Validating a next generation GreenFeed unit to measure methane emissions in sheep

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Abstract

Enteric methane (CH_4) from sheep is a major contributor to agricultural greenhouse gas (GHG) emissions in pasture-based systems such as New Zealand, and reliable, scalable measurement is essential for mitigation research, inventory development, and low-emission breeding. Respiration chambers (RC) provide high-precision continuous measurement but cannot be used under grazing conditions, creating demand for field-compatible alternatives such as the GreenFeed (GF) system. Although GF has been widely validated in cattle, sheep-specific validation, particularly for cattle-designed units equipped with new-generation tunable diode laser (TDL) sensors remains limited. This study therefore compared CH_4 emission estimates from a cattle-designed TDL-GF unit against RC measurements in sheep under controlled indoor conditions.

Twelve Romney ewe lambs (<1 year) were allocated to either a standard ryegrass–white clover pasture or a diverse pasture (ryegrass–clover–plantain–chicory) to generate variation in emissions. Methane production was measured in open-circuit RC over two consecutive 24-h periods (with one additional day due to technical issues) and subsequently estimated using GF over three days using eight scheduled 5-min spot samples distributed across a 24-h cycle. Agreement and bias were assessed using regression-based methods following the St-Pierre framework, with residuals (RC − GF) regressed against mean-centred GF values to test mean (intercept) and proportional (slope) bias. Carbon dioxide (CO_2) estimates were analysed as a diagnostic comparison.

Eleven animals generated paired RC–GF data (one lamb, #7306 did not engage with GF bait and produced no valid visits). GreenFeed CH_4 production showed a significant positive relationship with RC measurements ($R^2 = 0.65$; $P < 0.01$), indicating moderate agreement and substantial capture of between-animal variation. Mean bias for CH_4 production was not significant ($P = 0.27$),

but proportional bias was detected (negative slope; $P < 0.01$), demonstrating that disagreement between methods increased with emission level. For CH_4 yield, agreement was weaker ($R^2 = 0.38$; $P = 0.04$) with no significant mean bias ($P = 0.28$) but significant proportional bias ($P = 0.003$). In contrast, GF performed poorly for CO_2, showing weak association with RC ($R^2 = 0.28$; $P = 0.09$) and substantial mean and proportional bias (both $P < 0.001$), consistent with greater sensitivity of CO_2 to short-term variation and intermittent sampling in sheep. Diet influenced CH_4 residuals ($P = 0.02$), with improved agreement under the lower-fibre diverse pasture and greater residual variability under the higher-fibre standard pasture.

These results show that a cattle-designed GF unit equipped with TDL sensors can capture meaningful between-animal differences in CH_4 emissions in sheep without systematic mean bias, supporting its use for controlled research phenotyping and comparative evaluation of mitigation strategies. However, proportional bias and increased variability relative to RC indicate that intermittent sampling limits precision for absolute daily emission quantification, particularly when sampling density is low and under higher-fibre feeding conditions. Increasing the number and temporal spread of spot samples and validating protocols under grazing are likely to improve representativeness and robustness, while RC remain essential for high-precision quantification and method validation.

Table of contents

List of Table

List of Figure

List of Equation

List of Abbreviations

ADF	Acid Detergent Fibre
CCC	Concordance Correlation Coefficient (Lin's CCC)
CCAC	Climate and Clean Air Coalition
CH_4	Methane
CO_2	Carbon Dioxide
CP	Crude Protein
CV	Coefficient of Variation
DM	Dry Matter
DMI	Dry Matter Intake
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization of the United Nations
GF	GreenFeed System
GHG	Greenhouse Gas
GLEAM	Global Livestock Environmental Assessment Model
H_2	Hydrogen
H_2O	Water
ICC	Intraclass Correlation Coefficient
IPCC	Intergovernmental Panel on Climate Change
LMD	Laser Methane Detector
MCR	Methyl-Coenzyme M Reductase
ME	Metabolisable Energy
MFE	Ministry for the Environment (New Zealand)

MPI	Ministry for Primary Industries (New Zealand)
N_2O	Nitrous Oxide
NDIR	Non-Dispersive Infrared Sensor
NH_3	Ammonia
NDF	Neutral Detergent Fibre
NPN	Non-Protein Nitrogen
OECD	Organisation for Economic Co-operation and Development
O_2	Oxygen
PAC	Portable Accumulation Chamber
ppm	Parts Per Million
QTL	Quantitative Trait Locus
R^2	Coefficient of Determination (Squared Correlation)
RC	Respiration Chamber
RDP	Rumen-Degradable Protein
RFID	Radio-Frequency Identification
SF_6	Sulphur Hexafluoride (Tracer Gas)
spp.	Species
Stats NZ	Statistics New Zealand
TDL	Tunable Diode Laser Sensor
TMR	Total Mixed Ration
USDA	United States Department of Agriculture
VFA	Volatile Fatty Acid
VFI	Voluntary Feed Intake

WSC	Water-Soluble Carbohydrate
°C	Degree Celsius
3-NOP	3-Nitrooxypropanol

Chapter One General Introduction

1.1 Introduction

Methane (CH_4) is a potent greenhouse gas (GHG) with a global warming potential approximately 28 times greater than that of carbon dioxide (CO_2) over a 100-year timescale (Myhre et al., 2014). Although its atmospheric lifetime is shorter than that of CO_2, its short-term warming impact is substantially stronger. As such, CH_4 reduction has been identified as one of the fastest and most effective strategies for slowing global temperature rise in the coming decades (Hristov et al., 2025a).

Agriculture is a major source of CH_4 emissions, primarily through enteric fermentation in ruminant animals such as cattle, sheep, and goats (Calvin et al., 2023; Hammond et al., 2016c). In New Zealand, enteric CH_4 accounts for over one-third of total GHG emissions, making the livestock sector a central focus of mitigation efforts (MfE, 2023). Sheep farming remains a cornerstone of the agricultural economy, with approximately 23.6 million sheep producing meat and wool exports valued at over $4 billion annually (MPI, 2025; Stats NZ, 2025; USDA, 2023). Sheep alone contribute around 30% of national enteric CH_4 emissions (MfE, 2023). Extensive research on CH_4 emissions in sheep has been conducted in New Zealand, with most mitigation studies relying on respiration chambers (RC) and, historically, the sulphur hexafluoride tracer technique. In contrast, relatively little work has been undertaken under grazing conditions using field-applicable approaches such as the GreenFeed (GF) system.

This limits the evaluation of mitigation strategies under commercial pasture systems and highlights the need for sheep-specific validation of measurement technologies applicable in farm

environments, as RC cannot be used under grazing conditions (Hammond et al., 2016a; Jonker et al., 2016).

Given the large contribution of sheep to national CH_4 emissions, accurate monitoring under in-situ grazing conditions is critical for guiding industry-specific mitigation strategies aligned with New Zealand's CH_4 reduction targets under the Zero Carbon Act. Reliable measurement is central to national inventory reporting, climate policy development, and assessment of mitigation approaches such as feed additives and forage innovations (Garnsworthy et al., 2019; Hristov et al., 2025b). Measuring CH_4 emissions in small ruminants presents both practical and methodological challenges. Several *in vivo* measurement techniques have been developed, among which RC are regarded as the reference method (*gold standard*) due to their high precision and experimental control (Garnsworthy et al., 2019). However, their confinement requirements prevent use under grazing conditions. The GF system offers a field-compatible alternative by sampling exhaled gases during voluntary visits to a baited feeding station. While GF has been extensively validated in cattle (Huhtanen et al., 2015; O'Connor et al., 2024), validation in sheep remains limited, and few studies have directly compared GF derived CH_4 estimates with RC measurements (Jonker et al., 2018).

GreenFeed hardware has evolved substantially in recent years. Prior to 2021, two distinct units were produced using nondispersive infrared (NDIR) CH_4 sensors: one designed for cattle (i.e., large ruminants), and a more sensitive small ruminant (e.g., sheep, goat) unit with lower airflow rates capable of detecting emissions down to approximately 4 g CH_4/d. While effective for high emitters, the cattle NDIR units exhibited limited precision at lower emission levels (<20–30 g/d) due to sensor noise around 40 ppm. In 2021, these sensors were replaced with tunable diode laser (TDL) technology across all GF models (C-Lock-Inc., 2021). The TDL sensors provide substantially higher precision (<1ppm noise), improved stability, and reduced gas interference, enabling

detection limits as low as 0.25 g/d. This upgrade theoretically allows a single GF unit to be applied across species; however, this cross-species performance has not yet been empirically validated.

To address this gap, this study evaluated CH_4 emission estimates in sheep using paired measurements obtained from GF and RC under controlled conditions. This design enables direct comparison of the two systems, providing a robust basis for assessing agreement, bias, and practical suitability for small ruminant research.

1.2 Aim and Objectives

Aim

To compare CH_4 emission estimates in sheep using GF and RC methods.

Objectives

To achieve this aim, the study pursued the following objectives:

1. To compare CH_4 emission estimates produced by each method in terms of magnitude, repeatability, and bias.

2. To develop methodological recommendations for applying GF in CH_4 monitoring and inventory frameworks for sheep.

1.3 Hypotheses

- Null hypothesis (H_0): There is no significant difference in CH_4 emission estimates between GF and RC methods in sheep.

- Alternative hypothesis (H_1): There is a significant difference in CH_4 emission estimates between GF and RC methods in sheep.

Chapter Two Literature Review

2.1 Introduction to methane and livestock emissions

Methane (CH_4) is a potent greenhouse gas (GHG), trapping approximately 28 times more heat than carbon dioxide (CO_2) over a 100-year period (IPCC, 2014; McAuliffe et al., 2023). Although its atmospheric lifetime is relatively short (around 12 years), its high radiative efficiency results in a strong near-term warming effect (Shindell et al., 2012). Consequently, reducing CH_4 is widely recognised as one of the fastest strategies to slow, and potentially reverse, climate change in the coming decades (CCAC, 2021; Hristov et al., 2025a).

Globally, agriculture contributes around one-quarter of anthropogenic GHG emissions, with livestock production responsible for approximately two-thirds of agricultural CH_4 output (Gerber et al., 2013a; IPCC, 2014; Morgavi et al., 2011). In New Zealand, agriculture is the dominant emission source, and livestock-derived CH_4 alone accounts for about 43% of total national GHG emissions (MfE, 2022). Ruminant animals, such as sheep and cattle, produce CH_4 as a natural by-product of enteric fermentation of fibrous feeds, with between 2 and 12% of gross energy intake lost primarily through eructation (Johnson and Johnson, 1995; Murray et al., 1976). This energy loss varies with diet quality, feed intake, digesta passage rate, and rumen microbial structure (Gerber et al., 2013a; Knapp et al., 2014b; Patra, 2012).

The FAO's GLEAM model indicates that CH_4 emission intensity (kg CO_2-eq kg^{-1} protein) is highest in developing regions (Figure 2.1A) where productivity is low, whereas emission density (t CO_2-eq km^{-2}) peaks in regions with high livestock populations (Figure 2.1B; FAO, 2023).

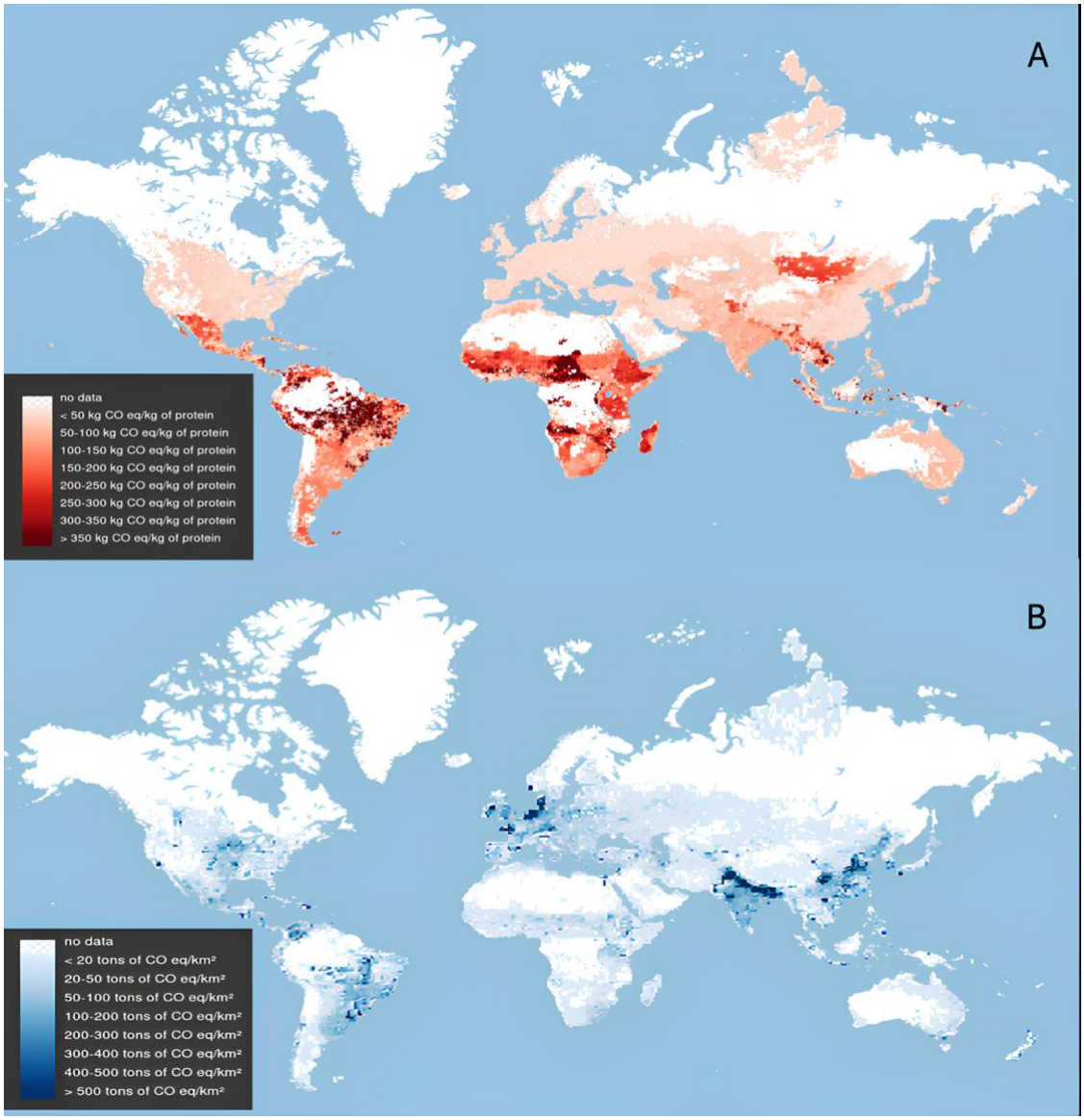

Figure 2.1 Global methane emission intensity (A - kg CO_2-eq /kg protein) and methane emission density (B - tons CO_2-eq /km2) emission density from livestock by region.

Source: GLEAM v3 (FAO, 2023): FAO GLEAM Public Dashboard

In this context, mitigation commitments under the Paris Agreement often differ between regions, with developing countries prioritizing reductions in emission intensity and developed nations targeting absolute emission reductions (Savaresi, 2016; UNFCCC, 2015). These approaches reflect differences in production efficiency, economic development, and food security priorities, and

emphasise the need for region-specific mitigation strategies. In pasture-based systems such as those common in New Zealand, sheep contribute a significant share of agricultural CH_4 emissions (Figure 2.2), highlighting the importance of species and system-specific measurement approaches under grazing conditions.

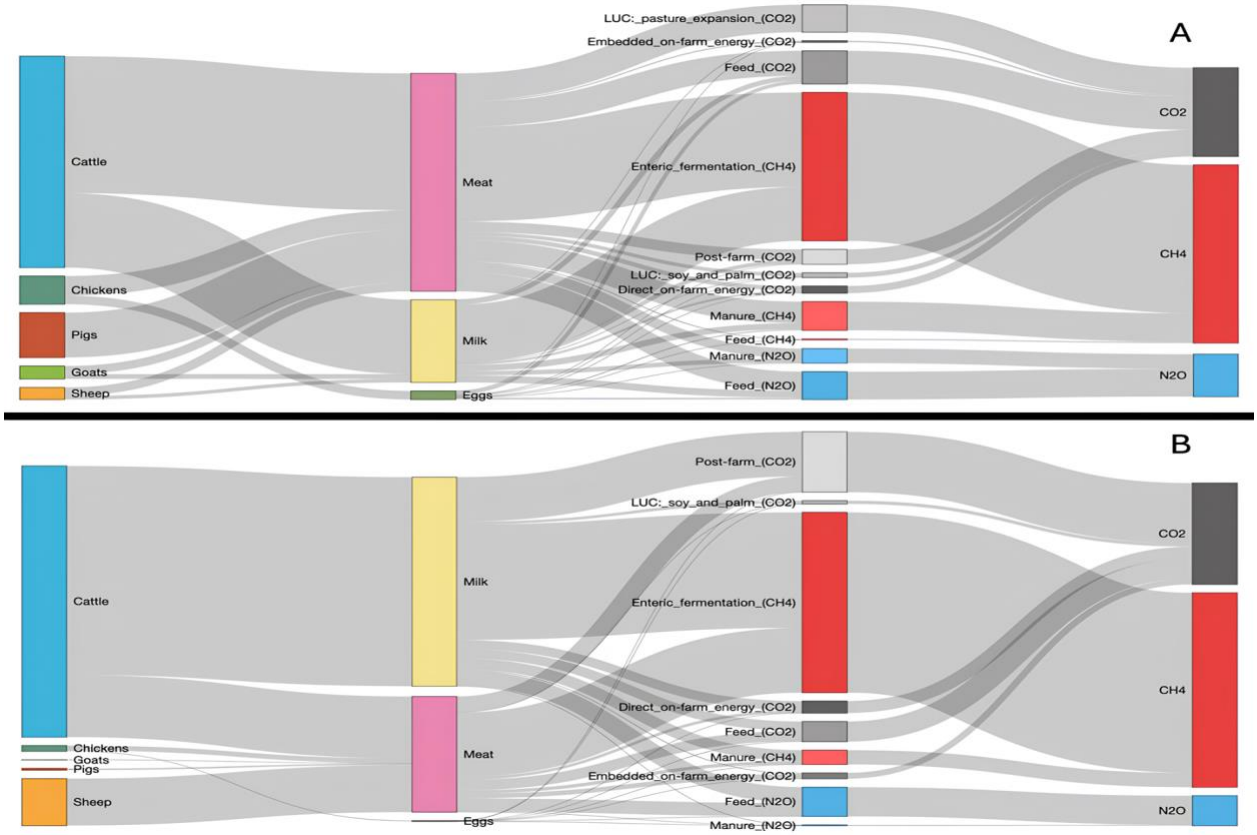

Figure 2.2 Sankey diagram of livestock-related emissions in 2015 by product (cattle, sheep, pigs, etc.), emission pathway (enteric fermentation, manure, feed), and gas (CH_4, N_2O, CO_2), comparing global patterns (top) with New Zealand (bottom).

Source: Adapted from GLEAM 3 https://foodandagricultureorganization.shinyapps.io/GLEAMV3_Public/

New Zealand's emissions profile is distinctive among developed countries, with agriculture contributing about half of total national GHG emissions and enteric CH_4 accounting for

approximately 43% (MfE, 2022). This has positioned livestock CH_4 at the centre of national climate policy, including the Climate Change Response (Zero Carbon) Amendment Act 2019, which established specific reduction targets relative to 2017 levels (MfE, 2019; Reisinger and Clark, 2018). Given CH_4's unique atmospheric behaviour, precise measurement is essential for national inventories and for evaluating mitigation strategies in pasture-based systems. Under the IPCC framework, Tier 2 approaches estimate emissions using country-specific average values for animal classes and feed types, while Tier 3 approaches apply more detailed models that account for variation in genetics, management, and production conditions. While current national reporting relies on Tier 2 emission factors, progress is being made toward Tier 3 approaches (Eugène et al., 2019; MfE, 2022). Beyond regulatory compliance, robust emissions measurement also supports New Zealand's agricultural export markets, where demand for verified low-emission production is increasing (Gerber et al., 2013a).

