Methane Emissions of Grazing Dairy Cows fed Graded levels of Concentrates

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Abstract

The GHG inventory of New Zealand currently assumes that all dairy cattle emit 21.6 grams of methane (CH_4) for every kilogram of dry matter (kg DM) of pasture eaten. However, supplement feeding has increased in New Zealand pastoral systems in recent decades to comprise ~18% of the total feed offered to New Zealand's dairy herd. Previous studies have shown different diets can alter the CH_4 emissions of dairy cattle and therefore the objective of this study was to determine the effect of increasing concentrate intakes on pastoral dairy cows' CH_4 emissions and milk production. Early lactation dairy cows (n = 72) were allocated (n = 18 per treatment group) to receive 0, 2, 4 or 6 kg DM of concentrates per day while grazing pasture *ad-libitum* over 63 days. Methane emissions were measured in the field for individual animals using the 'GreenFeed' automated emissions monitoring system. Changes to liveweight and body condition score, daily milk production and weekly milk composition were recorded and used to estimate individual animals' dry matter intakes. Liveweight change, milk production and estimated dry matter intakes were not found to significantly change with increased concentrate feeding rates. Methane production (g CH_4 / day) was not affected by concentrate feeding and was similar across all treatment groups, however CH_4 yield (g CH_4 / kg DM) and CH_4 intensity (g CH_4 / kg fat and protein corrected milk) linearly decreased with increasing concentrate inclusion in the diet (P = 0.041; P = 0.022, respectively). This was also confirmed by a significant and linear decrease of the methane to carbon dioxide ratio (CO_2 : CH_4) emitted by animals with increased concentrate feeding (P = 0.011). These results have demonstrated that CH_4 yields change when feeding increasing levels of concentrate feed to pasture-based dairy cattle in New Zealand, which differs from the current assumption for calculating the national GHG inventory. Responses in CH_4 emissions and milk production parameters were however relatively small in this study however, which was likely due to generous pasture offers that resulted in a large substitution of pasture as concentrate feeding rates increased.

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1. General introduction

Within New Zealand's primary industries, dairy milk production has been, and continues to be, a crucial contributor towards the nation's gross economic outputs. New Zealand's dairy industry produces approximately 20 billion litres of milk annually from dairy cattle (Bos Taurus), which has steadily grown by over 330% over the past 30 years (DairyNZ, 2023). This intensification of milk production has been significant for New Zealand's economy, with dairy contributing as much as $26 billion to New Zealand's export revenue in 2023 (MPI, 2023). However, this intensification has been strongly linked to increased carbon emissions from the dairy sector which have rose by 28% between 1990 - 2021 (MfE, 2021). This boom in milk production can be argued as having cemented dairy as one of New Zealand's most significant export industries as well as New Zealand's greatest contributor to the nation's GHG emissions profile (Reisinger et al., 2017).

In a global context, GHG can be broadly summarised as a group of gases which both tend to accumulate within the earth's atmosphere and induce an additive insulation effect which heats the earth (NZAGRC, 2023). Historically GHG, such as carbon dioxide (CO_2) or methane (CH_4), have cycled between the earth's surface and atmosphere to buffer and disperse the intense warming effect of solar radiation as part of stabilising global climates and supporting the planet's balanced ecosystems (Reisinger, 2018). However, increases of human activities following the industrial revolution (such as electricity generation, transportation, and food production) have greatly increased the rates of GHG production and accumulation within the earth's atmosphere. This continued rate of elevated GHG emissions has been shown to induce a greater greenhouse warming effect of the planet, initiating the widely analysed effects of global warming and climate change (MfE, 2021). Figure 1.1 shows the extent of global temperatures increases since the industrial revolution (circa 1850).

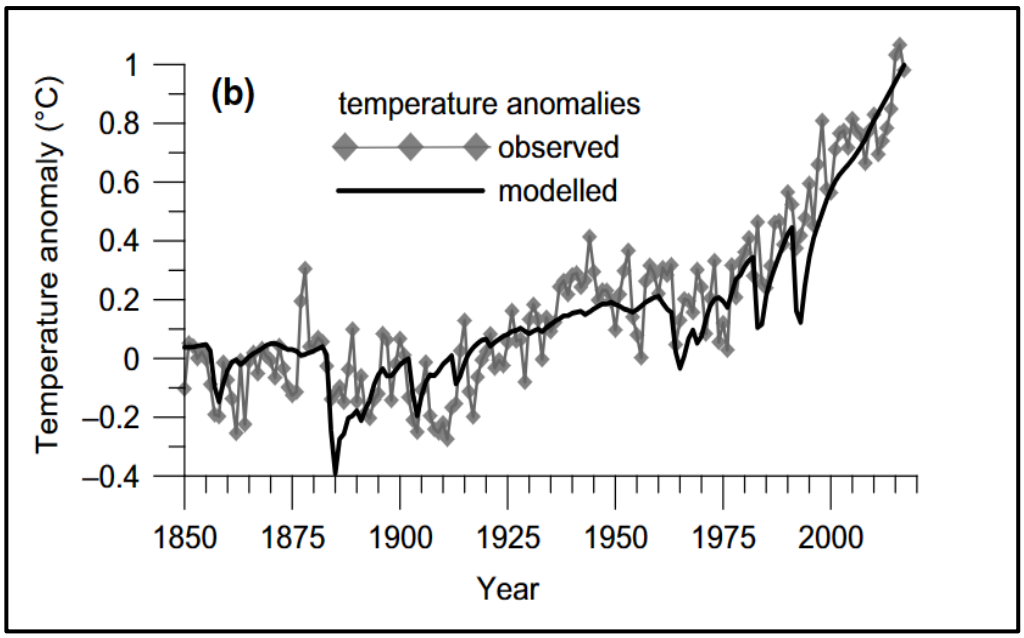

Figure 1.1 The upward trend in modelled and observed temperature (°C) post-industrial revolution. Sourced from Reisinger & Clark (2018).

While global average temperatures and CO_2 levels have naturally fluctuated throughout long-term (~100,000 years) climate cycles on earth, the concern of modern climate change and global warming is derived from both the speed and intensity of these increasing temperatures. Figure 1.2 further demonstrates how these increases of atmospheric CO_2 concentrations have coincided with a sharp and anomalous increases of atmospheric CO_2 concentrations post-industrial revolution to rise well above expected levels. (NZAGRC, 2023).

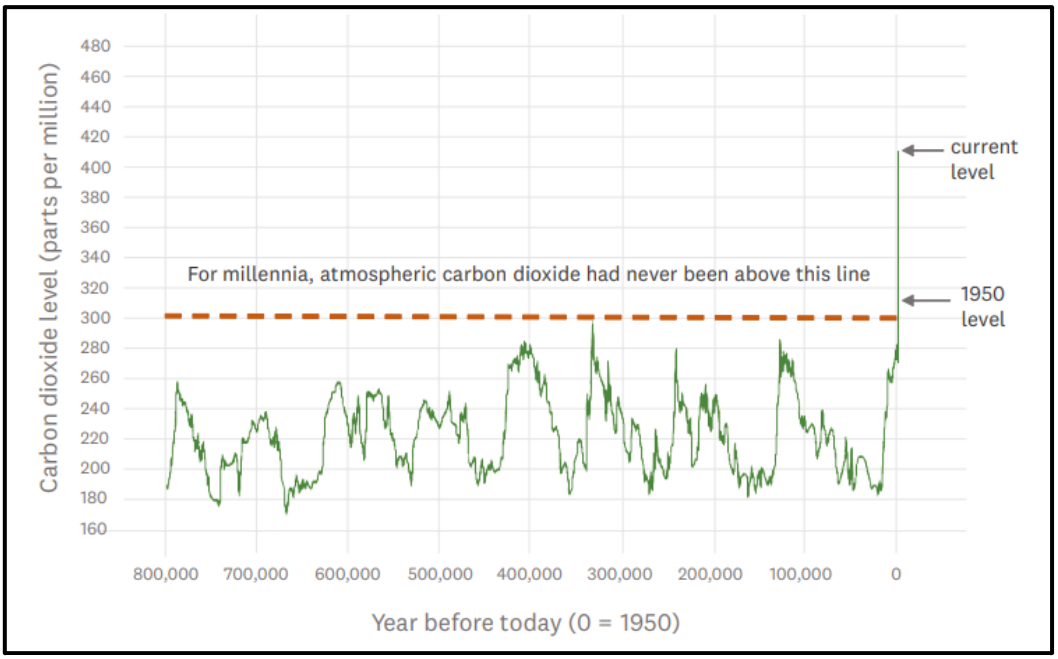

Figure 1.2 The atmospheric CO_2 levels (parts per million) since ~800,000 B.C. and the effect of post-industrial emissions on atmospheric GHG. Sourced from (NZAGRC, 2023).

The global warming effect from this increase of atmospheric GHG concentrations has also widely influenced the changing of climates across the world, including the patterns and intensities of rainfall and temperatures. For New Zealand, the current 1.1^{o}C increase in average global temperature (from pre-industrial revolution levels) has resulted in year-on-year record breaking hottest years since 2013, along with an increased frequency of intense weather events such as storms, cyclones and droughts (MfE & Stats NZ, 2023). Climate change and its effects will likely therefore challenge the sustainability and profitability of pastoral-based milk production systems, as the consumption of pastures and crops are the main driver of animal and financial performance for these systems (Beca, 2020; Neal and Roche, 2020). Potentially negative effects of climate change for New Zealand farmers includes increasing intensity and dispersion of rainfall which could alter annual growth patterns of forages. Likewise, increased temperatures could also stress plants and animals or allow new diseases to negatively impact on production levels and current farm system viability (Kenny, 2001). A wider outlook on New Zealand's society has estimated that the financial consequences of climate change may have cost up to NZ$720 million between 2007-2017, largely due to damage from droughts, floods and coastal erosion (Frame et al., 2018).

Within New Zealand's pastoral sector, enterically produced CH_4 comprises over 70% of the agricultural sectors' gross emissions, of which more than half is derived from dairy cattle (discussed further in section 2.2.1 *The New Zealand greenhouse gas inventory*) (MfE, 2021). However, while CH_4 acts as a potent GHG with 28-32 times the warming effect of CO_2, the gas is also short-lived, having an estimated half-life of approximately 12 years (Reisinger, 2018). Therefore, accurately measuring and mitigating emissions from dairy cattle will act as a crucial means to significantly decrease New Zealand's total GHG emissions.

Addressing climate change through the reduction of any anthropogenic emissions however requires many careful considerations to ensure that target emission levels can be achieved without unsustainably impacting environmental or human well-being (Moss et al., 2010). Targeting a reduction of enteric CH_4 from New Zealand's national dairy herd must therefore consider wider effects than just environmental relief. Dominated by pastoral landscapes (at over 50% of New Zealand's total landmass), the food and fibre sectors contributed $56.2 billion to New Zealand's export revenue in the 2022/23 financial year, with 44% of this being attributed to dairying (MPI, 2023). In this sense, while the emissions from dairy may contribute nearly a fifth of New Zealand's total emissions, dairy

also stands as New Zealand's largest export product and accounts for ~3% of New Zealand's gross domestic product (MPI, 2023).

Drastically de-intensifying or eliminating dairy production from New Zealand would therefore likely force production elsewhere to meet rising demands for dairy goods, which could be less GHG efficient than sustained production in New Zealand (Beauchemin et al., 2022). Currently, New Zealand is estimated to produce milk at ~0.8 kg CO_2 equivalent units per kg of fat and protein corrected milk (FPCM) as opposed to the global average of ~1.3 kg CO_2-equivalent per kg FPCM (Ledgard et al. 2020; Mazzetto et al., 2021). Similarly, the potential for economic and social losses to New Zealand from significantly de-scaling dairy would not adhere to the sustainable focus of international climate change mitigation agreements like the Paris Agreement (see section 2.2.*2 New Zealand's commitment to climate change*) (Leahy et al., 2020). Mitigation solutions for on-farm emissions must therefore strike a careful balance to ensure that CH_4 emissions of dairy cattle are reduced without incurring significant economic, social or other ecological fallouts. Resultant research focuses to accomplish these criteria in New Zealand have centred around robustly measuring the emissions of ruminants under different conditions as well as examining different methods of reducing CH_4 production in the ruminant's foregut (Leahy et al., 2019).

Currently, New Zealand's GHG inventory assessment has established that the rate at which dairy cattle emit CH_4 is linearly correlated with feed intake, measured as dry matter intake (DMI). The inventory assessment assumes that 21.6 g of CH_4 is produced from every kilogram of dry matter (kg DM) of pasture consumed by all dairy cattle in New Zealand (MfE, 2021). However, studies by Della Rosa et al., (2022a), Jonker et al., (2017) and Jiao et al., (2014) have found evidence to suggest that enteric CH_4 production rates may change in pasture-based diets when supplemented with alternative feeds. The present study will seek to further measure the CH_4 emissions from pasture-fed dairy cattle supplemented to differing levels with a standardised concentrate feed. By design, this aims to allow the detection and quantification of any changes to CH_4 emission rates (as g CH_4 / kg DMI) across differing diet compositions and quality which are common to New Zealand's dairy sector. It is hoped that this thesis will help to further the understanding of dietary effects on CH_4 production in dairy cattle for the investigation of sustainable CH_4 mitigation methods in New Zealand's dairy sector.

2. Review of Literature

2.1. Overview of New Zealand dairy systems

In the 2021/22 dairy season (June to June), the average New Zealand dairy herd consisted of 449 cows, milked twice daily on 158 hectares of pastoral land to yield 386 kg of milksolids (MS) per cow, per year (DairyNZ, 2023). Operating under pastoral grass-based farming principles, New Zealand dairy production systems typically operate on a model of cost efficiency by converting homegrown feed to milk (Martin & Sneddon, 2023). Foundationally, this pastoral farming model hinges on New Zealand's temperate maritime climate, which enables both the year-round growth and outdoor grazing of perennial pastures and other homegrown feeds (Holmes et al., 2002).

