sentry and cup anemometer (03001 RM Young Wind Sentry set, Campbell Scientific Inc, Logan Utah, USA) provided additional wind direction and speed. Air temperature (T107, Campbell Scientific Inc, Logan Utah, USA) and humidity (HMP55C, Campbell Scientific Inc, Logan Utah, USA) were measured each minute and averaged to (typically) 5 minutes. All data were

recorded to a data logger (CR5000, Campbell Scientific Inc, Logan Utah, USA) and downloaded daily. Weather parameters were required by WindTrax software to calculate fluxes using Lagrangian stochastic model (bLS).

3.3.5.   Animal Management

Period 1 (Feb7 – Feb13) involved 32 cattle allocated across 4 pens (n=8) with feed provided *ad libitum*. Each morning at 0900 cattle were walked to adjacent yards (80 m north) and each animal's N_2O canister replaced with a weighed full canister (N_2O was released as a tracer gas for emission measurements). Cattle were absent from the measurement pens for 30 to 60 minutes while this occurred, allowing zero check of open path (OP) equipment. For period 2 (Feb 14 – Feb 21) all internal fences and the laneway were removed and only the 16 cattle with prior GreenFeed experience were retained for the second week of measurements.

Immediately after period 2, the remaining animals (n=16) were transferred to the University of New England cattle research facility, individually housed in pens and offered feed equal to their individual mean daily feed intake as during period 2 (period 3). This feed consisted of the chaff and the GEM pellets in amounts equal to the average of each source consumed by each individual-animal during period 2. After at least 3 days of this fixed intake, cattle were placed in respiration chambers for 24 h during which this same feed allocation was made. On removal from chambers (start of period 4) the offering of chaff and GEM pellets to each animal was changed to equal the mean each animal had consumed in period 1. After at least 3 days on this intake level, individual-animal daily methane production was again measured over 24 h in the RC.

Table 3-1 Chemical composition of the dry matter of blended oaten/lucerne chaff and of pellets dispensed to steers by the GreenFeed emission monitor (GEM) unit throughout the experiment.

	Oat/Lucerne chaff	GEM Pellets
Dry Matter (%)	90.2	91.0
Neutral Detergent Fibre (%)	49.4	25.0
Acid Detergent Fibre (%)	31.0	7.0
Crude Protein (%)	15.1	16.1
Metabolisable Energy (MJ/kg) [A]	10.0	12.1
Digestible Organic Matter in the DM[B] (%)	64	76

[A]Calculation of metabolisable energy based on the Australian Fodder Industry Association equation (2011)
[B]Digestibility estimated using the Pepsin-Cellulase method

3.3.6.   Emission measurements

Four procedures were used to estimate total herd methane emissions ($kgCH_4/d$) in periods 1 and 2. These included micrometeorological approaches based on OP Laser and OP FTIR measures, tracer dilution using controlled release of N_2O, and the GEM unit for measuring daily methane production of individual-animals. Operational details for these procedures and for measurement of daily methane production by RC in periods 3 and 4 are described below.

Tracer dilution measured by OP-FTIR (Tracer-FTIR)

Total herd methane production was derived by comparison of the mixing ratio of methane from the animals and that of a tracer-gas, released at a known rate from miniature N_2O canisters attached to the animals, as measured downwind from the animals by OP FTIR spectroscopy. The methane flux was calculated as the product of known N_2O release rate by CH_4:N_2O ratio, where the CH_4 and N_2O were the measured mixing ratios above local background

concentrations (as determined in the upwind measurement-path). The OP FTIR spectrometer used was a Matrix IR-Cube, (Bruker Optik GmbH, Ettlingen, Germany) equipped with a cooler (-196°C, RicorK508, Ricor, En Harod Ihud, Israel) MCT detector (Infrared Associates Inc., Florida, USA, or Judson Industries, Montgomeryville, PA, USA). This was coupled to a 250 mm Schmidt-Cassegrain telescope (LX 200ACF, Meade Instruments Corporation, Irvine California, USA) expanding the beam from 25 to 250 mm diameter and reducing beam divergence to 2 mradians. The system was mounted onto a servo-motor controlled automated instrument mount (AIM, IAAC Pty. Ltd. Unanderra Australia) to allow computer controlled alignment of the beam between spectrometer and distant retro-reflectors. Each spectrum was analysed immediately after collection using the MALT analysis program to obtain path-averaged mixing ratios of N_2O and CH_4 (Griffith 1996).

OP FTIR instruments were located on the SW and NE corners of the pens, with each instrument able to orientate to two measurement-paths (Figure 3-1). Measurement-paths were approximately 7 m from the pens, with path lengths (instrument to retro-reflector) of 76 and 78 m at an average height of 1.4 m. Instruments alternated between the two paths every 5 minutes, with orientation synchronised to parallel paths, unless wind was predicted to be consistent from one direction, when the favored up- and down-wind paths were selected.

The tracer-gas (N_2O, engine boost grade, BOC) was released from pressurized canisters mounted on the back of animals. Each canister was fitted with a head encompassing a capillary tube (PEEKsil HPLC capillary tubing, 0.025 mm inner diameter, SGE Analytical Science Pty Ltd, Ringwood, Victoria,

Australia) to limit the flow-rate of tracer gas to around 10 g/h and filled with approximately 300 g of N_2O. The temperature of the canisters was monitored every 6 minutes using miniature temperature sensors (ThermocronTemperature model TCS, OnSolutions, Baulkham Hills, NSW, Australia).

The OP FTIR instruments were calibrated based on air samples collected in glass bulbs (600 ml) at each measurement path when the animals were absent from the pen and meteorological conditions were favorable (wind speeds > 2 ms^{-1}, and wind direction other than North to limit contamination from the animal yards).The collected air samples were analysed in the laboratory using a closed-path FTIR spectrometer calibrated to a known standard.Canisters were mounted in backpacks, glued onto the dorsal midline of the animal (29-30 animals in period 1 and 15-16 in period 2). The backpacks were lined with insulation to limit temperature variation of the canisters. Animals were trained to the backpacks and the weight of the canister before the start of the experiment. The canisters and temperature logging buttons were exchanged every 24 h at a nearby yard. The canisters were weighed at the start and end of each 24 h period to calculate the daily N_2O release rate. As the flow rate from the canisters is dependent on the gas temperature, the instantaneous release rate was calculated by weighting the average flow rate by the measured canister temperature and the known temperature dependence of the flow rate from the canisters, as determined in the laboratory (Bai 2010).

Methane emissions were derived from individual (5 minute average) mixing ratio measurements, based on the difference in measured CH_4 and N_2O mixing

ratio at the down- and up-wind measurement paths. These were pooled to provide 30 minute averaged total herd methane emission values.

bLS with the OP-FTIR (bLS-FTIR)

Total herd methane production from the OP FTIR was also derived using the backward Lagrangian Stochastic (bLS) technique (Flesch et al., 1995). In this inverse dispersion technique the emission rate was calculated from the increase in CH_4 mixing ratio, above local background mixing ratio, downwind of the animals. Mixing ratio data was averaged into 15 minutes intervals for a total of 96 possible segments through the 24 h day to match wind turbulence data then loaded into the software "WindTrax" (www.thunderbeachscientific.com) used for the calculation. In the bLS model thousands of trajectories are calculated upwind of the measurement-paths to mimic the transport of CH_4 from the pen to the measurement-paths for the given wind conditions. The pen is assumed to be a uniform area source in the model calculations.

The sonic anemometer provided the following wind and turbulence required to derive input variables for bLS calculation: friction velocity (u*), Obukhov stability length (L), surface roughness length (z_0), and wind direction (as described in Flesch et al., 2004). We followed the procedure of Flesch et al. (2005) and ignored error-prone periods when the wind was light (u* < 0.15 m/s), the atmospheric stability conditions were strongly stable or unstable (|L|<5 m), or when the fraction of pen area covered by the downwind laser footprint was < 75%.

bLS with the OP-Laser (bLS-Laser)

Total herd methane production was also measured using the bLS technique applied to OP Laser data. The Laser (GasFinder/Scanner, Boreal Laser Inc., AB, Canada) was mounted on a digital scanning motor (PTU D300, FLIR Motion Control Systems, Burlingame, CA, USA) and located 60 m south of the source area perimeter. The scanning motor was programmed to cycle the Laser between two paths giving an upwind and a downwind mixing ratio measurement for prevailing easterly winds (Figure 3-1). The termination of each Laser path consisted of an array of 6 corner cubes housed in an enclosure with a polycarbonate window (retro-reflector). The average path height was 1.6 m. The line-averaged CH_4 mixing ratio (ppmv) was recorded approximately every second for 60 s, and processed to generate 15 minutes average mixing ratios for each path. Measurements with return infrared radiation levels outside of the manufacturer recommended limits were excluded from the average as were periods when the Laser was transitioning to a new path.

The OP Laser was calibrated on-site. Prior to animals being moved to the site, air samples were taken for gas analysis along the Laser paths and transported to a laboratory for analysis on a gas chromatograph. Average mixing ratios measured by the gas chromatograph were compared to corresponding OP Laser mixing ratios, and a multiplicative Laser correction factor was used to equate the two. This correction factor was applied to all subsequent Laser data.

GreenFeed Emission Monitoring Unit

One GEM unit (C-Lock Inc; South Dakota, USA) was used in the experiment. This unit operates as an open faced respiration hood into which cattle place their head for short periods (3-6 min) while receiving a supplement in a controlled manner based on animal identity (ear-tag) detected by radio frequency identification. Air is drawn though the hood at an accurately determined rate and CH_4 and CO_2 mixing ratio measured by inbuilt sensors, with on-line processing used to calculate average flux during that visit expressed as gCH_4/d. Regular measures of background gas mixing ratios are also taken when no cattle are present. Before and after the experiment the CH_4 and CO_2 sensors were calibrated by infusing labeled standard gas straight into the sensors. After the study, pure CO_2 was released into the shroud at a gravimetrically determined rate and its recovery estimated from CO_2 mixing ratio and flow rate in the exhaust. As head position is critical for the accuracy of the technique, a proximity sensor mounted in the unit was used to underpin a data quality assurance system in which a CH_4 emission measurement was made only if there was a minimum of 3 minutes of data collected per animal visit, and the animal did not remove its head from the shroud in this time. Cattle were induced to place their head in the hood by a small regulated release of a highly palatable attractant from a feed-hopper. The attractant was commercially manufactured 6mm diameter feed pellets (Pryde's EasiFeed, Gunnedah, NSW, Australia, Table 3-1). GEM was programmed with a drop size of 30.5 g/drop and pellets were only dropped at 50 s intervals with a maximum of 6 drops per GEM visit with at least 3 h delay between

GEM visits for all animals. GEM visits and their corresponding individual CH_4 measurements were used to investigate the herd diurnal emission pattern.

Respiration chambers

Eight open circuit respiration chambers were used in periods 3 and 4 (Hegarty et al., 2012). Chambers (3.6 x 2.4 x 2.4 m) were constructed of 4 mm polycarbonate fixed to a galvanized frame. All chambers were sealed into a water-filled rebate in the concrete floor. A yard made of steel cattle panels (3 x 1.8 x 1.8 m) was bolted to the floor to confine the cattle within each polycarbonate chamber. An air flow system (outside shed – chamber – flow meter – high pressure fan) drew fresh air through each chamber (approximately 1400 L/min), with the rate of flow though the exhaust line from each chamber being measured by an SCI mass flow meter (Model ST75V, Fluid Components International, San Marcos, California, USA). A subsample of air from each chamber (2 L/min) was continuously drawn from the exhaust line adjacent to the site of each flow meter, dried using a refrigerated drier (4^oC) and passed through a multiplexer. The sample drying, analytical and data processing software were configured by AZCO Holdings (Auckland, NZ). The gas mixing ratios in these dried sample streams and a dried sample of concurrent ambient air were determined consecutively throughout the 24 h measurement period, with the dried sample pumped into a Servomex 4100 analyser fitted with GFx infrared sensors. The gas analyser was calibrated each morning using a standard mixed gas and recovery of methane through chambers was checked by introducing a standard pulse of methane (99%) before and after the experiment. All individual chamber emission data were corrected to 100% CH_4 recovery.

Prediction of emissions based on daily intake

Predicted daily emissions and methane yield (MY, gCH_4/kg DMI) were estimated based on dry matter intake (DMI) and gross energy intake (GEI) of animals, chemical analysis of the feed using the IPCC (2006) methodology in which methane production equals 6.5% of GEI. GEI was calculated from the chemical composition of the feed assuming a 19% loss of the apparently digestible energy based on the energy lost as combustible gases produced during feed fermentation and energy-containing compounds in urine (McDonald et al., 2011). Metabolisable energy (ME, MJ/kg DM) was calculated using the prediction equations recommended by the Australian Fodder Industry Association Laboratory methods manual (AFIA 2011) for roughages other than silages (ME = 0.203 * DOMD (%) – 3.001). Digestibility of the organic matter in the dry matter (DOMD) was estimated using the Pepsin-Cellulase method (AFIA 2011).

The average MY of cattle in the RCs (period 4 and 3) was used to predict total herd methane production for periods 1 and 2 (calculated as the product of measured MY of animals offered the same ration in periods 3 and 4 and the corresponding daily intake). As only 1 day of RC data was available for each animal at each intake, it was not statistically possible to compare 7 days means of daily methane production in periods 1 and 2 with the 1 day DMP determined in the RC in periods 3 and 4.

3.3.7. Statistical analysis

The daily emissions from period 1 and 2 were standardized to the units of gCH_4/head/d and mixed model analyses were conducted for daily methane production, total herd methane production and MY in Genstat (Payne et al., 2011),

using the residual maximum likelihood (REML) procedure. Period and measurement method, along with their interaction, were fitted as fixed effects. Days (within the periods) were taken as replicates, with the temporal autocorrelation between successive days being accounted for by adopting an autoregressive (of order one) error structure. 95% confidence intervals were calculated for the measurement techniques in each period, and compared with the RC estimates of total herd methane.

For the analysis of period 3 and 4 (RC), the independent animals (each in a separate chamber, run in two batches due to the restricted number of respiration chambers) were the replicates to calculate mean daily methane production and MY and to estimate total herd methane. The estimated means for total herd methane, daily methane production and MY were subjected to protected least significance difference testing (at the 5% level). Residual plots were used to check the validity of the underlying statistical assumptions of homogeneity of variances and normality.

3.4. **Results**

Rainfall during the experiment was 14.4 mm in period 1 and 0.4 mm in period 2. In period 1 wind conditions were poorly suited to OP studies with light mist and fog leading to condensation on mirrors and reflectors, particularly in the mornings. Wind was predominately from the east, with the exception being the 10th and 11th of February when the direction varied between 180 and 310 degrees (Figure 3-2). Wind speeds varied from 0 to 6 m/s during the study. Winds were lighter during period 1, and stronger and more consistently from the east

during period 2. This difference resulted in more favorable meteorological

conditions during period 2 compared with period 1.
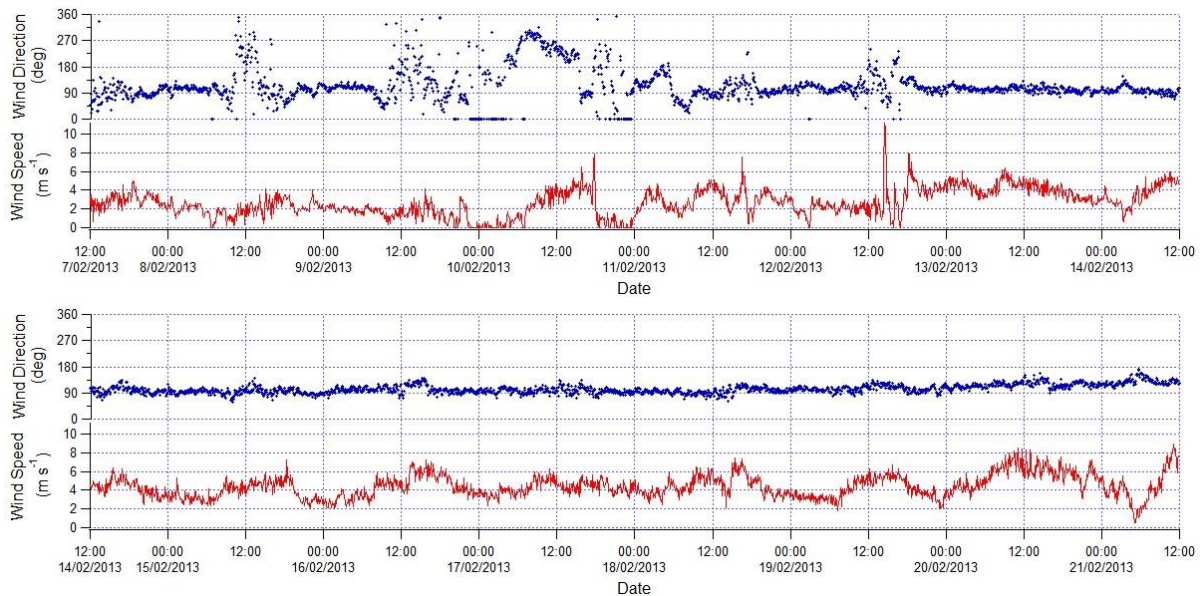
Figure 3-2 Wind speed and direction during emissions measurements (Top: period 1 from 7/2/2013 12:00 to 14/2/2013 12:00. Bottom: period 2, 14/2/2013 12:00 to 21/2/2013 12:00)

3.4.1.   Micrometeorological-based daily methane emission measurements

The ability of methods to detect mitigation of the total herd methane

emissions was tested by comparing emissions across periods. Mean total herd

methane with 95% confidence intervals (kg CH_4/d) are presented in Figure 3-3.

There was a significant effect of measurement technique, period and their

interaction ($p < 0.05$). All methods differ from each other in their estimates of total

herd emissions in period 1 ($p < 0.05$). In period 2, which had wind conditions more

conducive to atmospheric techniques, the bLS-Laser, bLS-FTIR and Tracer-FTIR

measures of total herd emissions did not differ ($p > 0.05$), although all were higher

than the GEM estimate ($p < 0.05$). All approaches except the bLS-Laser were able

to detect lower total herd emissions in period 2 (16 animals) than in period 1 (32 animals; Figure 3-3, p<0.05).

Total herd emissions in periods 1 and 2 were also estimated from the MY determined during RC measurements multiplied by the measured DMI of all cattle in each period. Best agreement between total herd emissions so calculated was with the GEM, being within the 95% confidence interval of GEM in both periods, while estimates from FTIR (periods 1 and 2) and Laser (period 2) systems were significantly higher than that derived from GEM (p>0.05) or estimated from the RC data. Estimates from bLS-Laser in period 1 were excluded for this comparison.

Method	tracer FTIR		bLS FTIR		GEM		bLS Laser	
	7.3	5.1	8.2	5.1	6.3	3.6	5.1	5.3
Period	1	2	1	2	1	2	1	2
p value	d	b	e	b	c	a	#	b
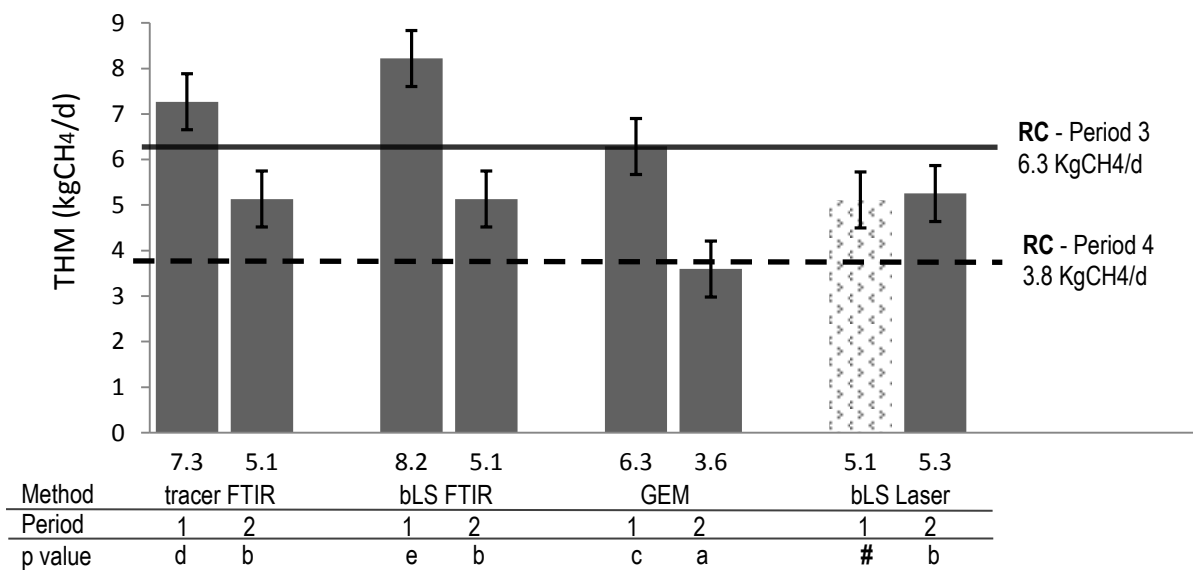
#: laser-bLS estimate in Period 1 was confounded by unsuitable climatic conditions (mirror fogging, low wind speed) meaning insufficient data was available to provide a reliable estimate.

Figure 3-3 Total herd methane emissions (kgCH$_4$/d) by period according to the four paddock methods (in bars): OP Fourier Transform Infrared Spectrometry using N$_2$O Tracer release (tracer-FTIR), bLS (bLS-FTIR), GreenFeed Emissions Monitor (GEM), OP Laser using bLS (bLS-Laser). 95% confidence interval indicated in the error bars. Dashed (period 4) and full (period 3) horizontal lines represents the estimates of total herd methane emissions based on the Respiration Chambers (RC).