2.2 Enteric methane: physiology, measurement relevance, and implications

The rumen functions as a warm, anaerobic fermentation chamber that supports a diverse and highly specialised microbial ecosystem. Operating at approximately 39 ℃ and pH 6.0–7.0, it provides optimal conditions for bacteria, protozoa, fungi, and methanogenic archaea to degrade fibrous plant material that the host cannot digest enzymatically (Hristov et al., 2013; Martinez-Fernandez et al., 2018). During carbohydrate fermentation, microbes produce volatile fatty acids (VFA), primarily acetate, propionate, and butyrate, which are absorbed and supply most of the animal's metabolizable energy (ME) (Hungate, 2013; Janssen and Kirs, 2008; Kamra, 2005; Wang et al., 2017). Hydrogen (H_2) is generated as a by-product of these pathways and plays a central role in

CH_4 formation (Janssen, 2010; Wang et al., 2017). Accumulation of H_2 inhibits microbial activity by disrupting fermentation balance; therefore, efficient removal mechanisms are essential (Janssen, 2010). Methanogenic archaea fulfil this role through the hydrogenotrophic pathway, reducing carbon dioxide with H_2 to form CH_4 and water:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

This is the dominant methanogenesis pathway in sheep and is largely driven by genera such as *Methanobrevibacter* and *Methanosphaera* (Hook et al., 2010; Janssen and Kirs, 2008). Alternative pathways using formate or methanol contribute less under typical pasture-based diets but may increase with higher legume inclusion (Ungerfeld, 2020; Wang et al., 2017). The final step of CH_4 formation is catalysed by methyl-coenzyme M reductase (MCR), a primary target of mitigation technologies including 3-nitrooxypropanol (3-NOP) and bromoform-containing supplements (Beauchemin et al., 2020; Hristov et al., 2013; Martinez-Fernandez et al., 2018). Methanogenesis is shaped not only by H_2 availability but also by microbial interactions. Many fibrolytic bacteria generate H_2 during carbohydrate breakdown, while some protozoa both produce H_2 and host methanogens intracellularly or on their surfaces. These close associations facilitate direct transfer of H_2 between species, a process known as interspecies H_2 transfer, which enhances methanogenic efficiency (Janssen and Kirs, 2008; Kelly et al., 2022; Leahy et al., 2010; Ungerfeld, 2020). As a result, protozoa-associated methanogenesis can account for a substantial proportion of total CH_4 formation (Newbold et al., 2015). Changes in diet, animal condition, or rumen environment can shift the relative abundance and activity of these microbial groups, influencing both the rate and metabolic pathways of H_2 disposal (Ungerfeld, 2020; Wang et al., 2017). Together, these physiological processes explain why CH_4 formation is an inherent and tightly regulated component of rumen fermentation. The balance between H_2 production, H_2 removal, and the structure of the

microbial ecosystem determines not only how much CH_4 is produced but also how dynamically CH_4 output responds to shifts in feeding, substrate availability, and rumen conditions (Janssen, 2010; Ungerfeld, 2020). A simplified overview of these physiological processes is shown in Figure 2.3, which illustrates the progression from feed intake, microbial fermentation and H_2 production, through methanogenesis by rumen archaea, to the release of CH_4 from the animal.

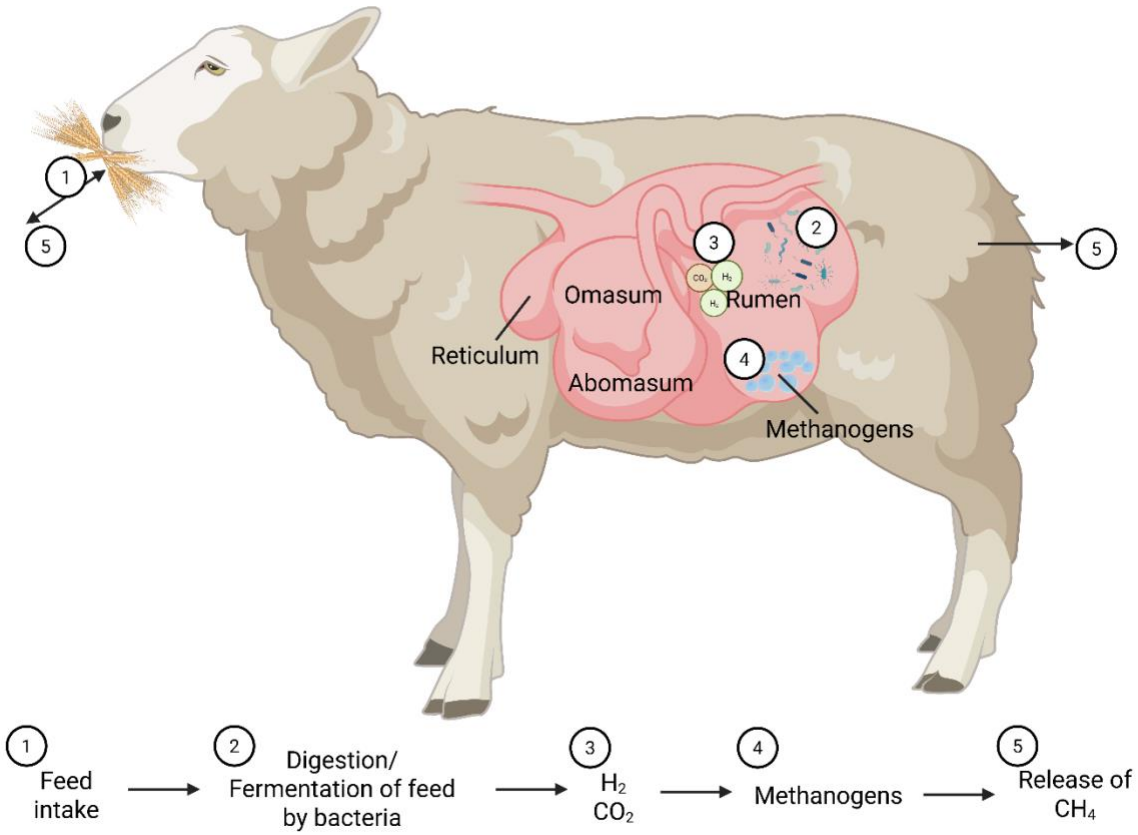

Figure 2.3 Enteric methane production: physiology and biochemistry of methanogenesis (Created in https://BioRender.com)

Methane production in sheep is not constant throughout the day but fluctuates according to changes in substrate availability, rumen fermentation dynamics, and microbial activity (Goopy et al., 2015; Muñoz et al., 2012). Feeding behaviour is one of the strongest drivers of these diurnal patterns.

After a meal, the increased availability of fermentable substrates stimulates microbial fermentation, leading to greater VFA and H_2 production, which in turn elevates CH_4 output. As fermentation slows between meals, CH_4 production temporarily declines (Goopy et al., 2015). The timing, size, and frequency of meals therefore produce characteristic peaks and troughs in daily emission profiles. The type and proportion of VFAs produced also influence methane patterns. Pathways leading to acetate and butyrate formation generate H_2, increasing the pool available for methanogenesis. In contrast, propionate formation consumes H_2 and competes with methanogens for reducing equivalents (Janssen, 2010; Ungerfeld, 2020). Diets dominated by fibrous pasture typically favour acetate production and therefore support higher CH_4 formation, while diets that enhance propionate shift hydrogen away from methanogenesis (Hristov et al., 2013; Janssen, 2010). These shifts do not only affect total CH_4 output but also shape the timing and amplitude of post-feeding emission peaks.

Rumen pH further modulates microbial activity and daily CH_4 dynamics. Diets high in rapidly fermentable carbohydrates can lower rumen pH, inhibiting fibrolytic bacteria and methanogens and reducing CH_4 output during these periods. When pH remains closer to neutral, fibre digestion and associated H_2 production proceed more efficiently, contributing to higher CH_4 formation (Janssen, 2010; Wang et al., 2017). Fluctuations in pH across the day therefore translate into shifts in microbial fermentation patterns and corresponding changes in CH_4 release. Digesta retention time provides another layer of influence. High-fibre forages increase rumen retention, allowing microbes more time to ferment structural carbohydrates and produce H_2. In contrast, more digestible or starch-based diets pass through the rumen more rapidly, reducing fermentation time and lowering CH_4 formation (Hristov et al., 2013; Janssen, 2010). Variation in retention time across feeding events or dietary phases contributes to changes in when CH_4 is produced, not just how

much is formed. Microbial population dynamics also create short-term variation in CH_4 output. Protozoa, which can harbour methanogens, play a substantial role in daytime CH_4 production through both H_2 generation and direct methanogen support (Leahy et al., 2010; Newbold et al., 2015). Shifts in protozoal abundance, bacterial composition, or fungal activity triggered by diet, feeding pattern, or rumen conditions can modify the timing and magnitude of CH_4 peaks (Goopy et al., 2015; Ungerfeld, 2020). Even when total daily CH_4 production remains stable, these microbial interactions can alter the shape of the emission curve across the day.

Together, these factors explain why enteric CH_4 follows clear diurnal rhythms rather than a smooth or linear pattern. A range of dietary strategies has been investigated to reduce enteric methane by altering fermentation pathways, limiting H_2 availability, or directly inhibiting methanogenic activity. Lipid supplementation, for example, can lower CH_4 output by suppressing protozoal populations, reducing fibre digestion, and providing alternative hydrogen sinks, although excessive inclusion in the diet may impair feed efficiency (Hristov et al., 2013; Jayanegara et al., 2014). Condensed tannins, present in some legumes and tree forages, can reduce methanogenesis by binding to proteins and modulating rumen microbial populations (Beauchemin et al., 2020). Nitrate-based supplements act as competitive electron sinks, redirecting reducing equivalents away from CH_4 formation, but require careful management to avoid nitrite accumulation and associated toxicity risks (Hristov et al., 2013). More targeted mitigation approaches have focused on inhibiting key methanogenic enzymes. The most established example is 3-nitrooxypropanol (3-NOP), which directly blocks methyl-coenzyme M reductase, the enzyme catalysing the final step in CH_4 formation and has demonstrated consistent reductions in CH_4 emissions across multiple trials (Hristov et al., 2013; Martinez-Fernandez et al., 2018). Seaweed species such as *Asparagopsis taxiformis*, which contain bromoform, offer similar inhibitory effects, though questions remain

regarding residue management, long-term animal health, and implementation under grazing systems (Beauchemin et al., 2020). Although these dietary strategies show promise, their effectiveness and practicality vary across production systems. For grazing sheep in particular, mitigation tools must perform reliably under variable pasture conditions and feeding behaviour. As such, accurate and representative CH_4 measurements are essential for evaluating dietary interventions and for supporting breeding, inventory reporting, and on-farm mitigation efforts (Hristov et al., 2013; Muñoz et al., 2012).

Because CH_4 fluctuations can be substantial across the day, CH_4 measurement systems must be able to capture both the magnitude and the timing of emissions to produce accurate and representative estimates. Respiration chambers (RC) remain the reference method for enteric CH_4 measurement because they quantify gas exchange continuously, generating complete emission curves that include post-feeding peaks and between-meal lows. This continuous monitoring means that RC measurements fully reflect the underlying biological rhythms of CH_4 production and provide high-precision data suitable for calibration, mitigation evaluation, and research requiring detailed emission profiles (Goopy et al., 2015; Muñoz et al., 2012). In contrast, GF systems record CH_4 only when animals voluntarily visit the unit. As a result, the accuracy of GF measurements depends heavily on how well the distribution of visits aligns with the animal's natural emission pattern. If visits are frequent and well spread across the day, GF can capture representative portions of the CH_4 curve (Hammond et al., 2016a). However, if visits cluster at particular times such as around feeding or during routine management activities, GF may oversample periods of either high or low-emissions, introducing bias into daily estimates. These challenges can be amplified under grazing conditions, where feeding behaviour and pasture availability influence both rumen fermentation and animal movement. The implications extend to animal ranking, mitigation

evaluation, and national inventory development. For breeding programmes, unbiased and repeatable measurements are essential to correctly identify animals with lower CH_4 phenotypes (Johnson et al., 2022). At national scale, country-specific emission factors depend on robust, locally generated data that accurately capture daily variation (Baasansuren et al., 2019). At the farm level, emerging certification and incentive schemes require reliable, transparent measurements to ensure fair reporting and adoption of mitigation practices (Starsmore et al., 2024). In each case, the validity of the CH_4 measurement method and its ability to reflect true emission patterns directly determines the credibility of downstream decisions. Overall, understanding how rumen physiology shapes CH_4 production patterns is fundamental for evaluating the strengths and limitations of measurement systems. Systems that can represent diurnal variation provide the most accurate view of true CH_4 output, while systems that rely on behavioural sampling require careful management, calibration, and interpretation to ensure that results reflect biological reality rather than artefacts of sampling behaviour.

2.3 Relevance of methane monitoring and genetic selection in sheep

Sheep farming is a cornerstone of New Zealand's economy and rural identity, but it also produces a large share of the country's GHG emissions. Although sheep release less CH_4 than cattle because they are smaller and eat less, their emissions per kilogram of meat or wool can match or even exceed cattle, especially in low-productivity systems (Gantner, 2016). Farm management has a large influence over CH_4 emissions with factors such as fibre-rich diets, slow growth, and longer periods to reach slaughter weight resulting in higher CH_4 emissions (Ungerfeld, 2020). In contrast, higher input farms that use improved genetics, maintain better pasture quality and have good

reproductive planning can lower CH_4 intensity by increasing output for the same or fewer animals (Meo-Filho et al., 2022; Wang et al., 2024).

Individual sheep naturally differ in how much CH_4 they produce, even when fed and managed the same way, and research in New Zealand and overseas shows these differences are partly genetic (Pinares-Patiño et al., 2013; Rowe et al., 2019).

Studies report that CH_4 emission traits are moderately heritable in sheep, with heritability estimates typically ranging from 0.13 to 0.33 depending on the trait definition (e.g. methane production, yield, or intensity) and the measurement method used (Jonker et al., 2017; Pinares-Patiño et al., 2013). This heritability has been proven in practice through AgResearch's CH_4 Selection Line Flock, where breeding from low-emitting animals achieved a 10% reduction in CH_4 yield without affecting growth, reproduction, or wool production (Jonker et al., 2017; Rowe et al., 2019). Encouragingly, links between CH_4 and production traits such as growth rate, carcass quality, and wool yield are generally neutral or slightly positive, meaning that breeding for low CH_4 does not harm productivity (Rowe et al., 2019). These genetic effects arise from differences in rumen function and microbes between animals, including how long feed stays in the rumen, how it ferments, and how many methanogens are present (Kittelmann et al., 2014). With new genomic markers, quantitative trait loci (QTLs), and improved "residual CH_4" measures that account for feed intake, selection accuracy continues to improve (de Haas et al., 2021). Together, these advances show that low-emission traits can be reliably built into multi-trait sheep breeding goals, supporting productivity while addressing environmental performance (de Haas et al., 2021).

2.4 Influence of diet and pasture systems on methane emissions

2.4.1 Forage composition and carbohydrate fermentation in methane production

The type, structure, and quality of pasture influence enteric CH_4 emissions from grazing sheep. In New Zealand, where sheep mostly graze, the forages they eat directly affect rumen fermentation, H_2 formation, and the total CH_4 produced. Typical temperate pastures are made up of perennial ryegrass (*Lolium perenne* L) and white clover (*Trifolium repens* L), and in recent times, often mixed with other species such as red clover (*Trifolium patens* L), plantain (*Plantago lanceolata* L) and chicory (*Cichorium intybus* L). These plant species differ in morphology, fibre, digestibility, nitrogen concentration, and secondary compounds, all of which shape their impact on enteric CH_4 (Waghorn et al., 2002).

Fibre rich forages like mature ryegrass contain high levels of neutral detergent fibre (NDF) and acid detergent fibre (ADF), which tend to favour acetate-dominated fermentation pathways that are associated with greater H_2 availability for methanogenesis. Structural carbohydrates such as cellulose and hemicellulose are broken down by fibre-digesting microbes to form mainly acetate and butyrate, and the formation of these VFA releases H_2, the key substrate used by methanogens to produce CH_4 (Bannink et al., 2006; Janssen, 2010). However, high fibre content is also linked with lower digestibility, which can limit the amount of fermentable substrate entering the rumen. At lower intake levels, this reduction in digestibility can offset the acetate effect, resulting in unchanged or even reduced CH_4 yield, whereas at higher intake levels the fermentation profile becomes more influential and may lead to increased CH_4 production. Slow fermentation and longer feed processing time in the rumen increase the CH_4 formation per kg of feed eaten, by allowing greater H_2 turnover, particularly at moderate to high intake levels. In contrast, non-structural

carbohydrates such as sugars and starches ferment more quickly and tend to shift the VFA profile away from acetate toward greater proportions of propionate and/or butyrate, depending on the substrate. These pathways utilise reducing equivalents and therefore compete with methanogenesis for available H_2, although they are always produced as part of a mixed VFA profile (Bannink et al., 2006). The speed and completeness of digestion also influence CH_4 output. Highly digestible feeds with less lignin and more water-soluble carbohydrates move through the rumen faster, which lowers retention time and limits H_2 build up (Ellis et al., 2012; Hassanat et al., 2017). By contrast, slow fermenting, high-fibre diets stay longer in the rumen and allow more H_2 turnover, which increases CH_4 production.

Legumes such as *Lotus* species which have higher crude protein (CP), break down faster, and often contain condensed tannins that can block methanogens or reduce protozoa numbers that host them. Legume-based diets are typically associated with higher voluntary intake, which contributes to reduced CH_4 yield through faster passage rate. While fermentation rate and secondary plant compounds also contribute to CH_4 dynamics, the increase in intake represents an important confounding factor and may play a more dominant role than fermentation effects alone in explaining reduced methane yield in legume-based diets. (Min et al., 2021; Waghorn and Clark, 2004; Woodward et al., 2004). Herbal species such as chicory and plantain are typically lower in fibre and may alter rumen fermentation patterns, but their effect on CH_4 yield is not driven by fermentation profile alone. These forages often promote higher voluntary intake, which itself contributes to lower CH_4 yield. Differences in digestibility and intake therefore interact to influence overall CH_4 production rather than a single direct shift toward propionate formation (Della Rosa et al., 2022; Durmic et al., 2016).

Seasonal growth and stage of plant maturity also influence CH_4 production, but their effects are not driven by digestibility alone. Young leafy pastures in spring and early summer tend to be more digestible and ferment more rapidly, which can reduce CH_4 yield per unit of intake. These pastures are also associated with higher voluntary intake, further reducing CH_4 yield. In contrast, more mature and fibrous forages, which commonly occur later in the season, generally have lower digestibility and longer rumen retention time, conditions that favour greater hydrogen availability and potentially higher CH_4 formation, although the net effect depends on how intake level and fermentation dynamics interact (Knapp et al., 2014a; Waghorn and Clark, 2004).

Forage quality indicators such as ME, CP, NDF, ADF, and lignin are closely associated with CH_4 yield, as they influence rumen fermentation patterns, intake, and passage rate. High energy, low fibre forages not only support better animal performance but also reduce CH_4 per unit of animal product. However, the extent to which pasture improvements deliver both environmental and economic gains depends on the inputs required to establish and maintain these species. In summary, the mix and quality of pasture species remain key levers for CH_4 mitigation in grazing systems. By choosing and managing pastures that are more digestible, fast fermenting, or naturally contain anti methanogenic compounds, farmers can reduce emissions while supporting productivity, although the management intensity required will vary across temperate grazing systems.

2.4.2 Nutrient synchrony and microbial efficiency in methane mitigation

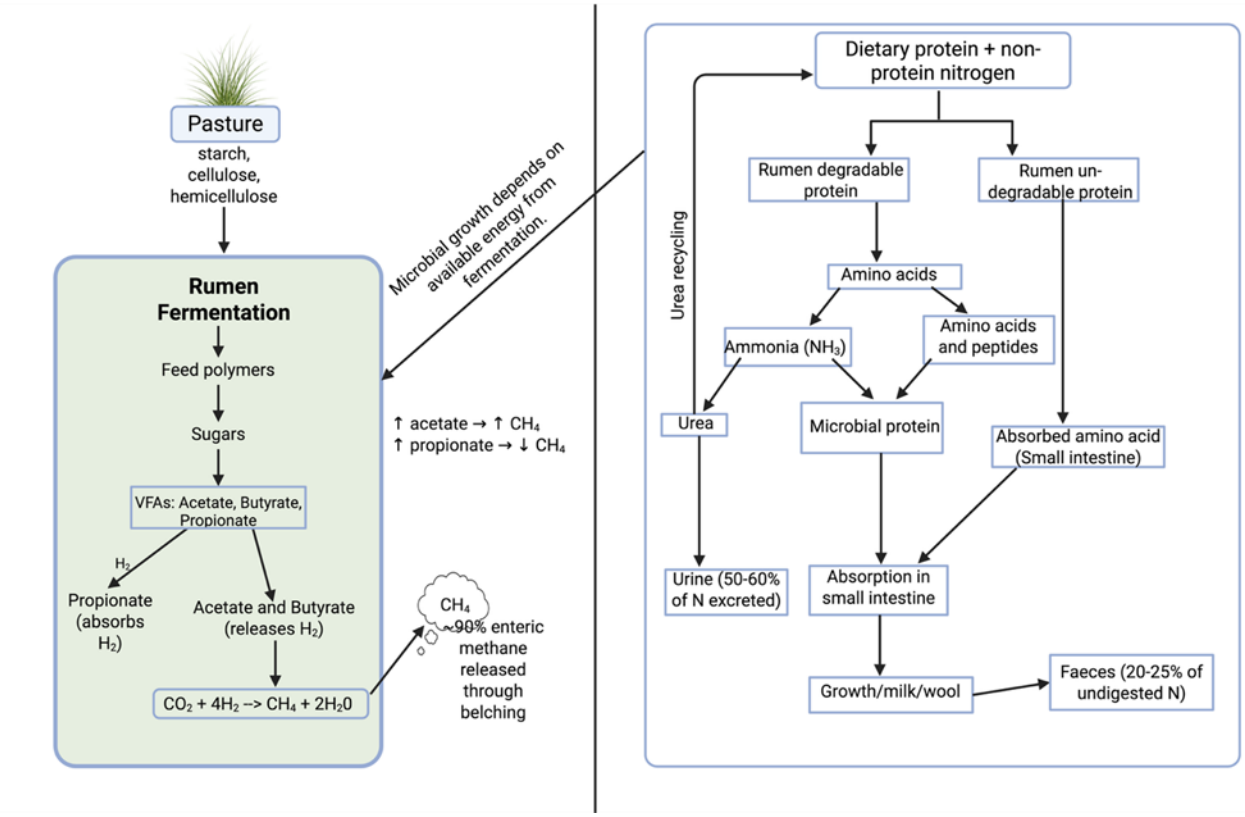

Figure 2.4 Integrated pathways of carbon fermentation, nitrogen utilisation, and methane production in the rumen (Created in https://BioRender.com).