Grazing animals *in-situ* allows for a cost-effective means of feeding animals, as it does not necessitate forages to be harvested prior to feeding, which is common across indoor systems and is typical of dairy systems in the Northern Hemisphere (Martin & Sneddon, 2023). Generally, it can be said that while grazing based dairy systems cost efficiently convert homegrown feed into milk, intensive feeding systems produce milk with significantly greater efficacy (FAO, 2022a). For instance, the average dairy cow in the United States of America, which is generalised as being intensively fed indoors, produces 795 kg MS produced per cow, per year (kg MS/cow/year) (Britt et al., 2021), which is roughly twice that of the average dairy cow in New Zealand.

Optimising the productivity of pastoral dairy farms by increasing milk production (as kg MS per cow or per hectare, per year) while maintaining on-farm costs has been and continues to be of great interest to New Zealand's dairy sector. Dairy has traditionally represented New Zealand's largest export industry, sending 90-95% of annual dairy production overseas (MPI, 2023). Despite producing only around 2.5% of the world's (cattle) milk, New Zealand's productivity accounts for ~30% of the global dairy trade (FAO, 2022b), which may increase by up to 58% between 2010 and 2050 with global trends of increased food consumption (Beauchemin et al., 2020).

2.2. Changing greenhouse gas emissions within New Zealand Dairy

Seeking to meet growing international demands for milk from grass-fed animals, particularly consumer markets across Asia (FAO, 2022b), the annual milk yield (kg milk/animal) of New Zealand dairy cattle has steadily lifted by 53% between 1992 and 2022 (DairyNZ, 2023). This intensification of national milk production is underpinned by a doubling of New Zealand's dairy herd between 1990 and 2023 (DairyNZ, 2023). Alongside this population increase, New Zealand pastoral farmers also employed several significant management practises to support increased animal productivity in this

period, such as an 800% increase of synthetic nitrogen fertiliser usage (MfE, 2021), increased supplementary feed usage (Rattray et al. 2007) and the introduction of North American Holstein-Friesian genetics to the New Zealand dairy herd (Reisinger, 2018).

As New Zealand's national dairy herd has expanded, both in number and in milk yields, the CO_2-equivalent emissions from dairy cattle were estimated to have risen 128% between 1990-2019. As of 2019, the CH_4 emissions from dairy cattle approximately contributed 14,014 kilotons of CO_2-equivalent units per year to the atmosphere (MfE, 2021), which in turn contributed ~17% of New Zealand's total annual carbon emissions. To bring this to scale, the enteric CH_4 emissions of dairy cattle alone were estimated to comprise over 40% of New Zealand's agricultural emissions (MfE, 2021). Figure 3 demonstrates how intensifying milk production in New Zealand has led to a linearly trending intensification of CH_4 emissions from dairy cattle.

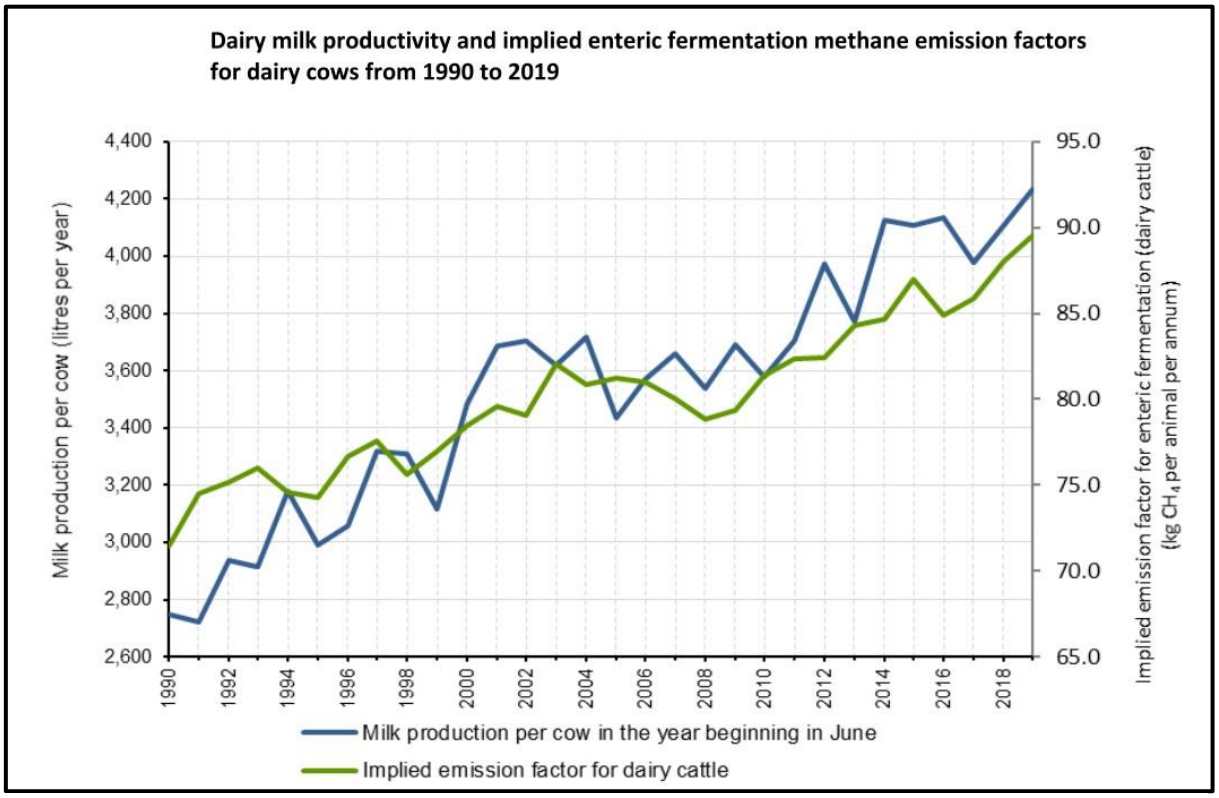

Figure 2.1 The relationship between increased annual milk production and increased annual methane emissions from New Zealand dairy cattle since 1990. Source from MfE (2021).

Although enteric CH_4 emissions will comprise the primary focus of this thesis, the 47% growth of emissions tied to soil nutrient cycles in between 1990 – 2019 also comprises a very significant portion of agricultural GHG emissions in New Zealand. In total, soil emissions comprised 19.6% of New Zealand's total agricultural emissions as of 2019 (Reisinger et al., 2017a; Ledgard et al., 2020). These

soil emissions are primarily derived from the volatilisation of urea to nitrous oxide (N_2O), with N_2O emissions from urine/dung patches and synthetic fertiliser contributing ~50% and ~18%, respectively, to soils emissions as of 2019. As nitrogenous fertiliser-based emissions increased by 520% and stocking rates doubled in this 20 year period, the significant increase of soil emissions is largely tangible to the intensification of dairy populations and productivity in this period. While these emissions are not examined further in this study, this correlation between increasing soil emissions alongside dairy's intensification highlights that an efficient use of productive resources may be crucial in New Zealand's wider management of agricultural emissions.

2.2.1 The New Zealand greenhouse gas inventory

For New Zealand, all GHG emissions are estimated and inventoried by sector on behalf of the Ministry for the Environment (MfE). Within New Zealand's current (2021) GHG inventory report, emissions are quantified and assessed by domestic and industrial sources between 1990-2019, and it is calculated that agricultural emissions contribute 48% of New Zealand's total GHG profile (MfE, 2021). As not all GHG are equal in their CO_2-equivalent warming potential, understanding the root sources, types and quantities of GHG being emitted from a sector are crucial to reducing emissions at wider scales.

Within New Zealand's agricultural emissions profile, CH_4 and N_2O, the respective primary GHG released from pastoral agriculture, were considered to have warming factors of 28-32 CO_2-equivalent and 260-300 CO_2-equivlant units, respectively (Reisinger, 2018). Resultantly, CH_4 and N_2O are considered to constitute 73% and 20% of the whole agricultural sectors' total emissions respectively (MPI, 2021). Enteric CH_4 therefore can be assessed to represent such a significant emission source within New Zealand's pastoral sector due to both its potency and its scale of production.

The most significant driving factor of enteric CH_4 production has long been established as DMI (Blaxter & Clapperton, 1965) and the current GHG inventory of New Zealand assumes that enteric CH_4 production is a linear function of DMI (at the animal level), as shown in equation 2.1.

Equation 2.1 Summary model for dairy cattle CH_4 inventory emissions (MfE, 2021):

$$\mathrm{CH}_{4\text{-Enteric-Dairy}} = DMI \times \frac{MCR}{1000}$$

Where:

$CH_{4\text{-Enteric-Dairy}}$ = The CH_4 emissions from enteric fermentation released from cows over a given period. Typically expressed over time as (kg CH_4 / cow / year).

DMI = Dry matter intake(s), a standardised measurement of feed intake which excludes water weight. MCR = Methane conversion rate (constant) of ingested feed. The MfE GHG inventory assessment assumes that 21.6 g of CH_4 is released per kg DMI by dairy cattle in New Zealand (Pickering et al., 2022).

When applied, this model has estimated that the average New Zealand dairy cow emits 89.5 kg CH_4 / year, assuming these cattle are fed pasture only (MfE, 2021). However, as 97% of the nation's dairy farms are not defined as pasture-only systems (DairyNZ, 2021a) and an estimated 18.8% of New Zealand's total dairy feed is currently derived from supplements (MPI, 2016; Sangster, 2022), the accuracy of this model needs to be tested. As mentioned in section 1.0 *General introduction*, research in historical and contemporary settings, both within New Zealand and internationally, has indicated that there may be a strong effect of diet quality on enteric CH_4 emission rates in ruminants (Blaxter & Clapperton, 1965; Beauchemin et al., 2008; Arndt et al., 2022). This study's investigation therefore lies in further examining the robustness of this MCR value of 21.6 g CH_4/kg DM eaten by dairy cattle by examining whether offering differing feeds in differing quantities to pasture-based dairy cattle may affect their MCR.

2.2.2 New Zealand's commitment to climate change

In response to international signals for climate change action, the central government of New Zealand has set national and international commitments to significantly reduce the nation's total carbon footprint. Most notably for New Zealand's dairy farmers are the nation's signing of the international Paris agreement (2015) alongside previous and subsequent domestic enactments to reduce the nation's GHG emissions by -50% by 2050 (Wright, 2016). In summary, the Paris agreement principally dictates that agreeing nations must reduce their environmental footprint in effort to limit global warming to no more than 1.5°C above pre-industrial revolution levels, without reducing their food productivity (Leahy et al. 2020). The Zero-carbon act has further legislated this overarching global target within New Zealand to set a series of refined domestic emission rate targets. These include the requirement of on-farm GHG emissions accounting by January 1st, 2025 (National Party, 2023; NZAGRC, 2023) as well as on-farm CH_4 reductions targets of -10% of 2017 emission levels by 2030, and between -27 to -47% by 2050, respectively (Reisinger et al., 2017a).

These emission reduction targets have been met with some controversy, with particular commentary emerging around the ambitiousness of these targets and New Zealand dairy's relatively small existing carbon footprint across the world (FAO, 2010; Ledgard et al., 2020). While these

legislative tools will ensure that farmers are able to produce sustainably in future, there are currently few farm-ready strategies for significant enteric CH_4 mitigation which can be implemented in pastoral farming systems (Leahy et al., 2020; National Party, 2023) and therefore these targets represent a large concern to New Zealand's livestock farmers (Reisinger et al. 2017b). This concern may be considerably magnified by the potential introduction of an emissions trading scheme to New Zealand (National Party, 2023) and increasing climatic variances which in turn may significantly affect the profitability and productive sustainability of New Zealand's pastoral farming sector (Frame et al. 2018). These concerns magnify the importance of researching, quantifying, and implementing applicable CH_4 mitigation strategies on New Zealand farms, as well as ensuring the accurate accounting of future on-farm CH_4 emissions for the future sustainability of New Zealand's dairy sector.

2.3. Overview of ruminant digestion and methane

As outlined in section 2.2.1 *The New Zealand greenhouse gas inventory,* CH_4 emissions from all cattle are assumed to be able to be estimated by multiplying DMI with a constant CH_4 conversion factor of 21.6 g/kg DMI. However, the extents and rates of digestion in the rumen, the end products of fermentation as well as the level of intake and have been discussed as significant factors of CH_4 conversion rates (Blaxter & Clapperton, 1965; Brask et al., 2015). To examine how different feeds and diets may alter CH_4 emissions from dairy cattle through the means listed above, a wider exploration into the digestive physiology, fermentative processes and the production of CH_4 in the rumen are firstly required.

The rumen and the associated stomach chambers of the ruminant differ greatly in structure and function from the foregut of monogastric animals, as shown in Figure 2.2. These physical differences largely stem from the ruminant's ability to convert large volumes of fibrous plant material into the necessary nutrients to support animal maintenance, growth, and milk/meat production (Van Soest, 1983).