OP FTIR

The CH$_4$ mixing ratio in the FTIR measurement path during period 1 (32 animals) was up to 400 ppb above local background mixing ratio when meteorological conditions were favorable (wind speed > 2 m/s and easterly). In period 2, with the number of animals reduced to 16 animals and meteorological conditions close to ideal, the increase in CH$_4$ mixing ratio above local background varied between 50 and 150 ppb (wind speed 6 and 2 m/s respectively, direction easterly, Figure 3-4). These downwind mixing ratios were well above the minimum measurement sensitivity of the instrument.
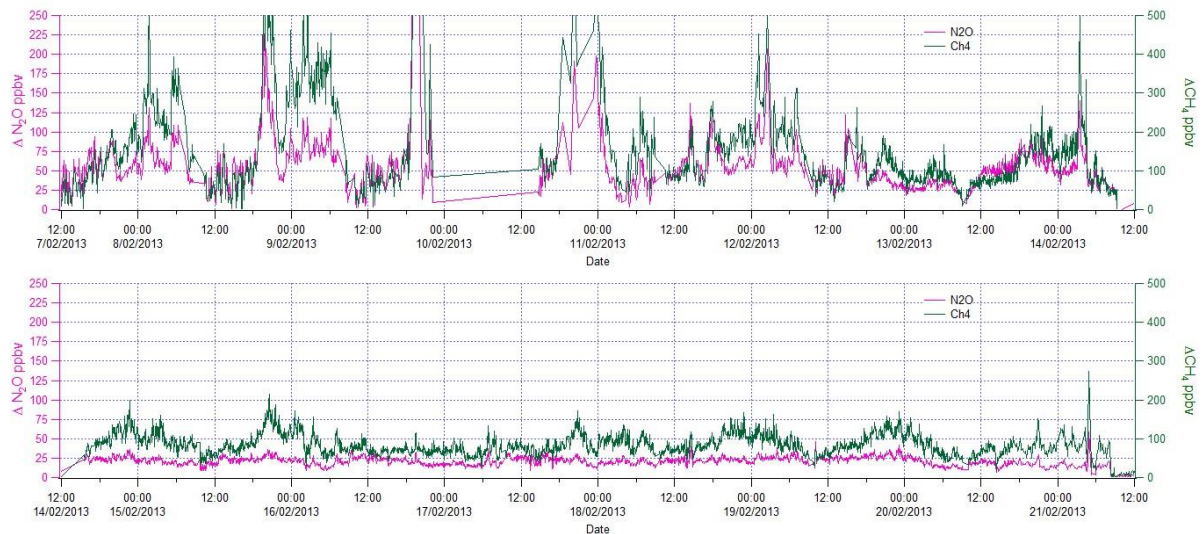
Figure 3-4 Increased CH$_4$ and N$_2$O mixing ratios being the difference in mixing ratios measured at the upwind and downwind OP FTIR instrument paths during emission measurements from period 1 and period 2 (N$_2$O was released from calibrated canisters as an external tracer).

The goal for the micrometeorological and the tracer techniques was to have complete emission measurements throughout the 24 h day, so that any diurnal emission patterns could be characterized. Our procedure was to condense all measurements in a period to a 24 h emission record based on the time of day of the observation. During period 1, FTIR mixing ratio measurements were made in 58 out of 96 possible 15 minutes intervals/d (60%). On days 3 and 4 of this period, very few mixing ratio data points were available. In period 2, with better meteorological conditions, mixing ratio measurements were obtained for 90% of 15 minutes intervals. A diurnal pattern in methane production was found from the bLS FTIR measurements with an R^2 of 0.8 for the spline. The lowest emissions were registered at 0600 h and maximum values at 1800 h. The emission range within the day (daily maximum to minimum) was 26.9% of the daily mean.

Estimates of total herd emissions were higher for bLS-FTIR than Tracer-FTIR in Period 1 ($p<0.05$), but no differences were detected between OP methods in their estimates during period 2. Both the Tracer-FTIR and bLS-FTIR techniques were able to detect between-period differences in total herd emissions associated with the lower number of animals in period 2 ($p<0.05$).

OP Laser

As with the FTIR techniques, the goal for the bLS-Laser technique was to have continuous emission measurements throughout the 24 h day. During period 1 only 29 laser-based 15 minutes flux segments/d were calculated out of 96 (31.7% data capture), with cattle off-site for 4 of these. Moisture build-up on the reflectors was responsible for 66% of the data coverage, with unsuitable meteorology (low wind speed, wrong wind direction, rain, stability) being

responsible for the remainder. Period 2 was much improved with regard to missing data, with 75% of the potential data collected. Laser operational problems accounted for 68 % of the missing data with the remaining 32% due to unsuitable meteorology.

There was excellent agreement between the Laser and FTIR based emission measurements in period 2. However, in period 1 the two were significantly different. Because the bLS-Laser and bLS-FTIR emission measurements were different in Period 1, and because the FTIR based measurements of daily methane production and MY were consistent between periods 1 and 2 while the bLS-Laser measurements were not, we conclude that the bLS-Laser measurements during period 1 were inaccurate and this is due to the Laser mixing ratio measurements (and not the bLS calculations).

3.4.2.   Individual-based daily methane emission measurement.

GreenFeed Emission Monitoring Unit

Individual-animal based data from the GEM was collected over periods 1 and 2 with an average of 3.7 visits/animal/d out of a possible 8 daily visits, with all animals providing valid CH_4 estimates. Steers consumed an average of 21.4 drops of pellets/d, at an average weight of 30.5 g (± 0.9 g) per drop; giving a mean daily pellet consumption of 653 g/d. Average length of the visits was 5.1 minutes (± 1.2 min) totaling 144.6 minutes of data collected/d in period 1 (8 animals) and 311.4 of data collected/d in period 2 (16 animals).

Daily methane production was calculated by averaging all emission data longer than 3 minutes collected at the GEM for each individual-animal on a daily basis and then averaging all individual-animal estimates for that day (to get the daily production). Daily methane production of individuals was 196.7 g/d (sd=14.3) in period 1 and 224.2 g/d (sd=12.7) in period 2. Individual estimates were scaled up to herd emissions using the known daily intakes of the non-measured animals for period 1 while in period 2 total herd emission was computed directly as the sum of all 16 animals being measured. GEM herd emission estimate was significantly lower in period 2 than period 1 (p<0.05).

The spline model used to investigate the diurnal pattern in the emissions (values within 12 minute intervals were averaged to get an average flux over that time segment) showed significant and cyclic diurnal patterns for daily emissions estimated by the GEM (p<0.01). The emission range within the day (daily maximum to minimum) of the fitted spline was 24.7% of the daily mean. Minimum daily methane production estimate was around 0600 h and maximum around 2000 h. Visits to the GEM showed a uniform pattern over the day across all cattle over the 2 periods. The spline model explained 93% of the variation in daily methane production averaged over each 12 minute period of the day using all the data collected within each segment.

Respiration chambers

One of the 16 steers did not complete the 24 h measurement period (due to problems in the water supply in its chamber) so 15 animal emissions were measured in period 3. All 16 animals were measured in period 4. There were no significant differences in daily methane production and MY between periods

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(p>0.05, Table 3-2), despite a 23% greater DMI and an 11% higher daily methane production in period 2. Individual DMI in the RC was over 95% of the offered feed.

Table 3-2 Mean individual-animal dry matter intakes (DMI), daily methane production (DMP) and methane yield (MY) measured by OP-FTIR using N_2O as tracer-gas (Tracer-FTIR) or using bLS (bLS-FTIR), OP-Laser using bLS (bLS-Laser), open circuit respiration chambers (RC), by GreenFeed Emission Monitoring (GEM). Predicted DMP equals 6.5% of Gross Energy Intake (IPCC 2006)

Method	n (Per 1 - Per 2)	DMI (kg/d)		DMP # (gCH4/d)		MY # (gCH4/kg DMI)	
		Per 1	Per 2	Per 1	Per 2	Per 1	Per 2
Tracer-FTIR	(32 - 16)	9.1	11.9	227 [bc]	321 [b]	25.3 [bc]	27.1 [b]
bLS-FTIR	(32 - 16)	9.1	11.9	257 [c]	321 [b]	28.7 [c]	27.1 [b]
bLS-Laser	(32 - 16)	9.1	11.9	160 [a]	329 [b]	18.0 [a]	27.8 [b]
GEM	(8 - 16)	9.7	11.9	196 [b]	225 [a]	21.5 [ab]	19.0 [a]
				Per 4	Per 3		
RC[†]	(16 – 16)	9.7	11.9	209	234	21.7	19.9
Predicted				193	252	21.2	

 # Significant interaction between method and period was observed and is reported in the text.
† In periods 3 and 4, the quantity of feed offered in RCs equaled the mean voluntary intake in periods 2 and 1 respectively.
Within columns, means with a common letter are not significantly different ($P<0.05$).
SED for DMP is 16.61 and SED for MY is 1.935.

3.4.3.   Feed Intake and predicted methane emissions.

In period 1 with 32 cattle (4 pens of 30 x 30 m each, with 8 cattle and 1 automated feeder/pen) the mean feed intake was 9.10 kg DM/head/d across all animals. In period 2, only 16 animals were present and they had the entire area in which to move (60 x 63 m) with 3 automatic feeders from which they could obtain feed. Associated with this change in stocking rate and of feeder accessibility, the average daily DM intake/head increased from 9.10 to 11.91 kg/d between period 1 and 2 (Table 3-2) with a CV of 4.6 and 5.4% within periods respectively. A predicted methane emission factor (calculated as a 6.5% of the GE intake) of 21.2

gCH$_4$/kg DMI was used in the calculation of predicted daily methane production (DMI*21.2, Table 3-2). Daily intake of the steers did not differ when they were being assessed in the field or at the RC (p>0.05) with refusals in the RC <5% of the offered feed.

3.4.4.   Daily Methane Production and Methane Yield

Significant between method differences in daily methane production and MY were apparent in period 1 and period 2 with a significant interaction between methods and periods (p<0.05). Period 2 was nearly ideal for method intercomparison with winds during this period generally moderate, steady and nearly ideal for micrometeorological calculations. During period 2 the OP methods (Tracer-FTIR, bLS-FTIR and bLS-Laser) did not differ in estimated daily methane production or MY (Table 3-2; p>0.05) but all OP systems estimates were approximately 45% higher than those estimated by GEM on the same 16 cattle. Emissions from the OP based methods in period 2 were also higher than predicted from IPCC (~30%) or observed through the RC (~40%), although a test for the significance of differences was not possible.

In contrast, period 1 (32 animals) was less ideal for comparisons due to poorer wind conditions, and mirror fogging that resulted in large data loss (particularly for the Laser system). The daily methane production estimated by bLS-Laser was significantly less than all other field methods (p<0.05). There were no differences between Tracer-FTIR and bLS-FTIR derived estimates of daily methane production and MY within either period (p>0.05). GEM and RC based

measures of daily methane production and MY were comparable to the predicted

values based on IPCC 2006 (Table 3-2).

3.5. **Discussion**

This study sought to assess the accuracy of techniques to measure

livestock emissions in a paddock and their ability to detect mitigation, with

mitigation in this case being achieved by a reduction in the source mixing ratio

through a reduced number of cattle being present. In periods 1 and 2 the stocking

density of cattle (85 to 42 cattle/ha, respectively) was substantially higher than

occurs in commercial grazing environments and was higher than theoretically

(McGinn 2013) or practically (Phillips et al., 2013) required for the OP

measurement systems. These stocking densities were chosen to provide high

downwind mixing ratio enhancement to provide confidence in the technique

comparisons. It should be noticed that the expected magnitude of the emissions

reduction achievable with real mitigation approaches is usually lower than the

detected 37% (Hristov et al., 2013).

Accuracy of field emission measurement techniques has previously been

assessed through controlled release verification studies (Flesch et al., 2004), by

repeated field vs. respiration chamber comparisons (Velazco et al., 2015) and by

comparison to tracer based methods (Laubach et al., 2014). In this study,

emission measures in periods 1 and 2 were benchmarked against emissions

measures from the same cattle fed the same intake of the same feed when in

respiration chambers (periods 3 and 4). While comparison significance test of

differences was not possible between field and chamber methods, it is apparent

there was a high level of consistency between daily emissions (gCH$_4$/head/d)

estimated from all OP methods in period 2 (321-329 g/d), but that these emissions were higher than the GEM measure (225 g/d; $p < 0.05$) which was itself comparable to those estimated from the RC (234 g/d) or calculated using IPCC methodology (252 g/d).

The differential between OP derived and predicted (RC, IPCC) daily methane production estimates is not readily explained. Weather conditions in period 2 were nearly ideal for micrometeorological calculations, so that very few sampling segments were missed over that week (10% for FTIR and 25% for Laser), so bias due to time-of-sampling in the diurnal emission cycle is unlikely to cause the differences. The good agreement between the different atmospheric techniques in period 2 (tracer and bLS), using independent mixing ratio measurements (FTIR and Lasers), and calculated by two different groups, gives added confidence in their accuracy. On the other hand, errors in the respiration chamber should also have been low since a controlled release of methane was made into all chambers before and after the RC periods, and emissions corrected to 100% methane recovery. MY observed from the RC measurements were also consistent with published values taken under similar conditions (Herd et al., 2014).

If one accepts that the atmospheric and RC techniques both accurately measured emissions, there are several possible reasons for a difference between the two. One possibility might be the contribution of manure emissions, which may vary in importance between the field and the RC environment. In the field, cattle were present for 21 days prior to emission measurements, and there would be some build-up of manure that was not replicated in the RC environment. The field surface may also have been wet due to rain and dewfall, which is different from

the RC environment although there were very few rainfall events during the study (15 mm in total). These differences could promote higher CH_4 emissions from the field although the general view is that any manure emissions would be small in comparison to enteric emissions (Flessa et al., 1996). In addition, when the animals were absent from the pen during periods 1 and 2, we did not observe enhanced CH_4 levels downwind of the pens, suggesting that emissions from the manure were small.

Another possible explanation for the difference between those measurements is the MY values of the herd were truly different between the field and RC measurements. The repeatability of MY has been shown to be less in measurements made 14 d apart than in those made on consecutive days (McEwan et al., 2012) so the possibility of the rumen changing between periods cannot be dismissed, however there is no reason to anticipate such variability in MY by individuals would change the mean MY of the population between periods, especially since the cattle had been on ration for over 40 days by the start of period 3.

Our comparison between the daily methane emission measurements from the atmospheric techniques and the simultaneous GEM measurements focus on period 2, when the conditions for the micrometeorological measurements were nearly ideal, and the three micrometeorological techniques gave statistically identical results. Unlike period 1, all (16) same animals were measured across the atmospheric and GEM techniques in period 2. In this case the daily methane production from GEM was about 30% lower than the atmospheric techniques estimates. Some GEM underestimation was expected: the GEM system do not

measure manure emissions (as does the atmospheric techniques) nor emissions from flatulence. In period 1 GEM determined daily methane production (196 g/d) was also within 10% of that predicted from RC (209 g/d) and IPCC (193 g/d) but variability within OP approaches was much greater and this can be ascribed to collection of more limited data due to variable wind conditions in period 1 (Figure 3-2). Laubach et al. (2013) found close alignment (<10% difference) between the sum of individual animals emissions (measured by SF_6 tracer) and herd emissions measured by OP Laser and FTIR, but large (33-68%) discrepancies with vertical profile /mass budget field methods. The importance of selective data use in field studies was subsequently highlighted by Laubach et al. (2014). The large amount of missing Laser data in period 1 (due to low wind and mirror fogging) means that little can be concluded from the lower ($p<0.05$) period 1 emissions estimated by Laser. Such observations suggest caution is needed in imputing daily fluxes when full 24h emission monitoring is not possible. However, daily methane production is increasingly being estimated by methods that do not record emissions continuously, but only consider data for hours (Goopy et al., 2011), minutes (Garnsworthy et al., 2012; Velazco et al., 2015).

In the case of the GEM, while the 3-5 minutes measures showed a high variability in short term emission rates, the sampling regime achieved great uniformity in sample frequency throughout the 24h day. This allowed the diurnal variation in flux rate to become apparent and provided an average estimate of daily methane production that was consistent with RC and IPCC predicted values, showing multiple short-term emission measures can be effectively used to estimate daily methane production. The diurnal cycle in emission rate observed by

Velazco et al. (2015) make apparent that failing to sample over the 24 h could introduce bias if measurements were to be restricted to a fixed time in the daily cycle (i.e. a non-robotic milking parlor where cattle are milked only in early morning and late afternoon or in field methods when wind speed is regularly too low to provide useable data at some times of day). In this study with cattle being able to access GEM all day and making regular day and night visits to the unit, the risk of bias was small.

Apart from accuracy, this study also sought to assess the capacity of these methods to detect and quantify mitigation, in this case evidenced by a reduction in total herd emissions between periods 1 and 2. The expected emission reduction (based on total DMI in periods 1 and 2 and the MY of cattle determined in RC on the diet) was 2.5 kg/d, being 37% of the period 1 emission. Significant mitigation was detected across these periods by Tracer-FTIR, bLS-FTIR and GEM ($p<0.05$), with the magnitudes of mitigation observed by FTIR (2.4 and 3.1 kg/d) and GEM methods (2.7 kg/d) in keeping with that expected from change in total feed intake. While it is encouraging to see mitigation being detected, estimates of mitigation calculated by difference contain the errors associated with both component measurements (s.e. of difference, (i.e. $mean_1 - mean_2$) = sqrt $[(s.e._1)^2 + (s.e._2)^2]$) (Raj 1968) so precision of the measures (control v mitigated emissions) is more important than accuracy in determining emission reduction if both measures share the same bias.

Considerable work has been done to assess the operational limits of OP methods (McGinn et al., 2014). This data also raises the possibility of quantifying mitigation where only relative emissions are known, that is where the absolute

emission may be uncertain or at risk of bias, but the difference between two measures taken from the same system may rightly quantify the emission mitigated. In this experiment, FTIR provided higher estimates of total herd emissions than were anticipated from intake and from MY determined in RC or predicted by IPCC, but the magnitude of the reduction across periods was in keeping with prediction. For potential users of the bLS technique with cattle, these results are an important finding. Recent bLS studies have monitored animal positions, assuming that animal position was critical to an accurate emission calculation (e.g., McGinn et al., 2010). However, our bLS-FTIR calculations that assume pen emissions are evenly distributed across the paddock were nearly identical to those measured with the tracer-FTIR technique in which the tracer is released from the animal (implicitly accounting for the animal positions). This shows that bLS studies in smaller paddocks and high stocking rates like ours can use the much simpler experimental approach where the paddock is treated as a uniform gas source, so that animal positions need not be monitored. This result appears to confirm similar finding from McGinn et al. (2014).

The individual-animal based estimates of methane production are consistent with those of other studies for beef steers of similar age and liveweight under grazing conditions (Boadi and Wittenberg 2002, McGinn et al., 2009). Respiration chamber produced results corroborate a close fit with GEM and the predicted emissions (IPCC 2006). When accurate emission measurements of emissions or mitigation by individuals are required (ie. for genetic selection), individual-animal based techniques with intermittent (GEM) or continuous monitoring (RC) should be used. If the mitigation strategy was to affect feeding

behavior, short term measures estimates of mitigation may fail to rightly quantify mitigation. Velazco et al. (2014) reported that dietary NO_3 affects the feeding frequency and so the interval between feeding and GEM measurement potentially skewing the daily methane production estimates. It is concluded that both the individual-animal and group-based techniques for measuring methane investigated in this experiment can be useful in quantifying enteric emission mitigation from livestock but the possibility of bias needs to be recognized in all measurement approaches.

3.6. **Acknowledgements**

Journal-Article Format for PhD Theses at the University of New England

STATEMENT OF AUTHORS' CONTRIBUTION

Atmospheric and GreenFeed measurement systems to quantify beef cattle methane emissions benchmarked using respiration chambers.

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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STATEMENT OF ORIGINALITY

Atmospheric and GreenFeed measurement systems to quantify beef cattle methane emissions benchmarked using respiration chambers.

We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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Chapter Four

Methane emissions and feeding behavior of feedlot cattle supplemented with nitrate or urea.

4. Methane emissions and feeding behavior of feedlot cattle supplemented with nitrate or urea

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4.1. Abstract

Nitrate may serve as a non-protein nitrogen (NPN) source in ruminant diets while also reducing enteric methane emissions. A study was undertaken to quantify methane emissions of cattle when nitrate replaced urea in a high concentrate diet. Twenty Angus steers were allocated to two treatment groups and acclimated to one of two iso-energetic and iso-nitrogenous finisher rations (containing NPN as urea or as calcium nitrate), with all individual feeding events recorded.   A single methane measurement device (C-lock Inc. South Dakota, USA) was exchanged weekly between treatments (2 x 1 week periods per treatment) to provide estimations of daily methane production (DMP gCH_4/d). A 17% reduction in estimated DMP (P=0.071) resulted from nitrate feeding, attributed to both a tendency for reduced DMI (P=0.088) and H_2 capture by the consumed nitrate. NO_3-fed cattle consumed a larger number of meals (14.69 v 7.39 meals/d; P<0.05) of smaller size (0.770 v 1.820 kg/meal) each day, so the

average interval between a feeding event and methane measurement was less in NO$_3$-fed cattle (3.44 v 5.15h; $P<0.05$). This difference could potentially have skewed estimated DMP and have contributed to the tendency ($P=0.06$) for NO$_3^-$-fed cattle to have a higher MY (MY, gCH$_4$/kgDMI) than urea-fed cattle. This study found short-term emission measurements made over 2 weeks (per treatment group) were adequate to show dietary nitrate tended to reduce emission and change the feeding pattern of feedlot cattle. Changes in feeding frequency may have confounded the ability of short-term methane measurements to provide data suitable for accurately estimating methane per unit feed intake.