Rumen fermentation relies on the coordinated supply of fermentable carbohydrates and rumen-degradable protein (RDP) to support efficient microbial growth (Figure 2.4). When energy and nitrogen are available in synchrony, microbes incorporate more NH_3 and amino acids into microbial protein, reducing excess ammonia accumulation and improving overall fermentation efficiency (Hristov et al., 2025b; Sinclair et al., 1993). Greater microbial capture of nitrogen may alter H_2 availability and fermentation patterns, which could influence CH_4 yield per unit of feed fermented, although responses are diet-dependent (Hristov et al., 2025a). At the same time, rumen microbes use nitrogen from dietary protein and from non-protein nitrogen (NPN sources such as urea and NH_3) to synthesise microbial protein when sufficient fermentable energy is available. Excess NH_3

is absorbed through the rumen wall and converted to urea, which may be recycled to the rumen or excreted in urine (Lapierre and Lobley, 2001). Efficient synchrony between energy and nitrogen use supports microbial growth, improves nutrient utilisation, and lowers methane emissions (Hristov et al., 2013; Sinclair et al., 1993).

Nitrogen use in the rumen is closely linked with microbial activity and energy supply, influencing both protein use and CH_4 production. Rumen microbes need NH_3, peptides, and amino acids as N sources to build microbial protein, which then supplies essential amino acids to the animal (Bach et al., 2005; Hartinger et al., 2018). If the supply of fermentable energy and N is not well matched, whether because N exceeds the available energy for microbial capture or because energy supply exceeds available N, microbial protein synthesis becomes inefficient. In the former case, ruminal NH_3 can accumulate and increase N losses, while in the latter case NH_3 may remain low but microbes become N limited. Both situations reduce microbial efficiency and alter fermentation in ways that can influence H_2 availability for methanogenesis (Dijkstra et al., 2013; Lu et al., 2019). This highlights why aligning N release with carbohydrate fermentation improves microbial protein synthesis. When energy and N are supplied in synchrony, microbes can use H_2 more efficiently for microbial protein synthesis, which may reduce the amount available for methanogenesis. Improved microbial capture of N therefore acts as a H_2 sink and can lower CH_4 emissions without necessarily requiring a shift in VFA patterns (Arias et al., 2020; Bhatt et al., 2019). The type and breakdown rate of dietary protein also matters. Fast-degrading proteins release NH_3 quickly, while slower degrading sources provide a steady supply over time, improving N use by microbes (Chen et al., 2022). Some plants contain tannins, which can bind to proteins and slow down their breakdown. This reduces NH_3 build-up and, in some cases, lowers CH_4 production (Min et al., 2021; Orzuna-Orzuna et al., 2021). Urea recycling helps maintain rumen N supply when fermentable energy

supports microbial use. When rumen NH_3 is high, recycling decreases because NH_3 is absorbed and converted to urea, meaning the process redistributes N rather than increasing the rumen N pool (Hristov et al., 2019). Overall, N supply influences microbial growth and protein synthesis, which can affect how efficiently rumen microbes utilise available nutrients. Although synchronising energy and nitrogen supply can improve microbial use of NH_3 under some conditions, evidence for consistent reductions in CH_4 is limited. In practice, these pasture driven fermentation effects can also be influenced through targeted supplementation, although responses in grazing sheep remain variable and highly context dependent.

2.5 Overview of methane measurement methods

Accurately measuring CH_4 from sheep is essential for testing how well mitigation strategies work, understanding differences between individual animals, and meeting national GHG reporting requirements. Several methods are available to measure CH_4 in ruminants, each provide distinct advantages but differ in accuracy, cost, throughput, and practicality (Bekele et al., 2022; Zhao et al., 2020b). The following subsections gives an overview of the main CH_4 measurement techniques used for sheep, focusing on RC and the GF system alongside the sulphur hexafluoride (SF_6) tracer technique, portable accumulation chambers (PACs), and laser methane detectors (LMD) techniques.

2.5.1 Respiration chambers: the 'gold standard'

Figure 2.5 Respiration chambers used for measuring enteric methane emissions in sheep at AgResearch, New Zealand.

Source: RNZ Insight, 2018

Respiration chambers are widely recognised as the '*gold standard'* for measuring enteric CH_4 emissions from sheep providing accurate, continuous high-precision gas exchange measurement under controlled conditions (Tedeschi et al., 2022; Zhao et al., 2020b). The principle involves housing the sheep in the chamber while continuously monitoring inlet and outlet air composition and flow rate (Tedeschi et al., 2022; Waghorn, 2014). By analysing CH_4 in the air entering and leaving the chamber, and combining this with precise airflow data and adjustment for temperature, pressure and humidity, researchers can calculate exactly how much gas the animal produces over

time. Sheep usually stay in the chambers for 24 to 72 hours, during which all emissions are collected. This allows detailed tracking of CH_4 production and other metabolic processes (Storm et al., 2012; Zhao et al., 2020b). Respiration chambers have been used for method validation (Jonker et al., 2018), nutritional experiments (Blaxter and Clapperton, 1965), genetic evaluations (Jonker et al., 2018), and testing additives and vaccines (Hristov et al., 2025b; Wright et al., 2004), and forming the basis for national GHG inventory (Tedeschi et al., 2022; Zhao et al., 2020b). Long-term RC datasets, such as those used by Jonker and colleagues, provided the heritability estimates required to construct low-methane breeding values now used in national sheep selection programmes in NZ (Hickey et al., 2022; Jonker et al., 2018).

The main advantage of RC includes exceptional accuracy, precision, and repeatability in measuring CH_4 exchange. Although whole-animal calorimetry systems can monitor feed intake continuously, most RC used for CH_4 studies do not include automated intake measurements (Patra, 2016; Zhao et al., 2020b). Their precision makes them essential for comparing and validating other tools such as the GF system, the SF_6 tracer technique and PACs (Jonker et al., 2018). Environmental variables such as temperature, pressure and humidity are measured to express methane at standard conditions (Hegarty, 2013). Although gas analysers operate at high analytical frequency, in most RC systems a single analyser is shared across 2-10 chambers. As a result, the analyser cycles between chambers, providing CH_4 readings for each chamber at intervals (typically every 2-45 minutes), rather than continuous per animal measurements (Della Rosa et al., 2021; Hammond et al., 2016b). These design features allow RC to achieve high measurement repeatability, with intraclass correlation coefficients typically around 0.80-0.90, indicating that repeated measurements on the same animal are highly consistent (Lassey et al., 2011). Coefficients of

variation frequently fall below five percent (Robinson et al., 2014; Storm et al., 2012), enabling detection of subtle differences caused by diet, genetics, or physiological status.

However, the controlled and highly instrumented nature of RC systems also introduces practical constraints that must be considered. They cannot be used under grazing or other production conditions and the animal has to be brought to the measurement device (Tedeschi et al., 2022). Although RC are designed to allow natural standing and lying behaviours while maintaining airtightness (Waghorn, 2014), the controlled environment differs from real pasture conditions, i.e., the pasture is not standing and there is no competitive and selective grazing when offered a new herbage break. Confinement can reduce voluntary feed intake by up to 25 percent (Waghorn, 2014), although not always occurring, which can artificially lower CH_4 production if not accounted for (Hammond et al., 2016b). Stress may influence CH_4 output indirectly through changes in feeding behaviour and dry matter intake (DMI), which is a primary determinant of daily CH_4 production (Hegarty, 2013; Lassey et al., 2011). To minimise these effects, sheep are provided with acclimation periods of one to three days, and researchers may apply correction factors or extend adaptation times to ensure that measured emissions better reflect normal behaviour (Knapp et al., 2014a; Robinson et al., 2014). Despite these challenges, RC remains essential for testing new CH_4 measurement technologies and providing the most accurate emission data possible, when properly calibrated and gas recovery determined.

2.5.2 Greenfeed system: practical applications and challenges in small ruminants

Figure 2.6 GreenFeed system showing sheep voluntarily entering the sampling hood for methane breath measurements under pasture conditions.

The GF system is an automated open-circuit breath-sampling technology originally developed for measuring enteric CH_4 emissions in cattle under research and farm conditions (Hristov et al., 2015). Interest in applying the system to small ruminants has increased as researchers seek methods that can operate under grazing and semi-natural environments. However, despite its widespread use in

cattle, the validation and evaluation of GF performance in sheep remain limited, with only a small number of studies addressing its suitability for small-ruminant CH_4 measurement (Mcgovern et al., 2025; Tadesse et al., 2024). The GF system measures enteric CH_4 by drawing exhaled air past a gas-sampling inlet while the animal consumes a small bait feed inside the hood of the unit. As the animal places its head into the feeding area, airflow is controlled to capture breath and eructation plumes, which are then analysed for CH_4 and CO_2 concentrations using integrated gas analysers. Each visit produces a short "spot sample," and multiple visits over several days are aggregated to estimate daily CH_4 emissions. The system records airflow, gas concentrations, visit duration, and timing, allowing emissions to be calculated using standardised equations (Hammond et al., 2016a; McGinn et al., 2021). This spot-sampling approach distinguishes GF from continuous measurement systems such as respiration chambers.

Early GF units used NDIR gas sensors, which had adequate sensitivity for cattle but were less precise at the lower CH_4 concentrations typical of sheep. The small ruminant GF has a lowered entry height and narrower hood dimensions compared to cattle GF, to ensure consistent sampling of breath from smaller animals. In 2021, C-Lock introduced a major hardware upgrade by replacing NDIR sensors with TDL analysers, making the GF system a universal unit suitable for both cattle and small ruminants. This update increased analytical sensitivity and removed the need to use small-ruminant specific units (C-Lock-Inc., 2021). However, validation of the GF system in small ruminants remains limited. Only a small number of studies have attempted to assess its feasibility in species other than cattle, and dedicated validation work in sheep is largely absent. Recent exploratory trials in goats indicate that GF can be used to capture short-term CH_4 emissions, but these studies also emphasise the need for species-specific behavioural training and validation against reference methods (Tadesse et al., 2024). Research in farmed deer has likewise highlighted

practical challenges related to visit behaviour and measurement duration, reinforcing the importance of evaluating GF performance across species that differ from cattle in feeding patterns and temperament (Bennett et al., 2022). Practical challenges arise when applying the GF system to sheep, largely because their behavioural patterns differ from those of cattle for whom the system was originally developed. Species differences suggest that sheep may show lower motivation to approach concentrate-based feeders, greater neophobia toward unfamiliar equipment, and shorter feeding bouts, all of which could influence visit frequency and measurement duration (Provenza and Balph, 1987). Earlier small-ruminant GF designs also required physical modifications to ensure the animal's head was correctly positioned within the sampling zone, indicating that system animal fit can affect data quality.

Because GF relies on voluntary visits, uneven or infrequent participation may reduce data completeness and accuracy. Although this has been documented primarily in cattle studies (Beck et al., 2021; de Mol et al., 2024), similar issues are plausible in sheep and require formal evaluation. Group dynamics such as social hierarchy effects on feeder access could also influence visit patterns, but this remains to be quantified in sheep. Collectively, these potential behavioural and management challenges highlight the need for species-specific validation before GF can be reliably used for sheep. To date, the practical application of the GF system in sheep has been limited, with only a small number of exploratory studies reporting its use (Mcgovern et al., 2025). Early work describing small-ruminant adaptations to the system focused primarily on engineering feasibility rather than formal validation of measurement accuracy. Some broader ruminant CH_4 datasets have included sheep measured using GF-type approaches (Belanche et al., 2023; Huhtanen et al., 2019), but these do not represent dedicated validation studies and therefore provide only preliminary insights. Related investigations in other small ruminants, such as goats (Tadesse et al., 2024) and

farmed deer (Bennett et al., 2022), highlight the need for species-specific behavioural and sampling evaluation before GF can be confidently applied in sheep. A few pilot nutrition or CH_4-phenotyping studies have trialed the system in in small ruminants under research settings (Mcgovern et al., 2025; Rivero et al., 2025; Tadesse et al., 2024), but no published work has yet established validation criteria or demonstrated its suitability for large-scale genetic or ranking applications in sheep.

Overall, while GF provides a promising approach for measuring CH_4 under grazing-type conditions, sheep-specific validation particularly against RC and with appropriate behavioural sampling protocols remains limited and requires further study.

2.5.3 Sulphur hexafluoride tracer technique

The sulphur hexafluoride (SF_6) tracer technique was one of the first portable methods developed to measure enteric CH_4 emissions in grazing animals (Johnson et al., 1994), and it remains widely used in field-based studies today. The SF_6 technique involves placing a small permeation tube containing a known quantity of SF_6 into the rumen of the animal. The tube releases SF_6 gas at a constant predetermined rate. As the animal breathes, both SF_6 and CH_4 are exhaled and are collected simultaneously through a halter-mounted sampling apparatus connected to an evacuated canister. After a 24-hour collection period, the gas samples are analysed in the laboratory, and CH_4 emissions are calculated from the ratio of CH_4 to SF_6, adjusted for the known release rate of SF_6 (Johnson et al., 1994). This principle allows researchers to estimate daily CH_4 production without confining animals (other than fitting the equipment and canister), making the method particularly attractive for grazing conditions. Applications of the SF_6 tracer technique have been extensive in ruminant research. It has been used to quantify CH_4 emissions in cattle, sheep, deer and goats under pasture-based management systems, enabling the evaluation of dietary interventions, feed

supplements, and breed differences. Because the method can be applied to free-ranging animals, it has been instrumental in large-scale studies aiming to capture emissions in farming environments. It has also played an important role in validating national GHG inventory methodologies by providing emission factors under field conditions.

The advantages of the SF_6 technique are primarily its portability and suitability for measuring CH_4 emissions from grazing animals under commercial or pasture-based conditions. It provides integrated daily emission estimates when gas is collected over several consecutive days, which is useful for characterising CH_4 output under field conditions. However, because several measurement days per animal are required to achieve accuracy, compared to RC, its overall throughput is not necessarily higher than chamber-based approaches once labour and analytical requirements are considered (Garnsworthy et al., 2019). Although SF_6 has been used in a range of countries and production systems, it is best suited to outdoor or well-ventilated environments; in housed systems, accumulation or 'pocketing' of SF_6 and CH_4 can bias concentration measurements, so careful system design and validation are essential and the risk of measurement error is higher (Garnsworthy et al., 2019; Pinares-Patiño et al., 2008).

The method has several important limitations. It is technically demanding and requires skilled personnel to prepare, calibrate, and analyse permeation tubes and gas samples. The accuracy of measurements can be influenced by factors such as variation in gas mixing within the rumen, leaks in the sampling system, or improper canister placement. Animal behaviour, such as rubbing or pulling at the halter equipment, may also compromise data collection. Furthermore, SF_6 is itself a potent greenhouse gas, raising environmental concerns about its continued use in large-scale studies (Pinares-Patiño et al., 2008). Although widely used, the method is less precise than RC and can be logistically intensive when applied to large herds over extended periods. The SF_6 tracer

technique provides a practical means of estimating CH_4 emissions under grazing conditions, offering a balance between field applicability and measurement reliability. While it lacks the precision of RC and raises sustainability concerns due to the use of a synthetic GHG, it remains a valuable method for studying CH_4 emissions in pasture-based systems and for supporting genetic and dietary research in large animal cohorts.

2.5.4 Portable accumulation chambers (PAC)

Portable accumulation chambers (PAC) provide a simple and rapid method for estimating enteric CH_4 emissions, making them especially useful for large-scale screening and genetic studies. How they work is based on placing an animal in a sealed chamber for a short duration, typically 30-60 minutes. During this time, the animal continues to breathe and belch normally, and gases exhaled accumulate within the chamber. At the end of the sampling period, the concentration of CH_4 and other gasses in the chamber is analysed, and emission rates are estimated by combining gas concentrations with chamber volume and measurement duration (E et al., 2021; Goopy et al., 2015; O'Connor et al., 2024). Because animals are only enclosed temporarily, PAC can be used repeatedly across many individuals within a single day, and 10-12 PAC are used at once, allowing for high-throughput measurement of CH_4 emissions. Portable accumulation chambers are primarily used for high throughput screening in genetic evaluation and breeding programmes, where relative differences among animals are more important than precise daily emission estimates. In New Zealand, PAC form the core phenotyping tool for the national low CH_4 sheep breeding programme, enabling thousands of animals to be screened efficiently and supporting the development of CH_4 breeding values (Hickey et al., 2022; Jonker et al., 2018).

They have also been employed in dietary intervention studies where relative differences between treatment groups are more important than accurate daily emission values (Goopy et al., 2014). In

some regions, PAC have been used in large-scale screening programmes, particularly for genetic evaluations and ranking animals for CH_4 traits (Jonker et al., 2018). The advantages of PAC lie in their portability, simplicity, and scalability. They are relatively inexpensive to build and maintain, compared to RC, SF_6 or GF systems, and they require minimal infrastructure, making them accessible to researchers in a wide range of settings. The short measurement duration (20-60 minutes) allows large numbers of animals to be tested each day, providing a high-throughput solution for population-scale studies. This efficiency is particularly valuable for genetic selection programmes, where thousands of animals need to be measured (Goopy et al., 2015). Portable accumulation chambers are also less technically demanding than tracer methods such as SF_6, reducing the training and analytical expertise required for their use.

Despite these strengths, PAC have several limitations. Because measurements are limited to short-term accumulation periods, they do not capture the full diurnal variation in CH_4 emissions, which can lead to biased estimates if extrapolated to daily values (E et al., 2021; O'Connor et al., 2024). Enclosure within the chamber, even briefly, can lead to behavioural adjustments that require consideration when interpreting CH_4 measurements, even though direct effects on CH_4 production have not been demonstrated. The method provides relative rather than absolute emission estimates, meaning it is most appropriate for ranking animals or comparing treatments, rather than for accurate quantification of CH_4 output (Jonker et al., 2018). Portable Accumulation Chamber offer a useful supplementary approach for CH_4 studies, provided their methodological limitations are recognised. While they lack the accuracy and comprehensiveness of RC, and provide only snapshot estimates rather than continuous data, PAC fill an important niche by enabling rapid and repeatable CH_4 assessments across large animal populations.

2.5.5 Laser methane detectors (LMD)

Laser CH_4 detectors (LMD) estimate enteric CH_4 using open-path infrared laser spectroscopy. Measurements require the operator to be close to the animal, and many protocols involve restraining or positioning the animal to obtain stable readings, limiting their use to controlled settings (Roessler and Schlecht, 2021). A handheld or tripod-mounted device is aimed at the muzzle of an animal, and the laser beam passes through the exhaled breath plume. Methane molecules absorb the infrared light at a specific wavelength, and the detector quantifies CH_4 concentration in parts per million per metre. By repeatedly scanning exhaled air plumes during respiration or eructation, operators can obtain point measurements of CH_4 concentration, which are then used to estimate emission rates (Chagunda et al., 2009). Applications for LMD are primarily in preliminary assessments and large-scale surveys where rapid, non-invasive data collection is required. They are used to rank animals or groups for relative CH_4 output, evaluate dietary treatments, and monitor emissions. Laser CH_4 detectors are also suitable for on-farm use in commercial settings, where they can provide quick feedback to farmers or researchers without the need for elaborate infrastructure. Because they are portable and easy to deploy, LMD have been tested in both indoor housing systems and outdoor pasture environments (Chagunda, 2013; Ricci et al., 2014). In some genetic studies, they have been applied as a screening tool to identify animals with relatively high or low CH_4 emissions before subjecting them to more precise measurement techniques (Kang et al., 2022; Pereira et al., 2023). The potential advantages of LMD relate mainly to their portability and ability to collect point measurements in open or semi-natural settings. However, obtaining reliable readings typically requires the animal to remain relatively still for several minutes, meaning some degree of close handling or restraint is often needed to maintain a stable distance and reduce head movement during scanning. Although each individual measurement is quick, producing a usable

estimate generally requires many repeated scans per animal, resulting in low overall throughput and substantial labour requirements. While LMD devices themselves are less expensive than RC or GF units, the operator time required makes the cost per animal high, limiting practicality for large-scale studies. Their accuracy is also highly sensitive to environmental conditions such as wind, humidity, and background CH_4 as well as operator positioning and animal movement, leading to considerable measurement variability (Bruder et al., 2017; Chagunda, 2013). Moreover, LMD provide only instantaneous concentration values rather than integrated emission rates, restricting their usefulness to qualitative or comparative assessments rather than absolute CH_4 quantification. Given these limitations LMD remain an exploratory tool whose role in CH_4 research is still being evaluated and debated, rather than a validated method for ranking.