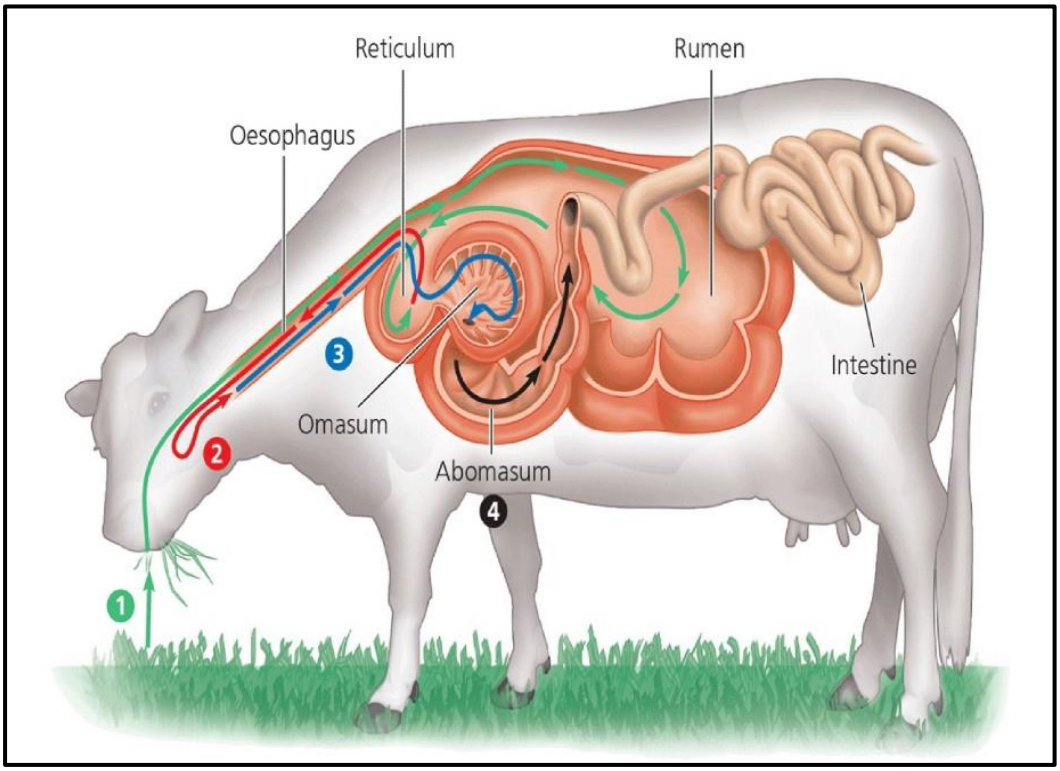

Figure 2.2 An overview of the ruminant foregut structure, showing the route of digesta. Sourced from Urry et al., (2021).

As shown in Figure 2.1, ingesta flows through the four compartments of the foregut and beyond into the small intestines in four steps. Step 1 shows the animal grazing and swallowing forage via the oesophagus into the first compartment of the foregut, the reticulum, which sits beyond the cardiac sphincter and is continuous with the second chamber the foregut, the rumen. This step (green arrows) also demonstrates how feed is mixed continuously between the reticulum and rumen during contraction cycles and bulk fermentation. Step 2 shows the return of mixed and partially fermented ingesta back to the reticulum where it is regurgitated, ruminated, and re-swallowed for further fermentation in the rumen. Steps 3 and 4 then show that sufficiently fermented ingesta that is of small enough particle size (typically 1-2mm^2 in cattle) will be recollected into the ventral region of the reticulum and diverted through to the omasum via the reticulo-omasal orifice. Large sheet-like laminae within the omasum remove 30-60% of fermented ingesta's water content (Van Soest, 1983) before passing ingesta into the abomasum (via the omasal-abomasal orifice) to denature remnant protein structures and washed-out microbes using acidic secretions and proteases (Akers & Denbrow, 2008).

As up to 89% of all CH_4 emitted from ruminants is produced in the rumen from microbial fermentation, and this CH_4 is released by exhalation and eructation of the animal by mouth and nose (Hook et al. 2010). This thesis will therefore further examine the relationships between ruminal

fermentation and mitigating CH_4 emissions, rather than focusing on the emissions tied to completed digestion which includes those tied to faeces and urinary outputs.

2.3.1 Structure and function of the rumen

The holistic function of the reticulorumen (the continuous rumen and reticulum) is to provide substrate (ingesta) and a supporting environment (the rumen) for the sustainment of microbial colonies (Church, 1993). In exchange, rumen microbes yield fermented precursors of animal energy, protein and lipid production which establishes a symbiotic relationship between the host animal and the microbial community within the rumen (Wolin, 1979; Van Soest, 1983). To best facilitate this microbial digestion, the rumen is (generically) divided into five sacs, which can be seen in Figure 2.3 below. Each of these sacs (defined by supportive folds and structural pillars) contributes towards the mixing of ingesta during contraction cycles for efficient fermentation of fibrous feeds, the eructation of gases resultant of fermentation and/or the sorting of fermented ingesta towards the reticulo-omasal orifice for further digestion (Van Soest, 1983).

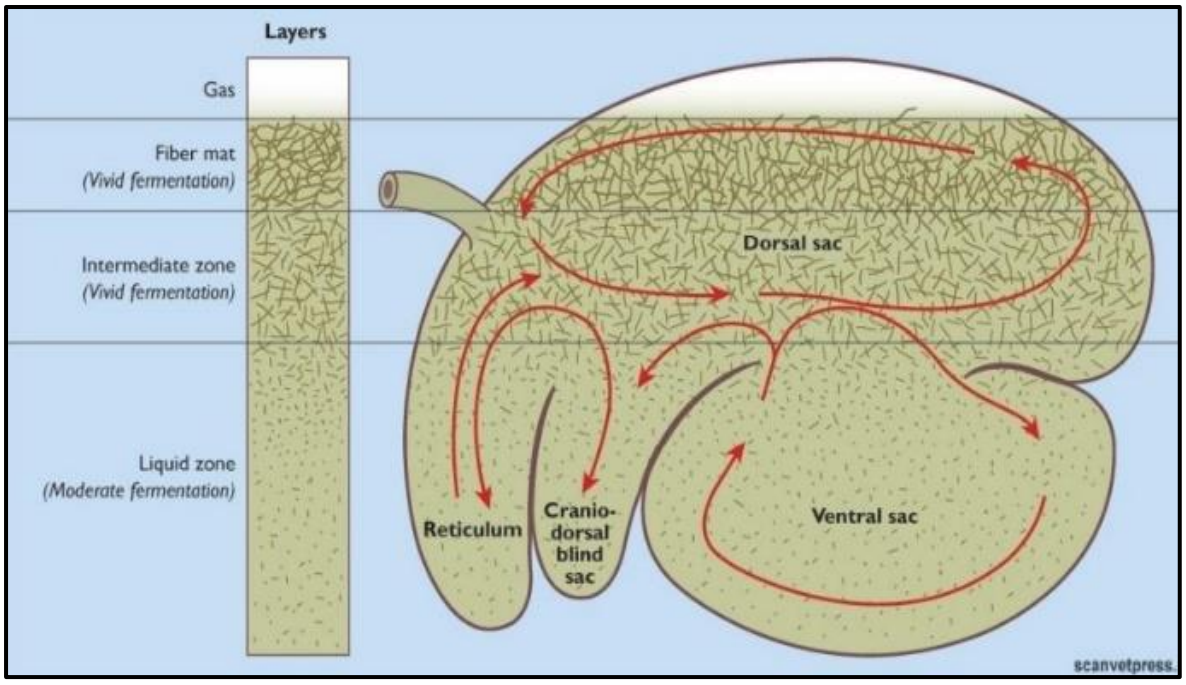

Figure 2.3 An overview of ingesta flow through the rumen during contractions (red arrows). Sourced from Sjaastad et al., (2012).

Moving caudally from the reticulum, the dorsal and ventral sacs of the rumen can be seen to comprise the two larger compartments of the rumen. The dorsal sac in particular contains the gaseous build ups released during fermentation and suspends the buoyant factions of fibrous ingesta. The ventral sacs of the rumen contain the liquid layer of the rumen and suspends the rumen's digestive microbes along with smaller, denser fractions of ingesta (see Figure 2.3). In particular for grazing

ruminants like *Bos Taurus,* which indiscriminately graze on less nutritive dense forages than browsing ruminants, the dorsal sac is further divided into dorso-caudal and dorso-cranial regions. This further increases the volume of the rumen within cattle for greater bulk collection of ingesta and the rapid expulsion of gases produced from this feeding behaviour. In an adult (500 kg) dairy cow, the combined reticulum and rumen may have a total volume of >80L and may comprise <16% of the animal's liveweight when filled (Church, 1993).

To ensure that this large lumen space is constantly facilitating an efficient level of fermentation and to allow gaseous build-ups to be eructated from the rumen regularly during active digestion, the sacs of the rumen enters phases of synchronised dilation and contraction (Ruckebusch & Tomov, 1973; Dijkstra, 2005). Figure 2.3 shows the completed actions of the biphasic A-wave contraction cycle (red arrows), which disrupts the fibrous mat within the dorsal sac for increased microbial attachment and initiates both the mixing and regurgitation of ingesta for rumination. B-wave contractions (not shown in Figure 2.3) then force the gases collected in the dorsal sac during A-wave cycles towards the oesophagus for eructation which prevents bloat in the animal (Church, 1993).

A-wave contractions commence with a sharp contraction of the reticulum, followed by a caudal pattern of contractions across the sacs of the rumen. This ensures that the phases of sac contraction and dilation occurs in tandem to encourage mixing of ingesta (e.g. as the dorsal sac contracts and disrupts fibrous ingesta there, the ventral sac is dilated to receive this mobilised ingesta) (Church, 1993). In turn, this resultant mixing allows finer ingesta fragments to be directed caudal-ventrally into the reticulo-omasal orifice, saturates larger ingesta fragments within the fibrous mat layer to trap these pieces in the liquid layer for greater fermentation and draws longer fractions of this fibrous mat layer into the dorsal region of the reticulum. Secondary contractions of the A-wave cycle then arrange this drawn ingesta past the cardiac sphincter and into the oesophagus where ingesta can be bolused and regurgitated to the oral cavity via anti-peristaltic motions of the oesophagus (and pressure differentials between the oral cavity and the thoracic inlet during contraction cycles) for rumination (see step 2, Figure 2.2) (Van Soest, 1983).

Rumination acts as a key mechanism to further degrade the fibrous fractions of ingesta using mastication and salivation. Continued mastication of ingesta bursts cell contents and likewise allows the separation of cell walls to increase saturation, microbial attachment, and fermentation upon re-swallowing. Rumination also provides a significant opportunity for the animal to provide more pH

buffers and saccharolytic enzymes into the rumen through salivation, as to buffer the pH of the rumen environment and further aid microbial activity (Sjaastad et al., 2010).

As A-wave contractions occupy the oesophagus during the rumination, this prevents the escape of the enteric gases yielded from fermentation. The removal of these gases ensures that the host animal does not experience bloat and likewise ensures that the dynamic equilibrium of fermentation is not disrupted within the rumen (Van Soest, 1983). A-wave contractions do however encourage the collection of CO_2 and CH_4 into the dorsal and caudo-dorsal blind sacs, as part of disrupting the liquid and fibrous layers of the rumen. These collected gases can be directed towards the relaxed cardiac sphincter for eructation once regurgitation has finished through B-wave contractions. These eructation's, which typically occur every 50 – 60 seconds in the active digesting *Bos Taurus* act as the main vector for CH_4 release from the rumen, with over 90% of CH_4 typically being eructated following active fermentation (Van Soest, 1983).

2.3.2 The rumen environment

Expanding from the structural layout of the rumen described in 2.2.1 Structure and function of the rumen, the epithelial linings of the reticulum and the rumen have developed to support microbial production, attachment and for animal nutrient absorption. Both organs' epithelia are described as stratified and squamous, with the corium cells of this membrane lining being arranged in a strong, scale-like formation which is more pronounced in the more resilient honeycomb structure of the reticulum's lining (Akers & Denbrow 2008). These epithelial linings also support the develop of papillae in both organs of the reticulorumen. However, the larger papillae of the reticulum are typically more involved in processes of catching ingesta (for direction to the oesophagus or omasum or for collection and containment of foreign bodies within the reticulum) in comparison to the smaller papillae of the rumen which performs much of the reticulorumen's nutrient absorption (Church, 1993).

Papillae, the long finger-like extensions which make up the brush border of the rumen, are a larger extension of villi which are common to the epithelia of many organs with the mammalian digestive tract. These brush borders increase surface area of digestive organs to facilitate greater permeability of aqueous nutrients into the aorta (Akers & Denbow, 2008), as this passive nutrient absorption is dependent on ionic concentration gradients between the liver, the portal vein, and the rumen (Church, 1993). Increased vascularity to the epithelia and mucosa of the reticulorumen supports the dispersion of this ionic gradient and increases the transportation rates of nutrients to

liver. In turn, this greatly increases the rumen's ability to rapidly absorb nutrients, including <80% of all substrates involved in energy production within the host animal (Van Soest, 1983).

Looking beyond the physical features of the rumen, a chemically and ecologically balanced environment must be maintained within the rumen to encourage sustained microbial function and animal digestion. Hallmarks of a healthy and sustainable rumen environment include the provision of temperatures between 38-42° C, a pH of 5-7, a potential for reduction (E_h) at -250 to -450 megavolts which denotes an overall tendency to donate hydrogen/electrons in the rumen (depending on pH) and an oxygen content of <0.6% (by volume of gases within the rumen) (Van Soest, 1983; Church, 1993). The rumen also turns over its contents completely approximately three times in two days (Wolin, 1979), replacing the liquid within the rumen <15 hourly (Van Soest, 1983). The combination of these factors were essential to ensuring that the colony of microbes within the rumen naturally developed to be both anoxic and short-lived, therefore ensuring this digestive colony cannot compete for the same energy substrates used for animal respiration (Wolin, 1979).