Key words: methane, cattle, measurement, feeding behavior, greenhouse gases

4.2. Introduction

Methane produced during the fermentation of the feed in the foregut of ruminants is Australia's largest anthropogenic source of methane. While there is a suite of developing mitigation strategies (Cottle *et al.,* 2011), the inclusion of nitrate salts in managed feeds is one of the most predictable in its mitigation efficacy, being used in lick blocks, liquid supplements, pelleted and TMR rations (van Zijderveld *et al.,* 2010, Li *et al.,* 2012). There is a growing need for measurement methods to verify emissions for inventory and quantify mitigation as Australian legislation is developed to pay livestock owners to reduce emissions under the Carbon Farming Initiative (www.countrycarbon.com.au). Increasingly, daily methane production (DMP; g CH$_4$/d) is being estimated from short-term

measures of methane flux made on-farm when animals come to feed (Zimmerman *et al.,* 2013, Velazco *et al.*, 2013a), water (Mc Ginn *et al.,* 2010) or for milking (Garnsworthy *et al.,* 2012; Larssen *et al.,* 2012) or are simply gathered from the paddock for measurement (Goopy *et al.*, 2011). The constraints of many of these emerging methods are being evaluated with the 2 main issues being the conversion of measured methane concentration to methane flux, and/or bias in estimated DMP due to emissions being determined at a fixed time daily (Hegarty, 2013). The commercially produced GreenFeed Emission Monitoring System (GEM; C-Lock Inc, South Dakota, USA) measures flux directly over periods of approximately 5 minutes as cattle access a bait station at programed intervals when these emissions are measured (Zimmerman *et al.,* 2013). Initial validation studies of the GEM have shown DMP estimates correlate highly with open circuit calorimetry measures in which all emissions throughout the day are measured (Hammond *et al.,* 2013), so the GEM system was selected to quantify mitigation of DMP by dietary nitrate in feedlot cattle. This study was conducted to verify that nitrate salts could be safely included in the TMR of feedlot cattle and would reduce the DMP and methane yield (g CH_4/kg DMI) as expected from published literature.

4.3. **Materials and Methods**

4.3.1.   **Animals, management and diets**

Twenty Angus steers (352.9 kg ±17.7 kg) were randomly allocated to two treatment groups (n=10) and housed in 2 feedlot pens. On feedlot introduction, steers were acclimated to the two iso-energetic and iso-nitrogenous finisher diets containing NPN as urea or as calcium nitrate (Table 4-1), by progressively increasing levels of grain and of NPN inclusion over four weeks. In the first week, the steers were fed a starter ration containing 30% rolled barley, 50% hay, 10% cottonseed meal (% as fed basis), and supplement containing NPN sources, limestone, minerals, and vitamins. Then the rolled barley content was increased gradually over 2 weeks (48% in second week and 65% in the third week) until the final diet composition was reached (75% rolled barley, 11% hay, 3% cottonseed meal and supplement containing NPN, limestone, minerals and vitamins). Urea and calcium nitrate stepped up from 0.25% and 1% in the starter ration to 0.89% and 2.57% in the finisher ration respectively. The rations were each offered through an automatic feeder fitted with RFID sensors to identify and record every individual meal (one feeder/pen; Bindon, 2001). Two steers were removed from the urea group due to variable feed intake which was considered a possible indicator of acidosis on Day 17, leaving 8 steers in that pen for the study. During the acclimation, steers had access to a GEM unit in their pen to train them in GEM use and visits were monitored daily. The single GEM device was exchanged between treatments on a weekly basis (2 x 1 week periods per treatment) throughout the acclimation and measurement periods to provide estimates of DMP.

Table 4-1 Chemical composition of the dry matter of finisher rations containing urea or nitrate and of pellets dispensed to feedlot cattle by the GreenFeed emission monitor (GEM)

	GEM Pellets	Urea Finisher	Nitrate Finisher
Neutral Detergent Fibre (%)	27	23	22
Acid Detergent Fibre (%)	9	11	9
Crude Protein (%)	16.8	11.5	11.4
Metabolisable Energy (MJ/kg) [A]	12.3	12.4	12.7
Crude Fat (%)	3.4	1.8	1.9
Digestible Organic Matter in the DM[B] (%)	76	82	80
Urea (% as fed)	0	0.89	0
Calcium Nitrate (% as fed)			2.57

[A]Calculation of metabolisable energy done with Australian Fodder Industry Association (2011) equation for grains and concentrates.
ME (MJ/kg DM) = 0.858 + 0.138 DOMD (%) + 0.272 EE (%)
[B]Digestibility determined by wet chemistry

The GEM uses a pelleted supplement as an attractant (Table 4-1), delivered to the steers based on their identity according to a programed supplement delivery schedule (www.c-lockinc.com). Animals need to place their head in the shroud of the GEM to access the delivered pellets. Air is drawn thought the GEM at a known rate and concentrations of methane are measured continuously. A proximity sensor mounted in the front of the GEM indicates if an animal's head is in position to avoid loss of eructated methane from the shroud. The programmed schedule restricts the frequency of the visits throughout the day by delaying the delivery of the attractant (pellets). In this study, pellets were only dispensed when cattle voluntarily placed their head in the shroud of the unit only after an interval of at least 4 hours since their last measurement; animals coming for a return visit within this time received no further supplement. For data to be accepted from a GEM visit, an animal needed to stay in front of the GEM for at

least 2 minutes to ensure inclusion of adequate eructation events, with the pellets being dropped every 40 seconds (50.8g ±0.8g pellets/drop) with a maximum of five drops per measurement period to encourage animals to stay for at least 2 minutes. An animal would only get the maximum number of drops in a feeding period if it stayed for at least 3 minutes. An animal staying for longer did not induce release of any additional pellets for the next 4 hours.

4.3.2. Feed intake, live weight gain and methane production

The ration was offered *ad-libitum* through automatic feeders fitted with RFID sensors to identify and record every individual meal (Bindon, 2001). Meal weight, time and duration of all individual feeding events were automatically recorded. All visits at the GEM unit were recorded as well as all feeding events. Pellet intake was included in the daily DMI according to the number of drops registered on a 24 hours basis. Daily feed intake was calculated as the sum of all feeding events within 24 hours (including GEM pellets). Animals were measured for live weight (LW) weekly before morning feeding and without fasting.

4.3.3. Methaemoglobin concentration in blood

Weekly blood samples were taken before morning feeding using lithium-heparin vacutainers (BD-Plymouth, UK). Samples collected by tail venepuncture were used to determine methaemoglobin (MetHb) concentration in blood by the spectroscopic method of Hegesh *et al*., (1970), within 30 minutes of sample collection. As MetHb increased over the study period, only data from the final (day 54) is reported (Table 4-2). MetHb is expressed as the percentage of total haemoglobin in blood.

4.3.4. Statistical analyses

Mixed model analyses were conducted in Genstat (Payne *et al.,* 2011) with animals fitted as a random effect and nitrogen source as a fixed effect. Final MetHb levels were analyzed using initial levels as a covariate. Feed intake, live weight gain, daily feed events, daily feed events size, and feed conversion ratio were assessed by one-way ANOVA. The significance level was set at *P*<0.05.

4.4. Results and Discussion

As inclusion of NO_3 in the diet can cause nitrite toxicity, so blood MetHb of all cattle was tested weekly as an indicator of animal welfare. At the final measurement (Table 4-2) when MetHb was highest, there was still no significant effect of nitrate on MetHb (*P*=0.217). None of the animals showed signs of methaemoglobinemia during the experiment, with the highest individual concentration of MetHb being 3.3% (of total haemoglobin) which was not a threat to animal health or wellbeing.

This short-term study was too brief to adequately quantify treatment effects on LW and DMI, so no difference in live weight gain (LWG) or DMI were detected (Table 4-2). However, these data were used to estimate emission intensity (g CH_4/kg LWG) and methane yield (g CH_4/kg DMI). A larger concurrent growth study of cattle on the same diets, was conducted in which DMI (10.32 v 11.02; *P*<0.001) and LWG (1.59 v 1.71 kg/d; *P*<0.01) were lower on the nitrate diet than on the urea diet (Hegarty *et al.,* 2013). There were no differences between NO_3-

fed and urea-fed cattle in methane intensity (gCH$_4$/kg LWG) or feed conversion ratio in the current experiment (P=0.76), with average values of 110 gCH$_4$/kg of LWG and 6.58 kg of feed/kg LWG respectively (Table 4-2).

Animal access and utilisation frequency of the GEM device were similar between groups, with the GEM unit located in each pen for 2 x 1 week periods. During the experimental period, 83% of animals used the GEM and supplied emission data for analysis. Groups differed little in the number of cattle using the GEM (7/8 in the urea-fed group and 8/10 in the NO$_3$-fed); the length of the visits (P=0.267, mean 3 minutes and 40 seconds); or the number of individual daily visits (P=0.340, mean 2.74 visits/day). The overall number of methane measures was 449 with 206 in the NO$_3$-fed group and 243 in the urea-fed group over the 2 weeks of measurements. Similar distribution between treatments was observed with lower frequency of visits during the night-time (9pm to 4am) in both treatments. The total time of methane data collection during the experiment was 12:50 and 14:30 (hh:mm) for NO$_3$-fed and urea-fed steers respectively.

Consistent and persistent reduction in ruminant DMP as a direct effect of dietary NO$_3$ inclusion has being extensively documented. The main contributing factors to this mitigation described in the literature are: 1) the reduction in voluntary intake (Holshof et $al.$, 2012, Hegarty et $al.$, 2013), and 2) the inhibition of the methanogenesis explained by preferential use of H$_2$ for nitrate reduction over reduction of CO$_2$ to CH$_4$ (van Zijderveld et $al.$, 2010). There was a evidence of both these mechanisms occurring in this study (P<0.1, Table 4-2).

To predict the likely total mitigation resulting from nitrate ingestion, the emission reductions anticipated from changes in feed intake and from

stoichiometric repartitioning of H_2 into NO_3 reduction were summed. The equation

of Moe and Tyrrell (1979) developed for cattle on high concentrate diets was used

to predict DMP, suggesting DMP of NO_3-fed cattle would have been 12% lower

than urea-fed cattle based on differences in DMI alone (Table 4-2; assumed lignin

and silica = 1.1% and 0.1% in DM respectively). While measured DMP tended to

be lower for NO_3- than urea-fed steers (169.9 v 204.6g CH_4/head.d. (P=0.071),

the observed 17.0% reduction in emissions, however, was not fully explained by

the reduced DMI associated with dietary nitrate. To estimate the additional

potential abatement resulting from ruminal reduction of dietary nitrate,

stoichiometry was applied where the reduction of 1 mol of nitrate to ammonia uses

the same amount of H_2 as does reduction of 1 mole of CO_2 to methane (Nolan *et

al.,* 2010). The efficiency of that reduction, however, is known to decline with

nitrate inclusion level as summarised by van Zijderveld *et al.* (2010) and Nolan *et

al.* (2010). A reduction efficiency of nitrate of 78% was assumed (Nolan *et al.*,

2010), giving a potential DMP abatement based on this stoichiometry of 40.2

gCH_4/head.d rather than 51.5 gCH_4/head.d (100% efficiency). Together, the

emission reduction expected from reduced feed intake (30g/d) and from rumen H_2

repartitioning in NO_3-fed relative to urea-fed cattle (41.6 g/d) is approximately

twice that observed during the 14 d short-term emission measures collected for

each group (34.7 g/head.d).

An increase in MY was apparent in NO_3-relative to urea-fed cattle (P=0.06.

Table 4-2) that has not been previously observed and is contrary to past

observation and expectation (van Zijderveld *et al.*, 2011, Velazco *et al.*, 2013b).

While we do not have continuous 24h gas collection data to verify if observed

differences in MY in this study actually occurred, the apparent rise in MY

associated with dietary NO_3 caused us to examine the feeding pattern and timing

of emission measurements relative to feeding events on both rations.

Table 4-2 Least square means for average dry matter intake, number and weight of feeding events, delay between feeding event and methane measurement, measured daily methane production, predicted methane production, methane yield, live weight, live weight gain, feed conversion ratio and methaemoglobin concentration in blood in feedlot cattle given diets containing urea or nitrate.

	Urea	Nitrate	P-value
Dry matter intake (kg/d) [A]	12.9	10.8	0.088
Number of meals per day (meals/d)	7.39	14.69	0.007
Meal weight (kg/meal)	1.82	0.77	<0.001
Delay between feeding event and GEM (h)	5.15	3.44	0.006
Measured daily methane production (gCH4/d) [B]	204.6	169.9	0.071
Predicted methane production (gCH4/d)[BC]	239	209	-
Methane yield (g CH4/kg DMI) [B]	15.8	16.9	0.064
Live weight (kg)	430	419	0.388
Live weight gain (kg/d)	2.00	1.61	0.188
Feed conversion ratio (kg feed/kg LWG)	6.47	6.69	0.824
Methane intensity (g CH4/kg LWG)	107.7	113.1	0.760
Final methaemoglobin (%)	0.827	1.307	0.217

[A] Dry matter intake measured over 4 weeks
[B] Daily methane production (measured and predicted) and methane yield calculated over 2 weeks
[C] Predicted methane production based on Moe and Tyrrell (1979). This estimate does not include any effect of nitrate on methane production except through reduced feed intake.

NO$_3$-fed steers consumed more meals/d of the finisher ration ($P<0.01$), with

each meal being of smaller size than for urea–fed steers ($P<0.001$; Table 4-2).

Consequently, the time interval between consuming a meal of finisher ration, and

a measure of methane production being made was significantly shorter for NO$_3$-

fed cattle (3.44 V 5.15h; $P<0.05$). Feed intake and time after feeding are known to

affect methane emission rate (van Zijderveld *et al.*, 2010), and emission rates

measured soon after feeding are higher than the daily mean (Crompton *et al.*,

2011). The shorter average interval between eating and commencement of short-term emission measures by NO_3-fed cattle could therefore have caused the measured emission of NO_3-fed cattle to be higher than the true daily average, contributing to the apparently lower than predicted mitigation from NO_3 and a higher MY.

An attempt was made to adjust emission data for the size and timing of meals relative to each emission measurement. A consumption index (CI) that was used as a covariate in analysis of DMP was calculated from the weight and time (relative to GEM visit) of each feeding event for each animal in the 1d or 3d prior to each GEM visit. CI was calculated by summing terms of (meal weight/interval between feeding event and GEM visit) for all feeding events recorded in 24 (CI1) or 72 hours (CI3) prior to the GEM measurement. The calculation procedure assumed linearity in the effect of meal size and time interval time on DMP Such that the larger the meal and/or shorter the interval the higher CI value. This linear approach to adjusting for differences in feeding pattern seems to be a poor predictor of the relationship between CI and DMP as no significant association between DMP estimates and CI was found. Further, the estimates of DMP that had been adjusted for CI1 or CI3 showed very little correlation with each other (r^2=0.17). Future Consumption Index evaluations should include non-linear models of methane emission versus time of feed events as described by Crompton *et al*., (2011).

This study indicates an emission measurement program relying on short-term emission measurements is useful in identifying reductions in emissions likely to result from dietary NO_3 as a nutritional mitigation strategy. The measured

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mitigation was less than expected based on measured DM intake and calculated rumen stoichiometry, which could reflect either emissions not being as predicted, or inaccuracy in estimation of DMP. Since this short-term emission measurement system (GEM) has had its emission estimates correlated with respiration chambers (Hammond *et al.,* 2013, Chapters 2 and 3 of thesis) we have little reason to doubt measurement accuracy. The significant diet effect on the interval between feed consumption and methane measurement, however, identifies the need for caution in extrapolating short-term emission measures into daily emission rates when quick acting rumen modifiers such as nitrate are being evaluated. We propose a non-linear consumption index should be developed to assist in future analyses of mitigation strategies if short-term methane emissions are made and feeding data are available.

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STATEMENT OF AUTHORS' CONTRIBUTION

Methane emissions and feeding behavior of feedlot cattle supplemented with nitrate or urea.

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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Chapter Five

Daily methane emissions and methane intensity of grazing beef cattle divergently selected for residual feed intake.

5. Daily methane emissions and emission intensity of grazing beef cattle genetically divergent for residual feed intake

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5.1. Abstract

Since daily methane production (DMP; g CH_4/d) is strongly correlated with dry matter intake (DMI), the breeding of cattle that require less feed to achieve a desired rate of average daily gain (ADG) by selection for a low residual feed intake (RFI) can be expected to reduce DMP and also emission intensity (EI; g CH_4/kg ADG). An experiment was conducted to compare DMP and EI of Angus cattle genetically divergent for RFI and 400-day weight (400dWT). In a 6-week grazing study, 64 yearling-age cattle (30 steers, 34 heifers) were grazed on temperate pastures, with heifers and steers grazing separate paddocks.

Liveweight (LW) was monitored weekly and DMP of individual cattle was measured by a GreenFeed emission monitoring unit (GEM) in each paddock. Thirty-nine of the possible 64 animals had emission data recorded for 15 or more days, and only data for these animals were analysed. For these cattle, regression against their mid-parent estimated breeding value for postweaning RFI (RFI-EBV) showed that a lower RFI-EBV was associated with heavier LW at the start of experiment. Predicted dry matter-intake (pDMI), predicted DMP and measured DMP were all negatively correlated with RFI-EBV ($P<0.05$), whilst ADG, EI and predicted CH_4 yield (pMY; g CH_4/kg DMI) were not correlated with RFI-EBV ($P>0.1$). Daily CH_4 production was positively correlated with animal LW and ADG ($P<0.05$). The associations between ADG and its dependent traits EI and pMY and predicted FCE (kg pDMI/kg ADG) were strongly negative (r=-0.82, -0.57 and -0.85, $P<0.001$) implying that faster daily growth by cattle was accompanied by lower EI and MY and improved feed conversion. These results show that cattle genetically divergent for RFI do not necessarily differ in ADG, EI or pMY on pasture and that, if heavier, cattle with lower RFI-EBV can actually have higher DMP while grazing moderate quality pastures.

Key words: methane, grazing cattle, measurement, RFI, greenhouse gases

5.2. **Introduction**

Reductions in the quantity of daily CH_4 production (DMP; g CH_4/d) and intensity of methane emissions (EI; g CH_4/kg ADG) arising from livestock are increasingly demanded by the public and governments (IPCC 2013), with strategies to achieve this mitigation needing to be validated in commercial

environments (Pickering *et al.* 2013). Given the strong positive relationship between dry matter intake (DMI; kg/d) and DMP (Grainger *et al.* 2007; Kennedy and Charmley 2012; Ramin and Huhtanen 2013), it can be expected that animals with lower feed intakes will also have lower DMP. Feed intake of forages is primarily regulated by the energy deficit of the animal and its rumen digesta load (Weston 1996) which are themselves affected by body size and physiological status of the animal (Allison 1985).

Variation exists among individual cattle in DMI required to maintain liveweight (LW; kg) and for growth (Koch *et al.* 1963; Herd *et al.* 2004a). Residual feed intake (RFI, kg DM/d) is a measure of the variation in feed efficiency of individuals above or below the feed use efficiency of the population, due to differences in digestion, metabolism, activity and thermoregulation (Herd and Arthur 2009). It is the difference (residual) between measured DMI and predicted DMI for given body size and level of production (Herd *et al.* 2003). *"Animals of superior (lower) RFI will produce the same specified average daily gain (ADG, kg/d) with less feed intake, and this is independent of the BW or ADG of the animal."* Residual feed intake is moderately heritable (Herd *et al.* 2003) and divergent selection has resulted in reduction in feed intake with no compromise in growth performance of young cattle (bulls, steers and heifers) with ad-libitum access to medium-to high digestibility rations and improvement in growth rate and feed conversion of steers on pasture (Herd and Pitchford 2011). In an experiment that measured cow feed intake at pasture over 3 years, cows bred for high RFI consumed 12% more, produced only 1% more weight of weaned calf and gained 16% less weight themselves, resulting in a cost of production that was 14%

greater and maternal productivity 12% lower compared to cows bred for low RFI (Pitchford *et al.* 2014).

Lower DMP has been measured in cattle of lower RFI in feedlot situations (Nkrumah *et al.* 2006, Hegarty *et al.* 2007) and under conditions of high intake of good quality pasture, but no difference was apparent when the same cattle consumed pastures of poorer quality (55% dry matter digestibility; DMD; Jones *et al.* 2011). This is consistent with findings of previous studies of beef cattle grazing extensive pastures that cattle genetically low for RFI have a greater ADG than do genetically-high RFI cattle, but do not necessarily eat less (Herd *et al.* 2002; 2004b; 2011). If no differences in DMI and DMP are evident but higher ADG is verified in low-RFI cattle, this should result in a lower (more favorable) feed conversion efficiency (FCE; kg DMI/kg ADG) and lower EI. Reduced emissions from selective breeding may be an effective long-lasting mitigation strategy delivering a permanent reduction of CH_4 emissions if productivity is not affected (Cottle *et al.* 2011; Pickering *et al.* 2015).