2.6 Accuracy, precision, repeatability, and bias in methane measurement: Respiration chambers vs. Greenfeed measurements

Comparing the performance of the GF system with RC is important for understanding CH_4 results from different measurement systems. Studies in cattle provide useful insight for sheep research, showing moderate to strong agreement between GF and RC data when calibration and sampling protocols are well controlled (Hristov et al., 2015; McGinn et al., 2021). However, GF often records slightly lower daily CH_4 than RC because it samples intermittently rather than continuously, relies on voluntary visits, and primarily captures eructated CH_4, not CH_4 from the rear (Hammond et al., 2015). These differences highlight the importance of frequent sampling and full day coverage when comparing GF data with continuous RC measurements (Manafiazar et al., 2017; McGinn et al., 2021).

Despite these limitations, GF can still provide accurate estimates of CH_4 yield (g CH_4/kg of DMI) and detect changes due to diet or genetics in real world conditions where using RC is less practical and expensive (Hristov et al., 2015; Tedeschi et al., 2022). However, GF also show differences in total CH_4 emissions caused by inconsistent visit frequency and varying motivation to access the GF unit (de Mol et al., 2024; MPI, 2020; O'Connor et al., 2024). Incomplete 24-hour coverage remains a major source of bias, as GF may capture post feeding peaks but miss lower emissions during the night or *vice versa*. The three main factors which affect how well GF and RC data agree are: number and duration of spot-samples per animal, feed intake differences, and animal behaviour. Because of this, GF results must be interpreted carefully, especially when calculating emissions per unit of feed or using them for national inventory purposes (Ma et al., 2024). Animal social factors, such as dominance and temperament, also affect how often animals visit the GF unit, which can influence data quality (de Mol et al., 2024; Starsmore et al., 2024). On the other hand, RC confinement can also reduce feed intake, which in turn may lower CH_4 output. Understanding these system specific limitations is essential when comparing results (O'Connor et al., 2021). The most reliable way to compare GF and RC is through within-animal studies, where the same animals are measured using both methods. Although simultaneous measurement is not possible, sequential measurement of the same individuals allows direct assessment of how the two systems compare.

Accurately measuring enteric CH_4 emissions from sheep depends not only on the equipment used but also on understanding a few key statistical principles that determine data quality. Accuracy refers to how close a measurement is to the animal's true CH_4 output. In practice, RC measurements are highly accurate but low-throughput, limiting the number of animals measured, whereas GF can potentially measure a much larger proportion of a flock/herd, improving capture of between-animal variation. Because absolute emissions cannot be directly measured, highly controlled systems like

RCs are used as reference points to check how accurate other methods are (Storm et al., 2012; Tedeschi et al., 2022). Precision, on the other hand, refers to the amount of variation in CH_4 measurements among animals within the same treatment group. Lower within-group variability indicates higher precision and improves the ability to detect true differences due to diet, genetics, or management. For method comparison, within-animal precision is also important because it reflects how reliably the same animal's CH_4 output is estimated across repeated measurements. For spot-sampling systems such as GF, within-animal precision improves as the number of valid visits samples increases, because averaging more measurements reduces random error associated with short sampling events. A method can be reasonably precise even if its absolute accuracy is limited, and precision is especially important for reliably ranking animals in breeding and genetic selection programmes (Hristov et al., 2018; Robinson et al., 2014). Repeatability is an animal-level metric describing how consistently an individual's CH_4 measurements reflect its true underlying emission level across repeated days. High repeatability indicates that most of the variation in repeated measurements is attributable to the animal rather than short-term environmental or measurement noise, which is essential for ranking animals in breeding programmes (Coppa et al., 2021; Robinson et al., 2014). Bias means any consistent error that causes measurements to deviate from the true value. Bias can be caused by instrument errors, calibration issues, or external factors such as uneven sampling or animal behaviour. For example, GF might underestimate emissions if animals do not visit the unit evenly throughout the day (Huhtanen et al., 2015; Manafiazar et al., 2017). Recognising and correcting bias is essential when comparing results or combining data from different measurement systems (Hristov et al., 2018; MPI, 2020; Tedeschi et al., 2022).

Several statistical tests have been proposed to evaluate both agreement and association of two techniques/methods (Bland and Altman, 1986; St-Pierre, 2016) Table 1). Considering RC is

considered the "*gold standard*", RC will hereafter be referred as the observed values, and the GF system will be referred as predicted values.

A commonly used evaluation method is the Bland-Altman analysis, which provide a visual comparison between measurement methods by plotting the difference between them against their average, helping to identify any fixed (mean bias) or proportional bias (bias increasing/decreasing with emission level) and provides limits of agreement for interpreting measurement differences (Bland and Altman, 1986). Bland-Altman analysis provides estimates of mean bias and limits of agreement (and associated confidence intervals where required), but because different statistical approaches quantify different aspects of agreement and differ in sensitivity to error, it is commonly complemented with regression based bias testing and additional performance metrics to provide a more complete evaluation of method performance.

Table 2.1 Main descriptive statistics to evaluate methods for methane (CH_4) emissions from sheep.

Statistic	Abbreviation	Description
Bias at minimum predicted CH_4	-	Estimated prediction bias at the lower end of the CH_4 range, obtained by inserting the minimum predicted CH_4 value into the regression equation: $[b_0 + b_1(P_{MIN} - P_{MEAN})]$, where b_0 = mean bias; b_1 = linear bias; P_{MIN} = minimum predicted CH_4; and P_{MEAN} = mean predicted CH_4. This quantifies whether the model tends to over or under predict CH_4 at low-emissions.
Bias at maximum predicted CH_4	-	Estimated prediction bias at the upper end of the CH_4 range, obtained by inserting the maximum predicted CH_4 value into the regression equation: $[b_0 + b_1(P_{MAX} - P_{MEAN})]$, where b_0 = mean bias; b_1 = linear bias; P_{MAX} = maximum predicted CH_4; and P_{MEAN} = mean predicted CH_4. This shows whether prediction error increases or decreases for high-emissions.
Coefficient of determination	R^2	Proportion of variation of observed CH_4 that can be explained by predicted CH_4.
Linear bias	-	Linear bias is estimated by the slope of the regression of the residuals (observed-predicted) on the mean-centred predicted values. It represents the change in the bias of the prediction (g/d) per unit change in the prediction (i.e., per g/d in predicted CH_4).
Mean absolute error	MAE	$(\sum\|O_i - P_i\|)/n$, where n = number of paired observed (O) and predicted (P) CH_4 values being compared.
Mean bias	-	Estimated as the average systematic difference between observed and predicted CH_4 values (observed–predicted). This mean bias can be computed either as: • the mean of the observed – predicted differences (Bland-Altman approach), or • the intercept from a regression of residuals on mean centred predicted values (regression-based approach). Both approaches quantify the same underlying mean bias.
Intraclass Correlation Coefficient	ICC	Estimated as the proportion of total variance in CH_4 measurements attributable to differences between animals rather than measurement error. Indicates how consistently a method ranks animals across repeated measurements; high ICC values reflect strong repeatability.
Coefficient of variation	CV	Ratio of the standard deviation to the mean of repeated measurements (%). Indicates relative precision.
Mean square prediction error	MSPE	$\sum (O_i - P_i)^2/n$, where n = number of paired observed (O) and predicted (P) CH_4 values being compared (g/d).
Root mean square error	RMSE	Square root of the mean square prediction error of the simple linear regression of observed on predicted CH_4 (g/d).
Concordance correlation coefficient	CCC	Ranges from 0 to 1, where 1 indicates perfect agreement and values closer to 0 indicate poor agreement between methods (Lin, 1989).

An important advancement to the Bland-Altman logic was proposed by St-Pierre, who demonstrated that plotting residuals (observed − predicted) against observed values leads to misleading evidence of linear bias, even when a model or measurement method is unbiased (St-Pierre, 2016). His derivation shows that, under an unbiased system, residuals and observed values are mathematically correlated, with an expected slope equal to $(1 - R^2)$. Therefore, apparent slopes in residual-versus-observed plots are not evidence of bias, they simply reflect model/method geometry (St-Pierre, 2016). Because of this, residuals should be plotted against predicted values, not observed values. When residuals are regressed on predicted CH_4, the expected slope under unbiasedness is zero. This provides a valid statistical test for mean bias (intercept) and linear bias (slope) after mean-centering the predictor to remove intercept-slope intrinsic correlation (St-Pierre, 2016).

Lin's Concordance Correlation Coefficient (CCC) measures precision and accuracy together and penalises results when there is consistent bias or shift in scale (Lin, 1989). Other measures, such as the Coefficient of Variation (CV) and Intraclass Correlation Coefficient (ICC), indicate how consistent CH_4 measurements are within and between animals over time (Difford et al., 2016). These approaches give more meaningful results than simple correlation (R^2), which can sometimes hide systematic errors even when methods have a high R^2 (Bland and Altman, 1986).

Several technical and biological factors can affect the quality of CH_4 data. Problems like instrument drift and poor calibration introduce bias into both GF and RC results (Storm et al., 2012). Animal factors, such as differences in feed intake during measurements with the two methods, social dominance, or stress responses, can also add variability. In GF systems, uneven sampling due to irregular visits may bias results toward specific feeding times (de Mol et al., 2024; McGinn et al., 2021). In GF systems, uneven sampling due to irregular animal visits may bias estimates toward

specific feeding times and reduce representativeness of daily emissions (de Mol et al., 2024; Della Rosa et al., 2025; McGinn et al., 2021). Under grazing conditions, external factors such as weather may indirectly influence data completeness by affecting animal visiting behaviour and the distribution of spot samples across the day; however, visits recorded under unsuitable conditions or failing system validity criteria are typically excluded during quality control (Bennett et al., 2022). Reducing these sources of error is essential. This can be achieved by following strict calibration routines, allowing animals time to adapt before measurement, and filtering data to remove outliers (Beck et al., 2021; de Mol et al., 2024; Pressman et al., 2025).

In summary, applying strong statistical and methodological controls improves the reliability of CH_4 measurement systems. These principles provide a solid foundation for validating new tools like GF against reference methods such as RC, ensuring CH_4 data are accurate, scientifically credible and suitable for use in national greenhouse gas inventories and treatment/animal ranking (Pressman et al., 2025; Robinson et al., 2014).

2.7 Summary of literature gaps and study rationale

Although major progress has been made in measuring enteric CH_4 emissions from livestock, small ruminants especially sheep are still underrepresented in global CH_4 research (Della Rosa et al., 2022). Most measurement systems such as GF and SF_6 were originally developed for cattle and have not been fully adapted to the unique physiology and behaviour of sheep (Zhao et al., 2020a). This gap is particularly important in countries like New Zealand, where sheep make up a significant share of agricultural CH_4 emissions (MPI, 2020). The lack of sheep specific validation of GF reduces confidence in CH_4 results with respect to this method (Beck et al., 2021). In addition, some biological and behavioural factors such as animal stress responses or social interactions may

influence CH_4 production, but their contribution to differences between RC and GF measurements remains unclear and has not been quantified in sheep. Most comparison studies account for feed intake and visit patterns, yet potential behavioural effects require further evaluation (de Mol et al., 2024; Robinson et al., 2014). These factors must be included in validation approaches to make sure differences between measurement methods are due to the tools themselves and not animal responses or sampling errors (de Mol et al., 2024; MPI, 2020).

Addressing these gaps requires new measurement protocols that are standardised, scalable, and designed with sheep biology and behaviour in mind. RC provide continuous 24-h CH_4 measurements and are therefore used as a reference method. In contrast, the GF system estimates daily CH_4 production from multiple short spot samples collected during voluntary visits, which may result in uneven sampling across animals and incomplete coverage of the diurnal emission pattern (MPI, 2020; O'Connor et al., 2024). Developing clear guidelines on sampling frequency, acclimation periods, and data adjustments will make results from different studies easier to compare and use (O'Connor et al., 2024). In New Zealand, the value of this work lies in its practical application, as reliable sheep-specific CH_4 data are essential for supporting effective on-farm mitigation strategies and improving the consistency of emissions reporting (Hickey et al., 2022; MPI, 2020; NZAGRC, 2022; Science Media Centre, 2025). By improving the accuracy and relevance of measurements, this study aims to build a stronger, evidence-based framework for managing CH_4 emissions in small ruminant farming systems. Addressing these gaps through a direct comparison of GF and RC in sheep forms the basis of the present study (see Chapter 3).

Chapter Three Research Methodology

3.1 Introduction

Agriculture contributes roughly one quarter of total anthropogenic greenhouse gas (GHG) emissions worldwide, with ruminant livestock being the dominant agricultural source of methane (CH_4) (Gerber et al., 2013b; IPCC, 2023). As countries intensify their commitments to mitigate climate change, the livestock sector faces increasing pressure to reduce its carbon footprint while maintaining productivity and food security. Reducing CH_4 emissions from human activities is one of the fastest ways to slow near-term warming (CCAC, 2021; Shindell et al., 2012), yet rising global temperatures may also amplify natural CH_4 release through self-reinforcing feedbacks (Dean et al., 2018; Schuur et al., 2015). These dynamics increase the need for accurate CH_4 quantification, central to global climate mitigation strategies.

Reliable CH_4 measurement underpins the development of effective mitigation practices, national inventories, and science-based policy frameworks. It also supports genetic selection, feed innovation, and on-farm management programmes aimed at enhancing efficiency and environmental performance. Achieving these goals requires precise, scalable measurement technologies suitable for both controlled research environments and field conditions.

Respiration chambers have been the primary reference method used in New Zealand to quantify enteric CH_4 emissions in sheep over the last two decades. RC provide continuous, high-precision gas exchange measurements under controlled conditions and are widely regarded as the “gold standard” method (Storm et al., 2012; Zhao et al., 2020a).

GreenFeed systems provide an alternative approach based on short-duration spot sampling of exhaled gases during brief animal visits to the unit (Hammond et al., 2016a; McGinn et al., 2021). While GF has been widely evaluated in cattle, comparatively fewer studies have assessed its performance in small ruminants, particularly in direct comparison with RC measurements. As a result, uncertainty remains regarding agreement, potential bias, and repeatability of GF-based CH_4 estimates in sheep. In the present study, GF measurements were conducted under controlled indoor conditions, allowing method comparison with RC while minimising confounding effects of grazing behaviour and environment.

In 2021, the manufacturer introduced a tunable diode laser (TDL) sensor across all new GF models (units 272 and higher), replacing the NDIR sensor. The TDL sensor offers a noise level below 1 ppm CH_4, high stability, and minimal cross-sensitivity to other hydrocarbons. These advances have improved detection limits to around 0.25 g CH_4 day^{-1} for cattle-type units and 0.05 g CH_4 day^{-1} for sheep units (C-Lock-Inc., 2021).

Therefore, the objective of the present study was to compare CH_4 emission estimates obtained from paired GF and RC measurements in sheep under controlled conditions, and to quantify agreement, bias, and repeatability when using a cattle-designed GF unit in sheep.

3.2 Materials and methods

The study was conducted at Massey University in Palmerston North, New Zealand (40°22’39.14”S, 175°36’26.17”E) in Autumn (30th April - 20th May, 2025). All procedures involved in this experiment were approved by the Animal Ethics Committee of Massey University (Authority no.: AEC 25-15) in accordance with the Animal Welfare Act (1999) and Massey University Code of Ethical Conduct for the use of Animals for Research, Testing and Teaching.

3.2.1 Animals, diets and experimental design

Twelve Romney female lambs [mean live weight (LW) 40.7 ± 3.50 kg; <1 year old] were sourced from Massey University's commercial research flock (Palmerston North, New Zealand). Prior to the experimental period, lambs were identified using electronic ear tags, vaccinated (Ultravac 7-in-1, Zoetis, Australia) and treated with a pour-on anthelmintic (Cydectin 0.5%, Zoetis, Australia). Lambs were weighed using a portable electronic scale (Prattley, Temuka, New Zealand), blocked by LW, and then randomly assigned to two treatments: either perennial ryegrass (*Lolium perenne* L) and white clover (*Trifolium repens* L) or a diverse pasture mix including perennial ryegrass, white clover, chicory (*Cichorium intybus* L) and plantain (*Plantago lanceolata* L) (see Table 3.1 for chemical composition). These diets were used to introduce variation in CH_4 production for method comparison.

Table 3.1 Composition of the feed (n = 4) and pellets used during the trial (average ± standard deviation)

Chemical composition *(DM-basis)*[1]	Standard pastures	Diverse pasture	Bait[2]
Dry matter (DM, *% as-fed*)	13.5 ± 0.200	9.72 ± 0.058	89.0
Crude protein, % DM	19.6 ± 0.346	26.7 ± 0.404	11.3
Fat, % DM	3.27 ± 0.802	4.00 ± 0.666	3.00
Neutral detergent fibre, % DM	45.5 ± 1.75	34.8 ± 1.22	24.3
Acid detergent fibre, % DM	27.0 ± 1.44	22.1 ± 1.61	9.90
Lignin, % DM	3.27 ± 0.473	2.90 ± 0.608	2.60
Gross energy, MJ/kg DM	16.2 ± 0.354	15.4 ± 0.551	17.0

[1]% DM (Dry matter) unless otherwise stated. [2]Commercial pellet – MealTime, manufactured by Denver Stock Feeds, Palmerston North, New Zealand (barley 39.0%, broll 35.1%, soybean meal 21.8%, molasses 3.0%, limestone 1.0%, and sheep premix 0.10%, as per manufacturer label) [3]g/100g DM unless otherwise stated.

The study was conducted over 21 days starting with 7 days of grazing to acclimatise sheep to the respective treatment cultivar, followed by 7 days of acclimatisation to indoor housing and feeding cut grass of the same treatment, one day of acclimatisation to housing in individual crates, three measurement days in RC and then three days with GF measurements. During acclimatisation and GF measurements, the lambs were housed individually in pens (approximately 2.0 × 1.5 m; concrete flooring with rubber matting). Lambs had *ad libitum* access to freshly cut pasture (harvested daily at ~7 cm height) and water. Pasture was offered twice daily, with 50% of the daily allowance provided at 09:00 h and the remainder at 16:00 h. The rate of pasture refusals was recorded. A fixed amount (10 g/d) of commercial pellets (Table 3.1) was provided to standardise the diet during GF unit and RC measurements. The overall environmental schedule and sequence of measurements are summarised in Figure 3.1. Prior to the GF measurement period, lambs were introduced to the GF unit and offered bait pellets to build familiarity with the equipment and ensure ready consumption of bait during scheduled sampling visits.

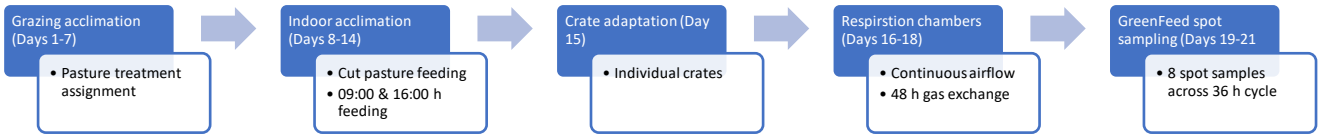

Figure 3.1 Experimental schedule and measurement sequence for validating a cattle-designed GreenFeed (GF) unit against respiration chamber (RC) measurements in sheep.

3.2.2 Measurements of methane production

Methane emissions were measured using two methods: open circuit RC and GF units (604 and 605). GreenFeed measurements were carried out at the Massey University Facility under controlled

indoor conditions while RC measurement was carried out at the Ruminant Methane Measurement Centre, Bioeconomy Science Institute, Grasslands Research Centre, Palmerston North, New Zealand (40°22'44.36"S, 175°36'38.66"E).

3.2.2.1 Gas measurement using respiration chamber

Each lamb underwent two consecutive 24-hour measurements in open-circuit RC. This was extended with an additional day because of technical issues on the previous day. Chambers were closed following the afternoon feeding (16:00 h), and lambs had continuous access to water and pre-weighed pasture supplied through feed troughs located inside the chambers. Chamber airflow was maintained at a constant rate and monitored continuously (Pinares-Patiño et al., 2011). Methane concentration in the air stream flowing in and out of the chamber was measured using a Servomex gas analyser (Servomex Group Ltd., Crowborough, East Sussex, United Kingdom). The CH_4 recovery performed by NIWA (Wellington) prior to chamber measurements was 96.2 % ± 2.57. Daily CH_4 production (g/day) was calculated as the product of airflow rate and the difference in CH_4 concentration between inlet and outlet air streams, corrected for temperature, humidity and pressure. Measured emissions were adjusted for the recovery of each individual chamber used to ensure consistency across animals.