If these biological and chemical conditions are not satisfied, the environment and/or the microbes will pragmatically attempt to rebalance the rumen to sustain optimal fermentation. For example, the rumen environment may be able to buffer against a low pH within the rumen by increasing passage rates through the foregut and/or through increased secretions of pH buffers in the saliva or bile (Wolin, 1979). Similarly, within the colony of rumen microbes, a permanent population of facultative anaerobes is sustained to consume excess oxygen, which may be trapped within fibrous structures ingested by the animal. Both these instances highlight symbiotic relationships existing between the host animal and the population of microbes living within the rumen, which ultimately ensures that rumen microbes can yield the end products which are desired by the host animal (Wolin 1979; Church, 1993).

2.4. Principles of ruminal fermentation

Most ruminal microflora, with particular emphasis on those which yield energy metabolites for the host animal, can be generalised as being anaerobic and highly competitive free-floating bacteria. As with all simple-celled organisms, these microbes' primary purpose is to ingest and modify substrates to produce energy (as Adenosine Triphosphate; ATP), for respiration and to yield constituents required for microbial reproduction (Dijkstra et al., 2005). However, while most ruminal bacteria are anaerobic, true anaerobic respiration utilises alternative oxidising agents (such as nitrates or sulphates) which are not freely available in the rumen due to this environment's negative E_h (Wolin,

1979). Furthermore, many of microbes within the rumen (which also commonly includes protozoa, fungi, and archaea) are simple celled and therefore lack the mitochondrion necessary to perform the complex chain of electron exchanges involved within the citric acid cycle for oxidative ATP production (Van Soest, 1983). Instead, most ruminal bacteria complete their metabolism through fermentation, an alternative process of anaerobic ATP generation.

Fermentation is initiated with the glycolysis, the catabolism of glucose through glycolysis, which begins after microbes (typically) attach to their ingesta source using external filaments (glycocalyxes or hyphae in the case of fungi) (Urry et al., 2021). Successful attachment of microbes then allows enzymatic exudes to start hydrolysing substrates down to monomers for cellular uptake and conversion to glucose (Church, 1993). Per unit of liberated glucose, glycolysis yields one unit of pyruvate, two units of ATP (for the fermenter) and two net units of nicotinamide adenine dinucleotide dehydrogenase (NADH) (Mathison et al., 1998). To complete fermentation from glycolysis, yielded pyruvate will be further reduced to end by-products like volatile fatty acids (VFA) by microbes. This acts as a means for microbes to yield the oxidising agents required for the re-synthesis of nicotinamide adenine dinucleotide (NAD) from NADH and rebalance cellular oxidising agents. This final resynthesis completes the fermentation of one monomer of glucose, allowing by-produced VFA to then be absorbed by the host animal for metabolism and oxidative ATP production (Akers & Denbrow. 2008).

Typically, over 200 different species of bacteria exist within the bovine rumen and this bacterial colony usually exceeds 50% of the rumens' total biomass (Dijkstra, 2005; Balance et al., 2014). Bacteria within the rumen are primarily classified by the types of substrates they preferentially metabolise, or by their most prevalently yielded end product(s) from fermentation. Following this generalisation, the most common type of ruminal bacteria can be described as being saccharolytic (complex carbohydrate or lignocellulosic polymer digesting), given that most of the ruminant's diet is comprised of complex carbohydrates (Baca-Gonzalez et al., 2020). Church (1993) proposed to define the rumen's microbial colonies into the eight following groups: cellulolytic (cellulose and hemicellulose digesting), pectinolytic bacteria (pectin and some hemicellulose digesting), amylolytic (starch digesting), simple-sugar digesting, intermediate acid-utilising (using lactate, formate or succinate digesting), proteolytic (amino acid separating and protein digesting), ammonia-producing, lipolytic (which typically degrade long-chain triglycerides and phospholipids into shorter-chain fatty acids) and methanogens (methane-producing archaea).

As a generalisation, the stoichiometry of fermentation and subsequent yield of cellular ATP is limited the rate of carbohydrate ingestion by the host animal (Dijkstra et al., 2005). Following the general dynamics of all diverse microbial colonies, bacterial counts within the rumen tend to rise sharply when fermentable substrates are introduced to the rumen, usually peaking within the first four hours following a feeding event at up to 10^{10} - 10^{11} cells per g of rumen contents (Church, 1993). Ruminal microbes are also very competitive in their feeding behaviours, both inter and intra-specifically, which can be observed through their specific order of attachment onto ingesta or metabolic substrates. For instance, cellulolytic bacteria will attach to fibrous material (cell walls) readily and more strongly than other saccharolytic bacteria. However, amylolytic and simple-sugar digesting bacteria will outcompete cellulolytic bacteria in their attachment to the ruptured contents of cells (Church, 1993). Holistically, these bacteria provide ecological services for each other, however this interspecific competition also encourages greater digestibility of ingesta within the rumen and greater total fermentation per kg DM, yielding higher metabolite production for host animals as a result (Van Soest, 1983).

2.4.1 Fermentation end products

When investigating the diets of dairy cattle, the componentry of feeds are commonly disseminated into the macronutrient groups of carbohydrates, proteins and lipids which are utilised by different rumen microbes in differing affinities or quantities (Church, 1993; Holmes et al., 2002). While the digestive function and metabolic pathways for ingested proteins and lipids are outlined in brief within this chapter, these macronutrients serve as relatively minor inputs to enteric CH_4 production within pastoral diets. The fermentation of carbohydrates is largely viewed as the most significant vector of enteric CH_4 production, and the examination of carbohydrate fermentation will be a primary focus of this thesis (Ransom et al., 2017).

For the productive ruminant, crude protein (CP) intakes are strongly correlated with muscular skeletal growth and milk production, as well as being essential for regulating rumen microbial populations (Holmes et al., 2002). Ruminants are unique animals in the sense that they are able to self-synthesise a broad spectrum of required and essential amino acids, through both the digestion of washed out rumen microbes and through the absorption of amino acids yielded by rumen microbes themselves (Church, 1993). As much of CP uptake occurs beyond the rumen, ingested CP is disseminated into pools of rumen-degradable protein, microbial protein and rumen non-degradable protein, along with the transformed pool of recycled non-protein nitrogen (Dijkstra et al., 2005).

Rumen degradable protein includes the readily available units of peptides, and amino acids which are hydrolytically liberated from ingested organic material by proteolytes, with the carbon skeletons of this process readily entering gluconeogenic metabolism in the host animal. Feeds high in rumen degradable protein are associated with high levels of rumen microbial stimulation, as microbes will readily assimilate free amino acids in the rumen and as glucogenic skeletons are of high energetic value to the ruminant animal (Church, 1993). Conversely, undegradable rumen protein along with washed out rumen microbes (the digested portion of microbial protein) describes the fraction of ingested proteins which escape the rumen. Undegraded proteins in the rumen can either be fully degraded in the abomasum or small intestines, to provide essential and non-essential amino acids to the host animal or may escape digestion in the ruminant animal entirely (Dijkstra et al., 2005). Finally, non-protein nitrogen describes a continuously circulating inorganic pool of ammonia, ammonium, nitrates and urea, which is derived from further catabolism of glycogenic amino acids in the rumen. When rumen degradable protein is insufficient for microbial requirements, non-protein nitrogen is readily utilised in the rumen to stimulate microbial production. When microbial protein is sufficient, non-protein nitrogen can also be absorbed by the liver and converted to urea and recycled between the liver, blood stream and salivary glands. Non-protein nitrogen can then return to the rumen in salvia where it is utilised for microbial uptake, or it is redirected to the kidneys for excretion in urine (Church, 1993; Van Soest, 1983).

Lipids (also written as ether extract or crude fat, expressed on a DM% basis) typically constitute a very small fraction of the foraging ruminant's diet, with leafy forages typically containing between 2-5% of their DM% as lipids (Church, 1993; Dijkstra et al., 2005). Phospholipids (the inner plant cell membrane components) and triglycerides (the waxy substances of cell walls) comprise the two major sources of lipids within forages. However, a host of fatty acids, including glycolic and linolenic acids, may be prevalent in oil seed by-products, pasture seed head, grains or other stock feeds (Rattray et al., 2007). Rumen microbes tend to aggressively hydrolyse and saturate lipids to long chain free fatty acids and glycerol before being passed beyond the rumen and absorbed in the small intestines (Dijkstra et al., 2005). While glycerol can be converted to VFA in the rumen and is associated with increased energy production in the host animal, hydrogenised free fatty acids which are absorbed in the ileum are strongly associated with increased body or milk fat production in the animal. Lipid ingestion is highly associated with increased energy production rather than fatty tissue deposition in the host animal (Holmes et al., 2002).

While protein and lipids can be argued as being minor contributors to energy yields within the rumen and for methanogenesis in pasture-based diets, it is important to recognise these macronutrients as integral factors of digestibility. Broadly referring to the rate and extent of disappearance between ingesta and excreta, digestibility is heavily determined by the composition and ratios of carbohydrates, fats and proteins in the diet. As such, many contemporary methods of calculating the digestibility and metabolisable energy contents of feeds (such using SCA, 1990) heavily factor protein, lipid and carbohydrate fractions of the diet into quantifying how much energy the ruminant animal can liberate from a foodstuff.

2.4.2 Carbohydrate fermentation

Carbohydrates typically comprise 50-80% of the dry matter content (DM, as %) of plant cells. The primary fermentative products of carbohydrates are VFA, with rumen- derived VFA contributing 66-85% to the host animal's energetic requirements (Ransom et al., 2017) and therefore acting as the predominant substrates for both microbial ATP synthesis and animal metabolite production. Carbohydrates can be broadly grouped into being slowly fermentable structural carbohydrates, which are the most common constituents in the walls of plant cells, as well as non-structural carbohydrates (NSC) which are mostly comprised of rapidly fermentable cell contents, as shown in Figure 2.4.

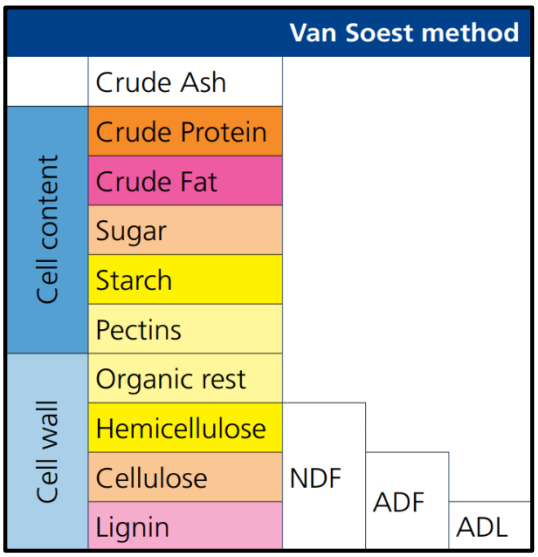

Figure 2.4 Diagram of the organic matter within the wall and contents of a plant cell (Foss, 2018).

Within Figure 2.4 neutral detergent fibre (NDF) is defined as including fibrous but fermentable hemicellulose alongside poorly fermentable cellulose and undigestible lignin, to act as a key measure of voluntary feed intakes. Conversely, acid detergent fibre (ADF) is defined as including only poorly

digestible cellulose and indigestible lignin and is useful for quantifying how much of the diet is indigestible. These can be contrasted with NSC which include pectins, sugars, fructans, organic acids (including skeletons of lipids and crude proteins) and starch (Van Soest, 1983; Holmes et al., 2002). As a broad generalisation, nutritively dense NSC fractions of the diet are released, fermented and metabolised to completion more readily and rapidly than fibrous fractions of the diet. In turn, the ratios of NSC : NDF within productive ruminants' diets can help to describe many important factors of voluntary feed intakes as well as the efficiency and rates of digestion within the rumen.

To initiate carbohydrate fermentation, rumen microbes attach to their preferred contents of organic matter within structural and non-structural carbohydrates and enzymatically catabolise these macronutrients to monosaccharides (or disaccharides) for fermentation. Cellulose and starches are catabolised into monomers of glucose for fermentation, while fructans, pectins and hemicellulose are readily broken down to various hexoses and pentoses (like fructose, sucrose and malate) before being uptaken (Van Soest, 1983). As a general rule, cytosols of bacteria take these monomers up, convert these to pyruvate and to complete fermentation for cellular ATP production (as described in section 2.4 *Principals of ruminal fermentation*). The major by-products of completed carbohydrate fermentation in the rumen are utilised by the host animal for oxidative ATP generation and are generalised as being acetate (C_2), propionate (C_3), and butyrate (C_4). While pyruvate acts as the major intermediary for the production of C_2, C_3 and C_4, different microbes within the rumen's eight major taxa favour the production of differing VFA. This diversity and responsiveness within the rumen's microbial colony allows the ruminant to efficiently ferment an array of complex plant-based diets. However, when it is further considered that differing taxa favour the fermentation of differing macronutrients, a trend of differing VFA outputs emerges across differing diets. A simple example of this is seen through increased C_2 production from increased cellulolytic bacterial fermentation within high fibre, forage-based diets as opposed to high starch diets, which tend to favour increased intermediate acid utilising bacteria activity and yields of C_3 and C_4 (Church, 1993)

While all three of the ruminant's primary VFA can act as intermediary substrates for energy production, each VFA differs slightly in their metabolic uptake and preferred pathways for the host animal use. Once absorbed to the liver via the portal vein, C_2 readily enters the citric acid cycle within animal cells for ATP synthesis. Likewise, surplus C_2 is a common precursor for fatty acid synthesis which is used for adipose tissue and milk fat production in the ruminant animal (Dijkstra et al., 2005). Butyrate also readily enters the citric acid cycle once absorbed, however C_4 is also readily converted to ketones during epithelial absorption from the rumen for local energy generation. Propionate differs

slightly in its usages from C_2 and C_4, wherein it is the only primary VFA which is gluconeogenetic and is a significant driver of satiety which C_2 and C_4 do not significantly affect. Propionate is 75% calorically denser than C_2 and can be used for production of sugars alongside ATP production. Therefore, increased C_3 production is strongly associated with increased milk or tissue production from increased lactose (and/or protein) production in milk or the efficient energy transfer of C_3 to ATP (Hungate, 1966). The completed fermentation pathways of the primary VFA considered in this thesis are expressed in Equation 2.2 (provided from Van Soest 1983):

Equation 2.2 The completed fermentation pathways of glucose to VFA in the rumen.

$$Glucose \leftrightarrow Pyruvate + Hydrogen$$
$$C6H12O6 \leftrightarrow 2C3H4O3 + 2H2$$

$$Pyruvate + Water \leftrightarrow Acetate + Carbon\,Dioxide + Hydrogen$$
$$C3H4O3 + 2H2O \leftrightarrow 2C2H4O2 + 2CO2 + 4H2$$

$$Pyruvate + Hydrogen \leftrightarrow Propionate + Water$$
$$C3H4O3 + 2H2 \leftrightarrow 2C3H6O2 + 2H2O$$

$$Pyruvate \leftrightarrow Butyrate + Carbon\,Dioxide + Hydrogen$$
$$C3H4O3 \leftrightarrow C4H8\,O2 + 2CO2 + 2H2$$

(→ indicates completed microbial fermentation)

(← indicates chemical transfer of VFA to pyruvate if required as an alternative energy substrate for microbes)

To quantify how VFA productivity changes with differing diets, the primary VFA produced by ruminants are commonly expressed in standard molar ratios. These ratios can be broadly generalised between diets, e.g. VFA produced from roughage-based diets which tend to favour C_2 production approximate a ratio of 70 : 20 : 10 (C_2 : C_3 : C_4) (Hassanat et al., 2013). Whereas the VFA ratios yielded in grain-based diets may theoretically increase C_3 production up to ratios of 50 : 40 : 10 (C_2 : C_3 : C_4) (Church, 1993; Sun et al., 2015).