As digestibility and animal metabolism explain only two-thirds of variation in RFI in divergently selected cattle (Richardson and Herd 2004) it was proposed that differences in energetic inefficiency with which animals lose digested energy as enteric methane could contribute to RFI divergence and so EI of cattle. The aim of this study was to measure and compare ADG and individual DMP and EI under grazing conditions of beef cattle genetically divergent for RFI. It was hypothesized that animals genetically superior for RFI, as indicted by having lower estimated breeding values for RFI , would exhibit a lower DMP and lower EI.

5.3. **Materials and Methods**

5.3.1. **Animals, pastures and management**

All protocols for the care of animals used in this experiment were approved by the New South Wales Department of Primary Industries (NSW DPI) Animal Ethics Committee, Orange NSW (approval number ORA 13/16/004). The Angus cattle were bred at the NSW DPI Agricultural Research Centre, Trangie NSW, Australia, by artificial insemination. Cows were from the post-weaning RFI-divergent selection lines described by Arthur *et al.* (2001) and stored semen was from 2 sires per line which had previously been used in these selection lines. All sires and dams had EBV for postweaning RFI (EBV-RFI) and 400-day-of-age weight EBV (EBV-400dWT), dated November 2009, and calculated by the Animal Breeding and Genetics Unit, University of New England, Armidale NSW, using pedigree and performance records from the Trangie Angus herd. Summary statistics for sire and dam EBV, and accuracy of the EBVs, are presented in Table 5-2. Mid-parent EBVs for each animal were calculated as the arithmetic mean of the sire EBV and dam EBV. The breeding program resulted in 64 weaned calves, consisting of approximately equal numbers of males and females, and that had a wide range in genetic merit for RFI and 400dWT, as indicated by the range in mid-parent EBVs being greater than 2 kg/d for RFI and greater than 30 kg for 400dWT(Table 5-2). Male calves were castrated at ~4 months of age and weaned cattle calves were transferred to NSW DPI Agricultural Research and Advisory Station, Glen Innes (New South Wales), where all animals were managed together on native pastures to reach approximately 300 kg LW by 6 months post-weaning

A 42 d grazing study was then conducted with steers and heifers managed in separate (adjacent) 14 ha paddocks. The study consisted of 3 periods, being a 30 d introduction to pasture and to the use of GreenFeed emissions monitors (GEM), followed by two 21 d experimental periods in which LW and DMP were measured and pastures were sampled. A GEM unit was present in each paddock throughout the introduction and 42 d grazing experiment ensuring continuous access for all cattle. During the 42 d grazing experiment all cattle were weighed weekly at 9 am, without a fasting period, and all animals had unlimited access to reticulated water in paddocks. After the first 21 d of measurement, heifers and steers were rotated between paddocks to reduce confounding of sex with paddock and GEM unit. The LW recorded on day 21 was used as the mid-test LW, with ADG over the test period being calculated from the linear regression of weekly LW against day of measurement. .

All animals were rumen sampled by oesophageal intubation on day 21 with rumen pH determined immediately after rumen fluid collection using a portable pH meter (Orion 230 Aplus, Thermo Scientific, USA). Samples (15 mL) were acidified with 5 drops of concentrated (98%) Sulfuric acid, then frozen at -20° C and kept for later determination of volatile fatty acid (VFA) concentrations in the laboratory. Samples were thawed and VFA concentrations determined by gas chromatography (Nolan *et al.* 2010) using a Varian CP-3800 Gas Chromatograph linked to a Varian CP-8400 auto-sampler and Varian Star integration software. Faecal samples were collected from the rectum of all animals on days 14 and 32, stored frozen and later dried to constant weight (60°C), ground through a 1mm screen and the concentration of silica determined by X ray fluorescence (Tighe and Forster 2014)

using a Bruker Tracer III-V PXRF (Bruker Corp., Billerica, Massachusetts, USA)

that has been calibrated against inductively coupled plasma emission

spectroscopy measures of mineral content (McLaren *et al.* 2012). Silica (Si) was

chosen as an indigestible internal marker (Rymer 2000) but as the daily silica

intake of individuals was unknown, results are only expressed as silica

concentration and not used to estimate DMD. This means the Si concentration is

an indicator of relative digestibility and the only assumption is that animals

differing in RFI-EBV did not differ in the Si content of the forage they consumed.

Visual pasture assessment for DM on offer (kg/ha), percentage of green in

the DM and botanic composition in both paddocks was conducted on days -7, 7,

21 and 35. On day -7, pasture DM availability was assessed with 12 square

quadrats ($0.25m^2$) cut to ground level per paddock and dried in a forced air oven

($60\ ^oC$) until constant weight. Visual estimates of pasture yield within the

quadrants were made before cutting. Observer estimates were compared with cut

standards and the association between visual assessments and cut quadrants

was high (r=0.91) so no corrections were applied. Pasture composition was

assessed visually by recording the three predominant species (Tothill *et al.* 1978).

Pasture data are presented as average values over period 1 (up to day 21) and

over period 2 (day 21 to day 42). Pasture quality was determined in eight (two per

period and per paddock) pooled samples taken during the experiment. Crude

protein (CP) was assessed by wet chemistry (AOAC 990.03 method), acid

detergent fibre (ADF) and neutral detergent fibre (NDF) by near-infrared

spectroscopy, metabolizable energy (ME) by the AFIA 2.2R method, and

digestible organic matter in the dry matter (DOMD) by wet chemistry (AFIA

method 1.7R) conducted by the NSW DPI Feed Quality Service, Wagga Wagga NSW, Australia (AFIA 2014). The gross energy (GE) content of the pasture was assessed using the same eight pooled samples (dried, ground to 0.5 mm and combusted) using a bomb calorimeter (Model c7000Ika, Werke, Stafen, FRG) with a 0.5 g sample and 30 bars oxygen pressure (Phillipson 1964).

5.3.2.   In-field CH_4 emissions measurements

Daily CH_4 production (DMP) was estimated from multiple short-term breath measurements using two GEM units (Velazco *et al.*, 2015). The GEM (C-Lock Inc., Rapid City, S. Dakota) is a feeding station where pelleted supplement is provided in a controlled manner to cattle (quantity/supplement event and number of supplement events/d), based on animal identity as detected by radio-frequency identification ear-tag. To access the supplement, cattle place their head in an open shroud where the pellets are dispensed. Air is continuously drawn through the shroud and past the neck of the feeding animal at a precisely measured rate, and the concentration of gases (CH_4 and carbon dioxide; CO_2) and of propane (periodically released as a reference gas) are measured in the exhaust air stream. Background gas concentrations are automatically determined when no cattle are present and periodic calibrations and recovery tests are performed to define sensor responses to known concentrations of CH_4 and CO_2. Recovery tests were made based on a gravimetrically determined release of CO_2 into the shroud. The recoveries of released CO_2 were 95.9 and 97.9% for the two units used in the present experiment and no correction of data was made for recovery. Methane and CO_2 sensor calibrations were performed weekly and the coefficient of

variations across calibrations were 2.4 and 2.7% for the CH_4 sensors, and 2.1 and 2.5% for the CO_2 sensors, of the two GEM units, respectively.

Several eructation events are detected during the GEM supplement delivery (mean visit duration = 4.32 minutes with 1-2 eructations per minute). A proximity sensor in the shroud monitoring head-position of the animal throughout each feeding event was used to exclude all measures where animals stepped away from the shroud during the CH_4 measurement. The emission rates over all useful feeding events (defined as those of at least 3 minutes length with the animals head correctly in position) were averaged within each day to provide an estimate of DMP for that day. The GEM units were programmed to deliver up to 4 drops of pellets every 45 seconds and then wait for at least 3 hours before a new supplement event could occur for the same animal to ensure both, a minimum measurement time (3 minutes) and measurements to represent the daily variation in emissions. The GEM unit delivered pellets (6mm diameter and 15mm length) containing sorghum, wheat and cottonseed meal (DM = 93%; ME = 11 MJ/kg DM; CP = 14% DM; NDF = 13%; ADF = 5%) and aniseed flavor as an attractant ((0.075% DM; Fluidarom 1957, Norel, Spain). Pellets were a little higher in ME content than the pasture and with aniseed aroma to persuade the animals to voluntarily visit the GEM unit. The average weight of pellet dispensed in each supplement-drop was 52.3±0.3 g (mean±sd) so the maximum daily pellet intake was 1.58 kg DM and this was to keep pellet intake at one fifth or less of predicted daily DM intake. According to the frequency of CH_4 measurements, animals were grouped in regular (15 or more days with DMP estimates) and non-regular users (less than 15 days with DMP estimates) for the analysis.

5.3.3.   Predicted DMI and FCE

Estimation of intake in a continuously grazed pasture is often difficult (Cottle 2013) with factors such as the differences in forage digestibility and rumen retention time among individuals affecting the accuracy of the estimation. The method chosen to predict DMI (pDMI, kg/d) was that of Minson and McDonald (1987) that derives DMI from the LW and ADG of cattle (Equation 1). Mid-test LW and ADG over the experiment were used in this equation.

$$pDMI = (1.185 + 0.00454 \times LW - 0.0000026 \times LW^2 + 0.315 \times ADG)^2 \quad \text{(Equation 5)}$$

The Minson and McDonald (1987) equation has been validated for Australian temperate and tropical pastures with the difference (reported by the original authors) between the predicted and the observed intakes being below 1%. Predicted feed conversion efficiency (pFCE) was calculated as pDMI/ADG.

5.3.4.   Prediction of CH_4 emissions

The Blaxter and Clapperton (1965) equation for estimating cattle CH_4 emissions was used to predict the CH_4 emissions based on pDMI and measured DOMD (%). The equation calculates the percentage of dietary gross energy intake (GEI) lost as CH_4 from the apparent digestibility of dietary energy (DOMD used as a proxy) and the level of intake (LOI) defined as a multiple of maintenance energy requirement (Equation 2). The Minson and McDonald (1987) and Blaxter and

Clapperton (1965) equations are used for grazing beef cattle in the Australian Greenhouse Gas Inventory calculations (DoE 2013).

CH$_4$ conversion rate (% GEI) = 1.30 + 0.112 x DOMD + LOI (2.37 - 0.050 x DOMD)   (Equation 6)

5.3.5.   Residual CH$_4$ Production

Residual CH$_4$ production (RMP) can be calculated as the difference between the measured DMP and predicted DMP (Herd *et al.* 2014). In the present study, four forms of RMP were calculated. The first three forms used predicted DMP calculated from the predicted DMI (pDMI) and published DMP prediction equations. The predicted GEI (pGEI) was calculated as the product of GE content in the pasture and pDMI (Equation 1). The first form of RMP, RMP$_{B\&C}$, was calculated as the difference between the measured DMP and the predicted DMP using Equation 2 and the animal's pGEI. The second form of RMP, RMP$_{IPCC}$, was calculated as the residual between measured DMP and 6.5% of the pGEI based on the Intergovernmental Panel on Climate Change (IPCC) recommended conversion factor for grazing cattle (IPCC 2006). The third form of RMP, RMP$_{J\&J}$, was similarly calculated using a conversion factor for cattle fed roughages of 6% of pGEI (Johnson and Johnson 1995). A fourth method of calculating RMP (RMP$_{MR}$) sought to avoid assumptions required in predicting intake. A multiple regression (MR) of measured DMP against mid-test LW and ADG was conducted, with RMP$_{MR}$ being the residuals for DMP above and below the multiple regression

line. The four forms of RMP ($RMP_{B\&C}$, RMP_{IPCC}, $RMP_{J\&J}$ and RMP_{MR}) were investigated in the present study.

5.3.6.   Statistical analyses

To test for possible effects of the CH_4 measurement procedure on LW gain, in a preliminary analysis, the ADG of GEM regular users (>15 DMP estimates; n=39 animals) and non-regular users (<15 DMP estimates; n=25 animals) were compared, with mid-test LW fitted as a covariate. The lack of effect indicated that regular usage of the GEM units did not significantly affect growth performance. In the subsequent analyses, only data for the 39 regular GEM users were used. For each of the 39 animals that had at least 15 days with valid GEM DMP estimates, the DMP estimates for each day were averaged to calculate average DMP for each animal over the test period. Predicted CH_4 yield (pMY) was calculated as measured DMP divided by pDMI. The number and the overall length of CH_4 measurement sessions by the 39 animals were also determined. Statistically-significant associations between traits were determined by calculating Pearson correlation coefficients. The magnitudes of phenotypic associations between selected traits were calculated as the regression coefficients using the PROC GLM procedure of SAS (1989), with sex included in the model. For the two methane traits (DMP, EI) calculated without predictions based on LW or ADG, an analysis of covariance was conducted fitting DMP or EI against sex, initial-LW, ADG and EBV-RFI in GLM models, to check associations with EBV-RFI at the same LW and ADG.

5.4. **Results**

5.4.1. **Pastures**

The quantity of pasture DM available in period 1 (to day 21) was higher than in period 2 (day 21 onward) in both paddocks, and steers had more DM available than heifers within the periods (Table 5-1, $P<0.05$). The proportion of green tissue in the DM tended to be higher in period 1 than period 2 ($P<0.1$). The dominant species were cool season perennials with *Dactylis glomerata* (cock's-foot) and *Festuca arundinacea* (tall fescue) always present in both paddocks. *Phalaris aquatica* was present in the first period but was then lost from the sward while *Paspalum dilatatum* increased its prevalence in period 2. Overall the pastures exhibited a decline in DOMD over time which may have been exacerbated by grazing and sub-optimal conditions for pasture regeneration (high temperature and low rainfall). The average GE content of the pasture was 16.13 MJ/kg DM without differences between paddocks or periods ($P>0.05$; coefficient of variation = 2.3%; Table 5-1).

Table 5-1 Pasture dry matter availability, green fraction, botanic assessment and chemical analysis of the pasture available to cattle divergently selected for residual feed intake. Values are means by sex and period.

	Steers		Heifers	
	Period 1	Period 2	Period 1	Period 2
Pasture availability (kg DM/ha)	1456 a	1212 b	1305 b	1049 c
Green fraction of the pasture mass (%)	34 A	27 B	33 A	27 B
Paddock area (ha)	14	14.5	14.5	14
Botanic assessment (ranking of the three most frequent species present in the pasture)				
Species 1st	*Festuca*	*Dactylis*	*Dactylis*	*Dactylis*
Species 2nd	*Dactylis*	*Festuca*	*Phalaris*	*Festuca*
Species 3rd	*Paspalum*	*Paspalum*	*Festuca*	*Paspalum*
Chemical analysis and gross energy content (pooled samples collected at the beginning and end of each period)				
NDF (% DM)	63	61	61	62
ADF (% DM)	35.5	35.0	34.5	34.5
CP (% DM)	12.4	13.8	16.0	11.2
DOMD (%)	61	60	63	58
ME (MJ/kg DM)	9.2	9.1	9.8	8.8
GE (MJ/kg DM)	16.20	16.20	16.35	15.77

(a,b) Means within rows with differing letters are significantly different ($P < 0.05$).
(A,B) Means within rows with differing letters tend to be significantly different ($P < 0.1$).

5.4.2. CH$_4$ traits

No differences were evident in measured DMP between steers and heifers. Steers had a predicted DMP (pDMP) 15% higher than heifers as a consequence of these predictions being calculated using their higher ADG. Measured EI was 38% less in steers compared with the heifers, being higher in the heifers as a result of similar DMP yet a lower ADG than steers. The pMY and the three RMP traits calculated based on prediction equations were lower for steers compared to heifers. When RMP was calculated as the residuals of the multiple regression of

DMP on LW and ADG (RMP_{MR}), no difference was evident between steers and heifers. Rumen fluid pH, concentration of total VFA and the three major VFA, and Si concentration in faecal DM did not differ between steers and heifers. The number of CH_4 measurements and total collection time per animal did not differ between steers and heifers but the average duration of each CH_4 measurement tended to be lower in the heifers ($P<0.1$) compared with the steers.

Table 5-2 Descriptive statistics for 39 Angus yearling cattle, and means by sex, for mid-parent EBV for residual feed intake (RFI) and weight at 400 days of age (400dWT), initial liveweight (LW), average daily gain (ADG), predicted dry matter intake (pDMI) and predicted feed conversion efficiency (pFCE), rumen fluid traits, faecal silica content, descriptors of GEM usage, measured and predicted daily methane production (DMP and pDMP), emission intensity (EI), predicted methane yield (pMY) and four measures of residual methane production (RMP).

	Mean	SD	Min.	Max.	Steers	Heifers	#
N					19	20	
Individual sire EBV-RFI (kg/d)	-0.97	-0.61	0.61	1.42			
Accuracy of individual sire EBV-RFI (%)	71	71	59	71			
Individual sire EBV-400dWT (kg)	40	68	33	53			
Accuracy of individual sire EBV-400dWT (%)	75	75	86	86			
Dam EBV-RFI (kg/d)	-0.16	0.71	-1.0	1.2			
Accuracy of dam EBV-RFI (%)	61	6	53	73			
Dam EBV-400dWT (kg)	40	13	24	64			
Accuracy of dam EBV-400dWT (%)	72	0.9	70	73			
Mid-parent EBV RFI (kg/d)	-0.05	0.87	-1.00	1.13	-0.18	0.08	ns
Mid-parent EBV 400dWT (kg)	45.5	7.9	31.5	66.0	47.6	43.5	ns
Initial LW (kg)	325	23	253	360	325	325	ns
ADG (kg/d)	0.93	0.31	0.37	2.00	1.15	0.72	**
pDMI (kg/d)	7.38	0.82	6.15	10.08	7.97	6.83	**
pFCE (kgDMI/kgADG)	8.63	2.53	5.04	16.91	7.07	10.11	*
pH	6.99	0.60	3.90	7.85	7.09	6.90	ns
Acetate (mmoles/L)	37.5	9.6	13.3	53.4	36.1	38.7	ns
Propionate (mmoles/L)	8.26	2.08	2.66	11.65	8.08	8.42	ns
Butyrate (mmoles/L)	5.60	1.51	1.83	8.76	5.75	5.46	ns
Total VFA (mmoles/L)	51.3	13.1	17.8	72.5	50.0	52.6	ns

							#
Silica (mg/g DM)	65.5	6.0	37.2	74.1	66.2	64.8	ns
Number of CH_4 measures per animal	68.5	18.9	16	100	66.3	70.1	ns
Average length of CH_4 individual measures (min)	4.35	0.42	3.79	5.99	4.46	4.22	†
Total collection time (min)	296	79	74	416	290	296	ns
DMP (gCH_4/d)	175	18	128	212	179	172	ns
pDMP (gCH_4/d)	167	17	142	219	179	156	**
EI (gCH_4/kg ADG)	210	81	97	485	159	258	**
pMY (gCH_4/kg DMI)	23.9	2.7	16.4	29.4	22.5	25.3	**
RMP_{IPCC}	16.6	19.2	-39.7	48.3	7.2	25.6	*
RMP_{MR}	0	16	-54	24	-1.8	1.7	**
$RMP_{J\&J}$	29	19	-27	58	20	37	*
$RMP_{B\&C}$	8.6	18.6	-48.6	40.6	-0.1	17	ns

[#] Means for steers versus heifers differ: ns $P>0.1$; † $P<0.1$; * $P<0.05$, ** $P<0.01$.

Lower mid-parent EBV-RFI was associated with heavier initial LW at the start of the test, greater pDMI, greater actual DMP and predicted DMP (pDMP), and greater RMP where RMP was calculated from a multiple regression of DMP on LW and ADG (RMP_{MR}), but not EI, pMY or with the three RMP estimates based on widely-used prediction equations (Blaxter and Clapperton 1965, Johnson and Johnson 1995 and IPCC 2006) (Table 5-3). The magnitude in change in DMP with change in EBV-RFI (measured as the regression coefficient "b") was b=-10.9±2.9 gCH_4/kg RFI (se; P<0.001). From analysis of covariance, after fitting sex, initial-LW and ADG, the magnitude of change between DMP and EBV-RFI was similar, b=-9.1±3.1; P=0.006). Higher mid-parent EBV-400dWT were associated with heavier initial LW, greater predicted DMI (pDMI) and predicted DMP (pDMP) but was not associated with variation in actual DMP, EI, pMY or in any of the four RMP traits. Neither EBV was associated with variation in rumen fluid pH, concentration of total VFA or the three major VFA (results not shown), or with Si concentration in faecal DM.

Table 5-3 Pearson correlation coefficients (r-values) for mid-parent (midp) EBV for residual feed intake (RFI) and weight at 400 days of age (400dWT) with initial liveweight (LW), average daily gain (ADG), predicted dry matter intake (pDMI) and predicted feed conversion efficiency (pFCE), rumen fluid traits, faecal silica content, measured and predicted daily methane production (DMP and pDMP), emission intensity (EI), predicted methane yield (pMY) and four measures of residual methane production (RMP), and between the traits.