After completing RC measurements, each lamb underwent three consecutive days of CH_4 measurement using two GF units (C-Lock Inc., USA) located within the Massey facility, following a sampling protocol similar to that described by (Hristov et al., 2015) for cattle in a tie-stall facility.

3.2.2.2 Gas measurement using GreenFeed

Unlike the standard voluntary visit-based GF protocol, in which animals approach the unit freely, sampling in this study followed a handler-led approach whereby each lamb was brought to the GF

unit individually at scheduled times. This design ensured controlled temporal coverage of the 24-hr feeding cycle and allowed direct comparison with RC measurements under consistent conditions.

Methane measurements were obtained for each lamb during week 3 using a large ruminant GF unit (C-Lock Inc., Rapid City, SD) equipped with a tunable diode laser (TDL) gas sensor. The sampling procedures followed those recommended by (Hristov et al., 2015). Briefly, CH_4 emissions data of 8 samples were collected over 3 days as follows: starting at 18:00, 00:00, and 06:00 h (d1), 12:00, 21:00 and 02:00 h (d2), and 09:00 and 14:00 h (d3) to obtain a representative sample of a 24-h feeding cycle. Breath sample gas for individual lambs was collected for 5 min, followed by a background air sample collection for 2 min. Briefly, the unit was placed in front of each lamb for 5 min at a time to get 8 spot samples of 5 min at 3-h intervals across 3 d ensuring all lambs were sampled. During each sampling, one bait drop (7.43 ± 0.439 grams per drop) was dispensed every 20 seconds, resulting in a total of twelve bait drops per sampling (89.2 g/sampling). We delivered 12 drops at all visits to be consistent with the pellet provided to the animals during the chamber measurements. When the lamb's head was positioned within the feed trough, the system continuously recorded airflow and CH_4 concentration at the inlet and outlet of the extraction system, allowing estimation of CH_4 emission per visit. All visits meeting the manufacturer's criteria for valid sampling, including minimum visit duration, stable airflow, and sensor stability, were retained for analysis, and no samples were excluded. The GF units were calibrated following the manufacturer's recommendations.

The order of measurement (RC followed by GF) was consistent for all animals, and no dietary or environmental changes occurred between measurement methods. During the RC measurements, lambs received 90 g/d of the same commercial pellets used as bait in the GF system (divided

between morning and afternoon feeding) to ensure dietary consistency and facilitate direct comparison between methods. This design ensured that observed differences in CH_4 estimates reflected measurement system characteristics rather than differences in diet, intake, behaviour, or environment. However, measurements were not simultaneous, therefore one needs to acknowledge that CH_4 emissions might not have been exactly the same during measurements with RC and GF.

3.2.3 Sampling and chemical analysis of feeds

Pasture offered to the lambs during the indoor measurement period consisted of freshly harvested perennial ryegrass (*Lolium perenne* L) and white clover (*Trifolium repens* L), or a diverse pasture mix (Table 3.1), cut daily from paddocks at approximately 7 cm sward height. The two different pastures were used to generate variation in CH_4 emissions and allow a better evaluation of the measurement method. Fresh pasture was offered twice daily at 09:00 and 16:00 h, and refusals were collected and weighed prior to each feeding. A small, fixed allocation of commercial pellets was also provided during both respiration chamber and GF measurement period to support consistent intake across animals and to ensure that lambs engaged with the GF unit readily. All animals had unrestricted access to clean drinking water.

For feed analysis, a representative sample of the offered pasture was collected daily. Each daily sample was freeze-dried to constant weight to determine dry matter (DM) concentration. Dried samples were then pooled within week to create a composite sample for each cohort. Composite samples of both pasture types and commercial pellets were submitted to the Massey University Nutrition Laboratory for chemical analysis. Total nitrogen (N) content was determined using the Dumas combustion method (AOAC Method 968.06; AOAC, 2016), and crude protein (CP) concentration was calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre

(ADF) were analysed using the Van Soest detergent system (AOAC Method 973.18; AOAC, 2016) and both are expressed including residual ash. Ether extract (EE) was analysed using Soxhlet extraction with hexane as the solvent (AOAC Method 920.39). Gross energy (GE) content was determined by adiabatic bomb calorimetry using a calibrated bomb calorimeter, standardised with benzoic acid.

3.2.4 Evaluation of methodology adequacy

This experimental design enabled within animal comparison of continuous CH_4 measurements obtained using RC and intermittent visit-based measurements obtained using the GF system under similar feeding and housing conditions. Agreement between CH_4 measurements obtained using the RC (reference method) and the new generation large ruminant GF (alternative method) was assessed using the statistical framework described by St-Pierre (2003). In this approach, GF values were treated as the predicted measurements ($\hat{y}_i$), while respiration chamber values were considered the observed reference (y_i). Residuals were calculated as the difference between observed and predicted values:

Equation 3.1 Residual calculation

$$e_i = y_i - \hat{y}_i$$

Where e_i is the residual for animal i, y_i is the RC measurement, and $\hat{y}_i$ is the corresponding GF estimate.

To evaluate bias, residuals were regressed against mean-centered predicted values from the GF system. Mean-centering was performed as:

Equation 3.2 Mean-centering of predicted values

$$x_{c,i} = \hat{y}_i - \overline{\hat{y}}$$

Where $x_{c,i}$ is the centered GF value and $\overline{\hat{y}}$ is the mean GF estimate across animals.

The bias model was:

Equation 3.3 Bias regression model

$$e_i = \beta_0 + \beta_1 x_{c,i} + \varepsilon_i$$

Within this regression, the intercept (β_0) represents the mean (systematic) bias, while the slope (β_1) represents proportional (linear) bias. A significant slope ($P \leq 0.05$) indicates that the magnitude of bias changes with increasing CH_4 production. The relationship between GF and RC measurements was further evaluated by regressing observed CH_4 values (RC) against predicted values (GF) and calculating the coefficient of determination (R^2) as an indicator of the strength of association between the two methods.

3.2.5 Statistical analysis

Daily CH_4 emission values were calculated for each lamb and each measurement method. For RC, daily CH_4 production (g/d) was obtained directly from the continuous 24-h measurements, and values were averaged across the two measurement days for each animal. For the GF, CH_4 emissions were estimated from multiple short duration visits and averaged across the three consecutive measurement days to derive a single mean daily CH_4 value per animal. Data were analysed using linear mixed models in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Measurement method (RC or GF) was fitted as a fixed effect, and animal was included as a random effect to account for repeated measurements on the same individual. Where appropriate, measurement batch was included as a fixed effect to account for staggered entry of animals into the RC and GF measurement periods. Least squares means were compared between methods, and statistical significance was declared at $P \leq 0.05$.

Agreement between methods was further evaluated using regression-based approaches and complementary performance metrics, as described in Section 3.2.4 (*Evaluation of methodology adequacy*). All statistical analyses were conducted assuming residuals were normally distributed, and model diagnostics were examined to confirm compliance with model assumptions.

3.3 Results

3.3.1 Animal traits and gas production

All lambs consumed a large proportion of the offered pasture throughout the measurement period (Table 3.2); however, one lamb (ID 7306) did not consume the GF bait pellets during the adaptation period, chamber days, or GF exposure instances, therefore did not generate valid GF visit data for CH_4 estimation. Animal inclusion for the paired RC-GF dataset is summarised in Figure 3.2.

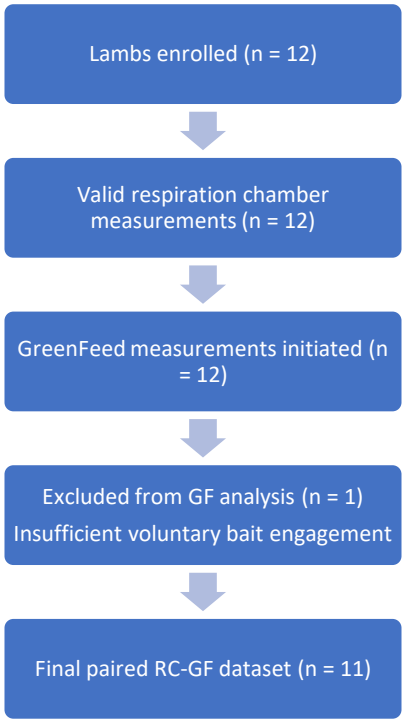

Figure 3.2 Flow diagram of animal inclusion for paired respiration chamber (RC) and GreenFeed (GF) CH_4 measurements.

Summary statistics for dry matter intake, live weight, and daily gas production measured using RC and estimated using GF are presented in Table 3.2. Mean live weight of lambs was similar between measurement methods, averaging 37.34 ± 3.63 kg during RC measurements and 37.9 ± 3.76 kg during GF measurements. Dry matter intake was slightly higher during the RC period (1.41 ± 0.21 kg/d) compared with the GF measurement period (1.28 ± 0.16 kg/d), with comparable ranges observed across animals. During the GF measurement period, mean visit duration was 177 ± 51.7 s (n = 11), with visit durations ranging from 122 to 321 s.

Mean daily CH_4 production measured using RC was 18.39 ± 4.22 g/d, with values ranging from 12.73 to 25.02 g/d (Table 3.1). Corresponding GF-derived CH_4 estimates averaged 17.86 ± 4.95 g/d and ranged from 12.44 to 27.90 g/d, indicating greater dispersion across animals. Carbon dioxide production measured in RC averaged 961 ± 75.0 kg/d, with a relatively narrower range (i.e. smaller standard deviation and difference between minimum and maximum values) across animals compared to GF-derived CO_2 estimates averaged 785 ± 398 kg/d (Table 3.1). These descriptive statistics provide the basis for subsequent analyses evaluating agreement, bias, and precision between GF and RC measurements.

Table 3.2 Summary statistics of variables derived from respiration chamber and GreenFeed (GF) measurements.

Item	*n*	Mean	SD	Minimum	Maximum
Respiration chamber					
Dry matter intake, kg/d	12	1.41	0.21	1.18	1.88
Live weight, kg	12	37.40	3.63	31.5	42.7
CH_4 production, g/d	12	18.40	4.22	12.7	25.0
CO_2 production, g/d	12	962	75.0	846	1064
GF estimation					
Dry matter intake, kg/d	12	1.28	0.16	1.05	1.71

GF visit duration, s[1]	11	177	51.7	122	321
Live weight, kg	12	37.9	3.76	31.50	43.0
CH_4 production, g/d	11	17.9	4.95	12.4	27.9
CO_2 production, g/d	11	785	399	187	1391

[1]Measurements commenced once the proximity sensor threshold of 400 was reached and continued until the animal voluntarily left the unit some animal remained in the Greenfeed (GF) unit waiting for more bait drops after the end of the 12 drops.

3.3.2 Agreement between GreenFeed and respiration chambers methane measurements

Linear regression of RC-measured CH_4 production against GF-estimated CH_4 production showed a significant positive relationship ($P < 0.01$; $R^2 = 0.65$; Figure 3.3A), indicating moderate agreement between methods across the observed range of emissions. Ewe lambs consuming different pasture types were included to generate variation in CH_4 production for method comparison. Diet significantly influenced the residuals ($P = 0.02$); however, bias was not estimated separately by diet, as this was not an initial objective of the study and the experiment was not statistically powered to support diet-specific bias estimation due to limited degrees of freedom.

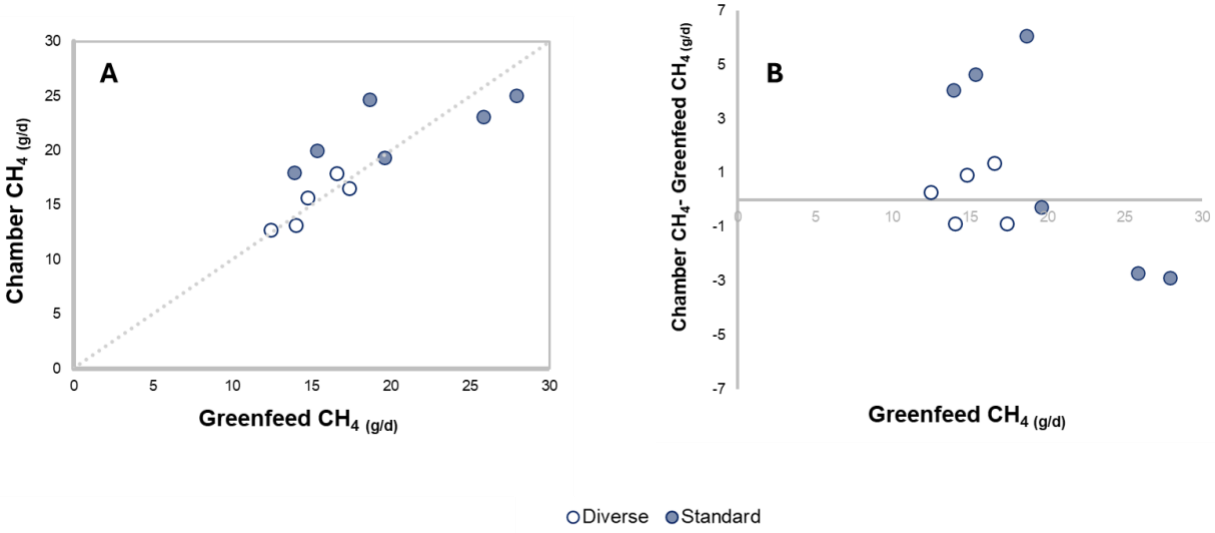

Figure 3.3 Agreement between methane (CH_4) emission estimates obtained using respiration chambers (RC) and cattle GreenFeed (GF) units in sheep.

A - Linear regression of RC-measured CH_4 production (g/d) against GF-estimated CH_4 production (g/d) (RC CH_4 = 6.43 ± 3.11 + 0.689 ± 0.168 × GF CH_4; $P < 0.01$; $R^2 = 0.65$). The dashed line represents the line of identity (y = x). **B -** Regression of residual CH_4 production (RC - GF; g/d) against mean-centered GF CH_4 values (Residual CH_4 = 0.695 ± 0.580 -0.551 ± 0.146 × centered GF CH_4; $P < 0.01$; RMSE = 1.91 g/d). Symbols represent individual animals, with open circles indicating diverse pasture and filled circles indicating standard pasture diets.

Regression of residual CH_4 production (RC - GF; g/d) against mean-centered GF CH_4 values, used to assess bias (Figure 3.3B). Mean bias, represented by the intercept of the regression, was not significant (0.695 ± 0.580 g/d; $P = 0.27$; Figure 3.3B). In contrast, a significant proportional (systematic) bias was detected, as evidenced by a negative slope (-0.551 ± 0.146; $P < 0.01$), indicating that the magnitude of the difference between methods increased with increasing CH_4 production.

Linear regression of RC-measured CO_2 production against GF-estimated CO_2 production showed a weak and non-significant relationship ($P = 0.09$; $R^2 = 0.28$; Figure 3.4A), indicating poor agreement between methods across the observed range of CO_2 emissions. Unlike CH_4, diet did not significantly influence residual CO_2 values ($P = 0.97$).

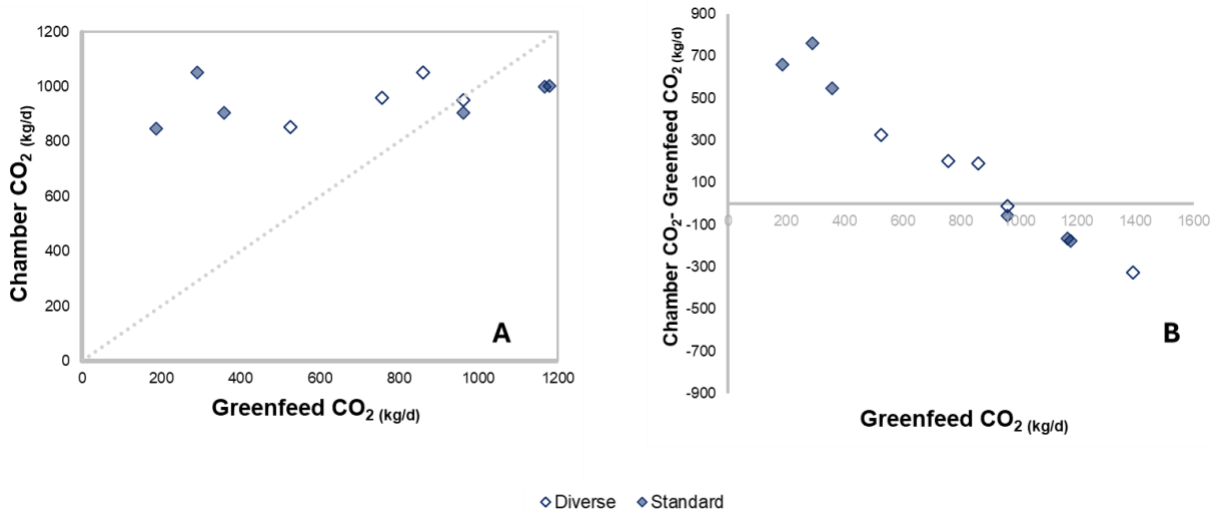

Figure 3.4 Agreement between carbon dioxide (CO_2) production estimates obtained using respiration chambers (RC) and cattle GreenFeed (GF) units in sheep.

A - Linear regression of RC-measured CO_2 production (kg/d) against GF-estimated CO_2 production (kg/d) (RC CO_2 = 882 ± 48.5 + 0.104 ± 0.0556 × GF CO_2; P = 0.09; R^2 = 0.28). The dashed line represents the line of identity (y = x). **B -** Regression of residual CO_2 production (RC - GF; kg/d) against mean-centered GF CO_2 values, (Residual CO_2 = 178 ± 22.5 - 0.89 ± 0.0613 × centered GF CO_2; P < 0.01; RMSE = 74.3 kg/d). Symbols represent individual animals, with open diamonds indicating diverse pasture and filled diamonds indicating standard pasture diets.

Regression of residual CO_2 production (RC - GF; kg/d) against mean-centered GF CO_2 values was used to assess bias between measurement methods (Figure 3.4B). A significant mean bias was detected, with a positive intercept (178.0 ± 22.5 kg/d; P < 0.001), indicating that, on average, respiration chambers measured higher CO_2 production than the GF system. In addition, a strong and significant systematic bias was observed, as indicated by the negative slope (-0.897 ± 0.061 kg/d per kg/d GF CO_2; P < 0.001), demonstrating that the discrepancy between methods increased markedly with increasing CO_2 production. The root mean square error (RMSE) of the model was

74.3 kg/d, reflecting substantial variability associated with intermittent, visit-based sampling of CO_2 by the GF system.

Linear regression of RC-measured CH_4 yield against GF-estimated CH_4 yield showed a significant positive relationship ($P = 0.04$; $R^2 = 0.38$; Figure 3.5A), indicating moderate agreement between methods across the observed range of yields. Diet effects on residuals were marginal ($P = 0.07$), and bias was not estimated separately by diet as previously explained.

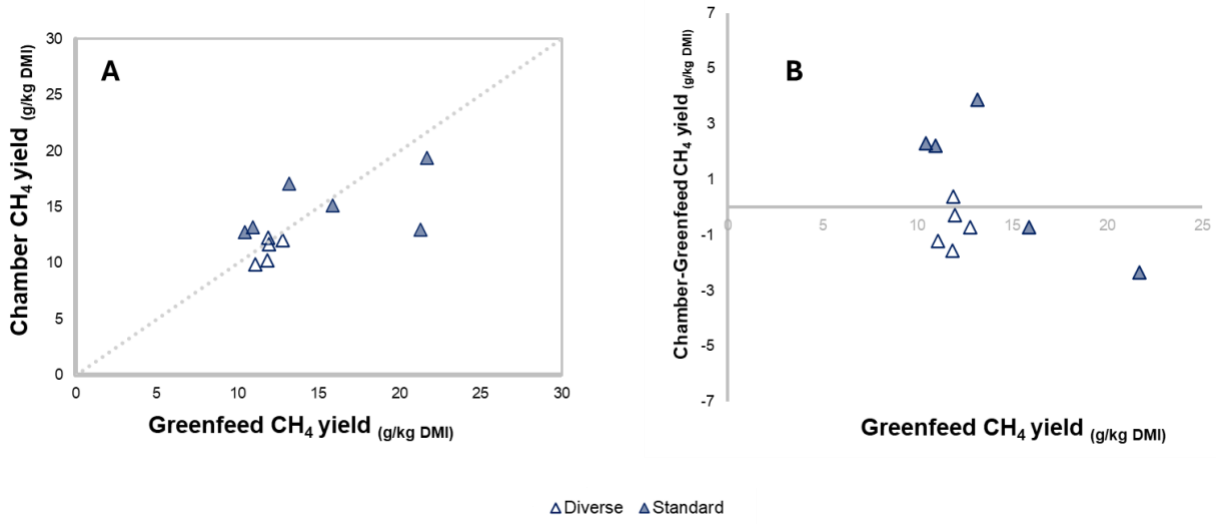

Figure 3.5 Agreement between methane (CH_4) yield (g/kg DMI – dry matter intake) estimates obtained using respiration chambers (RC) and cattle GreenFeed (GF) units in sheep.