2.5. Methanogenesis

Enteric CH_4 production (methanogenesis) was outlined in section 1.0 *General introduction*, as a by-product of ruminant fermentation. However, when examining Equation 2.0, methanogenesis can be further defined as an extension of carbohydrate fermentation (Church, 1993). At its centre,

methanogenesis describes the means in which methanogens produce CH_4 from carbon skeletons (such as CO_2 or formate) and surplus dissolved hydrogen (H_2), to perform respiration. (Wolin, 1979; Church, 1993).

When examining Equation 2.2, it is observable that during carbohydrate fermentation into C_2 and C_4 production, H_2 and CO_2 are released within the rumen. While surplus CO_2 production is inert and passable from the rumen without issue, H_2 production reduces the rumen's E_h and requires sinking to maintain the stoichiometric balance of the microbial environment. Methanogenesis can therefore offer an optimised pathway to the buffering of E_h to provide an ecological service to the rumen (Ungerfeld, 2020) while also opportunistically allowing greater ATP synthesis per carbon atom ingested by utilising CO_2 or alternative carbon skeletons of VFA production (Baca-Gonzalez et al., 2020)

Methanogenesis is specifically performed by methanogens, anaerobes which belong to the simple-celled archaea kingdom of micro-organisms. These archaea generally comprise between 0.3% - 3% of total ruminal biomass across 10 main taxa (Baca-Gonzalez et al., 2020). Within a meta-analysis of methanogen taxonomy across 32 animal species, Henderson et al., (2015) found that the taxa of bacteria and archaea within rumen colonies was fairly constant across 742 samples of rumen contents across global ruminant species. In particular, Henderson et al., (2015) noted that the *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* clades consistently comprised up to 74% of rumen archaeal biomass in most ruminant species and are the most prevalent methanogens within the *Bos* genus (Morgavi et al., 2010; Henderson et al., 2015). The absence of significant colonies of other methanogenic archaea outside of these primary two taxa reflects how methanogens favour and require a narrow range of fermentable substrates in few metabolic pathways. The predominant respiration pathways for *M. gottschalikii* and *M. ruminatium* form the generalised methanogenesis pathways are listed in equation 2.3 (Van Soest 1983):

Equation 2.3 The predominant methanogenic pathway of *M. Gottschalikii & M. Ruminantium* (Knapp et al., 2014)

$$Carbon\ Dioxide* + Hydrogen \rightarrow Methane + Water$$

$$CO2 + 4H2 \rightarrow CH4 + 2H2O$$

(→ indicates respiration)

*Formate or ethanol may also be facultatively utilised by most methanogens to perform respiration in lieu of CO_2 (Greening et al., 2019; Beauchemin et al., 2022)

When considering Equations 2.2 and 2.3 together, it is observable that stoichiometry of enteric CH_4 production is largely dependent on the bioavailability of H_2, which is in turn dependant of the VFA balances yielded from the animal's diet. In section 2.2.2, *The New Zealand greenhouse gas inventory*, DMI was stated as being the most significant determining factor of enteric CH_4 productivity. This is fundamentally underpinned by the notion that as feed intakes increase, carbohydrate intakes (as the predominant substrate of ruminants' diets) increase alongside total VFA yields in the rumen and resultantly total CH_4 production per day. However, equation 2.2 and section 2.4.2 *Carbohydrate fermentation* highlight that enteric CH_4 production is determinate of more than just DMI and factors which affect the bioavailability of H_2 or CO_2, such as diet compositions and subsequent VFA yields in the rumen, may alter CH_4 production (Crompton et al., 2011).

2.5.1 Patterns of methanogenesis

While enteric CH_4 is strongly determinate of DMI at a daily production level, researchers such as Crompton et al., (2011) and Jonker et al. (2017) have also established that CH_4 production dynamically responds to feeding rate. Grazing and browsing ruminants, like cattle or sheep, are crepuscular and typically feed most intensively at dusk and dawn. In response, it can be theorised these periods of increased feeding may correlate to periods of increased fermentation rates in the rumen and associated periods of increased bioavailability of both H_2 and CO_2 for methanogenesis (Muetzel et al., 2014).

This effect was examined by Crompton et al., (2011) by assessing the response of CH_4 emissions (as L/min in this study) across different feeding frequencies (number of meals per day). The study identified insignificant changes to DMI and total emissions per day with increasing feeding frequency. However, a strong relationship between the immediate rates of CH_4 production and time after feeding was identified, with emissions generally peaking at 40 – 140 minutes after eating. Jonker

et al., (2017) conformationally found that the rate of CH_4 emissions from dairy cattle peaked shortly after an intensive feeding period, and generally declined as animal gut fill was reduced. The summarised findings of Crompton et al., (2011) can be seen in Figure 2.5, which demonstrates this effect of feeding frequency on the pattern of CH_4 production within a day.

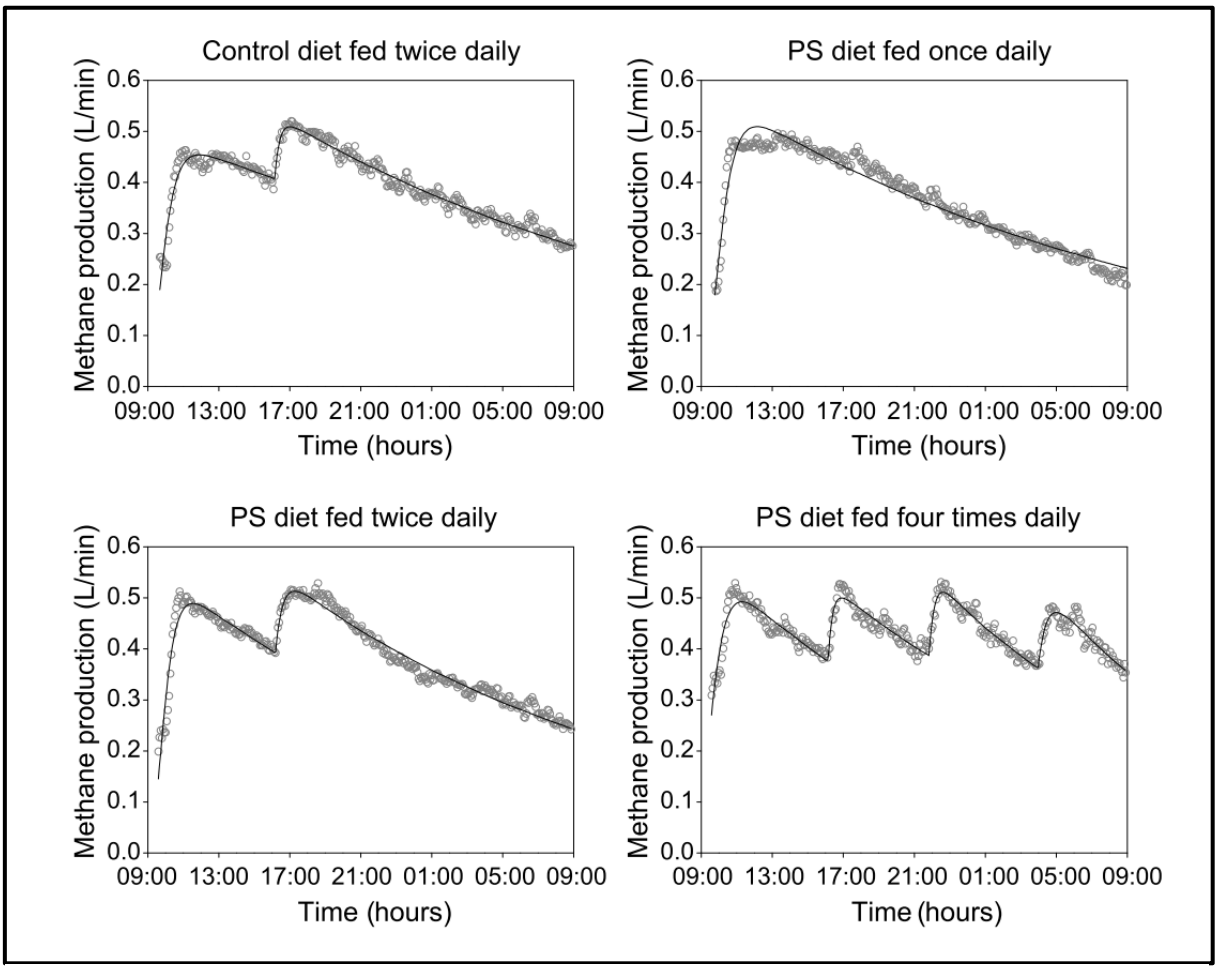

Figure 2.5 The effect of feeding frequency on methane production (litres/minute) in lactating dairy cattle. Sourced from Crompton et al., (2011) (PS: Protein supplemented diet).

Understanding when ruminants emit CH_4 is significant when measuring animal emissions and evaluating the effectiveness of any CH_4 mitigation technique, as capturing the curvilinear rises and troughs of an animal's emission rates is crucial to capturing and expressing its total daily emissions (Hammond et al., 2016a). This is particularly pertinent to farmed ruminants which display modified behaviours that affect the timing of feedings. For example, pastoral dairy cattle are generally not fed constantly and are often offered several smaller, more intensively eaten allocations of feed between milking events each day. This could therefore be expected to result in several smaller peaks of CH_4 production each day, which can be targeted for mitigation through various techniques.

2.6. Overview of contemporary methane mitigation techniques

While methanogenesis has long been argued as integral inefficiency to maintain healthy rumen function, researchers have held significant interest in disrupting the balance of CH_4 production in the rumen without significantly affecting animal performance or health since the 1970's (Blaxter & Clapperton, 1965; Ungerfeld, 2020; Beauchemin et al., 2022). A variety of mitigation methods which exploit various factors of methanogenesis have been investigated for efficacy, adoptability and wide-spread implementation in agricultural systems since 1995 (Boadi et al., 2004). Contemporary reviews of CH_4 mitigation methodologies suggest that a 20 to 40% reduction target of enteric CH_4 production is feasible in farmed ruminants without significant productive losses (Gerber et al., 2013; Hristov et al., 2018). However, short-term *in-vivo* studies have been able to demonstrate up to 98% reduction of enteric CH_4 production in sheep using methanogen inhibitors (Kinley et al., 2020), suggesting that very high reductions to methanogenesis are possible. However, the sustainability of some CH_4 mitigation techniques, such as inhibitory compounds, have been criticised for potentially affecting animal health, productivity or leaving residues in animal products which are unsafe for human consumption (Beauchemin et al., 2022).

Mitigation techniques of methanogenesis can be broadly disseminated into several categories. Many mitigation tools, such as vaccines or inhibitory compound usage, may functionally inhibit the metabolism of methanogens, whereas feeding alternative diets which may favour less C_2 production, changed substrate availability or contain bioactive secondary compounds may also alter the potential for methanogen function. Fundamentally, all CH_4 mitigatory tools can be categorised as agents which reduce the availability of H_2 and reduce the potential for CH_4 production (Boadi et al., 2004) or inversely inhibit methanogen activity by disabling their uptake of H_2. Functionally, these groupings describe whether a mitigatory tool favourably sinks and removes H_2 from the rumen by outcompeting methanogens for the reducing agent (Pacheco et al., 2014), or whether it inhibits methanogens function without a sink which is marked by surplus H_2 being belched from the rumen.

Current research focuses relevant to enteric CH_4 mitigation within New Zealand's pastoral sector include the usage of methane inhibitors, such as vaccines (Leahy et al., 2019). Methane vaccines function by causing treated animals to produce methanogen specific antibodies in the salivary glands. This in turn allows a constant delivery of these antibodies into the rumen during ingestion and active digestion (e.g. during rumination) when methanogenesis typically peaks. Successes of *in-vivo* CH_4 vaccine studies typically range between 8% - 66% decreases in CH_4 production (corrected for DMI) (Baca-Gonzalez et al., 2020; Hristov et al., 2022). However, despite these successes in controlled

experiments, effective implementation of many intensive mitigation methods into farm systems has been criticised. With unfeasibly labour and financial costs or high levels of animal handling, monitoring or controlled dosing often being required for CH_4 vaccination plans which may not suit many pastoral farmers (Beauchemin et al., 2020).