Trait[1]	Initial LW	ADG	pDMI	pFCE	pH	Total VFA	Faecal silica	DMP	pDMP	EI	pMY	RMP$_{IPCC}$	RMP$_{MR}$	RMP$_{J\&J}$	RMP$_{B\&C}$
Midp EBV-RFI	-0.40*	-0.20	-0.33*	0.04	-0.10	-0.13	-0.11	-0.55**	-0.34*	-0.04	-0.17	-0.20	-0.43**	-0.23	-0.22
Midp EBV-400dWT	0.56**	0.22	0.42**	0.02	0.16	-0.21	-0.01	0.24	0.44**	-0.01	-0.17	-0.17	0.03	-0.15	-0.17
ADG	0.07														
pDMI	0.46**	0.90**													
pFCE	0.17	-0.85**	-0.66**												
pH	0.35*	0.12	0.25	-0.10											
Total VFA	-0.31†	-0.06	-0.19	0.01	-0.24										
Faecal silica	-0.02	0.11	0.07	-0.15	-0.05	0.24									
DMP	0.35*	0.33*	0.41*	-0.17	0.06	-0.05	-0.17								
pDMP	0.51**	0.88**	1.00**	-0.64**	0.27†	-0.21	0.07	0.42**							
EI	0.09	-0.82**	-0.68**	0.97**	-0.14	0.05	-0.18	0.01	-0.67**						
pMY	-0.15	-0.57ns	-0.59**	0.52**	-0.21	0.14	-0.23	0.48**	-0.59**	0.70**					
RMP$_{IPCC}$	-0.11	-0.53**	-0.54**	0.46**	-0.18	0.13	-0.22	0.54**	-0.54**	0.64**	0.99**				
RMP$_{MR}$	0.08	0.00	0.00	0.06	-0.09	0.06	-0.21	0.91**	0.00	0.27†	0.79**	0.83**			
RMP$_{J\&J}$	-0.08	-0.49**	-0.49**	0.43**	-0.17	0.12	-.023	0.59**	-0.48**	0.61**	0.98**	1.00**	0.87**		
RMP$_{B\&C}$	-0.12	0.48**	-0.50**	0.42**	-0.19	0.13	-0.22	0.58**	-0.50**	0.61**	0.99**	1.00**	0.86**	1.00*	

[1]Units as per table 5- 2.   † $P<0.1$; * $P<0.05$, ** $P<0.01$

Phenotypic correlations between traits are presented in Table 5-3. There were significant though weak positive association between measured DMP and pDMI (r=0.41), and measured DMP and pDMP (r=0.42). The associations between DMP and ADG were positive though weak (r=0.33) but between EI and ADG was strongly negative (r=-0.82) implying that faster daily growth by cattle was accompanied by lower EI, but these parameters are not independent. Predicted MY was negatively correlated with ADG (r=-0.57) and positively correlated with measured EI (r=0.70) implying that MY was lower in animals that grew faster and in animals that had lower EI. Animals predicted to have a higher (more favourable) FCE had a lower predicted MY (r=0.52). The correlations between the three measures of RMP calculated from prediction equations (RMP_{IPCC}, $RMP_{J\&J}$ and $RMP_{B\&C}$) were all positive and very high (r values all >0.99) indicting they were measuring the same variation in RMP, but also all underestimated actual DMP as shown by the positive values for their means in Table 5-2. The correlations between these 3 measures of RMP and RMP from multiple regression (RMP_{MR}) were lower (r=0.83 to 0.87) indicating that they described between-animal variation in DMP less well than did RMP_{MR} which was derived from calculation based on the actual test data, not predictions. Variation in rumen fluid pH, concentration of total VFA or the three major VFA (results not shown), or with Si concentration in faecal DM was not associated (P>0.05) with variation in any of the 8 CH_4 traits reported, except for rumen pH which had a weak positive relationship with pDMP (r=0.27; *P*<0.1).

5.5. **Discussion**

Selective breeding to reduce RFI has been effective in achieving significant reductions in the DMI of cattle without compromising ADG (Arthur *et al.* 2014). Because of a strong association between DMP and the quantity of feed consumed (Kennedy and Charmley 2012), reducing DMI/head by moderate selection pressure on RFI has been shown to be accompanied by a reduction in DMP of cattle consuming moderately-to-highly digestible rations (Hegarty *et al.* 2007; Jones *et al.* 2011). This experiment provides contrary evidence with animals of superior (lower) EBV-RFI (higher feed efficiency) not exhibiting reduced DMP in the grazing environment, while acknowledging that DMI was not measured. More efficient animals do not necessarily eat less for a given production level (Herd *et al.* 2009) and that could explain the lack of differences in DMP. Quantifying DMI under grazing conditions would help to clarify the mechanism behind RFI differences between individuals and RFI's effect on CH_4 emissions. However, this result is consistent with the conclusion that genetic differences in DMP of sheep and cattle are often not apparent unless animals are on a high plane of nutrition (Pinares-Patino *et al.* 2003; Jones *et al.* 2011). More readily fermentable diets supporting greater DMI are often associated with lowered rumen pH, reduced acetate:propionate ratio in fermentation products so a reduced hydrogen surplus available for methanogens causing reduced methanogenesis (Lana *et al.* 1998).

The DMI of cattle in this experiment was not measured, but was predicted to have been higher for cattle with lower EBV-RFI due to their heavier LW. This, in turn, caused prediction of a higher DMP (pDMP) for the cattle with lower EBV-RFI, as was observed, and still present after adjustment for variation in LW and ADG. Emissions intensity is a composite trait calculated as the ratio of DMP to ADG and was only associated (negatively, at P<0.1) with genetic variation in RFI (EBV-RFI)

after adjustment for LW and ADG in the present study. Previous experiments with similar Angus cattle genetically-divergent for RFI and grazing similar pastures to the ones in this experiment reported no differences in intake but superior ADG associated with lower EBV-RFI (Herd *et al.* 2002; 2004b; 2011). No such association with ADG was found in this experiment which may explain the lack of association between EI and EBV-RFI. The negative directions for the associations of the four measures of RMP with EBV-RFI (statistically-significant only for RMP$_{MR}$) provides further evidence that the DMP of the cattle with lower EBV-RFI was not lower than would be expected for their size and growth rate (175 v 167 g CH$_4$/d; measured v predicted DMP, Table 5-1) . Richardson and Herd (2004) reported genetically low-RFI cattle had an improved ability to digest feed-DM which would be expected to increase MY. The lack of association between EBV-RFI and faecal Si concentration in the current study indicates no association with whole tract DM digestibility with EBV-RFI, even though the digestibility could not be quantified. Also, the lack association of total and major rumen VFA concentrations with EBV-RFI suggests that the cattle of lower EBV-RFI did not ferment any more DM in the rumen than did cattle with higher EBV-RFI.

Selection for low-RFI was effective in the abatement of CH$_4$ emissions when pasture quality and quantity were not restricted (Jones *et al.* 2011) and in feedlot situations (Nkrumah *et al.* 2006; Hegarty *et al.* 2007). The lack of reduction in observed DMP, observed EI and pMY in our study confirms the complexity of applying RFI-EBVs generated from cattle tested on high-quality grain-based diets to predict DMI and DMP in lower-quality feed environments common in many pasture-based livestock enterprises. The implication is that breeding for lower RFI as a CH$_4$ mitigation strategy on the basis of an expected reduction in intake and hence DMP, or expected improvement in growth rate and hence reduction in EI,

has only been validated where it is known that the cattle actually eat less feed or grow faster. In many pasture-based livestock grazing enterprises where pasture quality and quantity are restricted, DMP may not be lower in low-RFI cattle and the modelled benefits calculated in studies such as Basarab *et al.* 2013 (Canada) and Alford *et al.* 2006 (Australia) may overestimate the cumulative mitigation benefit following selection for RFI.

Methane EI is increasingly proposed as a mechanism to value livestock emissions in the beef production chain because it relates the emissions with the level of saleable product from the animal (Hristov *et al.* 2013). In our study, the association between DMP and level of production was positive but weak (r=0.33) and the faster ADG by cattle was accompanied by a stronger reduction in EI (r=-0.82), in agreement with Hegarty *et al.* (2010). The strong association between EI and ADG is due largely to EI being a ratio trait with ADG in the denominator. The regression relationship between DMP and ADG had a slope of 24 ± 13 g/d (mean±se) and intercept of 393 ± 42 g CH_4/d (mean±se) so that a 1-kg/d increase in ADG from 1 kg/d to 2 kg/d was associated with an almost halving of EI (from 417 to 220 g CH_4/kg ADG). While pasture intake was not measured, pFCE and pMY were negatively (favourably) correlated with ADG and strongly positively (favourably) correlated with measured EI. This implies that animals with a higher level of production will emit relatively less CH_4 (lower MY and lower EI), as reported by Waghorn and Hegarty (2011), and that more feed efficient animals (lower FCE) can be expected to have lower MY and lower EI while maintaining or increasing animal product output.

In this experiment EBV for RFI and 400dWT were associated with differences in LW achieved at yearling age on drought - affected pastures (verified

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by a decline in DOMD over time and a reduction in the pasture availability and green fraction of the pasture mass). While DMI of grazing cattle was not determined in this study, new options for assessing pasture intake while grazing through use of the GEM to deliver known quantities of indigestible markers or isotopically identifiable compounds in supplement offer promise that DMI can be determined indirectly in the future (Jones *et al.* 1981; Pereira *et al.* 2013; Velazco *et al.* 2015). Such data is needed to confidently predict the effects of genetic improvement of RFI on DMP, on MY and RMP in commercial cattle herds. Considering both total emissions and EI have been proposed as mechanisms for valuing livestock's GHG emissions in the carbon economy, the implications of this experiment are that selection for lower RFI may not always lead to reduction in quantity of daily emissions. However, where it can be demonstrated to be accompanied by a reduction in feed intake or an improvement in growth rate then a reduction in EI can be expected to result from using lower RFI genotypes in both grazing and feedlot enterprises. Further, breeding and feeding programs that increase ADG (without concurrent reduction in MY) can be expected to increase total emissions but reduce EI in a beef enterprise.

5.6. **Acknowledgements**

STATEMENT OF AUTHORS' CONTRIBUTION

Daily methane emissions and methane intensity of grazing beef cattle divergently selected for residual feed intake

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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STATEMENT OF ORIGINALITY

Daily methane emissions and methane intensity of grazing beef cattle divergently selected for residual feed intake

We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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Chapter Six

Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements.

6. Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements

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6.1. Abstract

Spot measurements of methane emission rate (n=3,048) by 24 Angus steers fed mixed rations from GrowSafe feeders were made over 3-6 min periods by a GreenFeed (GEM) emission monitoring unit. The data were analysed to estimate daily methane production (DMP; g/d) and derived methane yield (MY; g/kg DMI). A one compartment dose model of spot emission rate versus time since the preceding meal was compared with the models of Wood (1967) and Dijkstra *et al.* (1997) and the average of spot measures. Fitted values for DMP were calculated from the area under the curves. Two methods of relating methane and feed intakes were then studied: the classical calculation of MY as DMP / dry matter intake (DMI; kg/d); and a novel method of estimating DMP from time and size of preceding meals using either the data for the 2 meals preceding a spot

measurement only, or all meals for 3 d prior. Two approaches were also used to estimate DMP from spot measurements: fitting of splines on a 'per-animal per-day' basis and an alternate approach of modelling DMP after each feed event by least squares (using Solver), summing (for each animal) the contributions from each feed-event by best-fitting a one-compartment model. Time since the preceding meal was of limited value in estimating DMP. Even when the meal sizes and time intervals between a spot measurement and all feeding events in the previous 72h were assessed, only 16.9% of the variance in spot emission rate measured by GEM was explained by this feeding information. While using the preceding meal alone gave a biased (underestimate) of DMP, allowing for a longer feed history removed this bias.

A power analysis taking into account the sources of variation in DMP indicated that to obtain an estimate of DMP with a 95% confidence interval within 5% of the observed 64d mean of spot measures would require 40 animals measured over 45d (2 spot measurements/d) or 30 animals measured over 55d. These numbers suggest that spot measurements could be made in association with feed efficiency tests made over 70d. Spot measurements of enteric emissions can be used to define DMP but the number of animals and samples are larger than are needed when day-long measures are made.

Keywords: methane measurement, models, splines, GreenFeed

6.2. **Implications**

Short-term (spot) measurements are being used to verify on-farm mitigation of livestock enteric methane but their accuracy and precision are poorly defined.

Modelling in this study showed the spot emission rate was poorly correlated with feeding pattern in beef cattle, even allowing for all feeding events in the previous 72h (r = 16.9%). However, study of the sources of variation by a power analysis provided a basis for design of future experiments with spot measurements, showing detection of 10% treatment differences is possible in a spot sampling program made in association with feed efficiency tests over 70d.

6.3. **Introduction**

Measurement of enteric emissions from ruminants in their production environments is increasing (Hegarty, 2013). The simplicity of obtaining short-term (spot) measurements of enteric methane production has caused these methods to be developed for verifying mitigation on-farm (DoE, 2013) and for development of genetic parameters for methane production (Pickering *et al.*, 2013). Typically, the arithmetic average of spot measures is used as the estimate of daily methane production (DMP; g CH_4/d) yet the accuracy and precision of this approach has not been studied. Emission rates are known to change over momentary, diurnal and longer seasonal patterns (Crompton *et al.*, 2011; Ulyatt *et al.*, 2002; Munger and Kreuzer, 2008), requiring representative sampling. If the protocol does not incorporate sampling of emissions at least over the diurnal feeding and activity cycle, a scaling-up coefficient (as used by Garnsworthy *et al.*, 2012) or adjustment factors (such as for animal activity and time spent in each activity as used by Chagunda *et al.*, 2009) may be required to avoid bias in estimating DMP.

On-farm measurement of livestock DMP is likely to occur without knowledge of the dry matter intake (DMI) although herd intake may be determined (Jones *et al.*, 2011). In more controlled animal experiments, where individual

animals have their feed intake patterns measured and/or controlled, it is possible to attempt to relate methane measurements to intake patterns (e.g. Jonker *et al.*, 2014). Velazco *et al.* (2014) supplemented cattle with nitrate and reported unexpected DMP results using the GreenFeed (GEM) system which coincided with differences in time interval between feeding and GEM measurement which may have skewed the estimates of DMP. This finding stimulated a more intensive examination of the relationship between DMP and feeding history of the animal. Post-feeding emission curves have a similar shape to lactation versus time curves, with a relatively fast build up to peak production followed by a slow decline (Wood,1967; Djikstra *et al.*, 1997). Alternately, a non-linear, pharmacokinetic, one compartment oral dose model can be fitted (3 parameters reflect the area under the curve, elimination rate and absorption rate) to estimate daily flux (JMP, 2014).

The work reported addressed three objectives relating to understanding the dynamics of methane production and its relationship to recent feeding events. Specific objectives were to determine; (1) whether the simple arithmetic mean is the best way to estimate DMP from multiple spot emission measures; 2) how much DMP variation is explained by the timing and size of recent meals; and (3) the number and distribution of short term methane measures required to detect between group differences in emissions when no feed intake data is available.

6.4. Materials and Methods

6.4.1. Experimental design

This experiment was approved by the University of New England Animal Ethics Committee (AEC 14/002). Angus cattle lines divergently selected lines for low or high residual feed intake (RFI) established at the Trangie Agricultural Research

Center, New South Wales, Australia (Arthur *et al.*, 1996) provided 20-month old steers (n = 30) and heifers (n = 34) of starting LW 406.9 (± 35.8 SD) generated by approximately 2.5 generations of divergent selection. Steers and heifers were allocated in two separate feedlot pens. Three heifers and one steer were removed from the study before measurements commenced due to initial inappetence, so that 29 steers and 31 heifers were available for RFI and potentially for methane emission measurement. The total duration of the test (excluding the induction to the feedlot ration) was 70 days and heifers and steers were swapped between pens on day 35. Twenty-four animals having the most methane data (>85 or more measures of >3 min length) were chosen from these 64 animals for intensive study of the effects of feeding pattern on DMP (17 heifers and 7 steers; 9 high RFI and 15 low RFI genetic merit animals).

6.4.2.   Animals and feeding

Over 14d cattle were adjusted to a total mixed ration based on barley, cottonseed and hay (Table 6-1) provided for *ad libitum* consumption, with the ration being dispensed through GrowSafe automatic feeders (GrowSafe Systems Ltd. Airdrie, AB, Canada). Each pen had 4 individual automatic feeders, enough to provide *ad-libitum* feeding for the animals' body weight (Bindon, 2001). These feeders recorded the number of feeding events and the duration and weight of feed consumed at each feeding session (called a 'feed event') and were activated by RFID identification whenever an animal entered the feeding stall. A meal was defined as the period from which a new animal was detected in the automatic feeder and continued until the animal left. Weekly subsamples of the feed were frozen and pooled for later analysis of nutrient content (Table 6-1). Chemical

analysis of the feed was conducted by Wagga Wagga Agricultural Institute (New South Wales Department of Primary Industries, Australia).

6.4.3. Methane measurements

DMP was estimated from multiple short-term breath measurements using the GEM (manufactured by C-Lock Inc., S.Dakota, U.S.A.). The GEM is a feeding station where pelleted supplement is provided to cattle in a controlled manner (quantity/feed event and number of feed events/d) based on their identity detected by an RFID ear-tag. To access the supplement, cattle placed their head in an open shroud into which pellets are dispensed from a hopper. Air was continuously drawn through the shroud and past the neck of the feeding animal at a precisely measured rate. The concentrations of CH_4 and CO_2 and of propane (reference gas) were measured in the exhaust gas. Background gas concentrations were measured when no cattle were present and periodic calibrations and recovery tests performed to define sensor responses to known concentrations of methane and CO_2 and to ensure that >96% of the CO_2 test gas released into the GEM shroud was recovered in the exhaust gas stream. A spot measurement period of 3 to 5 minutes typically detected several eructation events and is called a 'spot sample' hereafter. To avoid data when animals stepped away from the shroud during methane measurement, a proximity sensor in the shroud, that monitors head-position of the animal throughout each feeding event, was used to identify this happening with such data being excluded. The emission rates over all useful feeding events (at least 3 minutes length with head in position) during a day were averaged to provide a mean DMP for that day. The pellets delivered in the GEM unit were 6mm in diameter and the system was programed to deliver up to 4 drops, each of 53g DM, separated by 45 seconds and then wait at least 3 hours to

allow a new supplement session for an individual animal. GEM pellets were formulated to closely match the ingredients and nutrient content of the main diet (Table 6-1) and also contained 0.075% aniseed XL flavoring (Fluidarom 1957, Norel Spain) to increase pellet palatability.

Table 6-1 Chemical analysis of the finisher feed-lot ration fed to steers and heifers during the 70-day study of residual feed intake

	Ration	
	Finisher ration	GEM pellets
Dry Matter (DM, %)	90.2	93.1
Neutral Detergent Fibre (%)	18	13
Acid Detergent Fibre (%)	8	5
Crude Protein (%)	12.3	17.7
DOM (%)	86	84
DOMD (%)	84	82
Inorganic Ash (%)	5	9
Organic Matter (%)	95	91
Metabolisable Energy (MJ/kg DM)	13.5	12.9
Crude Fat (%)	3.8	2.5

Finisher ration composed of barley (73%), Cottonseed (10%), Hay (8%) liquid supplement (5%) water (4%) as mixed.

6.4.4. Data processing

The data from the GrowSafe feeders and GEM units were transferred to an Excel spreadsheet with the following columns: Tag number, pen/GEM unit, date and time of feed event, length of feed event, CO_2 (g/d), CH_4 (g/d) and total mixed ration (TMR) intake (g). The GEM emission data were interspersed with GrowSafe feeder data, with data rows arranged in chronological order for each animal. Visual basic (VB) routines were written to calculate the time interval before each spot methane measurement and each recent feed event and then associated with the amount eaten at each feed event. The VB code calculated the time intervals and meal size of all feed events for up to 3 days before each spot methane measurement. The maximum number of feed events in the 3 days before a GEM spot measurement for any animal was 71. Methane measurements within the first 3 days of feeding the finisher ration were omitted from the analyses.

6.4.5. Data analysis

Relationships between spot emission rate and intake were studied using different approaches reliant on increasing levels of feed information inputs as described below.

Relationship between spot methane production rate and time since last feeding event.

The relationships between spot methane production rate (g CH_4/d) and time since just the preceding feeding event (intake 1) were analysed for each animal by including all spot measurements in three curve fitting functions as follows: 1) One compartment oral dose model $((abc)/(c-b))*(e^{-bt}-e^{-ct})$, 2) Wood model with b>0 $(a*t_1^b*e^{-ct})$, and 3) Dijkstra *et al.* (1997): Crompton *et al.* (2011) model $(a*e[(b*(1-e^{(-c*t1)})})/c -dt])$ where a, b and c are the best fit curve parameters and t is time (minutes) since last GrowSafe feed event. In addition, a spline was fitted through spot data (verses time since last feed) over all days for each animal to provide an alternate mean of averaging spot emission rates.

Relationship between spot methane production rate and time and weight of last two feeding events (Two intake quadratic model)

The spot methane data was analysed as:

$$y= a + b1*intake1 \text{ (g)} + b2*intake2 \text{ (g)} +c1*t1(min) +c2*t2(min) +d1*t^2 +d2*t^2$$

The areas under the emission rate (g/d) curves from zero to 1440 minutes post-feeding (divided by 1440 minutes) gave the estimated DMP after a feed event on

average. The areas were estimated by calculating the trapezoids under the curves.

Relationship between spot methane production rate or methane yield and time and weight of all feeding events in the preceding 72h (Three day intake models)

Preliminary analyses (not presented) indicated that the DMP and methane yield (MY; g/kg DMI) effects of each individual GrowSafe feed event extended for more than two days, as found for sheep (Robinson et al., 2011). Hence, for each MY estimation, all feed events from the previous three days were identified and aligned. The one-compartment dose model (JMP, 2014) was coded into Excel, summing the fitted MY from each individual feed event accounting for the time between a given methane measurement and the quantity of, and time delay since, each meal in the previous 72h. The model allowed different MY patterns to be fitted for each animal. Solver (MSExcel) was used to fit the model coefficients, by minimising the residual sums of squares. The area under this curve to infinity estimated total MY (total methane produced from each kg of intake). The area after 3 days was very small, justifying the decision to only account for feeds in the past 3 days. There were marked variations in DMP and MY values, so outliers over 2.5 standard deviations (on the log-scale) from the mean were culled. This resulted in only 103 readings being omitted (<3%; less-so because the original standard deviation was inflated by these outliers).