A - Linear regression of RC-measured CH_4 yield (g/d) against GF-estimated CH_4 yield (g/d) (RC CH_4 yield = 7.27 ± 2.67 + 0.436 ± 0.185 × GF CH_4 yield; P = 0.04; $R^2 = 0.38$). The dashed line represents the line of identity (y = x). **B -** Regression of residual CH_4 yield (RC - GF; kg/d) against mean-centered GF CH_4 yield values, (Residual CH_4 yield = -0.574 ± 0.711 -0.564 ± 0.185 × centered GF CH_4 yield; P = 0.01; RMSE = 2.36 g/d). Symbols represent individual animals, with open triangles indicating diverse pasture and filled triangles indicating standard pasture diets.

Regression of residual CH_4 yield (RC − GF; g/kg DMI) against mean-centered GF CH_4 yield values was used to assess bias between measurement methods (Figure 3.5B). Mean bias, represented by the intercept of the regression, was not significant (-0.706 ± 0.608 g/kg DMI; P = 0.28), indicating no systematic over or underestimation of CH_4 yield by the GF system relative to respiration chambers across animals. However, a significant systematic bias was detected, as indicated by a negative slope (-0.743 ± 0.179; $P = 0.003$), with CH_4 yield by GF becoming overestimated at high CH_4 yield.

3.4 Discussion

This study evaluated agreement between a cattle-designed GF unit equipped with TDL sensors and RC for measuring CH_4 emissions in sheep under controlled indoor conditions. GreenFeed demonstrated moderate agreement with RC for CH_4 production ($R^2 = 0.65$), capturing a substantial proportion of between-animal variation in emissions. No significant mean bias was detected for CH_4 production or yield, indicating that GF did not consistently over- or underestimate emissions at the population level; however, significant proportional bias was observed across both CH_4 metrics, and agreement for CO_2 production was poor. Diet composition influenced residual CH_4 behaviour, with greater variability observed under higher-fibre standard pasture conditions. These findings are discussed in relation to the behavioural, biological, and methodological factors that shaped GF performance in sheep.

3.4.1 Behavioural and sampling-related sources of variability in GreenFeed measurements

The preference of the ewe lamb 7306 to avoid consuming the pellets highlights a key limitation of spontaneous GF CH_4 measurement in ruminants. Valid CH_4 measurement using GF system

depends on the animal's voluntary visits of sufficient duration, typically at least 2 minutes, which are incentivised by the consumption of bait pellets (C-Lock-Inc., 2021). In order to obtain reliable estimates, sheep must not only visit the unit consistently but also correctly position their body in front of the machine, and place their head in the sampling hood within a defined proximity threshold relative to the sampling point (sheep at a proximity of 400 to 2500 in the web interface; (C-Lock-Inc., 2021).

Refusal to eat pellets directly compromises data acquisition, which can lead to missing or unusable CH_4 measurements even when basal pasture intake remains normal (Jonker et al., 2020; Tadesse et al., 2024). This observation supports previous findings that effective sample size substantially reduced in GF based studies and that bias may be introduced when non-participation is non-random and is associated with inherent differences in feeding/visiting behaviour (de Mol et al., 2024; Tadesse et al., 2024). Reporting of key GF engagement metrics is inconsistent: in a review of published studies, fewer than half reported actual voluntary visit data and only ~30–40% reported visit duration or visits/day, limiting reproducibility and interpretation of derived emission estimates (Della Rosa et al., 2021). This issue is particularly relevant in sheep, where valid-measurement success can be low; for example, only 28.9% of pre-selected grazing ewes achieved consistent ≥2-min visits suitable for valid CH_4 mass flow calculations (Weerasinghe et al., 2025). In this study, a high success rate of 91.6% was achieved, with ewe lamb 7306 being excluded due to insufficient valid visits. It is worth mentioning that all sheep used in this trial were hand reared, which likely contributed to the high level of engagement with the GF unit. Under commercial grazing conditions, substantially lower success rates would be expected, as shown by (Weerasinghe et al., 2025). We therefore recommend that future GF based CH_4 studies should report key system settings, including proximity sensor thresholds, which are adjustable within the GF user interface.

In the New Zealand grazing context, where animals are generally more vigilant and tend to remove the head from the hood to check for predators, we found it necessary to reduce the proximity setting to 400 (lower end of the manufacturer recommendation) in order to obtain valid CH_4 estimates comparable with RC measurements.

Live weight was comparable between RC and GF measurement periods, indicating that differences in gas emission estimates were unlikely to be driven by variation in animal size. Controlling for live weight is important in CH_4 studies, as it is closely linked to maintenance energy requirements and feed intake, both of which influence daily CH_4 production in ruminants (Hegarty, 2013; Knapp et al., 2014b). Based on descriptive statistics, dry matter intake was marginally higher during RC measurements than during GF measurements, though this difference was not formally tested. This difference in intake highlights a fundamental challenge inherent to method-comparison studies of enteric CH_4 emissions. Respiration chamber and GF measurements cannot be conducted simultaneously under identical conditions, as the air mixing environment in the chambers are very different than outside the chamber where the GF has to operate. Furthermore, chambers require confinement and controlled feeding, whereas the GF system operates under behaviour-influenced, visit-based sampling. As a result, small differences in DMI between measurement periods are difficult to avoid, even when the same animals and diets are used. Such discrepancies have been widely acknowledged in previous method-comparison studies and reflect differences in housing, feeding environment, animal behaviour and random day to day variation rather than systematic bias between measurement techniques (Knapp et al., 2014b; Tedeschi et al., 2022). Intake is a primary determinant of daily CH_4 production, with higher feed intake generally associated with greater absolute CH_4 emissions due to increased substrate availability for rumen fermentation (Hammond et al., 2016b; Hegarty, 2013). Despite these modest intake differences, similarity in

animal size and broadly comparable intake ranges provide a consistent biological context for subsequent comparison of CH_4 estimates obtained using RC and the GF system. This consistency in animal size and feeding level supports the interpretation that subsequent differences in CH_4 estimates between methods reflect measurement system characteristics rather than confounding biological factors.

A key observation from the descriptive analysis was that similar mean CH_4 estimates were accompanied by greater between-animal variability in GF-derived values. This pattern is a common feature of method-comparison studies and does not necessarily indicate systematic bias or poor performance of the alternative measurement approach. Rather, the greater between-animal variability in GF-derived estimates reflects fundamental differences in how CH_4 emissions are sampled by the two systems. Respiration chambers provide continuous, integrated measurements of total gas exchange over a full 24-hour cycle, capturing both post-feeding emission peaks and periods of lower CH_4 production, as well as CH_4 released via both eructation and the lower gastrointestinal tract, including flatus (Murray et al., 1976). In contrast, the GF system primarily measures eructated CH_4 during short-duration breath sampling events. In the present study, sheep did not have continuous access to the GF unit but were led to the system at scheduled intervals, resulting in repeated, semi-forced visits. As a result, daily GF-derived estimates are inherently sensitive to visit timing, visit frequency, and animal behaviour during these sampling periods. Variability in the extent to which scheduled visits coincide with post-feeding CH_4 peaks is therefore likely to increase dispersion in GF-derived estimates, even when mean values align closely with those obtained from continuous measurement systems (Hammond et al., 2016b; Huhtanen et al., 2019). Similar patterns have been reported previously in cattle, where GF-derived CH_4 estimates showed comparable means but greater variability relative to RC measurements, particularly when

visit distributions were uneven across the day (de Mol et al., 2024; McGinn et al., 2021). Although fewer validation studies exist for sheep, the same sampling principles apply, and increased variability in GF-derived CH_4 estimates is consistent with the visit-based nature of the system. Importantly, increased variability does not preclude the use of GF data for method comparison, mitigation studies, or animal ranking, provided that the number of animals per treatment are increased to maintain statistical power, sampling protocols are appropriately designed and sufficient valid visits are obtained. Recognising the distinction between central tendency and dispersion is therefore essential for interpreting GF-derived CH_4 estimates and for contextualising subsequent analyses of agreement, bias, and precision between measurement methods.

The recorded GF visit durations provide additional context for interpreting variability in GF-derived gas emission estimates. Mean visit duration exceeded the minimum threshold required for valid sampling, indicating that most retained visits were of sufficient length to support CH_4 estimation. However, the observed range in visit duration reflects inherent between-animal differences in feeding behaviour and engagement with the unit. Shorter visits reduce the time available for airflow and gas concentration stabilisation, increasing sensitivity to transient fluctuations in respiration, eructation and head position. Consequently, variability in visit duration can contribute to increased dispersion in CH_4 estimates derived from GF measurements, even when mean emission rates are comparable with RC values. Similar effects have been reported in cattle studies, where shorter or more variable visit durations were associated with greater between-animal variability in GF-derived estimates (Hammond et al., 2016a; Huhtanen et al., 2015). In the present study, inclusion of only visits meeting manufacturer validity criteria mitigated this effect, but residual variability associated with visit dynamics remains an inherent feature of intermittent, behaviour-dependent measurement systems.

Carbon dioxide estimates provided a useful diagnostic contrast between RC and GF measurement systems. Respiration chambers produced tightly clustered CO_2 values, consistent with continuous, controlled measurement of whole-animal gas exchange under stable airflow conditions. In contrast, GF-derived CO_2 estimates showed substantially greater between animal variability together with strong mean and proportional bias relative to chamber measurements (Figure 3.4A-B). This pronounced disagreement reflects not only the intermittent nature of visit-based sampling but also the physiological characteristics of CO_2 production, which is closely linked to rapid fluctuations in respiration rate and animal activity. Unlike CH_4, which arises primarily from rumen fermentation and displays predictable post-feeding emission patterns, CO_2 output varies on short time scales that are poorly captured by brief breath sampling events, thereby amplifying both variability and systematic bias in GF-derived estimates. Carbon dioxide production is driven by body metabolism and respiration rate, while measurement accuracy is influenced by airflow dynamics and the efficiency with which exhaled air is captured by the sampling system. As such, CO_2 is particularly sensitive to short-term fluctuations in animal posture, head position, visit duration, and breath capture efficiency during sampling events, because CO_2 output responds rapidly to changes in respiration rate and physical activity, whereas CH_4 emissions arise primarily from rumen fermentation and vary more slowly over time (Hammond et al., 2016a; Huhtanen et al., 2015).

The substantially greater dispersion in GF-derived CO_2 estimates likely reflects a combination of intermittent, visit-based sampling and limitations in accurately resolving low CO_2 fluxes in sheep using a system designed for larger ruminants. Given the lower absolute CO_2 emissions of sheep relative to cattle, some individual spot samples may have approached background concentrations, increasing sensitivity to breath capture efficiency and dilution effects. Consequently, while RC provide robust integration of whole animal CO_2 exchange, the present results indicate that the GF

system is not well suited for precise quantification of CO_2 production in sheep, and CO_2 estimates derived from GF should be interpreted with caution. The contrasting behaviour of CO_2 estimates between methods highlights the greater sensitivity of CO_2 to intermittent sampling, while the comparatively smaller increase in variability observed for GF-derived CH_4 indicates that CH_4 emissions are more robust to visit-based measurement structure. Respiration chambers integrate gas exchange continuously over time and effectively average out short-term fluctuations in respiration and activity, whereas GF systems rely on discrete sampling windows that are more sensitive to capture efficiency and background dilution, particularly when CO_2 concentrations approach background levels. As a result, variability in GF-derived CO_2 estimates is likely driven by measurement sensitivity rather than true fluctuations in respiratory activity (Tedeschi et al., 2022). Importantly, the diagnostic value of CO_2 variability lies in its ability to highlight sensitivity of the GF system to visit dynamics and airflow capture, with direct implications for protocol design and data filtering. Ensuring adequate visit duration, and sufficient temporal coverage across the day is therefore critical for minimising variability and improving interpretability of GF-derived gas emission estimates. These considerations are particularly relevant for small ruminants, where shorter feeding bouts and more vigilant behaviour may amplify sampling heterogeneity relative to cattle-based applications.

3.4.2 Biological and methodological factors influencing GreenFeed performance in sheep

The present study evaluated the performance of a cattle-designed GF system equipped with new-generation TDL sensors for measuring enteric CH_4 emissions in sheep under controlled indoor conditions, using RC as the reference method. GreenFeed-derived CH_4 production and yield exhibited significant positive relationships with RC measurements, indicating that the system

captured a substantial proportion of between-animal variation in emissions (Hammond et al., 2016a; Huhtanen et al., 2015). The absence of significant mean bias indicates that GF did not consistently over or underestimate CH_4 emissions relative to RC, as similarly reported in earlier method-comparison studies (McGinn et al., 2021). However, the evidence of proportional bias across CH_4 metrics, together with poor agreement for CO_2, highlights fundamental limitations associated with intermittent, visit-based gas sampling in sheep (Tedeschi et al., 2022). These findings demonstrate that while GF shows strong potential as a comparative tool for CH_4 phenotyping in sheep, methodological and biological factors currently constrain its reliability for absolute emission quantification (de Mol et al., 2024).

The moderate agreement observed between GF and RC CH_4 production estimates (R^2 = 0.65) indicates that GF was generally effective at ranking animals according to daily CH_4 output, consistent with previous GF validation studies demonstrating stable between-animal ranking despite inherent variability (Hammond et al., 2016a; Huhtanen et al., 2015). This level of association is comparable to that reported in cattle-based validation studies using earlier GF sensor generations, where coefficients of determination typically range between 0.60 and 0.85 (Huhtanen et al., 2015; McGinn et al., 2021). Although direct validation studies in sheep remain limited, the present results suggest that the improved sensitivity of TDL-equipped GF units can reasonably track inter-animal differences in CH_4 production in small ruminants under controlled conditions (C-Lock-Inc., 2021). From a practical perspective, this supports the potential use of GF systems for large-scale screening applications, such as genetic selection for low-emitting animals or comparative evaluation of mitigation strategies, where relative differences among animals are of greater importance than precise absolute emission values (de Haas et al., 2021; Pickering et al., 2015).

Despite this encouraging performance at the population level, the agreement between methods was moderate rather than strong, potentially influenced by both differences in measurement approaches and normal day-to-day biological variation arising from non-simultaneous measurements (Storm et al., 2012; Zhao et al., 2020b). Respiration chambers integrate gas exchange continuously over the full diurnal cycle, while the GF system sampled emissions across the day through brief visits that together accounted for only a limited proportion of total daily measurement time (Muetzel and Clark, 2015; Storm et al., 2012). In contrast, GF estimates are derived from short-duration spot samples obtained during scheduled animal visits (Hristov et al., 2015). Although the sampling schedule employed in this study was designed to cover the 24-hour feeding cycle, intermittent measurements remain inherently sensitive to the timing of visits relative to fermentation dynamics (Huhtanen et al., 2019). This methodological contrast likely contributed to the substantial variability observed in GF estimates and underpins the systematic bias detected across CH_4 production values (Tedeschi et al., 2022).

The regression of residual CH_4 production against mean-centered GF values revealed no significant mean bias but a significant proportional bias, indicating that the direction and extent of disagreement between methods depended on the level of CH_4 production. In practical terms, this means that the GF system tended to overestimate CH_4 emissions at some emission levels and underestimate them at others, rather than exhibiting a consistent offset across animals. Such proportional bias cannot be corrected through simple calibration adjustments, as it reflects a fundamental interaction between the sampling methodology and the biological processes governing CH_4 production (Tedeschi et al., 2022). Similar patterns of proportional bias have been reported in cattle GF validation studies, where intermittent sampling was shown to underestimate high-emitting animals and overestimate low emitters under certain feeding regimes (Hammond et

al., 2016a; Huhtanen et al., 2015). The present findings extend this observation to sheep and highlight that systematic distortion is likely intrinsic to visit-based breath sampling when fermentation dynamics vary substantially over time (Storm et al., 2012).

Methane yield exhibited weaker agreement between methods ($R^2 = 0.38$) compared with absolute CH_4 production, despite similarly lacking significant mean bias. This reduction in agreement is biologically and statistically expected, as CH_4 yield compounds uncertainty associated with both CH_4 emission estimates and DMI measurements (Hegarty, 2013; Knapp et al., 2014a). Any error or variability in either component propagates into yield calculations, amplifying disagreement between methods. This phenomenon has been observed in previous ruminant CH_4 studies, where relationships for yield are consistently weaker than those for total production (Pinares-Patiño et al., 2013). The presence of significant proportional bias in CH_4 yield further reinforces that GF-derived yield values should be interpreted cautiously, particularly when used to compare animals across a wide range of emission intensities or dietary conditions (Hegarty, 2013).

In contrast to CH_4 metrics, GF performance for CO_2 production was poor, with weak and non-significant agreement with RC measurements and the presence of both substantial mean and proportional bias. The large variability and high RMSE associated with GF CO_2 estimates reflect the strong sensitivity of CO_2 emissions to short-term physiological and behavioural fluctuations, including respiration rate, physical activity, head position within the sampling hood, and stress responses (Hegarty, 2013; Storm et al., 2012; Tedeschi et al., 2022). Unlike CH_4, which is primarily driven by ruminal fermentation processes and exhibits slower emission dynamics associated with feeding events, CO_2 production reflects whole-animal metabolic rate and respiration. However, both gases respond to intake and activity, indicating that the pronounced disagreement observed for CO_2 in the present study is unlikely to arise from biological variability alone. The ability of

intermittent breath sampling to provide representative CO_2 estimates has been demonstrated in cattle, where emissions are substantially higher and well within the detection range of GF sensors. In sheep, by contrast, lower absolute CO_2 fluxes likely resulted in some spot samples approaching background concentrations, increasing sensitivity to dilution and capture efficiency and amplifying measurement noise. Consequently, the poor agreement and large variability observed for GF-derived CO_2 estimates in this study reflect limitations in sensor resolution at small-ruminant emission scales rather than the visit-based sampling principle itself. These findings indicate that while GF systems can perform adequately for CO_2 measurement in cattle, they are not well suited for reliable quantification of CO_2 production in sheep.

Diet composition played an important role in shaping residual CH_4 behaviour and provides key biological insight into the observed systematic bias. The diverse pasture diet was characterised by lower NDF content, higher crude protein, and greater moisture compared with the standard ryegrass–white clover pasture. These compositional differences are likely to influence rumen fermentation dynamics and digesta physical structure (Janssen, 2010; Van Soest, 1994). Diets with lower fibre content and higher water solubility tend to promote faster rumen homogenisation and more uniform microbial fermentation, resulting in smoother temporal patterns of CH_4 production (Goopy et al., 2014; Janssen, 2010). Under such conditions, intermittent breath sampling is more likely to capture representative emission levels regardless of visit timing (Hegarty, 2013; Storm et al., 2012).

In contrast, the higher fibre content of the standard pasture increases rumen structural heterogeneity and slows feed particle breakdown, leading to more pronounced temporal variation in fermentation activity and CH_4 release (Grovum and Williams, 1973; Van Soest, 1994). As fibrous material is gradually degraded, CH_4 production fluctuates depending on passage rate, rumen stratification, and

microbial access to substrates (Janssen, 2010). These time-dependent dynamics increase the sensitivity of GF estimates to when an animal visits the unit relative to feeding events, contributing to greater residual variability and proportional bias (Hegarty, 2013; Storm et al., 2012). This biological mechanism is consistent with the observed dietary influence on CH_4 residuals and may contribute to differences in how well intermittent measurement approaches perform under specific feeding conditions.

While fibre fractions, CP and GE were characterised for the experimental pastures, soluble carbohydrate fractions and ash content were not measured, which limits complete interpretation of fermentation dynamics between diet types. Water-soluble carbohydrates and rapidly fermentable non-structural carbohydrates play a key role in determining rumen fermentation rate, volatile fatty acid profiles, and hydrogen partitioning, all of which influence both the magnitude and temporal pattern of CH_4 production (Bannink et al., 2006; Janssen, 2010). Differences in soluble carbohydrate availability and mineral composition between pasture types may have influenced overall rumen fermentation characteristics and CH_4 production, although direct assessment of temporal emission patterns was beyond the scope of the present study. Ash content influences effective DM concentration and energy dilution, potentially affecting intake behaviour and microbial efficiency (Van Soest, 1994). Although the measured fibre and protein fractions captured the major structural drivers of CH_4 formation, inclusion of detailed carbohydrate fractionation, mineral composition, and direct measurement of diurnal emission profiles in future studies would improve mechanistic understanding of diet-specific CH_4 dynamics and their interaction with intermittent measurement systems such as GF.