Inhibitory compound treatments, such as 3-nitrooxypropanol (commonly abbreviated as 3-NOP) or bromoform, inhibit the metabolism pathways of methanogens to effectively starve the archaea of ATP so long as these compounds are present in the rumen (Hristov et al., 2015). Again, inhibitory compounds have proven to be very effective within *in-vivo* studies, typically reducing CH_4 emissions by 35% - 98% (Hristov et al., 2015; Kinley et al., 2020). For New Zealand's pastoral farming systems however, the successful adoption of these inhibitors can again be called into question, with 3-NOP needing to be eaten in each mouthful to be an effective CH_4 mitigator (Leahy et al., 2019). Re-dosing ruminants in housed systems may be a feasible practise (e.g. by blending the compound into feed), however this may prove to be a substantially more difficult task in pastoral systems (Beauchemin et al., 2022). Careful storage of some inhibitory substances may also be barrier for implanting in pastoral systems, with natural *Asparagopsis* feed-additives requiring freezing to contain the active bromoform within the seaweed until feeding (Lee et al., 2018). The widespread adoption of more passive CH_4 mitigation techniques, while potentially less effective and non-additive when implemented with other mitigation tools (Gerber et al., 2013; Beauchemin et al., 2022), may however provide New Zealand pastoral farmers with a sustainable first step towards reducing their emissions.

2.7. Overview of nutritionally manipulating enteric methane

When discussing the effectiveness of CH_4 mitigation techniques, CH_4 productivity can be statistically reported and interpolated in several statistical terms. This thesis will report enteric CH_4 emissions on gram bases of daily CH_4 production (M_p; g CH_4 / day) , CH_4 yield (M_y; g CH_4 / kg DMI; also denoted as the CH_4 conversion rate or MCR in section 1.0 *General introduction*), and as CH_4 intensity (M_i; g CH_4 / kg of fat and protein corrected milk). Each of these terms are defined further in section 3.1. *Introduction and research objective*, however it is important to acknowledge the importance of discussing these terms together, as changes to one metric of CH_4 emissions may or may not correlate (or have opposite direction) with others. These three terms are reported together as the major findings of this thesis in Table 4.4.

2.7.1 The relationship between nutrition & methane production

All of the enteric CH_4 manipulation methods mentioned in section 2.6 *Overview of contemporary methane mitigation techniques* fundamentally act by reducing the potential for methanogens to complete cellular respiration by altering the availability or uptake of H_2. Likewise, in section 2.5 *Methanogenesis* and *2.5.1 Patterns of methanogenesis*, CH_4 production was discussed as being an ecological service which helped maintain the rumen environment's stoichiometry alongside being strongly linked to both the extents, types and rates of fermentation occurring in the rumen (Van Soest, 1983; Robertson & Waghorn, 2002; Boadi et al., 2004; Pacheco et al., 2014). Therefore, like any other mitigation tool which farmers could employ, each of these factors of methanogenesis can be strategically leveraged through nutrition to reduce CH_4 production in the ruminant animal.

Beauchemin et al., 2020 theorised that enteric CH_4 production equates to a loss of 2-12% of gross energy (GE) from the rumen and that at a macro level, manipulating enteric CH_4 through dietary strategies can be described as 'clawing back' some of this inefficiency of fermentation. This has been supported by a review written by Arndt et al., (2022) which theorised that CH_4 emissions can be sustainably reduced by up to 17% by increasing feeding levels in pastoral or housed ruminants.

Increasing the efficiency at which farmed ruminants convert GE into ME, or convert feed into meat and milk rather than CH_4, is often related to decreasing the ratio of $C_2 : C_3$ from feeding diets of increased feed value. For pastoral dairy farmers in New Zealand, increasing the feeding value of the diet can be commonly associated with practises of increasing forage quality or supplementing concentrated feeds to productive animals. At this agricultural level, increasing the feed value of the diet, often expressed as the megajoules of metabolisable energy per kg DM (MJ ME / kg DM), can be strongly correlated with a greater overcoming of the animal's hierarchal maintenance energy requirements and an associated increase in partitioned energy for animal performance (per kg DM eaten) (Rattray et al., 2007; Capper et al., 2009; Beauchemin et al., 2020). At this macro level, it can be assumed that diets of greater feeding value (or feeding level) can increase animal outputs (e.g. milk production) per level of DMI and M_p, reducing M_y by making ruminants more efficient eaters and producers (Pinares-Patino et al., 2009; Knapp et al., 2014).

This effect of increasing feed value to dilute maintenance energy requirements is however over-simplistic in the sense that it does not consider the effects of changing diet composition/quality on overall digestibility in the rumen, which in turn affects M_p and particularly M_y. For instance, Gerber et al., (2013) and Veneman et al., (2015) found that when increasing the feed value of cattle's diets by including >4% of DMI as lipids, a significant depression of DMI was reported due to an increased level of satiety from lipid feeding. As a result, in both studies, increasing feed value was found to

significantly decrease animal performance due to DMI reductions and although M_p decreased with DMI, digestibility of the diet increased alongside increases to M_y and intensity leaving these animals less CH_4 efficient. The digestibility of the ruminant's diet, as a stronger indicator of feed utilisation, DMI, the end products of fermentation, rumen pH and ingesta passage rates may therefore offer a comprehensive explaining variable of how nutrition can manipulate enteric CH_4 production.

2.7.2 The relationship between digestibility & methanogenesis

Apparent digestibility, whether expressed as total DM% digestibility (DMD, as g / 100g feed), organic matter digestibility (OMD, as g / 100g feed), or as the digestible organic matter within DM% (DOMD, as g / 100g feed), measures and quantifies the extent of ingesta disappearance through the ruminant's digestive tract. In section 2.2.1 *The New Zealand greenhouse gas inventory*, DMI was stated as the main driver of daily CH_4 production. However, Brask et al., (2015) identified the extent of feedstuff fermentation within the rumen as the primary factor of M_y, which in turn can significantly influence daily CH_4 production. In an examination of feedstuffs' effect on M_y, Janssen (2010) identified ingesta passage rates through the rumen, the carbohydrate composition of ingesta and subsequent VFA ratios produced in the rumen and rumen pH as shared major factors of both H_2 production and resultant methanogenic activity in the rumen.

As stated in section 2.3.2 *The rumen environment*, solid contents within the rumen can be expected to turnover three times in every two days (Wolin, 1979). Quantifying the disappearance rate of ingesta through the rumen is a complex matter, encompassing many interconnected variables of feed and animal characteristics (Holmes et al., 2002) which are beyond the scope of this thesis. For the purpose of enteric CH_4 mitigation however, increasing the NSD : NDF ratios of the diet is associated with a decrease of H_2 availability for methanogen activity through the factors identified by Janssen (2010).

Ingesta passage rates tend to decline in feeds which are structurally and chemically complex. This applies to many (but not all) forage-based diets which are generalised as containing greater amounts of structural carbohydrates and lower ratios of NSC : NDF. Feeds which require greater digestive effort to degrade and ferment, in turn leads to both slower fermentation rates and therefore longer rumen retention times. Conversely, grain-based diets which usually contain greater ratios of NSC : NDF and consist of smaller particles, can be more readily degraded for rapid fermentation and passage beyond the rumen (Knapp et al., 2014). Ingesta passage rates can also be positively influenced by increasing feeding frequency and DMI by decreasing the animal's total apparent digestion of the

diet (Janssen, 2010). However, as readily fermentable components of the diet (like NSC and CP) are far less likely to be passed beyond the rumen undegraded, declines of apparent digestibility are more likely to stem from significant decreases of structural carbohydrate fermentation. These effects of feed composition and intake rate on ingesta flow rates greatly influence the rate of fermentation in the rumen and M_Y through a series of effects.

At high enough levels, increased feeding rates and/or increased DMI by offering more palatable and digestible diets, could be expected to increase CO_2 and H_2 availability immediately after feeding, due to a greater availability and faster fermentation of readily fermentable substrates (like NSC) in the rumen (Crompton et al., 2011). However, this feed behaviour could decrease total ruminal fermentation of the diet, decreasing CH_4 emitted per kg DMI. In addition, increasing the DMI or feeding frequency of high NSC feeds would likely release VFA into the rumen environment faster and of lower ratios of C_2 : C_3 (also written as acetate : propionate ratio, or A : P) to sink H_2 away from CH_4 production (Boadi et al., 2004). Increasing the NSC contents of feed can therefore be expected to yield lesser CH_4 / kg DMI as an isolated effect, however, as VFA production rates are considered to be the main driver of rumen pH (Wang et al., 2020), a rapid release of VFA from diets' high in NSC may exceed the rumen's ability to buffer pH, further affecting the rumen's potential for enteric CH_4 production.

As stated in section 2.3.2 *The rumen environment*, the rumen attempts to maintain a pH of around 6 or greater (Tapio et al., 2017) and it is generally accepted that rumen pH can sustainably drop to as low as 5.5 – 5.6 without triggering acidosis (Jansen, 2010; Hassanat et al., 2013). Mediation of low rumen acidity is achieved through constant removal of acidic products via nutrient absorption as well as the constant addition of pH buffers from saliva (Boadi et al., 2004). However, the removal of acidity can be overcome by rapid VFA releases, especially as the need for rumen motility (which drives the passive transfer of liberated VFA to the rumen's epithelia for absorption), decreases as diets become more digestible (Church, 1993). Likewise, saliva inputs to the rumen can decrease with the reduced requirement for rumination of highly digestible feeds. Together, these effects may trigger significant (temporary) drops of pH in the rumen immediately after feeding highly digestible diets. Wang et al., (2020) explored this concept by fermenting high forage (70% forage by DM) and high concentrate (35% concentrate by DM) ingesta in six cannulated Holstein-Friesian cows. The high forage diet decreased rumen pH from 6.9 – 6.06 at four hours after feeding, while the high concentrate diet reduced rumen pH from 6.84 to 5.76 in the same time period. With both methanogens and cellulolytic bacteria widely noted as being particularly pH sensitive (Mathison et al., 1998; Sun et al.,

2015; Beauchemin et al., 2020), sharp declines of pH can significantly inhibit the production of C_2 and CH_4.

In agreement, Knapp et al., (2014) and Sun et al., (2022) found that total-tract NDF digestibility may decrease by up to 50% when rumen pH decreases to 5.5. This was further tested by Wang et al., (2020) to find that the C_2 : C_3 ratios of high forage diets increased from 4.21 - 4.67 after four hours of fermentation, while the C_2 : C_3 ratios yielded from the high concentrate diet reduced from 2.56 - 1.91 in four hours. The increase of rumen pH after four hours of fermentation in the high forage diet is partially explained by the expected rumen motility and rumination required to break-down this feed, which efficiently removes VFA and acidity from, and adds buffers to, the rumen (Wolin, 1979; Church, 1993). In the high concentrate diet, an increased potential for C_3 production and H_2 sinking from dietary starches alongside sharp decreases in rumen pH after feeding inhibited methanogen and cellulolytic activity for significant periods after feeding without adversely affecting these animals so long as the rumen is still able to buffer and mediate acidity.

To summarise the effects and relationships between digestibility and enteric CH_4 production, digestibility increases within the ruminant's diet are typically seen as increases of plant cell contents to plant wall contents and can be instrumentally gauged by the NSC : NDF ratios within the diet. For the production of enteric CH_4, the formation of both H_2 and CH_4 are negatively associated with increased C_3 production, low rumen pH and increased ingesta flow rates which are all associated with diets of increased digestibility at high enough inclusion levels (Ulyatt et al., 1976; Janssen, 2010, Sun et al., 2022). High inclusion rates of high NSC feeds are able to destabilise the rumen's homeostasis and significantly induce these effects of increased ingesta flow and decreased pH to disrupt the potential for H_2 and CH_4 production. However, the extent to which this can be achieved is disputed among researchers, with Janssen's (2010) review surmising that unless grain comprises half the ruminant's diet, CH_4 production is likely to be unaffected and that small inclusions of high NSC feed might drive further fermentation and CH_4 in the rumen.

2.8. Dietary scenarios to alter methane production

In recent years, many researchers have examined how diets of differing composition and digestibility affect M_p, M_y and M_i in productive ruminants. Diverse pastures (Jonker et al., 2019), herbs such as narrow-leaf plantain (Della Rosa et al., 2022a), bulb crops such as fodder beet (Jonker et al., 2017), leafy brassicas such as forage rape (Sun et al., 2015; Della Rosa et al., 2022b) or grain products such as corn silage (Hassanat et al., 2013), have all been examined for their effects on enteric CH_4

production. When examining each of these studies, total DMI/d, inclusion rate (as kg DM/d or % of DMI) of treatment feeds, indicators of digestibility including NSC : NDF ratios within the diet, rumen pH, changes in animal productivity (as liveweight change or milk production change) and changes to emissions (e.g. the ratio of CO_2 to CH_4 emitted) comprise some key identified traits to contextualise how these feeds may impact CH_4 productivity. Similarly, factors of age, breed, genetics, changes to animal liveweight or productivity and differences in CH_4 measurement techniques may be identified sources of error, both random and fixed, when comparing results across these studies and may all impact the variances of M_p, M_y and M_i under different dietary conditions (Beauchemin et al., 2020).