General linear model

Daily intakes, measured DMP and MY were plotted over the entire feeding period revealing notable patterns over time in each trait. For each measured DMP, the

average daily intake for the 3 previous days was used to calculate MY. Splines over time (with 3 d.f.) were fitted for the diurnal effect and days. Animals were fitted either as random effects, or as a fixed effect and the interaction estimated (i.e., allowing the animals to have different patterns over time). The predicted DMP and MY values for the animals were also estimated using the equations used in the Australian national greenhouse gas inventory report for lot fed beef cattle (DoE, 2014; Moe and Tyrell, 1979). Lignin (1.1%) and silica (0.1%) contents were assumed to estimate the cellulose content in the ration to solve the Moe and Tyrell (1979) equation.

6.5. Results and Discussion

6.5.1. Raw data, feed events and diurnal variation in emissions

There were 18,700 rows of raw data, being individual methane production measures of 24 cattle over 64d. The number of feed events in the 3 days before a methane measurement was normally distributed with a mean of 32 ± 9.6 (median 31, mode 33) and a range from 8-71. As meal frequency increased the size of each meal diminished from 1228g/d (±1006) at 1 meal/3d to 190 g/d (±180) with 71 meals/3d. For DMP, day (as a spline term) was the dominant effect, with R^2 of 23.3%. The diurnal effect (Figure 6-1) lifted this to 27.7%, and then adding 'animal' as a fixed effect gave a final R^2 of 36.1%.
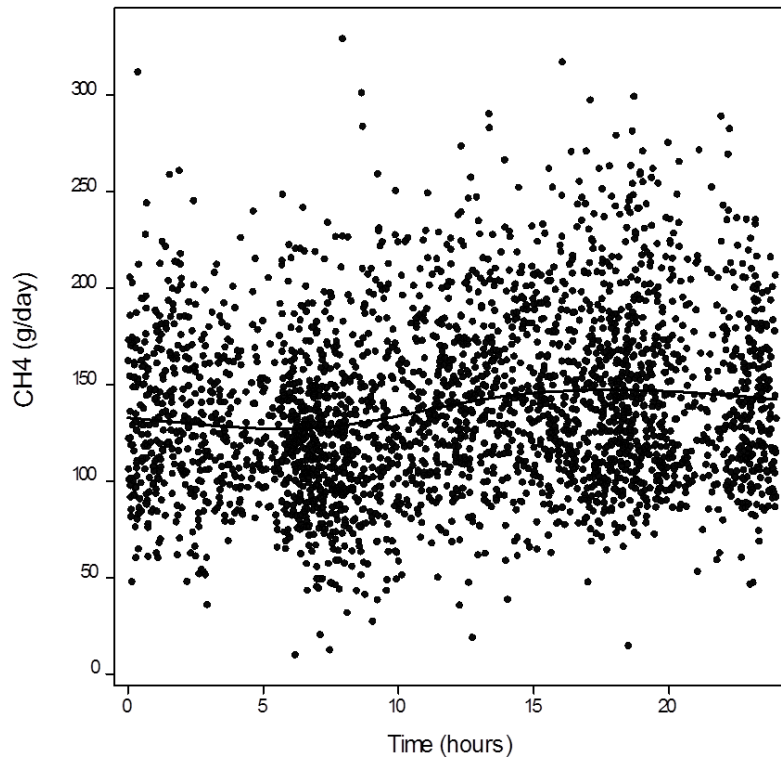
Figure 6-1 Diurnal spline fit for DMP for spot emission rates (expressed as gCH$_4$/d) from 24 Angus cattle on a feedlot total mixed ration.

6.5.2. Curve fitting

Simpler models. The simple models of spot DMP versus time since the most recent feed event explained little variance in spot DMP, reflecting the high variability in spot-measures and that these models did not include the size of the feed event or time and size of earlier feed events. For example, the R^2 of the one dose compartment model varied from 0.01 to 0.23 for the 24 cattle, averaging 0.10). Daily intake is recognised as a good predictor of DMP (Kennedy and Charmley, 2012), but association of spot emission rate with the most recent feed event or events was poor.

When the arithmetic mean spot emission rate for the trial was regressed against the fitted DMP, estimated as the area under the curve from models relating emission rate to time since last feed, the proportion of average spot rate

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variance explained (Figure 6-2), was R^2=0.54 for the one dose curve fit. The estimated areas under the curves equating to average DMP for the time curves are given in Table 6-2.

Table 6-2 Estimated daily methane production calculated as the arithmetic mean of all individual measurements for the animal ('spot average') or derived from the areas under various curves or as predicted by DoE (2014)

| Tag | sex | NFI line | DMP | | | | | |
			Spot average	One dose	Wood	Dijkstra	Spline	DoE (2014)
H077	*H	Low andNFI	123.3	81.4	82.7	123.3	127.3	196.9
H094	H	High NFI	128.8	76.2	77.3	76.9	148.3	208.0
H119	H	Low NFI	130.3	99.0	100.1	36.1	137.8	206.8
H146	H	High NFI	134.1	93.2	98.6	114.3	138.4	200.1
H164	H	High NFI	137.1	89.3	84.7	89.4	161.3	230.5
H187	H	High NFI	131.7	83.2	86.6	83.1	134.0	197.5
H190	H	Low NFI	136.3	72.3	75.5	110.5	135.6	143.2
H191	H	Low NFI	115.6	89.2	89.2	89.2	120.8	184.5
H199	H	Low NFI	183.9	146.9	117.5	128	176.4	209.0
H237	H	Low NFI	125.8	93.2	98.9	65.9	126.8	192.5
H242	H	High NFI	134.4	115.6	82.5	85.7	129.8	192.2
H249	H	High NFI	125.3	72.4	75.0	75.0	162.0	195.0
H251	H	Low NFI	123.7	104.6	105.1	105.1	123.0	183.2
H277	H	Low NFI	146.7	101.1	102	102.0	148.0	201.7
H281	H	Low NFI	133.5	78.5	79.1	78.5	133.3	190.5
H283	H	High NFI	142.1	102.2	133.7	133.4	139.2	191.4
H285	H	Low NFI	121.6	71.5	66.7	68.4	119.4	190.8
H129	S	Low NFI	124.8	78.3	73.5	78.5	122.5	169.4
H222	S	Low NFI	135.6	72.8	73.0	76.9	138.4	202.3
H255	S	Low NFI	137.2	106.1	103.0	106.1	138.6	183.4
H260	S	High NFI	151.9	109.5	105.8	113.7	150.3	212.6
H262	S	Low NFI	160.6	134.0	134.0	134.0	154.9	212.3
H279	S	High NFI	150.0	86.9	90.1	82.8	146.8	177.1
H300	S	Low NFI	139.4	82.6	79.8	89.1	138.8	178.4

*H: heifer, S: steer, andNFI: net feed intake
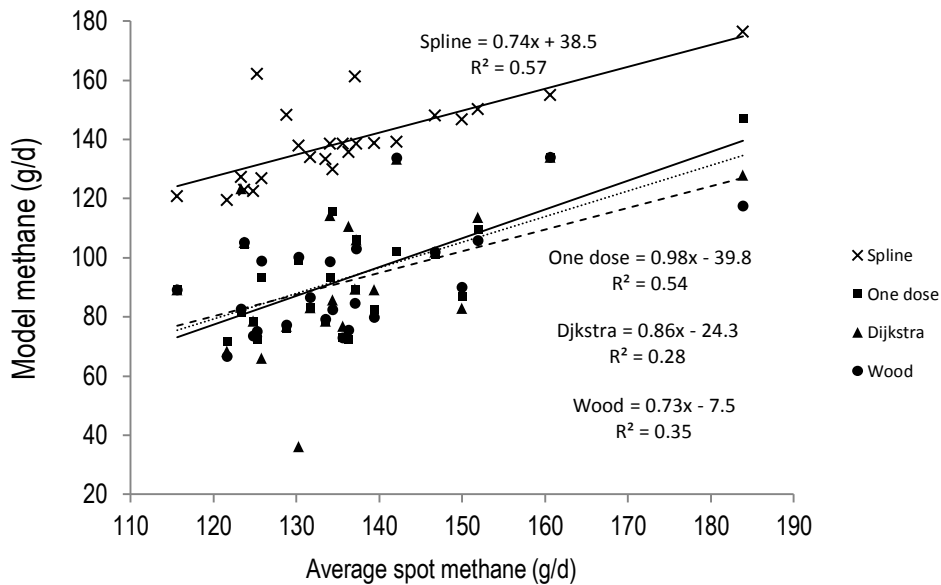
Figure 6-2 Estimates of daily methane production based on the integrated areas under curves derived from four models plotted relative to the average spot emission from each of 24 animals over 64 days.

The spot average (and spline) DMP estimates were lower and dissimilar to DoE (2014) estimates, which use Moe and Tyrell's (1979) equation, and are the basis of Australia's GHG inventory reporting. However, DoE (2014) estimates appear too high (e.g. Benchaar *et al.*, 1998) and therefore are of limited use as a benchmark DMP value. The correlations of DoE (2014) estimates to the spot average, one dose, Wood, Djikstra and spline models were 0.29, 0.37, 0.31, 0.02 and 0.52 respectively. The correlations between the average of spot estimates of methane and the curve estimates in Table 6-2 are shown in Figure 6-2. The spot average was more highly correlated to areas under fitted curves than to DoE (2014) estimates but the values were about 40 g/day higher than estimated from the areas under the various curves. The one dose compartment model using only time since the immediately preceding feed event fitted the data better than the two intake quadratic model and had the highest correlation to the average spot estimates (r=0.74). This was the model used for subsequent analyses.

Two intake models. The intake and time of eating since measurement regressions against spot DMP measures were also not good fits, when only one or two intakes were included in the regression. The two intake quadratic regressions and Djikstra curves of spot methane versus time since feeding had shapes that did not resemble the expected biological situation of a quick rise to peak and then steady decline. From time zero to 1 day post feeding, the quadratic curves continued to rise while the Djikstra curves rose and fell twice (data not shown).

Three day intake models. Solver was used to fit 'the average' (across-animals) one-dose model to DMP, scaled to a one kg feed-event (so the feed amount was factored in and directly-scaled). MY was the integral under this curve and was very close to the 'average' (on a per-animal-per-day basis) estimated MY values.

The variation in the DMP data resulted in a fitted model in which only 16.9% of variation was explained by modelling GEM spot measurements in relation to the size of, and interval from, each meal in the previous 3d. The fitted model allowed a lag period (from time of intake to start of methane production), but this always fitted as zero and rise to peak emissions was very rapid. Figure 6-3 shows the instantaneous emission rate over time for 'the average animal' for three days, following a one kg feed event. The integral under this MY curve was 12.1g/kg DMI.
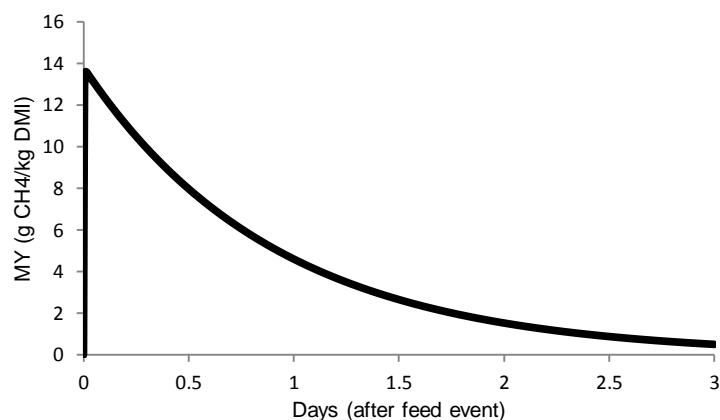
Figure 6-3 Output for the 'average' animal based on each kg of intake from integration over 3 days of the spot emission rates (gCH4/d) estimated from the one compartment dose model.

This model identified marked animal differences about the mean MY value of 12.1, where MY was calculated as the ratio of DMP on a given day and the average DMI on that day and the previous two days. To better understand the changes in emission attributes over time, DMI, DMP and MY over the trial were plotted and splines were fitted. The R^2 for DMP fitted by splines was 24.1%, and Figure 6-4 shows steady increases in DMP calculated from spot measurements over time, which were consistent between animals. The average DMP values were 101.1 g/d at day 0, 136.4 g/d at day 30 and 190.1 g/d at day 65, so DMP increases were 1.53 g/d/d from days 30 to 65, or 1.37 g/d/d for the whole trial. This trend in increasing calculated DMP with time was mostly due to changes in daily intake over time, with the same spline model for DMI having R^2 of 41.1%, as shown in Figure 6-5. Taking the ratio of DMP on a given day and DMI (averaged over day of measurement and two previous days) to calculate MY, the same model gave an R^2 of 18.1%, as shown in Figure 6-6.
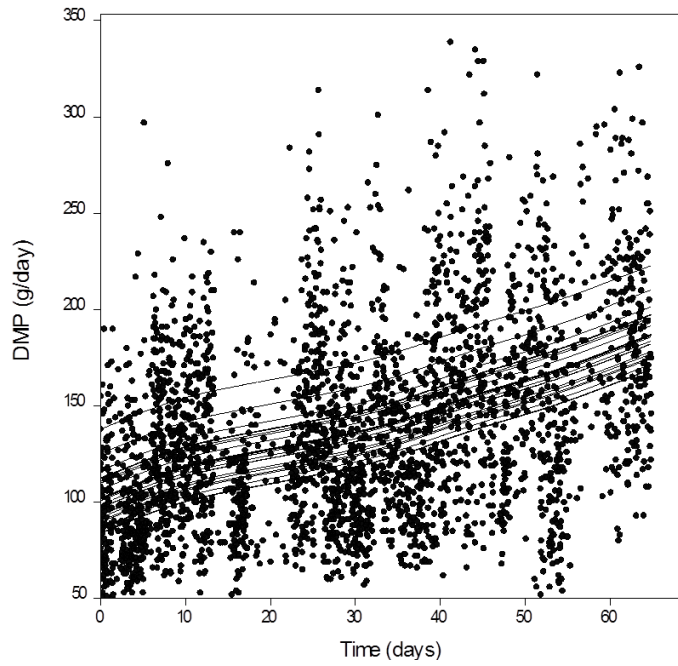
Figure 6-4 The fitted spines for estimated daily methane production of individual animals (n=24) with splines for individual animals.
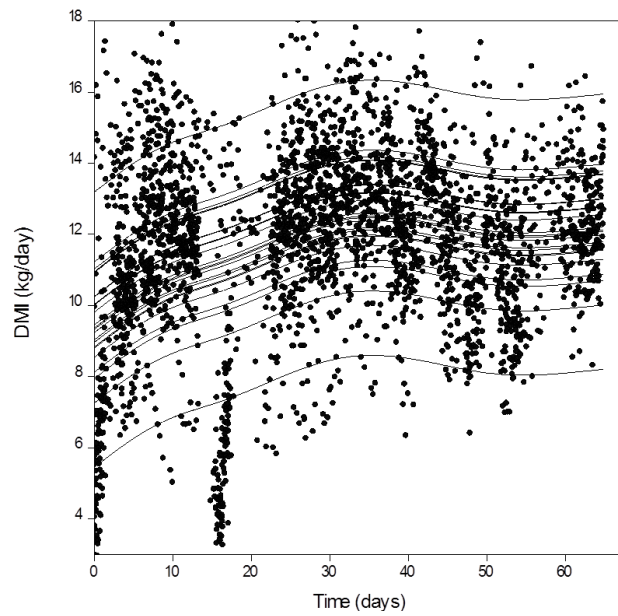
Figure 6-5 The fitted spines for estimated dry matter intake of individual animals (n=24) with splines for individual animals.
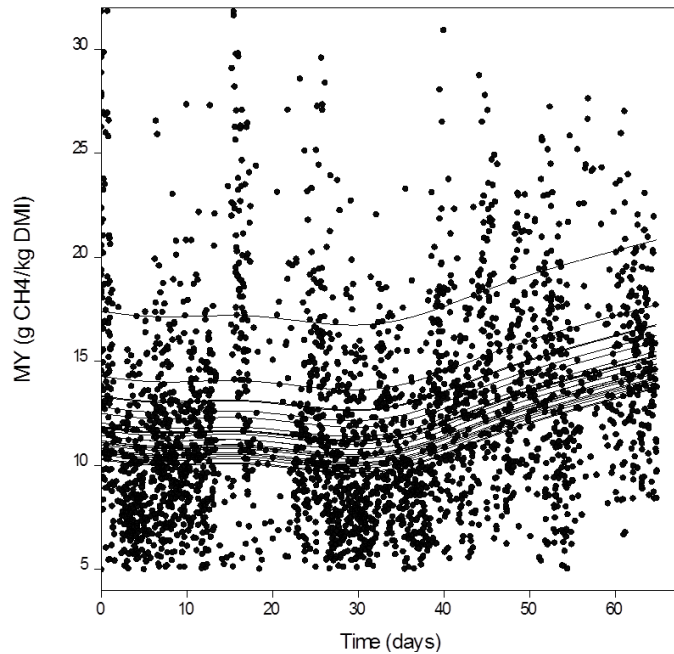
Figure 6-6 The fitted splines for estimated methane yield of individual animals (n=24) with splines for individual animals.

MY was steady until around day 40. MY for the first 37 days averaged 11.5 g/kg DMI; for the last 26 days it showed a steady increase, with an average of 13.9 g/kg DMI. There was the same degree of between-animal variability as was noted with the 3-day model (a few animals had very high MY values). While these MY values are lower than other studies (e.g. Ramen and Huhtanen, 2014) they match observed data (MY 13.1 g/kg DMI) for similar concentrate diets (Hegarty *et al.*, 2006) and with these same 24 animals measured in respiration chambers on the same diet (MY 15.5 g/kg DMI; Herd *et al.*, unpublished data) two months later. The accuracy of using GEM has been shown (Velazco *et al.*, 2014) through comparison with respiration chambers, so there is no reason to doubt the accuracy of the GEM as the CO_2 recoveries were high (95.9 and 97.9% of the gravimetrically determined quantities of CO_2 released into the shroud were recovered by the two GEM units used in the experiment). All model methane estimates and the average of spot estimates were lower than those predicted for

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feedlot cattle using Moe and Tyrell (1979). As noted previously, the equation of Moe and Tyrell (1979) has been shown to have a very high error of prediction, a high general bias and explain a relatively low proportion of variance in methane production (Benchaar *et al.*, 1998; Ellis *et al.*, 2007).

A comparison of the solver and average spline estimates of MY for each animal with those from DOE (2014) are shown in Table 6-3. The correlations between solver and spline MY estimates was 0.91 and with DoE (2014) MY estimates was 0.83. The correlation of spline and DoE (2014) MY estimates was slightly lower at 0.78.

Table 6-3 Average methane yield (gCH$_4$/kg DMI) of animals estimated by solver, splines and DoE (2014)

| Tag | sex | MY | | |
		Solver	Spline	DoE (2014)
H077	H	10.4	11.4	18.0
H094	H	9.8	11.6	17.6
H119	H	9.5	11.0	17.6
H146	H	11.0	11.5	17.9
H164	H	9.3	11.0	16.9
H187	H	10.7	11.4	18.0
H190	H	17.8	17.8	21.7
H191	H	10.3	11.2	18.6
H199	H	12.8	13.8	17.6
H237	H	11.0	11.6	18.2
H242	H	11.6	12.1	18.2
H249	H	10.7	13.9	18.1
H251	H	11.3	11.7	18.7
H277	H	12.0	12.2	17.8
H281	H	11.6	12.4	18.3
H283	H	11.9	12.1	18.3
H285	H	10.6	10.6	18.3
H129	S	13.0	13.5	19.5
H222	S	10.7	11.5	17.8
H255	S	12.1	12.9	18.6
H260	S	11.1	11.9	17.4
H262	S	12.2	11.5	17.4
H279	S	14.2	14.7	19
H300	S	13.2	13.8	18.9

Power analysis

The precision of estimated DMP or MY values, with or without available feed intake data, depends on the inter-play between the different sources of variation and the numbers of replicates within each component. Taking our DMP estimate based on spot emission data as an example, we first fitted a 'base' mixed model with no fixed effects and 'animals' and 'days' as the random effects (to estimate their variance components). As listed in Table 6-4, the residual variances were large (vs. their respective standard errors). As shown previously (Figure 6-5), however, 'days' do not appear to be a simple random effect, as there is an increasing trend in DMP over time. Table 6-4 also shows the variances for a second mixed model where the observed trend is accounted for by including a linear term as the fixed effect.

Table 6-4 Variance components (s.e.) from the general linear mixed models of MY and MP

Fixed effect	DMP		MY	
	Nil	Days (linear)	Nil	Days (linear)
Residual (between-measures; within-animals and within-days)	1689 (45)	1687 (45)	14.84 (0.40)	14.84 (0.40)
Animal	160 (52)	164 (53)	2.69 (0.83)	2.67 (0.83)
Day	935 (176)	349 (71)	6.51 (1.23)	5.48 (1.05)

Removing the trend in days reduced the estimated variance components for days from 935 to 349 for DMP and 6.5 to 5.5 for MY. Even when de-trended, the variance components for days are notably larger than those for animal. Other cattle methane studies (e.g. Blaxter and Clapperton, 1965; Boadi *et al.*, 2002; Harper *et al.* 1999; Pinares-Patino *et al.*, 2003; Vlaming *et al.*, 2008) indicate that between day variance in DMP is more likely to be closer to our lower value (i.e. when the effect of our linear trend is removed), so we used the between day

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variance value of 349, rounded to 350, in a power analysis of experimental designs for DMP estimated from spot measurements.