The methodological contrast between continuous RC measurements and intermittent GF sampling is therefore fundamental to interpreting agreement outcomes (Storm et al., 2012). While GF

systems offer substantial logistical advantages in terms of and potential on-farm deployment, they inherently trade temporal resolution for practicality (Hammond et al., 2016a). Continuous systems capture full diurnal emission profiles, whereas visit-based systems rely on representative sampling coverage to approximate daily totals (Hammond et al., 2016a; Storm et al., 2012). The present study demonstrates that even when visits are distributed across the day, limited sampling density may have contributed to systematic disagreement between methods, particularly under high-fibre feeding conditions. Given that recommended GF protocols typically involve substantially higher numbers of daily visits, the relatively small number of spot samples obtained in this study may not have fully captured diurnal emission variability. This highlights the importance of optimising sampling frequency, visit scheduling, and bait delivery strategies when adapting GF systems for sheep (Hegarty, 2013).

Several limitations of the present study should be considered when interpreting the findings. The relatively small sample size limited statistical power to estimate diet-specific bias models and contributed to uncertainty around regression parameters. The three-day GF measurement period, while consistent with previous validation approaches, may not have fully captured day-to-day variability in CH_4 production, potentially inflating noise and proportional bias (Hammond et al., 2016a). The fixed order of measurement (RC followed by GF) could have introduced minor acclimation or behavioural effects, although dietary and environmental conditions were held constant to minimise such influences. Additionally, one animal failed to engage with the GF bait system, illustrating practical challenges associated with voluntary sampling approaches in sheep. Lastly, the GF system used in this study was designed for large ruminants and may not have been sensitive enough to the lower CO_2 concentrations produced by sheep compared to cattle. Although the smaller hood geometry in the small ruminant GF brings the animal's nose closer to the

proximity sensor and may improve gas capture, the accurate performance observed for CH_4 measurements suggests that the primary limitation observed for CO_2 was likely related to sensor sensitivity rather than breath capture efficiency.

Future research should focus on extending measurement duration, increasing animal numbers, and evaluating performance across seasons and a broader range of diet types to better characterise the stability of GF-derived estimates (Storm et al., 2012) and also compare the performance of the small and large ruminant GF simultaneously. Investigations into the role of rumen passage rate, feed fermentability, and visit frequency optimisation would provide valuable insight into how intermittent sampling protocols can be refined for small ruminants (Hegarty, 2013). Further refinement of sheep-specific GF configurations, including optimization of bait delivery strategies and visit scheduling, may improve sampling representativeness and reduce behavioural variability, particularly under grazing conditions. While CH_4 measurement performance in the present study was encouraging, current visit frequency and bait recommendations were largely developed for cattle, and species-specific differences in visitation behaviour may influence data yield and robustness in sheep-based applications (Tadesse et al., 2024; Weerasinghe et al., 2025).

In practical terms, the present findings suggest that cattle-designed GF systems equipped with TDL sensors can serve as useful tools for estimating CH_4 emissions in sheep, particularly for capturing between-animal variation and evaluating treatment effects under controlled conditions. Mean CH_4 production and yield did not differ significantly between GF and RC measurements, demonstrating encouraging agreement at the population level. Although variability and evidence of proportional bias were observed, these patterns have also been reported in cattle-based validation studies and should be interpreted cautiously given the limited dataset in the present study. In contrast, poor agreement for CO_2 underscores the need for caution when using GF systems to infer metabolic or

intake-related parameters in sheep (Hammond et al., 2016a). With further methodological refinement and targeted validation, GF technology holds strong promise as a scalable CH_4 measurement approach for small ruminant.

The present study successfully met its objective of comparing CH_4 emission estimates obtained using GF and RC methodologies in sheep. The findings demonstrate that GF systems equipped with new-generation TDL sensors can capture between-animal variation in CH_4 production without consistent mean bias, supporting their application in comparative and screening contexts (Hammond et al., 2016a; McGinn et al., 2021). However, the presence of systematic proportional bias and elevated variability highlights important methodological constraints associated with intermittent sampling (Huhtanen et al., 2015). These outcomes address existing uncertainties surrounding the use of cattle-designed GF systems in small ruminants and provide practical guidance for their interpretation and future refinement (Weerasinghe et al., 2025). Collectively, this work contributes to improving the reliability of scalable CH_4 measurement approaches needed to support mitigation research and inventory development in sheep production systems (Hegarty, 2013).

Chapter Four Summary and Future *Research*

4.1 Key findings

The GF system demonstrated moderate agreement with RC for daily CH_4 production, explaining approximately two-thirds of the observed between-animal variation in emissions. The moderate R^2 indicates that while GF captured substantial between-animal variation, a proportion of unexplained variability reflects inherent limitations of intermittent sampling relative to continuous chamber systems. This indicates that GF was effective at capturing biologically meaningful differences among sheep, supporting its ability to capture meaningful between-animal differences in CH_4 output and treatment effects. No significant mean bias was detected for CH_4 production or CH_4 yield, suggesting that GF accurately estimated emissions relative to RC across animals. However, evidence of proportional bias was observed for both CH_4 production and yield, indicating that the magnitude of disagreement between methods varied across emission levels. GreenFeed-derived CH_4 estimates also exhibited greater variability than RC measurements, attributable to the behaviour-dependent nature of short-duration sampling events, variation in visit timing, and sensitivity to airflow dynamics. In contrast to CH_4 metrics, agreement between methods for CO_2 production was poor, with substantial mean and proportional bias and high variability, indicating that GF was not suitable for reliable CO_2 quantification in sheep. Diet composition influenced residual CH_4 behaviour, with improved agreement between GF and RC under lower-fibre diverse pasture conditions, while greater variability in agreement was observed under higher-fibre pasture.

4.2 Scientific and practical implications

These findings demonstrate that cattle-designed GF units equipped with TDL sensors show clear potential for measuring enteric CH_4 emissions in sheep. The ability of GF to capture substantial between-animal variation supports its use in CH_4 phenotyping, genetic controlled research phenotyping and comparative evaluation of mitigation strategies. This supports the reliability of GF-derived CH_4 estimates at the population level. However, greater variability and indications of proportional bias highlight limitations associated with intermittent sampling when estimating absolute CH_4 emissions, suggesting that more animals per treatment need to be measured. Intermittent sampling inherently trades temporal resolution for practicality, while biological fermentation dynamics further amplify distortions in visit-based estimates. From an applied perspective, these results align with the growing need for scalable measurement tools while emphasising the importance of protocol optimisation. In particular, the limited number of spot samples per sheep likely constrained the precision and representativeness of daily CH_4 estimates. Increasing the number and temporal distribution of spot samples would be expected to improve precision and better capture diurnal emission dynamics. While appropriate visit duration, proximity sensor settings, and animal acclimation are necessary to obtain valid measurements, sampling frequency remains a primary determinant of representative daily estimates. Respiration chambers remain essential for high-precision emission quantification and method validation, whereas GF technology offers a valuable complementary approach capable of extending CH_4 measurement to populations under production-relevant contexts.

4.3 Strengths and limitations of the study

A key strength of this research was the paired within-animal experimental design, allowing direct comparison of continuous RC measurements with intermittent GF estimates under identical dietary and housing conditions. The use of new-generation TDL sensors provided an evaluation of the latest GF technology, while inclusion of contrasting pasture types introduced biologically relevant variation in CH_4 production. Some limitations should be acknowledged. The relatively small sample size constrained statistical power for diet-specific bias modelling, and the three-day GF measurement period, based on eight 5-min spot samples per animal, may not have fully captured day-to-day emission variability. Future studies should therefore be designed and adequately powered to address these aspects. Measurements could not be conducted simultaneously across methods, introducing unavoidable intake and behavioural differences between periods. Voluntary engagement with the GF system remains a practical challenge in sheep, as demonstrated by non-participation of one animal, due to dietary preferences. Despite these limitations, the consistency of observed patterns across CH_4 metrics provides robust evidence of the fundamental strengths and constraints of intermittent sampling approaches in small ruminants.

4.4 Future research directions

Future studies should prioritise extended GF measurement periods, larger animal cohorts, wider intake ranges and include direct comparison with small-ruminant GF units to improve precision, address the cause of the proportional bias, and determine which GF configuration is most suitable for sheep. Validation under grazing conditions is essential to assess system performance in commercial production environments where feeding behaviour and visit distribution may differ substantially from controlled indoor settings. Further investigation into the influence of rumen

fermentation kinetics, diet fermentability, and passage rate on intermittent sampling accuracy would support development of optimised GF protocols for sheep. Refinement of visit scheduling, bait delivery strategies, and sensor positioning may reduce behavioural variability and improve the consistency of GF-derived CH_4 estimates in sheep under experimental conditions.

4.5 Overall conclusion

This study provides the first controlled validation of a cattle-designed GF system equipped with new-generation TDL sensors for CH_4 measurement in sheep. GreenFeed technology was shown to effectively capture between-animal variation in CH_4 production without systematic mean bias, demonstrated strong potential as a method for estimating CH_4 emissions in small ruminants. However, greater variability and evidence of proportional bias relative to RC measurements highlight limitations associated with intermittent, visit-based sampling. While GF provided unbiased estimates of mean CH_4 emissions at the population level, variability across emission ranges indicates that methodological refinement is needed to improve precision and consistency in individual-level measurements.

References

Arias, R. A., Guajardo, G., Kunick, S., Alvarado-Gilis, C., and Keim, J. P. (2020). Effect of Two Nutritional Strategies to Balance Energy and Protein Supply in Fattening Heifers on Performance, Ruminal Metabolism, and Carcass Characteristics. *Animals (Basel)* **10**.

Baasansuren, J., Fukuda, M., Ngarize, S., Osako, A., Pyrozhenko, Y., Shermanau, P., and Federici, S. (2019). 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories.

Bach, A., Calsamiglia, S., and Stern, M. D. (2005). Nitrogen Metabolism in the Rumen*. *Journal of Dairy Science* **88**, E9-E21.

Bannink, A., Kogut, J., Dijkstra, J., France, J., Kebreab, E., Van Vuuren, A., and Tamminga, S. (2006). Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *Journal of Theoretical Biology* **238**, 36-51.

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., and Wang, M. (2020). Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Animal* **14**, s2-s16.

Beck, M. R., Gunter, S. A., Moffet, C. A., and Reuter, R. R. (2021). using an automated head chamber system to administer an external marker to estimate fecal output by grazing beef cattle. *Journal of Animal Science* **99**, skab241.

Bekele, W., Guinguina, A., Zegeye, A., Simachew, A., and Ramin, M. (2022). Contemporary Methods of Measuring and Estimating Methane Emission from Ruminants. *Methane* **1**, 82-95.

Belanche, A., Hristov, A. N., van Lingen, H. J., Denman, S. E., Kebreab, E., Schwarm, A., Kreuzer, M., Niu, M., Eugène, M., and Niderkorn, V. (2023). Prediction of enteric methane emissions by sheep using an intercontinental database. *Journal of Cleaner Production* **384**, 135523.

Bennett, C., Ward, J., and Jonker, A. (2022). Can GreenFeed automated emissions monitoring systems measure methane output from young red deer grazing pasture?

Bhatt, R. S., Soni, L., and Sahoo, A. (2019). Methane production and microbial protein synthesis in adult sheep fed total mixed ration as mash and as complete feed block. *Carbon Management* **10**, 241-253.

Bland, J. M., and Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307-10.

Blaxter, K. L., and Clapperton, J. L. (1965). Prediction of the amount of methane produced by ruminants. *British Journal of Nutrition* **19**, 511-522.

Bruder, T., Rouillé, B., Yan, T., and Chagunda, M. (2017). Towards a robust protocol for enteric methane measurements using a hand held Laser Methane Detector® in ruminants.

C-Lock-Inc. (2021). GreenFeed Emissions Monitoring System — Hardware Update: Adoption of Tunable Diode Laser (TDL) Gas Analyzers. C-Lock-Inc., Rapid City, SD, USA.

Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P. W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., and Blanco, G. (2023). IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.

CCAC, U. (2021). "Global Methane Assessment: Benefits and Costs of Mitigating Methane Emissions."

Chagunda, M. G. G. (2013). Opportunities and challenges in the use of the Laser Methane Detector to monitor enteric methane emissions from ruminants. *Animal* **7**, 394-400.

Chagunda, M. G. G., Ross, D., and Roberts, D. J. (2009). On the use of a laser methane detector in dairy cows. *Computers and Electronics in Agriculture* **68**, 157-160.

Chen, P., Li, Y., Shen, Y., Cao, Y., Li, Q., Wang, M., Liu, M., Wang, Z., Huo, Z., Ren, S., Gao, Y., and Li, J. (2022). Effect of Dietary Rumen-Degradable Starch to Rumen-Degradable Protein Ratio on In Vitro Rumen Fermentation Characteristics and Microbial Protein Synthesis. *Animals* **12**, 2633.

Coppa, M., Jurquet, J., Eugène, M., Dechaux, T., Rochette, Y., Lamy, J. M., Ferlay, A., and Martin, C. (2021). Repeatability and ranking of long-term enteric methane emissions measurement on dairy cows across diets and time using GreenFeed system in farm-conditions. *Methods* **186**, 59-67.

de Haas, Y., Veerkamp, R., De Jong, G., and Aldridge, M. (2021). Selective breeding as a mitigation tool for methane emissions from dairy cattle. *Animal* **15**, 100294.

de Mol, R., Bannink, A., Dijkstra, J., Walker, N., and van Gastelen, S. (2024). The effect of feeding and visiting behavior on methane and hydrogen emissions of dairy cattle measured with the GreenFeed system under different dietary conditions. *Journal of Dairy Science* **107**, 7769-7785.

Dean, J. F., Middelburg, J. J., Röckmann, T., Aerts, R., Blauw, L. G., Egger, M., Jetten, M. S., De Jong, A. E., Meisel, O. H., and Rasigraf, O. (2018). Methane feedbacks to the global climate system in a warmer world. *Reviews of Geophysics* **56**, 207-250.

Della Rosa, M., Khan, M., Bosher, T., Maclean, P., and Jonker, A. (2025). Effect of number of measurement days on variance in methane and carbon dioxide emissions measured using GreenFeed units in grazing dairy cows and growing heifers. *Animal Production Science* **65**.

Della Rosa, M., Sandoval, E., Luo, D., Pacheco, D., and Jonker, A. (2022). Effect of feeding fresh forage plantain (Plantago lanceolata) or ryegrass-based pasture on methane emissions, total-tract digestibility, and rumen fermentation of nonlactating dairy cows. *Journal of Dairy Science* **105**, 6628-6638.

Della Rosa, M. M., Jonker, A., and Waghorn, G. C. (2021). A review of technical variations and protocols used to measure methane emissions from ruminants using respiration chambers, SF6 tracer technique and GreenFeed, to facilitate global integration of published data. *Animal Feed Science and Technology* **279**, 115018.

Difford, G. F., Lassen, J., and Løvendahl, P. (2016). Interchangeability between methane measurements in dairy cows assessed by comparing precision and agreement of two non-invasive infrared methods. *Computers and Electronics in Agriculture* **124**, 220-226.

Dijkstra, J., Reynolds, C., Kebreab, E., Bannink, A., Ellis, J., France, J., and Van Vuuren, A. (2013). Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. *In* "Energy and protein metabolism and nutrition in sustainable animal production", pp. 45-58. Wageningen Academic.

Durmic, Z., Moate, P., Jacobs, J., Vadhanabhuti, J., and Vercoe, P. E. (2016). In vitro fermentability and methane production of some alternative forages in Australia. *Animal Production Science* **56**, 641-645.

E, O. C., McHugh, N., Boland, T. M., Dunne, E., and McGovern, F. M. (2021). Investigation of intra-day variability of gaseous measurements in sheep using portable accumulation chambers. *J Anim Sci* **99**.

Ellis, J. L., Dijkstra, J., France, J., Parsons, A. J., Edwards, G. R., Rasmussen, S., Kebreab, E., and Bannink, A. (2012). Effect of high-sugar grasses on methane emissions simulated using a dynamic model. *Journal of Dairy Science* **95**, 272-285.

Eugène, M., Sauvant, D., Noziere, P., Viallard, D., Oueslati, K., Lherm, M., Mathias, E., and Doreau, M. (2019). A new Tier 3 method to calculate methane emission inventory for ruminants. *Journal of environmental management* **231**, 982-988.

Gantner, V. (2016). Ruminants and climate change.

Garnsworthy, P. C., Difford, G. F., Bell, M. J., Bayat, A. R., Huhtanen, P., Kuhla, B., Lassen, J., Peiren, N., Pszczola, M., Sorg, D., Visker, M. H. P. W., and Yan, T. (2019). Comparison of Methods to Measure Methane for Use in Genetic Evaluation of Dairy Cattle. *Animals* **9**, 837.

Gerber, P. J., Henderson, B., and Makkar, H. P. (2013a). "Mitigation of greenhouse gas emissions in livestock production: a review of technical options for non-CO2 emissions."

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., and Tempio, G. (2013b). "Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities."

Goopy, J. P., Donaldson, A., Hegarty, R., Vercoe, P. E., Haynes, F., Barnett, M., and Oddy, V. H. (2014). Low-methane yield sheep have smaller rumens and shorter rumen retention time. *British Journal of Nutrition* **111**, 578-585.

Goopy, J. P., Robinson, D. L., Woodgate, R. T., Donaldson, A. J., Oddy, V. H., Vercoe, P. E., and Hegarty, R. S. (2015). Estimates of repeatability and heritability of methane production in sheep using portable accumulation chambers. *Animal Production Science* **56**, 116-122.

Grovum, W. L., and Williams, V. J. (1973). Rate of passage of digesta in sheep. 4. Passage of marker through the alimentary tract and the biological relevance of rate-constants derived from the changes in concentration of marker in faeces. *Br J Nutr* **30**, 313-29.

Hammond, K., Waghorn, G., and Hegarty, R. (2016a). The GreenFeed system for measurement of enteric methane emission from cattle. *Animal Production Science* **56**, 181-189.

Hammond, K. J., Crompton, L. A., Bannink, A., Dijkstra, J., Yáñez-Ruiz, D. R., O'Kiely, P., Kebreab, E., Eugène, M. A., Yu, Z., Shingfield, K. J., Schwarm, A., Hristov, A. N., and Reynolds, C. K. (2016b). Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. *Animal Feed Science and Technology* **219**, 13-30.

Hammond, K. J., Humphries, D. J., Crompton, L. A., and Reynolds, C. (2015). Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulphur hexafluoride tracer. *Animal Feed Science and Technology* **203**.

Hammond, K. J., Jones, A., Humphries, D. J., Crompton, L. A., and Reynolds, C. K. (2016c). Effects of diet forage source and neutral detergent fiber content on milk production of dairy cattle and methane emissions determined using GreenFeed and respiration chamber techniques. *Journal of Dairy Science* **99**, 7904-7917.

Hartinger, T., Gresner, N., and Südekum, K.-H. (2018). Does intra-ruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation. *Journal of Animal Science and Biotechnology* **9**, 33.

Hassanat, F., Gervais, R., and Benchaar, C. (2017). Methane production, ruminal fermentation characteristics, nutrient digestibility, nitrogen excretion, and milk production of dairy cows fed conventional or brown midrib corn silage. *Journal of Dairy Science* **100**, 2625-2636.

Hegarty, R. S. (2013). Applicability of short-term emission measurements for on-farm quantification of enteric methane. *Animal* **7**, 401-408.

Hickey, S. M., Bain, W. E., Bilton, T. P., Greer, G. J., Elmes, S., Bryson, B., Pinares-Patiño, C. S., Wing, J., Jonker, A., and Young, E. A. (2022). Impact of breeding for reduced methane

emissions in New Zealand sheep on maternal and health traits. *Frontiers in Genetics* **13**, 910413.

Hook, S. E., Wright, A.-D. G., and McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea* **2010**, 945785.

Hristov, A. N., Bannink, A., Battelli, M., Belanche, A., Cajarville Sanz, M. C., Fernandez-Turren, G., Garcia, F., Jonker, A., Kenny, D. A., Lind, V., Meale, S. J., Meo Zilio, D., Muñoz, C., Pacheco, D., Peiren, N., Ramin, M., Rapetti, L., Schwarm, A., Stergiadis, S., Theodoridou, K., Ungerfeld, E. M., van Gastelen, S., Yáñez-Ruiz, D. R., Waters, S. M., and Lund, P. (2025a). Feed additives for methane mitigation: Recommendations for testing enteric methane-mitigating feed additives in ruminant studies. *Journal of Dairy Science* **108**, 322-355.

Hristov, A. N., Bannink, A., Battelli, M., Belanche, A., Sanz, M. C. C., Fernandez-Turren, G., Garcia, F., Jonker, A., Kenny, D. A., and Lind, V. (2025b). Feed additives for methane mitigation: Recommendations for testing enteric methane-mitigating feed additives in ruminant studies. *Journal of Dairy Science* **108**, 322-355.

Hristov, A. N., Bannink, A., Crompton, L. A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C. K., Bayat, A. R., Yáñez-Ruiz, D. R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K. J., and Yu, Z. (2019). Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. *Journal of Dairy Science* **102**, 5811-5852.