Della Rosa et al. (2022a) examined the effect of feeding narrow-leaf plantain or perennial ryegrass on CH_4 production in non-lactating dairy cattle. The study found that feeding plantain (DMD% = 75.7%) as opposed to perennial ryegrass (DMD% = 83.5) decreased M_p by 23.2% and M_y by 14.8%, without significantly affecting rumen pH or the C_2 : C_3 ratio within the rumen. In this instance, decreases to M_y were supported by the increased ratios of NSC : NDF within the pure plantain diet, with 1.16 g NSC / g NDF being observed in this study's plantain diet and 0.46 g NSC / g NDF being observed in this study's ryegrass diet. Lower DMD was responsible for driving decreased M_y in this instance and this study demonstrated that feeding a high feed value diet of less digestibility significantly decreased M_y.

Jonker et al., (2019) investigated the differences in CH_4 productivity from dairy cattle grazing diverse (containing ryegrass, white clover, lucerne, chicory and plantain) and traditional (perennial ryegrass-based) pastures. The study found that animals grazing diverse pastures had a statistically significant 10% increase in M_y, compared with similar animals grazing traditional pastures. Although the diverse pasture swards were similar in chemical composition to the traditional swards, greater digestibility of plants like chicory or plantain in the mixed pasture may have contributed to greater digestibility in the rumen, greater fermentation and greater CH_4 releases per kg DMI. However, this is offset by a significant +9% increase in milk production measured in animals grazing diverse pastures despite no significant change in DMI, numerically decreasing M_i.

In contrast to these studies, Della Rosa et al., (2022b) and Hassanat et al., (2013) reported that as the inclusion rate of forage supplements increased in grazing-based diets, this resulted in a curvilinear decrease in M_y. As a general trend across these studies, as higher levels of supplements were added into animals' diets, DMI and associated M_p values rose before rapidly declining at higher levels of supplementation. Della Rosa et al., (2022b) found that substituting perennial ryegrass with

forage rape in lambs significantly decreased rumen pH from 7.1 to 6.7 and 6.2 at 75% and 100% inclusion rates, with this turning point of pH decreasing C_2 : C_3 ratios in the rumen from 4.1 to 2.9 in both 75% and 100% inclusion rates groups. At low to moderate inclusions of forage rape in the diet (<50% of DMI / day), Della Rosa et al., (2022b) found insignificant numerical increases to M_p and DMI, with the high NSC : NDF ratio of forage rape encouraging greater intake and fermentation in the rumen until rumen pH was significant affected. However, including forage rape at 75% and 100% of lambs DMI were found to decrease M_y by 11% and 44%, respectively.

Research by Hassanat et al., (2013) confirmed these points by demonstrating a reduction in M_y from lactating dairy cattle without affecting M_p or milk production, by substituting lucerne silage with corn silage within a total mixed ration diet. A 27% inclusion of corn silage (as % total DM) significantly increased DMI, M_p and insignificantly increase of M_y. However, a 54% inclusion rate of corn silage was found to destabilise the rumen environment to decrease M_y from 20.3 g CH_4/kg DMI in the control group to 17.7 g CH_4/kg DMI in treated animals. These levels of corn silage inclusion also saw significant increased C_3 production by 33% (from 19.9% of total VFA in the control treatment, 21.4% of total VFA at 27% inclusion of corn silage and 26.4% of total VFA at 54% inclusion of corn silage). This was observed alongside a decrease in mean pH from 6.31 in the control treatment, to 6.27 and 6.07 at 27% and 54% corn silage inclusion rates, respectively.

2.9. Supplementation & methanogenesis

Supplement feeding (supplementation) in pastoral farming can be broadly described as the action of providing any additional non-pasture feed to grazing livestock (Rattray et al., 2007; DairyNZ, 2023). Supplements commonly used in New Zealand include conserved forages (e.g. pasture or maize silage), homegrown fodder crops (e.g. brassicas, lucerne or chicory), grains or grain products such as concentrates, or by-products of other food/fibre/manufacturing enterprises (e.g. palm kernel extract or distiller's grain) (DairyNZ, 2016). High feed value supplements, like concentrates, are typically used in pastoral dairy systems to support animal energy requirements at peak lactation or to support increased liveweight gain after drying off (Rattray et al., 2007). However, supplements can also be used to support animal energetic requirements during periods of low pasture growth (e.g. feeding pasture silage in summer to support mid-season lactation or growing winter crops to reduce grazing intensity on farm prior to peak pasture growth in spring) (Martin & Sneddon, 2023). Grazing animals' productive response to supplement feeds are denoted through the effects of addition and substitution, which describe and quantify how supplement feeding may affect pasture intakes and total daily DMI. Substitution is measured as a ratio of reduction in pasture intake (relative to no

supplementation) divided by supplement intake (kg DM pasture decrease / kg DM supplement fed), with high substitution rates describing a poor DMI response to supplement feeding. Conversely, addition describes an increase of animals' total daily DMI through supplement feeding (Bargo et al., 2003).

While supplements are typically used to provide feed of adequate quality or quantity in the shoulders of the pastoral season; the tactical usage of supplements in the interests of farm profitability and productivity is a strongly debated topic (Penno et al., 1996; Bargo et al., 2003; Rattray et al., 2007). Before utilizing supplement feeds, farmers should rationally always consider the cost and benefits of tactical supplement usage. These may consist of assessing animal productive responses to supplement feeding (e.g. the additions of kg liveweight gained or kg milk produced per kg DM of supplement offered), as well as substitution rates of pasture (Penno et al., 1996). Literature on this topic has consistently demonstrated that supplement feeding in periods of high pasture quality and quantity typically yield poor responses to milk production (per kg DM supplement fed) and high levels of substitution (Holmes et al., 2002; Rattray et al., 2007). As a result, a poor response to supplements and a poorer utilization of lush spring pasture (for spring block calving farmers) may often prove a cost ineffective exercise for farmers seeking profitable milk production (Holmes et al., 2002; Bargo et al., 2003). In many scenarios therefore, greater milk yield responses may be attained from supplement feeding in mid-late lactation animals, when pasture growth and quality is typically throughout the summer months in New Zealand. Irrespective of lactation stage, animal response to supplement usage and substitution must always be considered when tactically deciding to utilize supplement feeds. For instance, popular by-products like palm kernel expeller, may seem like a cost-effective means to many farmers to support animals in mid lactation, however the typically high NDF content of palm kernel expeller (DairyNZ, 2023) may further restrict the feed intake of animals and drive a very high rate of substitution. Likewise, feeding low quantities of high protein supplements, such as soybean meal, may often be cost ineffective in pasture-based dairy systems where C_3 production typically limits milk production as opposed to crude protein intakes (Holmes et al., 2002).

While typical effects of feed composition on substitution rates and milk yield responses from supplement feeding are well established within New Zealand's pastoral sector, the effects of supplementation on CH_4 production are still uncertain. As explored through section 2.8 *Dietary scenarios to alter CH_4 production,* different feeds with differing compositions can influence M_p and M_y differently. Supplementation of dairy animals, particularly with concentrate-based diets still therefore holds a somewhat uncertain potential for affecting animal CH_4 emissions (Olijhoek et al., 2018).

Olijhoek et al., (2018), in agreement with Church (1993), found that including cereal-based concentrates into the diet at or above 35% of DMI overcame the rumen's ability to buffer pH and significantly decreased M_y as rumen pH approached 5.5. Likewise, Janssen (2010) suggested that inclusion rates of concentrates less than 50% of the total diet may not affect CH_4 production and quantifying enteric CH_4 reductions under concentrate supplemented diets within pastoral settings can be challenging and may require further research (Beauchemin et al., 2009; Knapp et al., 2014).

2.10. Measurement of enteric methane

As mentioned in section 2.2.2 *New Zealand's commitment to climate change*, the accurate measurement of enteric CH_4 plays an important role in reporting CH_4 emissions at the farm level. Likewise, precise measurements also allow accurate quantification and ranking of any treatment effects on enteric CH_4 production rates and patterns (Della Rosa et al., 2021). The desired outcomes of all studies involving the measurement of enteric CH_4, whether *in-vitro, in-vivo* or *in-situ,* invariably centres around the accurate collection of emissions data from either a known quantity of fermented substrate or from an individual animal's M_p from a known DMI or time period.

The gold standard of all instruments used to measure *in-vivo* CH_4 emissions have historically been respiration chambers (RC), with other common measurement devices being sulphur-hexafluoride tracer gas slow-release boluses (SF_6) (Johnson et al., 1994) or spot measurement technologies such as the GreenFeed unit (GF) (C-Lock Industries, n.d.). The latter two technologies, while potentially being less precise than RC units, notably allow both *in-vivo* and *in-situ* study designs to be possible, providing opportunities for many findings which were previously unattainable with RC alone (Waghorn & Jonker, 2020)

Significant interest and discussion around the accurate use of SF_6 and GF systems in contrast to RC systems has followed in recent decades. Understanding how each of these different CH_4 measurement instruments operates and how they may best fit differing research methodologies is therefore an important factor in New Zealand's wider effort to quantify the emissions from ruminants (Jonker & Waghorn, 2020). Likewise, the exploration into measurement equipment and techniques which allow *in-situ* research in tandem with *in-vivo* RC units may allow more rigorous field testing, troubleshooting and ranking of different enteric CH_4 mitigation strategies (Huhtanen et al., 2015; Hammond et al., 2016a; Zhao et al., 2020).

2.10.1 Comparison of methane measurement technologies

Respiration chambers, or calorimetry chambers, have been utilised by agricultural researchers for over 120 years, having first being designed to collected gaseous emissions from individual animals as a partial metric of energy balances (Hammond et al., 2016a). Contemporarily, RC units are commonly referred to as the gold standard of CH_4 measurement equipment (Huhtanen et al., 2015) because all gasses emitted are captured and analysed with precise equipment (Della Rosa et al., 2021). Used in tandem with gas concentration measuring instruments, open-circuit respiration chambers ventilating ambient air through a given vessel at a precisely measured rate. This allows both real-time measurements of gas concentrations (on a volumetric basis) and a spatial basis (i.e. on a time basis) to be recorded from an individual animal (Pinares & Waghorn, 2014). Continuously drawn sub-samples from chamber air inlets and outlets are analysed using near-infrared sensors in this process as to record changes in gas concentrations from ambient air fed into the chamber and air circulated out of the chamber, which includes expelled gases. Knowing these concentrations of inlet and outlet gases, along with the air exchange rate (adjusted for temperature, humidity and pressure) of chambers in turn allows for precise calculation of M_p rates (Jonker & Waghorn, 2020). Figure 2.6 shows the schematics of a single cattle respiration chamber in use at the New Zealand Agricultural Greenhouse Gas Research Centre, note that the air outlet draws ambient air into the chamber and air outlet is connected to a near-infrared sub-sampler (not pictured).

Because M_p can be recorded from individual animals at a fine spatial resolution (as ppm CH_4/L of sub-sampled air recorded every ~3 minutes per chamber treatment effects on M_p can be isolated and observed to examine how significantly M_p peaks immediately after a feeding event. Likewise, M_p can be summarised to a daily output and daily (quantified as g CH_4/d of sub-samples passing through sensors) and can use to interpolate M_y and M_i if feed offerings (as kg DM/d) and both milk production and composition are also recorded during a study (when applicable). Other commonly recorded gases for analysis within RC unit experiments include CO_2 to infer any changes of CO_2 (as an additional marker of enteric fermentation) or the molar ratio of CH_4 : CO_2, along with O_2 and H_2 to examine any inhibition of CH_4 in the rumen (Zhao et al., 2020).

Common RC designs allow gaseous concentrations of inlet/exhaled gases to be accurately monitored in each chamber at 3 – 5 minute intervals to form 24-hour continuous datasets (Pinares & Waghorn, 2014). Similarly, Della Rosa et al. (2021) found across 230 reported studies that animals were typically placed in RC units from between 1 - 3 continuous days at a time to ensure that data across chamber measurement days were strongly correlation ($R^2 = 0.94$) and that DMI could be reliably

recorded using RC, as the first day of placement within individual chambers may stress animals and reduce intakes.

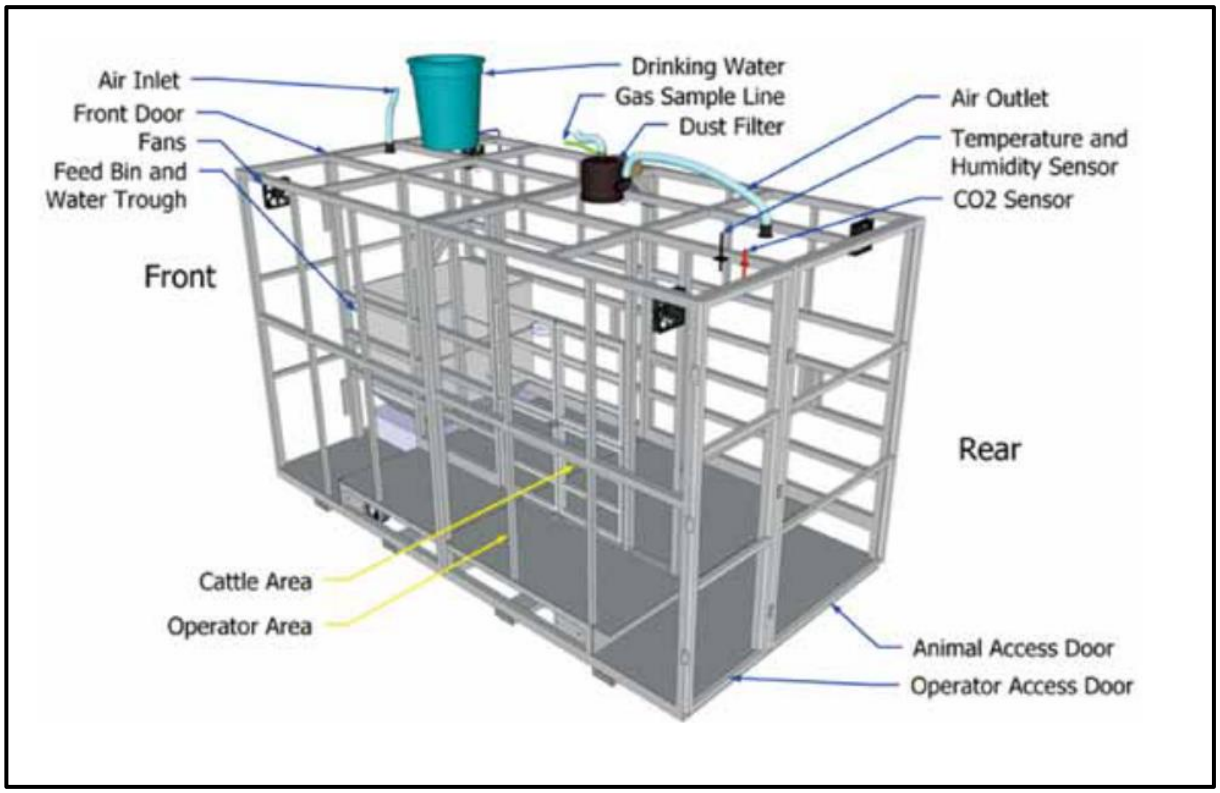

Figure 2.6 The layout of a cattle respiration chamber at the New Zealand Agricultural Greenhouse Gas Research Centre, AgResearch Grasslands, Palmerston North, New Zealand. Sourced from Pinares & Waghorn, 2014).