For the power analyses of DMP, we rounded the linear trend values to 1700 for the residual variation and 160 for the variance components for animals. The power analysis investigated the precision of the estimated mean using the 95% two-tailed confidence interval, taking the variance formulae in Cox and Solomon (2003) as shown below.

s.e. (mean) = sqrt [σ^2 / ($n_a . n_d . n_r$) + τ_a / n_a + τ_d / n_d], where

σ^2 is the residual variance,

n_a, n_d and n_r are respectively the numbers of animals, days and replicates, and

τ_a and τ_d are the variance components for animals and days respectively.

The targeted precision was 5% of the estimated 64d mean DMP or MY. Hence we expect 95% power for any future experiments with combinations of numbers of animals, days and replicates where this target is met. Figures 6-7 and 6-8 show these patterns for DMP and MY respectively, assuming two spot measures are made per animal per day (as occurred in this study).
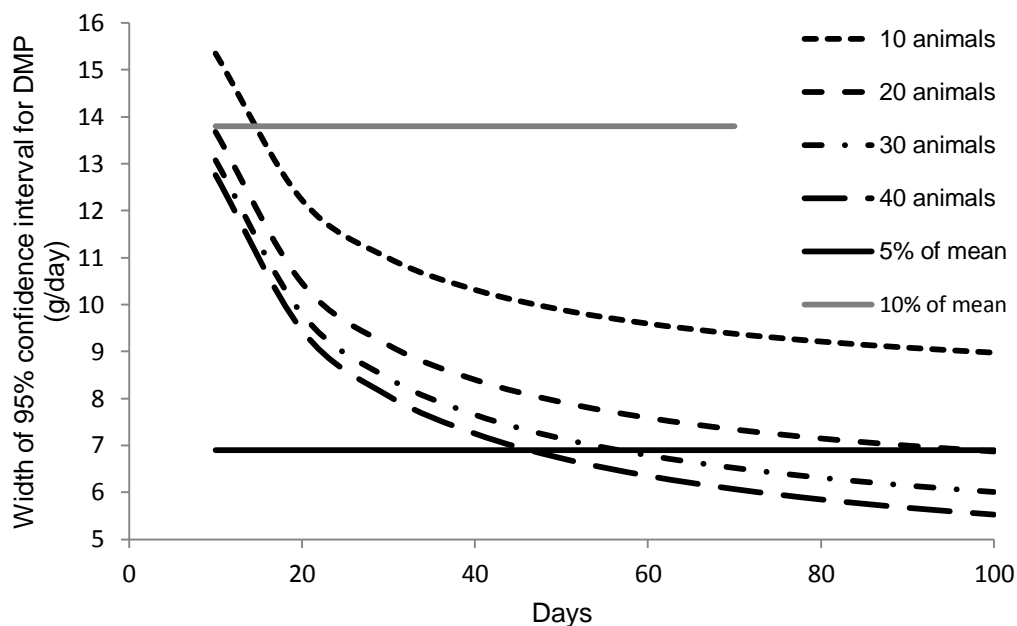
Figure 6-7 Estimated width of 95% confidence intervals (either side of mean) for daily methane production vs. numbers of days, for different numbers of animals (dashed lines) and the targeted 5% of the observed mean (solid black line) or 10% (solid grey line). Two spot measures of methane production rate per day are assumed.
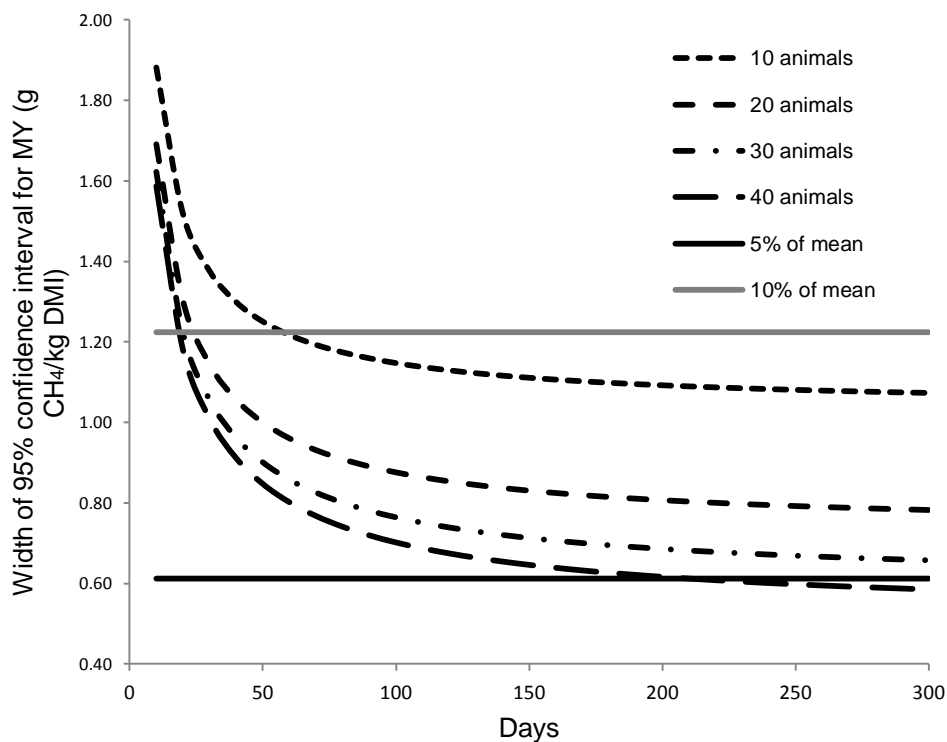
Figure 6-8 Estimated width of 95% confidence intervals (either side of mean) for daily methane yield vs.

numbers of days, for different numbers of animals (dashed lines) and the targeted 5% of the observed mean

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(solid black line) or 10% (solid grey line). Two spot measures of methane production rate per day are assumed.

As shown in Figure 6-7 it is realistically infeasible to achieve the targeted precision for DMP from a population of only 10 animals. With 20 animals 98 measurement days are needed. Doubling the number of animals to 40 results in 47 days being required (a time reduction of 52%). Conversely, for a 50-day trial 36 animals are needed to achieve the targeted precision; vs. 20 animals for a 100-day trial (a 44% reduction in the number of animals). So whilst the between-day variation was higher than between-animals, the overall differences in the required numbers of days or animals are not all that pronounced, so the numbers of animals and days are reasonably interchangeable in respect to power of the test. They both contribute equally to reducing the residual variance. The design of each future experiment will, of course, depend on the available budget and logistic limitations, but our formula and figures can be used as a guide for experimental design.

The maximum number of spot measures per animal per day (n_r) is set by the researcher but whether cattle utilise all the measuring opportunities is the animal's choice. However, increasing n_r only had a relatively minor effect here – for 20 animals, the required number of days to achieve target precision only reduces from 98 (for n_r = 2) to 91 for n_r = 5 and to 89 for n_r = 10. For 40 animals, the required numbers of days were 47, 45 and 44 for n_r = 2, 5 and 10 spot measures/d respectively. It would seem prudent for future researchers to perhaps assume n_r of 2, knowing that if they do achieve a higher number this will improve their precision (but only slightly). So in summary, the precision improves rapidly as the number of animals multiplied by days increases up to 30 and obtaining more

measures per day (which is more difficult to control as it depends on animal behaviour) has little effect on precision.

Feed events known

In overview, a strong association between DMP and daily DMI was apparent in keeping with published assessments (Kennedy and Charmley, 2012; Ramin and Huhtanen, 2013) however, a key objective of this study was assessing the association of individual spot measures of enteric emissions with feeding history. Our analyses found that if only the time of the preceding feed event is recorded before spot methane measurements then taking the arithmetic average of all spot measures is not much inferior to fitting various time models, such as the Wood, Dijkstra or one dose models. Of those models tested, the one dose compartment model was as good as any and had the advantages of the fitted curve parameters being more easily numerically integrated to estimate areas under the curve and a shape consistent with the expected biochemistry.

Including data (time and amount) of all feed events in the 3 days before each spot measurement, which consisted of up to 71feed events/3 days and obtaining a least squares solution for DMP or MY reduced the (underestimation) bias of the data but still left most variance in DMP or MY unexplained by the model. It is suggested that when feed events are recorded that use is made of the data for all feed events and not just those immediately prior to methane measurements.The fitting of splines further improved the goodness of fit and identified an increasing trend in MY as the trial progressed. Spines were fitted because of the variable nature of the methane emissions during the trial. All independent variables (DMP, DMI and MY) were converted to a 'per-animal per-day' basis. The fitted terms in the statistical model were 'animal' (notably different)

and 'days'. DMP rose steadily following the patterns in DMI, while MY increased during the trial. The causes of this increase in MY are not known. One possibility is that while daily DMI increased over the first 30d, periods of rumen acidification may have suppressed methanogenesis as methanogens are sensitive to acidity (Russell, 1998). Once daily feed intake stabilised, a more neutral pH could have been maintained allowing methanogens to increase and methanogenesis and MY to progressively rise.

The alternate (and novel) approach of modelling the measured patterns of DMP after each feed event using Solver was studied because the analysis was not a simply derived regression. Instead, the contributions from each feed-event were summed (for each animal) by best-fitting the one dose compartment model. The equation (Fig. S1) is based on each kg of intake, and the integration (over 3 days, which was the time period found necessary) resulted in an average MY value of 12.1 (g CH_4 per kg of intake). The spline and solver approaches gave similar answers; but the more traditional spline method identified patterns in the measurements over time.

Feed events unknown

With grazing animals, the timing and size of feed events is typically unknown. For this situation, we have provided the estimates of variance components and calculations for estimating how many animals and days of sampling would be needed to estimate sample means of DMP and MY with a desired precision. This data can then be used to calculate the sampling regime required to detect a desired percentage difference in emissions between treatment groups. Measuring

animals for less than 2 weeks is likely to result in non-significant results unless animals are measured for spot methane more frequently than occurred in this trial.

The power analyses suggested that spot measurements made over a 70d period, as is used in RFI tests, would be sufficient to estimate treatment means of DMP and of MY to a precision of within 5-10% of the true mean. Spot measurements of enteric emissions can be used to define DMP but the number of animals and samples are larger than are needed when day-long measures are made, such as in respiration chambers.

6.6. **Acknowledgments**

STATEMENT OF AUTHORS' CONTRIBUTION

Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

	Author's Name (please print clearly)	**% of contribution**
Candidate	Jose Ignacio Velazco	30
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	David Mayer	25
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Candidate   27/2/2015

              Date

Principal Supervisor   27/2/2015

              Date

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STATEMENT OF ORIGINALITY

Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements

We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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Chapter Seven

GENERAL DISCUSSION

7. General discussion

7.1. Introduction

On-farm CH_4 emissions have been identified as the largest contributors to the carbon footprint in livestock production systems (O'Mara, 2011), and significant investment is being made in mitigation by manipulation of rumen fermentation and ecology, animal and plant breeding and management of the production system (Cottle *et al.*, 2011, Doreau *et al.*, 2014).

Development of national GHG inventories and verification of mitigation has been largely been made using respiration chambers (RC) and SF_6 tracer technique. A requirement for evidence of effective on-farm mitigation under commercial production conditions (Action on the Ground - AotG 2014) and a desire to establish the phenotype of thousands of ruminants for breeding programs (Pickering *et al.*, 2013) has fueled the development of new techniques to measure methane emissions. Many of these approaches have not been properly tested to give confidence in the accuracy of the estimation of DMP they provide but spot measures of methane concentration or methane flux are increasingly being used to estimate DMP (Hegarty 2013).

This thesis sought to investigate the applicability of multiple short-term measures of methane flux to estimate DMP, using the GreenFeed Emissions Monitor (GEM) as the measurement device. While this thesis uses the GEM, it is anticipated that some of the principles will have application to the understanding of short-term emission measurement procedures such as portable accumulation chambers (Goopy *et al.,* 2010) and breath sensor techniques such as those of Garnsworthy *et al.* (2012a) and Chagunda *et al.* (2009).

The structure of this general discussion is built to address the research questions formulated at the beginning of this thesis, namely:

A. Are the estimates of DMP based on multiple measures of CH_4 flux over 3-6 minutes, different from RC measures of DMP from the same cattle consuming the same quantity of the same ration? (Section 7-2)

B. Can multiple 3-6 minutes measures of CH_4 flux be used to detect a reduction in the emissions expected when a mitigation strategy is implemented? (Section 7-3)

C. What would be the required number and timing of 3-6 minute measures of CH_4 flux to detect 10% mitigation at $P<0.05$ and what would be the optimal sampling regime to define the phenotype of an individual animal? (Section 7-4)

It should be noticed that all conclusions presented in this chapter apply to the use of GEM system under the described circumstances and the guidance in terms of number of animals, days and measures/day apply to randomized designs. Other experimental designs where animal to animal variation is accounted for are out of the scope of this thesis.

7.2. **Verifying accuracy of DMP estimated by multiple short-term emission measures relative to DMP measured by respiration chambers**

This first research question addresses the accuracy of the method and capability of multiple short-term measures to estimate DMP. Estimates derived from multiple short-term emission measures rely on the accuracy of each individual measure and adequate sampling frequency to account for diurnal or longer-term periodicity in emissions.

7.2.1. Accuracy of individual emission measures

In this thesis, the accuracy of the GEM sensor responses was evaluated based on periodical (typically weekly) calibration and recovery tests performed with the units (Table 7.1). Methane and CO_2 calibrations were performed during all experiments to define sensor responses to known concentrations of high quality CH_4 and CO_2 mixed gas. The within-test CV of both CO_2 and CH_4 sensors was below 3% showing accuracy of sensors was within the desired range for estimating gas concentrations. While accuracy of the air flow estimation (being measured by a pitot tube) was not measured directly, it was checked indirectly by release of a known quantity of CO_2 and comparing the recovery (flow x concentration) with weight of CO_2 released. This test also indicated if a gas was being lost from the shroud.

Table 7-1 Variation on the CO_2 and CH_4 sensor response to a labelled standard gas infusion during calibration in the three GEM units used in this thesis.

Experimental chapter	GEM unit id	Number of calibrations	CV in the CO_2 sensor	CV in the CH_4 sensor
2	11	6	1.0%	3.3%
3	11	2	1.6%	0.3%
4	11	4	0.7%	3.8%
5	30	5	2.1%	2.4%
5	31	8	2.5%	2.7%
6	30	9	2.7%	2.8%
6	31	9	2.8%	1.1%

Recently performed recoveries of released CO_2 on the three GEM units used (units 11, 30 and 31) were 98.3, 95.9 and 97.9% respectively, indicating a high level of concordance between the gravimetrically determined quantity of CO_2 released into the shroud and that calculated from the CO_2 concentration and air flow rate through the units. It should be noticed that both recovery and calibration

protocols were progressively improved throughout the thesis in attempt to standardize the protocols. The main development in calibration was the capacity to automate the procedure allowing more frequent calibrations reducing human error and labour. Currently the automatic calibrations are performed in a pre-scheduled daily regime reducing the CV between calibrations to less than 1%. The recovery test has also been improved by modifying the CO_2 release system (again reducing any potential human error).

A component of measurement accuracy is defining a valid short-term or spot measure and the minimum duration of each individual measure requires consideration. The minimum duration of a valid measure was investigated using a data set of 946 short-term measurements of emission rate from a minimum duration of 1 minute to a maximum of 12 minutes in a feedlot trial. When variability of the estimates was analysed by segments of time, the highest variability was found in the segment 1 – 3 minutes (CV= 0.73). When the subsequent segments were investigated, the CV ranged between 0.54 (all measures over 3 minutes) to 0.47 when measures of only 5 minutes or longer were computed. There was therefore, little benefit of increasing the minimum duration of the measurement from 3 to 5 but the number of valid measurement dropped from 194 to 40 as a consequence of increasing the minimum duration of the measurement. Therefore, the minimum duration for a valid measurement in this thesis (set at 3 minutes) was considered to offer a high number of valid measures while the variability of the estimates remains unchanged. In addition, to achieve a longer measurement session, the supplement delivery schedule would need to be programmed to "retain" the animal in position to be measured and this only can be achieved by increasing the level of supplement delivered as attractant.

Cattle eructate on average every 1.5 mins and take 25-40 breaths per min (Ulyatt *et al.*, 1999; Mortola and Lanthier, 2005). Distinct emission peaks carrying both CO_2 and CH_4, at 40-60 second intervals, were apparent when cattle were measured by a GEM. The chosen duration of at least 3 minutes ensured eructation events to be detected and therefore included in the measurement as well as exhaled breath. Background concentration as well as increased CO_2 and CH_4 levels in eructation events are evident in Figure 7-1.
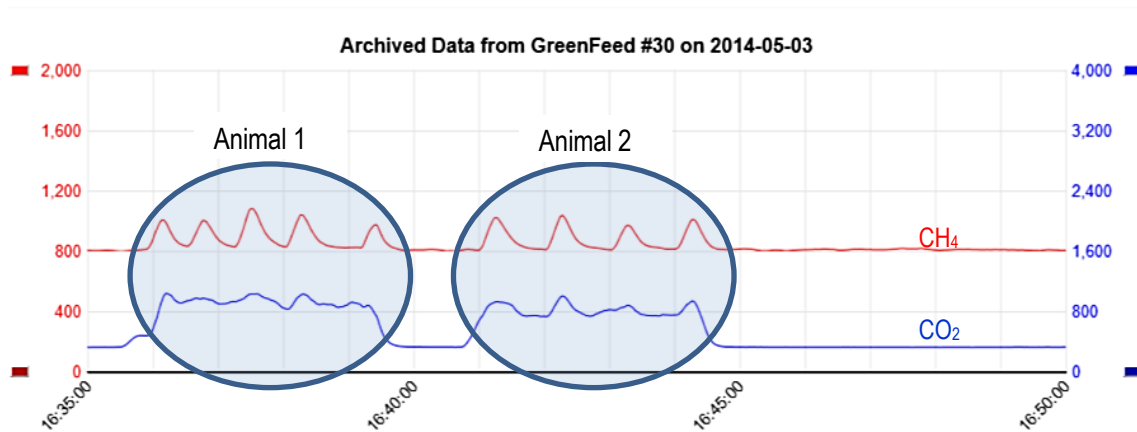
Figure 7-1 Methane (red) and Carbon dioxide (blue) emissions of two different animals (16:35 to 16:40 and 16:41 to 16:45) measured at the feedlot using the GEM system. Eructation events appear as a sharp peak and units are arbitrary. Steady lines before 16:35, between 16:40 and 16:41 and after 16:45 corresponds to background.

7.2.2. Accounting for diurnal variation

Any DMP estimate should be assessed with the awareness that emission rates change over momentary, diurnal and longer seasonal patterns, requiring representative sampling if that variability is to be considered. Within-day variance in emission rate is recognised (Lockyer and Jarvis 1995) and was consistently evidenced in this thesis (Figures 2-3 and 3-6 shows diurnal variation in emissions detected using the GEM) but the analysis of variances conducted as part of the

power analysis found the number of days of measurement required to define emission rate was little affected by the number of samples within day (Chapter 6 and section 7-4). This reflects a higher variation in emission rate between days than within days, which may reflect the weak diurnal variation in emission rates of animals fed ad-libitum, in contrast with animals exhibiting episodic grazing (Lockyer and Jarvis 1995). Despite this, it is hard to recommend other than to try to capture diurnal variation in emissions such as it is, by sampling in all stages of the day. In production systems with more episodic feeding this may be even more important, hence use of scaling-up coefficients like that proposed by Garnsworthy *et al.* (2012) or adjustment factors for animal activity and time spent in each activity as suggested by Chagunda *et al.* (2009). This is to avoid bias in the estimation of DMP while the current research identified more days rather than more measures/d as being desirable.

The GEM offers the capability of restricting animals' visitation by setting the maximum number of visits per day and the minimum time between visits. That means an animals' consecutive measures are going to be distant in at least this minimum period of time. The risk of selective data use causing bias in field studies was highlighted by Laubach *et al.* (2014), implying that caution is needed when estimating daily fluxes when the daily cycle of emissions is not uniformly monitored.

A pattern in diurnal emission rate of the herd was observed in independent experiments (Chapters 2 and 3) and there was concordance between GEM and Open-path FTIR description (timing and magnitude) of emission fluctuations across time as presented in Table 7.2.

Table 7-2 Hourly maximum and minimum total herd methane emission average and amplitude of the difference (percentage of the daily maximum to minimum in the daily average) for two independent CH_4 flux measurement systems (GEM and bLS-FTIR) during the experiment reported in Chapter 3.

	GEM	FTIR
Maximum (h:mm)	20:00	18:00
Minimum (h:mm)	06:00	06:00
Amplitude of the difference (daily max to min)	24.7%	26.9%

We also sought to explore variation in emission rate throughout the feeding cycle of cattle. Methane formation, accumulation and release initiates almost immediately after the diet enters the rumen, and we expected time between the last feed and the measurement to be critical when feeding highly fermentable carbohydrates. Using sheep, Diani *et al.* (2011) and more recently Guyader *et al.* (2014) using dairy cattle, investigated the effect of the inclusion of nitrate salts in the diet and found most of the methane emissions reduction attributed to nitrate salts occurs during the first 3 hours post-feeding. When testing a dietary CH_4 mitigation strategy (inclusion of nitrate salts in the TMR of steers, Velazco *et al.*, 2014), it was evident that nitrate results in a significant change in the animal's feeding behaviour. This change in feeding pattern coincided with unexpected emission results suggesting that differences in time interval between feeding and GEM measurement could have skewed the estimates of the DMP. It was concluded that a significant diet effect on the interval between feed consumption and methane measurement deserves attention and highlighted the need for caution in extrapolating short-term emission measures into daily emission rates when quick acting rumen modifiers are evaluated.