Hristov, A. N., Kebreab, E., Niu, M., Oh, J., Bannink, A., Bayat, A. R., Boland, T. M., Brito, A. F., Casper, D. P., Crompton, L. A., Dijkstra, J., Eugène, M., Garnsworthy, P. C., Haque, N., Hellwing, A. L. F., Huhtanen, P., Kreuzer, M., Kuhla, B., Lund, P., Madsen, J., Martin, C., Moate, P. J., Muetzel, S., Muñoz, C., Peiren, N., Powell, J. M., Reynolds, C. K., Schwarm, A., Shingfield, K. J., Storlien, T. M., Weisbjerg, M. R., Yáñez-Ruiz, D. R., and Yu, Z.

(2018). Symposium review: Uncertainties in enteric methane inventories, measurement techniques, and prediction models. *Journal of Dairy Science* **101**, 6655-6674.

Hristov, A. N., Oh, J., Firkins, J., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H., Adesogan, A., Yang, W., and Lee, C. (2013). Special topics—Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of animal science* **91**, 5045-5069.

Hristov, A. N., Oh, J., Giallongo, F., Frederick, T., Weeks, H., Zimmerman, P. R., Harper, M. T., Hristova, R. A., Zimmerman, R. S., and Branco, A. F. (2015). The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. *Journal of visualized experiments: JoVE*, 52904.

Huhtanen, P., Cabezas-Garcia, E., Utsumi, S., and Zimmerman, S. (2015). Comparison of methods to determine methane emissions from dairy cows in farm conditions. *Journal of dairy science* **98**, 3394-3409.

Huhtanen, P., Ramin, M., and Hristov, A. (2019). Enteric methane emission can be reliably measured by the GreenFeed monitoring unit. *Livestock science* **222**, 31-40.

Hungate, R. E. (2013). "The rumen and its microbes," Elsevier.

IPCC (2014). "Climate Change 2013: The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change," Cambridge, UK and New York, NY, USA.

IPCC (2023). Global Carbon and Other Biogeochemical Cycles and Feedbacks. *In* "Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change" (C. Intergovernmental Panel on Climate, ed.), pp. 673-816. Cambridge University Press, Cambridge.

Janssen, P. H. (2010). Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal feed science and technology* **160**, 1-22.

Janssen, P. H., and Kirs, M. (2008). Structure of the archaeal community of the rumen. *Applied and environmental microbiology* **74**, 3619-3625.

Jayanegara, A., Wina, E., and Takahashi, J. (2014). Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen: influence of addition levels and plant sources. *Asian-Australasian journal of animal sciences* **27**, 1426.

Johnson, K., Huyler, M., Westberg, H., Lamb, B., and Zimmerman, P. (1994). Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. *Environ Sci Technol* **28**, 359-62.

Johnson, K. A., and Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science* **73**, 2483-2492.

Johnson, P. L., Hickey, S., Knowler, K., Wing, J., Bryson, B., Hall, M., Jonker, A., Janssen, P. H., Dodds, K. G., and McEwan, J. C. (2022). Genetic parameters for residual feed intake, methane emissions, and body composition in New Zealand maternal sheep. *Frontiers in genetics* **13**, 911639.

Jonker, A., Antwi, C., Gunter, S., Hristov, A., Martin, C., Minnée, E., Renand, G., and Waghorn, G. (2020). Chapter 3: The 'GreenFeed' automated methane measurement system to determine enteric methane emissions from ruminants. pp. 17-32.

Jonker, A., Hickey, S., Pinares-Patiño, C., McEwan, J., Olinga, S., Díaz, A., Molano, G., Maclean, S., Sandoval, E., and Harland, R. (2017). Sheep from low-methane-yield selection lines created on alfalfa pellets also have lower methane yield under pastoral farming conditions. *Journal of Animal Science* **95**, 3905-3913.

Jonker, A., Hickey, S. M., Rowe, S. J., Janssen, P. H., Shackell, G. H., Elmes, S., Bain, W. E., Wing, J., Greer, G. J., Bryson, B., MacLean, S., Dodds, K. G., Pinares-Patiño, C. S., Young, E. A., Knowler, K., Pickering, N. K., and McEwan, J. C. (2018). Genetic parameters of methane emissions determined using portable accumulation chambers in lambs and ewes grazing pasture and genetic correlations with emissions determined in respiration chambers1. *Journal of Animal Science* **96**, 3031-3042.

Jonker, A., Molano, G., Sandoval, E., Taylor, P., Antwi, C., Olinga, S., and Cosgrove, G. (2016). Methane emissions differ between sheep offered a conventional diploid, a high-sugar diploid or a tetraploid perennial ryegrass cultivar at two allowances at three times of the year. *Animal Production Science* **58**, 1043-1048.

Kamra, D. N. (2005). Rumen microbial ecosystem. *Current science*, 124-135.

Kang, K., Cho, H., Jeong, S., Jeon, S., Lee, M., Lee, S., Baek, Y., Oh, J., and Seo, S. (2022). Application of a hand-held laser methane detector for measuring enteric methane emissions from cattle in intensive farming. *J Anim Sci* **100**.

Kelly, W. J., Mackie, R. I., Attwood, G. T., Janssen, P. H., McAllister, T. A., and Leahy, S. C. (2022). Hydrogen and formate production and utilisation in the rumen and the human colon. *Animal Microbiome* **4**, 22.

Kittelmann, S., Pinares-Patino, C. S., Seedorf, H., Kirk, M. R., Ganesh, S., McEwan, J. C., and Janssen, P. H. (2014). Two different bacterial community types are linked with the low-methane emission trait in sheep. *PloS one* **9**, e103171.

Knapp, J. R., Laur, G., Vadas, P. A., Weiss, W. P., and Tricarico, J. M. (2014a). Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *Journal of dairy science* **97**, 3231-3261.

Knapp, J. R., Laur, G. L., Vadas, P. A., Weiss, W. P., and Tricarico, J. M. (2014b). Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science* **97**, 3231-3261.

Lapierre, H., and Lobley, G. (2001). Nitrogen recycling in the ruminant: A review. *Journal of Dairy Science* **84**, E223-E236.

Lassey, K., Pinares-Patiño, C., Martin, R., Molano, G., and McMillan, A. (2011). Enteric methane emission rates determined by the SF6 tracer technique: Temporal patterns and averaging periods. *Animal feed science and technology* **166**, 183-191.

Leahy, S. C., Kelly, W. J., Altermann, E., Ronimus, R. S., Yeoman, C. J., Pacheco, D. M., Li, D., Kong, Z., McTavish, S., and Sang, C. (2010). The genome sequence of the rumen methanogen Methanobrevibacter ruminantium reveals new possibilities for controlling ruminant methane emissions. *PloS one* **5**, e8926.

Lin, L. I. (1989). A concordance correlation coefficient to evaluate reproducibility. *Biometrics* **45**, 255-68.

Lu, Z., Xu, Z., Shen, Z., Tian, Y., and Shen, H. (2019). Dietary energy level promotes rumen microbial protein synthesis by improving the energy productivity of the ruminal microbiome. *Frontiers in microbiology* **10**, 847.

Ma, X., Räisänen, S. E., Wang, K., Amelchanka, S., Giller, K., Islam, M. Z., Li, Y., Peng, R., Reichenbach, M., Serviento, A. M., Sun, X., and Niu, M. (2024). Evaluating GreenFeed and respiration chambers for daily and intraday measurements of enteric gaseous exchange in dairy cows housed in tiestalls. *Journal of Dairy Science* **107**, 10913-10931.

Manafiazar, G., Zimmerman, S., and Basarab, J. A. (2017). Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using

GreenFeed emissions monitoring system. *Canadian Journal of Animal Science* **97**, 118-126.

Martinez-Fernandez, G., Duval, S., Kindermann, M., Schirra, H. J., Denman, S. E., and McSweeney, C. S. (2018). 3-NOP vs. halogenated compound: methane production, ruminal fermentation and microbial community response in forage fed cattle. *Frontiers in microbiology* **9**, 1582.

McAuliffe, G. A., Lynch, J., Cain, M., Buckingham, S., Rees, R. M., Collins, A. L., Allen, M., Pierrehumbert, R., Lee, M. R., and Takahashi, T. (2023). Are single global warming potential impact assessments adequate for carbon footprints of agri-food systems? *Environmental Research Letters* **18**, 084014.

McGinn, S. M., Coulombe, J. F., and Beauchemin, K. A. (2021). Technical note: validation of the GreenFeed system for measuring enteric gas emissions from cattle. *J Anim Sci* **99**.

Mcgovern, F. M., Johnson, P. L., McHugh, N., Tortereau, F., Marie-Etancelin, C., Navajas, E. A., De Barbieri, I., Åby, B. A., Rowe, S. J., and Conington, J. (2025). Assessing methane production and feed efficiency in both growing and mature ovine animals in six countries across Europe, South America and Oceania. *Small Ruminant Research*, 107677.

Meo-Filho, P., Berndt, A., Pezzopane, J. R., Pedroso, A. F., Bernardi, A. C., Rodrigues, P. H., Bueno, I. C., Corte, R. R., and Oliveira, P. P. (2022). Can intensified pasture systems reduce enteric methane emissions from beef cattle in the atlantic forest biome? *Agronomy* **12**, 2738.

MfE (2019). Climate Change Response (Zero Carbon) Amendment Act 2019. Vol. 2025. New Zealand Government.

MfE (2022). "New Zealand's Greenhouse Gas Inventory 1990–2020."

MfE (2023). "New Zealand's Greenhouse Gas Inventory 1990–2021," Wellington, New Zealand.

Min, B. R., Pinchak, W. E., Hume, M. E., and Anderson, R. C. (2021). Effects of condensed tannins supplementation on animal performance, phylogenetic microbial changes, and in vitro methane emissions in steers grazing winter wheat. *Animals* **11**, 2391.

Morgavi, D. P., Eugène, M., Martin, C., and Doreau, M. (2011). Reducing methane emissions in ruminants: is it an achievable goal. *Challenging strategies to promote the sheep and goat sector in the current global context. CIHEAM/CSIC/Universidad de Leon/FAO, Zaragoza.(Options Méditerranéennes: Série A. Séminaires Méditerranéens, n. 99)*, 65-73.

MPI (2020). Guidelines for estimating methane emissions from individual ruminants using: GreenFeed, "sniffers," hand-held laser detector and portable accumulation chambers. Ministry for Primary Industries, Wellington, New Zealand.

MPI (2025). Situation and Outlook for Primary Industries: June 2025. (M. f. P. Industries, ed.), Wellington, New Zealand.

Muetzel, S., and Clark, H. (2015). Methane emissions from sheep fed fresh pasture. *New Zealand Journal of Agricultural Research* **58**, 472-489.

Muñoz, C., Yan, T., Wills, D., Murray, S., and Gordon, A. (2012). Comparison of the sulfur hexafluoride tracer and respiration chamber techniques for estimating methane emissions and correction for rectum methane output from dairy cows. *Journal of dairy science* **95**, 3139-3148.

Murray, R., Bryant, A., and Leng, R. (1976). Rates of production of methane in the rumen and large intestine of sheep. *British journal of nutrition* **36**, 1-14.

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., and Mendoza, B. (2014). Anthropogenic and natural radiative forcing. *Climate Change 2013-The Physical Science Basis*, 659-740.

Newbold, C. J., De La Fuente, G., Belanche, A., Ramos-Morales, E., and McEwan, N. R. (2015). The role of ciliate protozoa in the rumen. *Frontiers in microbiology* **6**, 1313.

NZAGRC (2022). "Greenhouse gas emissions on New Zealand farms — Seminar workbook." New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC), Wellington, New Zealand.

O'Connor, E., McGovern, F. M., Berry, D., Dunne, E., McEwan, J., Rowe, S., Boland, T., Morrison, S., Aubry, A., and Yan, T. (2024). Comparison of greenhouse gas emissions from sheep measured using both respiration and portable accumulation chambers. *animal* **18**, 101140.

O'Connor, E., McGovern, F. M., Byrne, D. T., Boland, T. M., Dunne, E., and McHugh, N. (2021). Repeatability of gaseous measurements across consecutive days in sheep using portable accumulation chambers. *Journal of Animal Science* **99**.

Orzuna-Orzuna, J. F., Dorantes-Iturbide, G., Lara-Bueno, A., Mendoza-Martínez, G. D., Miranda-Romero, L. A., and Hernández-García, P. A. (2021). Effects of Dietary Tannins' Supplementation on Growth Performance, Rumen Fermentation, and Enteric Methane Emissions in Beef Cattle: A Meta-Analysis. *Sustainability* **13**, 7410.

Patra, A. K. (2012). Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. *Environmental monitoring and assessment* **184**, 1929-1952.

Patra, A. K. (2016). Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. *Frontiers in veterinary science* **3**, 39.

Pereira, A. M., Peixoto, P., Rosa, H. J. D., Vouzela, C., Madruga, J. S., and Borba, A. E. S. (2023). A Longitudinal Study with a Laser Methane Detector (LMD) Highlighting Lactation Cycle-Related Differences in Methane Emissions from Dairy Cows. *Animals* **13**, 974.

Pickering, N. K., Oddy, V., Basarab, J., Cammack, K., Hayes, B., Hegarty, R., Lassen, J., McEwan, J., Miller, S., and Pinares-Patiño, C. (2015). Animal board invited review: genetic possibilities to reduce enteric methane emissions from ruminants. *animal* **9**, 1431-1440.

Pinares-Patiño, C., Hickey, S., Young, E., Dodds, K., MacLean, S., Molano, G., Sandoval, E., Kjestrup, H., Harland, R., and Hunt, C. (2013). Heritability estimates of methane emissions from sheep. *animal* **7**, 316-321.

Pinares-Patiño, C. S., Machmüller, A., Molano, G., Smith, A., Vlaming, J. B., and Clark, H. (2008). The SF6 tracer technique for measurements of methane emission from cattle – effect of tracer permeation rate. *Canadian Journal of Animal Science* **88**, 309-320.

Pinares-Patiño, C. S., McEwan, J. C., Dodds, K. G., Cárdenas, E. A., Hegarty, R. S., Koolaard, J. P., and Clark, H. (2011). Repeatability of methane emissions from sheep. *Animal Feed Science and Technology* **166-167**, 210-218.

Pressman, E. M., Kelly, L., Akter, A., and Kebreab, E. (2025). Comparison of strategies for spot sampling enteric methane and hydrogen emissions to evaluate methane-reducing feed additives in dairy and beef cattle. *Journal of Dairy Science* **108**, 11053-11070.

Provenza, F., and Balph, D. (1987). Diet learning by domestic ruminants: theory, evidence and practical implications. *Applied Animal Behaviour Science* **18**, 211-232.

Reisinger, A., and Clark, H. (2018). How much do direct livestock emissions actually contribute to global warming? *Global change biology* **24**, 1749-1761.

Ricci, P., Chagunda, M. G. G., Rooke, J. A., M Houdijk, J. G., Duthie, C.-A., Hyslop, J., Roehe, R., and Waterhouse, A. (2014). Evaluation of the laser methane detector to estimate methane emissions from ewes and steers. *Journal of animal science* **92 11**, 5239-50.

Rivero, M. J., Khan, A. A., Akpensuen, T. T., Meo-Filho, P., Pérez-Márquez, S., and Jones, A. (2025). Evaluating the Efficacy of a Novel Multi-Component Feed Additive for Methane Mitigation and Performance Enhancement in Sheep. *Ruminants* **5**, 17.

Robinson, D. L., Goopy, J. P., Donaldson, A. J., Woodgate, R. T., Oddy, V. H., and Hegarty, R. S. (2014). Sire and liveweight affect feed intake and methane emissions of sheep confined in respiration chambers. *Animal* **8**, 1935-44.

Roessler, R., and Schlecht, E. (2021). Application of the laser methane detector for measurements in freely grazing goats: impact on animals' behaviour and methane emissions. *Animal* **15**, 100070.

Rowe, S., Hickey, S., Jonker, A., Hess, M., Janssen, P., Johnson, T., Bryson, B., Knowler, K., Pinares-Patino, C., and Bain, W. (2019). Selection for divergent methane yield in New Zealand sheep-a ten-year perspective.

Savaresi, A. (2016). The Paris Agreement: a new beginning? *Journal of Energy & Natural Resources Law* **34**, 16-26.

Schuur, E. A., McGuire, A. D., Schädel, C., Grosse, G., Harden, J. W., Hayes, D. J., Hugelius, G., Koven, C. D., Kuhry, P., and Lawrence, D. M. (2015). Climate change and the permafrost carbon feedback. *Nature* **520**, 171-179.

Science Media Centre, N. (2025). NZ’s new targets to manage methane—expert reaction. Vol. 2025.

Shindell, D., Kuylenstierna, J. C., Vignati, E., Van Dingenen, R., Amann, M., Klimont, Z., Anenberg, S. C., Muller, N., Janssens-Maenhout, G., and Raes, F. (2012). Simultaneously mitigating near-term climate change and improving human health and food security. *Science* **335**, 183-189.

Sinclair, L., Garnsworth, P., Newbold, J., and Buttery, P. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. *The Journal of Agricultural Science* **120**, 251-263.

St-Pierre, N. (2016). Comparison of model predictions with measurements: A novel model-assessment method. *Journal of Dairy Science* **99**, 4907-4927.

Starsmore, K., Lopez-Villalobos, N., Shalloo, L., Egan, M., Burke, J., and Lahart, B. (2024). Animal factors that affect enteric methane production measured using the GreenFeed monitoring system in grazing dairy cows. *Journal of Dairy Science* **107**, 2930-2940.

Stats NZ (2025). Livestock numbers fall over the last 10 years while area planted in fruit increases. Statistics New Zealand.

Storm, I. M., Hellwing, A. L., Nielsen, N. I., and Madsen, J. (2012). Methods for Measuring and Estimating Methane Emission from Ruminants. *Animals (Basel)* **2**, 160-83.

Tadesse, D., Puchala, R., Yirga, H., Patra, A. K., Gipson, T. A., Min, B. R., and Goetsch, A. L. (2024). Determining Appropriate Numbers and Times of Daily Measurements Using GreenFeed System to Estimate Ruminal Methane Emission of Meat Goats. *Animals* **14**, 835.

Tedeschi, L. O., Abdalla, A. L., Álvarez, C., Anuga, S. W., Arango, J., Beauchemin, K. A., Becquet, P., Berndt, A., Burns, R., De Camillis, C., Chará, J., Echazarreta, J. M., Hassouna, M., Kenny, D., Mathot, M., Mauricio, R. M., McClelland, S. C., Niu, M., Onyango, A. A., Parajuli, R., Pereira, L. G. R., Del Prado, A., Paz Tieri, M., Uwizeye, A., and Kebreab, E. (2022). Quantification of methane emitted by ruminants: a review of methods. *J Anim Sci* **100**.

UNFCCC (2015). "Paris Agreement," Paris, France.

Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Frontiers in microbiology* **11**, 589.

USDA (2023). "New Zealand Sheep Production and Trends." U.S. Department of Agriculture, Washington, D.C.

Van Soest, P. J. (1994). "Nutritional ecology of the ruminant," Cornell university press.

Waghorn, G. (2014). Technical Manual on Respiration Chamber Designs.

Waghorn, G., and Clark, D. (2004). Feeding value of pastures for ruminants. *New Zealand Veterinary Journal* **52**, 320-331.

Waghorn, G., Tavendale, M., and Woodfield, D. (2002). Methanogenisis from forages fed to sheep. *In* "Proceedings of the conference-New Zealand grassland association", pp. 167-172.

Wang, S., Giller, K., Kreuzer, M., Ulbrich, S. E., Braun, U., and Schwarm, A. (2017). Contribution of ruminal fungi, archaea, protozoa, and bacteria to the methane suppression caused by oilseed supplemented diets. *Frontiers in microbiology* **8**, 1864.

Wang, W., Zhang, X., Wei, H., Wang, S., Ye, Y., He, L., Zhang, K., Lu, Y., Zhang, Z., and Huang, Y. (2024). Effects of feeding systems on the growth performance, carcass characteristics, and meat quality in sheep: A meta-analysis. *Animals* **14**, 2738.

Weerasinghe, R. N. N., Almeida, A. K., Horne, D. J., Burkitt, L., Kenyon, P. R., and Cranston, L. M. (2025). Adaptation of grazing sheep to GreenFeed emission monitoring units under New Zealand ryegrass-white clover pastures. *New Zealand Journal of Animal Science and Production* **85**, 27–31.

Woodward, S., Waghorn, G., and Laboyrie, P. (2004). Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduce methane emissions from dairy cows.

Wright, A. D. G., Kennedy, P., O'Neill, C. J., Toovey, A. F., Popovski, S., Rea, S. M., Pimm, C. L., and Klein, L. (2004). Reducing methane emissions in sheep by immunization against rumen methanogens. *Vaccine* **22**, 3976-3985.

Zhao, Y., Nan, X., Yang, L., Zheng, S., Jiang, L., and Xiong, B. (2020a). A Review of Enteric Methane Emission Measurement Techniques in Ruminants. *Animals (Basel)* **10**.

Zhao, Y., Nan, X., Yang, L., Zheng, S., Jiang, L., and Xiong, B. (2020b). A Review of Enteric Methane Emission Measurement Techniques in Ruminants. *Animals* **10**, 1004.