Spot measures of methane production by equipment, such as GEM units, Portable Accumulation Chambers or hand-held lasers, are usually made in association with an unknown pattern of pasture feed intake by the measured animals. In more controlled animal experiments, where individual animals have their feed intake patterns measured and/or controlled, it is possible to attempt to relate methane measurements to intake patterns (e.g. Jonker *et al.*, 2014). The methane production versus time curves has a similar shape to lactation curves with a relatively fast build up to peak production followed by a slow decline. The fitting of Wood (1967), one dose (JMP 2014) and Djikstra *et al.* (1997) curves were investigated in Chapter 6.

Time since the last meal was of surprisingly limited value in estimating the spot emission rate. Even when the meal sizes and time intervals between a spot measurement and all feeding events in the previous 72h were assessed, only 17% of the variance in spot emission rate measured by GEM was explained. This implies that accurate feeding data (always difficult and expensive to be collected) will add little value to individual short-term measures of methane production.

7.2.3.   Demonstrated accuracy of DMP estimates

One of the most significant findings to emerge from this study was the high level of agreement between RC measurements of CH_4 emissions and multiple short-term measurement estimates. Three experiments comparing DMP have confirmed that estimates between methods differ by only 5% to 8% ($P>0.05$), implying that multiple short-term measures of emission rates are complementary to and consistent with RC-derived measures, providing capability to measure a greater number of animals in their production environment over extended periods

of time. The association of DMP and DMI has been recently reviewed in a meta-analysis by Charmley *et al.* (2015) using all recent Australian data collected from open-circuit RCs. The authors proposed a simple relationship of 22.3 gCH$_4$/kg DMI should be applied to estimate daily CH$_4$ emissions from southern Australian beef cattle when animals are consuming less than 30% concentrate (R^2=0.79). The MY estimated using the GEM system under similar conditions in this thesis (23.2 gCH$_4$/kg DMI in Chapter 2 and 20.3 gCH$_4$/kg DMI in Chapter 3) indicates agreement between multiple short-term measurement estimates and equations based on calorimetry is within 10%.

7.2.4.   Implications and opportunities

Estimates of DMP from multiple short-term measures of emission rates are complementary to and consistent with RC-derived measures, offering capability to measure a greater number of animals in their production environment over extended periods of time. GEM units showed that repeated short-term measures accurately estimate DMP (within 5 to 8% of the RC measures, *P*>0.05) and thus provide a valid means of quantifying livestock emissions. This has application in verifying on-farm mitigation claims for carbon trading schemes and in generating high volumes of individual animal data to enable genomic selection of cattle based on enteric emission rates.

An interesting area for future research is the relationship between eructation pattern and animal type and physiological state, feed composition, fermentability of the diet and intake level and pattern since eructation events are critical to the short-term measurement techniques.

7.3. Quantifying CH$_4$ mitigation strategies using multiple short-term measures of DMP

If, as proven, short-term emission measures accurately estimate DMP, it is reasonable to expect they can accurately quantify the efficacy of methane mitigation technologies. This second research question addresses the capability of multiple short-term measures to verify abatement of enteric emissions resulting from implementation of mitigation strategies. This can involve verifying differences in emissions among dietary treatment groups or among animals genetically selected for divergent emission. Both approaches were addressed in this thesis, and in order to test the practicality and usability of the GreenFeed system, the experiments were carried out under typical Australian beef extensive (grazing) and intensive (feedlot) commercial production conditions.

7.3.1.   Animal CH$_4$ emission response to dietary Nitrate.

Strategies to mitigate GHG emissions from the beef industry are unlikely to be adopted if production or profitability is reduced. The inclusion of nitrate salts (Chapter 4) in the TMR of feedlot steers on methane emissions led to surprising emission effects (higher MY) and changed the feeding pattern, apparently affecting the emissions. This provided the motivation to examine the feeding pattern and timing of emission measurements relative to feeding events.

Any between-group differential in interval between eating and commencement of short-term emission measures could have biased the GEM estimates of DMP limiting the extent of the results as discussed in chapter 4. Nitrate's mitigation effect occurs during the first three hours after the ingestion of the nitrate and therefore timing of emission measurements relative to feeding

events will become critical for accurately quantifying mitigation when continuous 24h gas collection data is not possible. In this and all cases where the diet intervention changes the CH_4 emissions, more frequent measures should be needed to quantify emissions and eventual mitigation.

7.3.2. Quantifying herd scale mitigation using multiple short-term measures of CH_4.

A more fundamental approach to verify the effectiveness of mitigation was investigated in Chapter 3. In this study, short-term measures where applied to verify the reduction in total herd methane production resulting when the number of cattle was reduced (16 v 32 head). The individual-animal based estimates of DMP were consistent with those of other studies for beef steers of similar age and liveweight under grazing conditions (Boadi *et al.*, 2002, McGinn 2009) representing an opportunity to assess the accuracy of herd scale methods as reported by Laubach *et al.* (2008).

Significant mitigation was detected across periods with the magnitude of mitigation in keeping with that predicted based on total feed intake, and MY as estimated from the RC. RC results corroborate a close fit with GEM and the predicted emissions (IPCC 2006) for individual emissions (221.5, 210.5 and 222.5 gCH_4/d overall means for RC, GEM, IPCC respectively) and the decline in mitigation when cattle were removed also aligned closely across measurement methods (RC = 37%; GEM = 40%; Open Path FTIR = 41%).

Some practical observations on the GEM system warrant consideration.

1) It takes less than a week for animals previously trained in the usage of the GEM to restore their previous GEM usage pattern. This observation is consistent

with that from Waghorn *et al.* (2013) and 2) in a feedlot environment, the attractiveness of the delivered pellets must be high enough to reach a desirable visitation pattern because animals are already being offered *ad libitum* concentrate and therefore the attractiveness of a small allocation of pellets is questionable. The inclusion of aniseed flavour was not solidly evaluated (e.g. different levels of inclusion) but aniseed was used and was considered effective in increasing the attractiveness of the attractant supplement. In the other extreme, when nitrate salts were included in the pellets, very few animals fully accessed the available pellets, compromising the experiment. In New Zealand, lucerne pellets were shown to be as readily consumed from the GEM as grain-based pellets under grazing conditions (Pinares-Patino and Waghorn 2012, Waghorn *et al.*, 2013). If energy intake is not to be altered by the inclusion of supplement in the diet, inorganic pellets could potentially be used as attractant in the GEM system or further effort made to use water as the attractant.

In an attempt to avoid the usage of an energy containing supplement as attractant, a prototype GEM unit delivering water was designed and built by C-Lock Inc. and tested concurrently with the supplement delivering GEM device in experiment 1 of Chapter 2. Water was used as the attractant in place of pellets. The observation that eructation pattern was different when water was used to attract animals (Figure 2-2) identifies the need to use different data screening of emission profiles from a water unit than from a supplement unit. While the GEM-water prototype showed some weaknesses, the fact it does not introduce exogenous energy to bias DMP and the fact that all animals must drink, means further development appears warranted.

7.3.3. Assessing CH$_4$ emissions in cattle genetically divergent for feed efficiency

A further study to validate practical mitigation by using the GEM compared methane emissions and emissions intensity of grazing beef cattle genetically divergent for RFI. In this experiment, animals were measured in an open paddock using the GEM. In terms of practicality, just over 60% of the animals became frequent users in contrast to the previous experience where the recruitment rate was always over 80%. The number of cattle in the paddock per GEM unit was the highest tested in this thesis (34), but the recommended maximum number of animals per GEM unit is yet to be established. Based on our experience, 20 to 24 animals per unit seem to be adequate but needs to be tested with a proper experimental design.

The recruitment rate (ie. proportion of cattle frequently accessing GEM unit) deserves attention when planning an experiment since the required number of cattle units is to be typically calculated during experimental design but the final number of GEM users is unknown unless units are used in manual mode. In that case, researchers can use GEM system in tie stalls for measuring at prescribed time points (not tested in the present dissertation). The effects on recruitment of the production system (intensive vs extensive), prior experience with the GEM, supplementation history and general handling warrants further investigation as recruitment rate represents a key factor in the experimental design. Our study concluded faster daily growth by cattle was accompanied by lower methane intensity, in agreement with Hegarty *et al*. (2010) but only 39 of the 64 possible users were effectively measured so the assessment of RFI impacts was weaker than expected from the original design.

7.3.4. Implications and opportunities

Estimates of DMP from multiple short-term measures of emission rates are effective in detecting and quantifying reductions in emissions when the mitigation study does not affect feeding behaviour (eg. by reducing cattle population). When the mitigations strategy affects the timing, frequency or level of feed intake as did nitrate, short-term emission measures were not adequate tools to quantify mitigation. A more frequent regime of measures may account for those differences in methane emissions and should be investigated in future research.

7.4. Optimizing the measurement regime to verify mitigation and to define the long term phenotype of an animal

This section evaluates how to optimize the short-term measurement regime based on the variance structure of the data collected in chapters 5 and 6. Larger sets of data are needed to be confident in the estimated components of variance so this analysis is an initial approach and more investigation of different data sets (diets, breeds, physiological status, environments, extended periods of time) is required.

7.4.1. Experimental design for detecting mitigation between treatment groups.

In Chapter 6 the number of animals and days of measurement required to quantify the mean DMP and MY of a treatment group (n=10) was defined (Figures 6-7 and 6-8). To obtain a DMP estimate within 10% of the long term mean required only 13d of spot samples but further refinement of the estimate to within

5% of the long term mean was beyond the capability of the GEM, taking months. In the following section this power analysis is continued to develop experimental design recommendations for detecting mitigation between treatment groups. In a power analysis, the targeted differences between treatments needs to be established and, based on the known variance structure of the estimate, the minimal number of animals and number of observations needed to detect the expected difference with a given level of significance (typically $P<0.05$) calculated. In our case, data from a feed efficiency test (64 days length, Chapter 6) was used to calculate the variance structure of CH_4 emissions estimated using short-term measures and then, different herd size and trial duration scenarios were investigated.

All individual feed intakes were automatically recorded so MY was calculated on a daily basis and the variance structure of MY as well as DMP investigated in chapter 6. The number of measures/d and the minimum duration of the test were examined. Little changes in the number of days of test required when a low (2 measures/d) and a high (5 measures/d) frequency of measurement were compared, as a consequence of between-day variation exceeded within day variation. The expected P-values for a set number of animals (from 10 to 40 per treatment group) and specified test duration (from 10 to 70d) based on 2 measurements/animal/d are presented in Table 7.3 (DMP) and Table 7.4 (MY). Differences in the estimated mean CH_4 emissions between treatment groups to be detected were set at 5, 10 and 15% of the mean with an expected significance level of $P<0.05$.

Table 7-3 Expected *P*-values, for detecting significant differences between DMP means of two groups of animals with two short-term measures per day for a set number of animals per treatment group and test duration.

Number of days	Number of animals per treatment			
	10	20	30	40
5% expected difference				
10	0.481	0.429	0.408	0.397
20	0.376	0.301	0.270	0.252
30	0.325	0.236	0.199	0.179
40	0.294	0.198	0.158	0.136
50	0.274	0.172	0.130	0.108
60	0.260	0.154	0.111	0.089
70	0.249	0.141	0.097	0.075
10% expected difference				
10	0.149	0.105	0.090	0.082
20	0.070	*0.034*	*0.024*	*0.019*
30	*0.044*	*0.015*	*0.009*	*0.006*
40	*0.032*	*0.008*	*0.004*	*0.002*
50	*0.025*	*0.005*	*0.002*	*0.001*
60	*0.021*	*0.004*	*0.001*	*0.000*
70	*0.018*	*0.003*	*0.001*	*0.000*
15% expected difference				
10	*0.026*	*0.013*	*0.009*	*0.008*
20	*0.005*	*0.001*	*0.001*	*0.000*
30	*0.002*	*0.000*	*0.000*	*0.000*
40	*0.001*	*0.000*	*0.000*	*0.000*
50	*0.001*	*0.000*	*0.000*	*0.000*
60	*0.000*	*0.000*	*0.000*	*0.000*
70	*0.000*	*0.000*	*0.000*	*0.000*

Table 7-4 Expected *P*-values, for detecting significant differences between the MY means of two groups of animals with two short-term measures per day for a set number of animals per treatment group and test duration.

Number of days	Number of animals			
	10	20	30	40
5% expected difference				
10	0.647	0.611	0.596	0.588
20	0.571	0.510	0.483	0.468
30	0.533	0.455	0.419	0.398
40	0.509	0.419	0.376	0.350
50	0.493	0.394	0.345	0.316
60	0.481	0.375	0.322	0.291
70	0.473	0.361	0.304	0.271
10% expected difference				
10	0.354	0.304	0.285	0.275
20	0.254	0.188	0.162	0.149
30	0.211	0.137	0.110	0.095
40	0.186	0.110	0.082	0.068
50	0.171	0.093	0.065	0.051
60	0.160	0.081	0.054	*0.041*
70	0.152	0.073	*0.046*	*0.034*
15% expected difference				
10	0.162	0.123	0.109	0.102
20	0.089	0.051	*0.040*	*0.034*
30	0.063	*0.029*	*0.020*	*0.016*
40	0.051	*0.020*	*0.012*	*0.009*
50	*0.044*	*0.015*	*0.008*	*0.006*
60	*0.039*	*0.012*	*0.006*	*0.004*
70	*0.036*	*0.010*	*0.005*	*0.003*

The results presented in Tables 7.3 and 7.4 suggest, with the given variance structure, it is impractical to detect a 5% difference in DMP or MY between treatment groups using spot emission measures. To identify such a small mitigation effect, the number of animals per treatment (over 40) and the duration of the experiment (over 300d) is well over the normal experimental scale, with

obvious budget implications. In the other extreme, detection of 15% differences between treatment groups seems to be quite feasible with similar resource requirements to those used in measuring other traits such as growth and/or feed efficiency. Quantifying a significant 10% difference in mean DMP between treatment groups is a challenging but reasonable target, that can be achieved either with 10 animals measured over 30 days or 20 animals over 20 days implying a 30% reduction in the duration of the test when the number of animals is doubled. If the 10% difference is to be detected in MY, at least 30 animals per treatment group are needed over at least 70 days.

Interestingly, both number of animals and number of days of measurement affect the probability of finding a difference in similar manner, meaning researchers have flexibility in managing the size of treatment groups and/or duration of study to create a test of the desired power. The design of future experiments will, of course, depend on the available budget and logistic limitations, but under similar conditions, our analysis can be used as a guide for experimental design.

No substantial advantages in terms of number of animals or test duration are to be expected if the number of daily measures is increased (to at least up to 5 measures/d) based on the current calculations. In the example above (10% difference in DMP between groups), increasing the number of daily measures from 2 to 5 do not diminish the test duration nor the number of animals required per treatment group (data not shown). The number of short-term measures per animal per day relies on animal behaviour and therefore is outside the control of the researcher. However, by programming the GEM system, the researcher is able to set the minimum and maximum number of supplementation/measurement opportunities per day, but the animal can choose to access these or not.

Increasing the number of measures per day had only a minor effect relative to increasing the number of days. The assumption of 2 spot measures/animal/d seems reasonable keeping in mind that achieving a higher number of daily estimates will slightly improve the precision of the DMP estimates but will increase the total amount of eaten supplement.

The optimal experimental designs to detect a significant difference between estimated MY of groups using the SF_6 tracer technique and the RC was calculated by Vlaming (2008) using between-animal variance. The between-animal CV used in the calculations corresponds to reported variation for the SF_6 tracer technique (15 to 20%) and calorimetry (5 to 10%) in keeping with the literature. This approach ignores other sources of variation (between-days and technique variance for example). The greater variance in MY estimates using the SF_6 tracer technique was partly attributed to the difficulty in obtaining accurate measurement of DMI and to measurement error. With the assumption of targeting the detection of 10% difference between the MY means, 5 to 17 animals per treatment were needed using calorimetry and 37 to 64 using the SF_6 tracer technique. If the 10% difference in MY is to be detected using multiple short-term measures, at least 30 animals per treatment group are needed to be measured twice a day over 70 days.

7.4.2. Optimizing the methane phenotype determination of individual animals.

In this thesis, two data sets were used to calculate the minimum number of estimates needed to phenotype the long-term methane emissions of an animal as may be required to develop estimated breeding values. The data sets correspond to 64 animals grazing over 42 days and these same cattle after transfer to a

feedlot for 70 days with all valid short-term measures of CH$_4$ over 3 minutes being considered. The analysis ("an estimation of sample sizes") is based on an acceptable margin of error (MoE) for sampling, a level of confidence to be associated with the final estimates, and an estimated coefficient of variation for each particular sample. These calculations were advised by Dr Bruce McCorkell (a biometrician from NSW DPI) and assume the confidence level for sampling in this situation would be 90% (ie. the measured value of methane production should be within 10% of the long term estimate). The calculated CVs for the collected feedlot (chapter 6) and pasture (chapter 5) emissions data were 40% and 30% respectively. The MoE for each individual methane measurement was chosen as ± 10 gCH$_4$ (subjective estimate only). A measurement error of ± 10 gCH$_4$/d was chosen based on the previously described accuracy of the GEM system (being approximately double the instrument error of 3% exhibited during CO$_2$ recovery tests, to allow for additional environment induced variance in measurement accuracy). The average CH$_4$ values for these animals were 173 g/d when grazing and 142 g/d while in the feedlot. Measurement error expressed as a percentage of the means is, therefore, 100*(10/142) = 7%, and 100*(10/173) = 6% respectively. Sample sizes required assuming a margin of error and a desired level of confidence are calculated as:

Equation 7 Estimation of sample size

$$N = \frac{(z^2 \times CV^2)}{\left(\dfrac{MoE}{\mu}\right)^2}$$

where z is the value associated with the chosen confidence interval; CV was 40% (feedlot) or 30% (pasture) and MoE/μ is the ratio between the margin of error and

the mean. Margin of error is the maximum permitted deviation of the estimate from the true mean. As tabulated (table 7.5), approximately 68 spot measures are needed to provide an estimate of the DMP phenotype of an animal in a feedlot situation and 60 spot measures under grazing conditions to be 90% confident of the estimate being within 7.5% of the true mean. Table 7.5 shows how the sample size varies with the different combinations of confidence intervals and accepted margin of error.

Table 7-5 Number of consecutive short-term measures by margin of error (MoE - as a proportion of the mean) and level of confidence to be associated with the final estimates using feedlot (top) and grazing (bottom) data sets.

	Confidence interval			
MoE (%)	70	80	90	95
Feedlot data set				
5	61	93	153	217
7.5	27	41	68	97
10	15	23	38	54
Grazing data set				
5	54	81	134	190
7.5	24	36	60	85
10	13	20	34	48

The main implication of Table 7.5 is that, in a feedlot situation, an animal needs 54 spot emission measurements to be 95% confident that the estimated mean is within 10% of the true (long term) DMP phenotype. Very little was found in the literature on the question of optimal sampling, number of measures and/or sampling time. The results of this study indicate that the required minimum number of measures (n=60) to describe an animal's phonotype within 10% of the

long term mean DMP, can be achieved by sampling an animal twice a day over 30 days, or 5 times a day over 12 days. The more intense sampling schedules could confound the estimates under grazing conditions because a higher amount of supplement is required to attract the animals into the measuring device. Within those ranges, all combination of sampling regimes should deliver an estimation within 10% of the long term phenotype, with the awareness that less intense sampling regimes can possibly increase the number of animals utilising a GEM unit.

Unless DMP is the only trait to be analysed for genetic improvement, the CH_4 emissions regime will need to fit in with other test structures such as growth rate tests and/or feed efficiency tests. For example, if methane intensity or MY are to be included in the selection of more efficient animals, the feed intake, growth rate and DMP determinations should be made simultaneously. There is a minimum data requirement for all traits so the optimization of the test duration will seek to provide the data at the lowest cost. A 35 day test was suggested by Archer *et al.* (1997) to be sufficient to phenotype an animal's feed intake (critical for the calculation of methane yield). In that case, 2 samples/d would be appropriate to phenotype the DMP and therefore to calculate MY. If DMP is to be related to growth rate, a minimum 70 day test length with cattle weighted every two weeks was suggested to optimize the growth estimate so a less exhaustive regime would satisfy the minimum data requirements to estimate the methane emission intensity.

Under grazing conditions, where short-term estimates of DMP were shown to be less variable, a 70 day test for growth rate can easily be concurrent with the CH_4 determinations. It should be noticed that the measurement of DMP using

GEM technology is dependent on the delivery of attractant pellets and the possible interference of the supplement on the CH_4 emissions warrants further investigation. The other factor to consider under grazing conditions is the potential modification of grazing habits related to the pre-scheduled delivery of supplement, as it is unknown if the animals to be measured perturb their natural routine by using the GEM and if that can affect the CH_4 emissions.

7.4.3. Implications and opportunities

Information presented in this section may be useful to future research assisting in the experimental design a) to define the minimum number of animals and test length to verify a significant mitigation effect and b) to optimize the sampling regime to define an animals' phenotype to underpin genetic selection. The use of sampling over an extended number of days will enable the verification of long term stability in the methane emissions based on discreet measures. Multiple short-term measures appear to be accurate, appropriate, economical and less laborious than other measurement options. Variability of the measures between production environments (feedlot/grazing) was shown to play a key role in the measurement program requirement to establish an animals' phenotype and warrants further investigation. Since no research has been carried out with experimental designs accounting for animal to animal variation (such as change over or latin squares), the proposed guidance is of limited validity in those cases.

STATEMENT OF ORIGINALITY

Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements

We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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