The high rate of accurate data collection in RC (i.e. 1 recording / 5 minutes excluding 2 hours for feeding times may yield up to 264 unique datapoints per day for individual animals) alongside a regular calibrating of gas analysing sensors, allows this methodology to detect the circadian rhythms of animals' emissions with the ability to significance test the upper and lower limits of an animal and/or their treatment's effect with a high level of confidence (Della Rosa et al., 2021; Beauchemin et al., 2020). Such inherent accuracy and repeatability were crucial in establishing the clear linear relationship describing how enteric CH_4 emissions may be estimated by DMI (using different feeding amounts as a treatment) as g CH_4 / kg DMI (Pacheco et al., 2014; Zhao et al., 2020). However, the limitations of RC units start to become evident once a high volume of animal throughput is desirable (Jonker et al., 2020) or when infrastructure costs are to be minimised. The throughput of animals can be relatively slow with RC units, however as fewer animals and shorter time spans (e.g. 2-days per measurement) are required to accurately and precisely detect animal emissions, RC units could be argued as being a cost-effective and efficient testing methodology in an established research facility

(Della Rosa et al., 2021). Respiration chambers do however necessitate the removal of animals from their natural environment, which may potentially impact their DMI and skew calculations of M_p as a result (although M_y estimates remain accurate through RC measurements). In particular, RC units are limited for usage when seeking to investigate parameters relevant to *in-situ* studies, as RC units require forages to be cut and carried to chambers as opposed to allowing animals the ability to graze forages. Similarly, estimates of M_i in lactating animals are only able to be ascertained at a snapshot within an animal's productive season, as it is often not cost-effective to operate continuous long term/life-time studies through RC units (Jonker et al., 2020; Jonker & Waghorn, 2020).

While RC units provide a crucial methodology to validate a potential mitigation tool's effectiveness, or for accounting the enteric emissions of ruminants, they cannot robustly test mitigation tools designed for *in-situ* use in isolation. Likewise, RC units are unsuitable for repeat measure analyses studies as they are limited in their ability to continuously detect changes to animal metabolic parameters over time (e.g. identifying changing metabolic requirements as lactation stage changes), or to measure emissions from large groups of animals simultaneously. Alternative measurement methods, like SF_6 or GF, are therefore presented as potentially viable options for quantifying emissions from ruminants under some study conditions which RC are not optimised for (Jonker & Waghorn, 2020).

The SF_6 tracer technique provided the first recognised means to measure CH_4 emissions from individual animals within the field (Johnson et al., 1994). This technique utilises a known and controlled slow release of inert SF_6 from a permeation tube which is bolused into an animals' rumen.

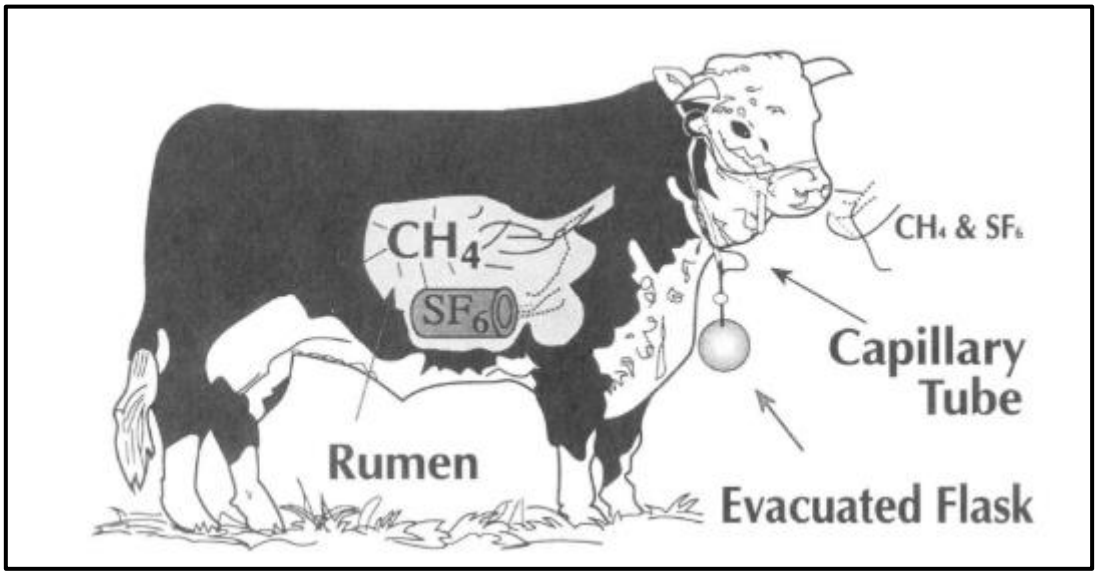

Figure 2.7 The original layout of a SF_6 bolused and haltered grazing cattle beast. Sourced from Johnson et al., (1994).

Expired SF_6 from this tube is exhaled and eructated by the animal constantly over time. Using tubing positioned adjacent to the mouth and nostrils, expired gases are directly siphoned into a collection canister suspended on the animal, either via a halter around the animal's head or on a harness on the animal's back. Figure 2.7 depicts Johnson et al., (1994)'s original permutation tube and halter arrangement.

Once filled, cannisters can be replaced on animals and collected SF_6 (as a tracer of CH_4) and CH_4 within a full cannister can then be analysed using gas chromatography. Molar mass ratio calculations are then used in combination with the known daily release rates of SF_6 and collected gas concentrations ascertained from chromatography to estimate CH_4 emissions over time (Johnson et al., 1994). Della Rosa et al., (2021) noted that the SF_6 technique can be comparably accurate to RC measurements in its ability to estimate M_p, and SF_6 studies typically require four days of continuous measurements to ascertain a high level of confidence in results. The technique also allows both simultaneous recordings to be made across an unlimited sample group (depending on equipment availability) within a single research event (Velazco et al., 2016) and for experimental animals to move freely within *in-situ* or *in-vivo* studies (e.g. to measure DMI in a partially controlled environment). However, the spatial resolution of measured M_p rates using the SF_6 technique can only be set to the rate at which gas collection vessels are replaced and the precise estimation of M_p is generally accepted as being lesser than that of RC measurements. Therefore, SF_6 studies can typically only assess M_p at a 24-hour resolution and cannot capture the circadian patterns of animal emissions.

C-Lock's GF units can be described as being versatile in their CH_4 researching applications, with similar attributes to both RC and SF_6 techniques. Developed during the early 2000's, GF units provide what can be described as an unobtrusive means to measure enteric CH_4 (Jonker et al., 2019), by measuring CH_4 through an integrated calculation of air flow and concentration (flux) which mimics the open-gas circuitry of RC technology (Jonker & Waghorn, 2020). Unlike RC units however, GF units are portable for *in-situ* research, collecting spot samples of animals' CH_4 emissions in sporadic and repeatable short-term measurement events (Hammond et al., 2016a). As spot sample measurements can be collected freely by animals across the day, studies utilising GF units can capture the circadian patterns of emissions from individual animals like RC units and likewise allows unrestrictive animal movement and animal to recording equipment interaction like SF_6 studies (within reason). Up to 40 animals per GF unit is recommended within *in-situ* experimental designs, as to ensure the collection of a statistically significant amount of data across a mob of animals is achievable in an optimally short measurement period (Della Rosa et al., 2021).

Functionally, GF units are comprised of two integrated devices: an automated, single stall feeder mounted to a fixture (i.e. a trailer when used in grazing studies) with an attached, covered CH_4 measuring device. An example of a trailer-mounted GF unit is depicted in Figure. 2.7, in which a cattle beast is visiting. This animal depicted in Figure 2.8 is currently eating from a feed-plate within gas measurement hood (green hood) which is situated below the feed hopper (silver box). The vertical gas collection pipe is seen ascending from behind the hood, from which gas samples are siphoned away and are analysed by a near-infrared sensor within the unit (obscured from view).

Figure 2.8 A trailer-mounted GreenFeed unit driven by a solar charged battery. Sourced from C-Lock Industries (n.d.).

The operation of a GF unit is initiated by the animal placing their head underneath this green hood where an electronic identification tag reader and proximity sensor will trigger the delivery of a small amount of feed to the animal from the hopper (using a rotating cup) and create a measurement session. This action encourages the animal to stay within the unit, defining the unobtrusive aspect of this measuring device, as all data entries collected from the GF unit require willing engagement from animals (Jonker et al., 2019). Once a measurement session is initiated, small amounts of feed are continuously delivered at 15-45 second intervals to maintain the animal's interest in staying beneath the hood, simultaneously an extraction fan will draw any exhaled gases away from the animal while they feed (Jonker & Waghorn, 2020). All collected gas is siphoned through a manifold behind the hood,

up and into the vertical air collection pipe for both flow meter recordings. For gas concentration analyses, small sub-samples of siphoned gases are drawn from the collection pipe for separate analysis using infrared sensors before all collected gas is purged from the system. Recorded flow rates and CH_4 concentrations are finally uploaded to C-Lock's private servers at the end of each spot-measuring event via a small computer within the GF unit (C-lock Industries, n.d.).

Usage of GF units can yield statistically correlative results to data ascertained from RC devices under correct management of the units, e.g. by encouraging animals to regularly visit units throughout each day and encouraging all animals to visit at all hours of the day across the study timeline, thereby facilitating a high level of data collection across the potential fixed effects of the study (animals emissions > hour of day > day of study) (Della Rosa et al., 2021). Huhtanen et al., (2019) built a regression model comparing the relative accuracy of the GF system to that of RC systems across six comparative studies, which saw a very high correlation of M_y measured between studies using GF and RC technologies ($r^2 = 0.92$) (Huhtanen et al., 2019). Similarly, Lee et al., (2022) noted that GF has the capability to estimate M_p emissions accurately, provided that sampling frequency is high and dispersed throughout the day to capture any spatial effects of emissions. This finding in particular is reinforced by Hristov et al., (2015), who emphasised the necessity of animal training to increase animal visitation rates and ensure that circadian rhythms of M_p can be captured across hours of day within a study.

To ensure the retrieval of accurate and confidence-bearing data, prior studies performed with GF units have previously suggested that cows will need to stay under the hood of the GF unit for a minimum of three minutes per measuring session (Jonker et al., 2017). However, Arthur et al., (2017) identified that spot samples recorded using GF units were only significantly inaccurate below two minutes in duration. Subsequent studies have been able to successfully generate statistically significant results using a minimum visit duration of two minutes (Bennett et al., 2021). This ensures that there is sufficient time for the unit to detect at least one eructation event per measurement session and allows the GF gas concentration samplers to distinguish between collected ambient CH_4 and CO_2 (sampled ppm from air), expired CH_4 (heightened ppm from the animal's breath) and eructated CH_4 (very high ppm) (C-lock Industries, n.d.). However, researchers using GF must also consider that the only incentive for animals to visit GF units for this ideal duration is the provision of feed. A balance must therefore be made to ensure that enough feed is delivered to animals over a long enough period to encourage them to remain under the hood of the units, without providing so much feed as to significantly alter animals' diets, which could affect their daily emission results (Jonker

& Waghorn, 2020). Correctly applying settings to control the delay of feed drops within a visit to GF units, along with limits to total drops per visit, delays between valid visits and total visits per day are essential to preventing dominant behaviours of GF visitation by some animals within a study group and further helps to ensure that data captured from GF units is spatially balanced across experimental units and across time of day (Hristov et al., 2015).

Automatic calibrations of near-infrared sensors for the precise outputs of average M_p per visitation, manual gas recoveries to validate automatic calibrations and calibrating of feed delivery rates (as grams per drop of feed and interval between drops) are essential to ensuring the integrity of data collected from GF devices during a study (Hristov et al., 2015). The immediate estimation of CH_4 emissions from information captured during spot samples is performed using Equation 2.3, before CH_4 / L of collected gas can be converted to g CH_4 emitted / time.

Equation 2.3 The calculation of CH_4 spot samples (g CH_4/L) used by GreenFeed devices.

$$CH_{4-Volume} = F_c * C_R * \sum_{tp} \Delta_t * \left(CH_{4avg} - CH_{4bkgrnd}\right) * Q_{air}$$

Where:

$CH_{4\text{-}Volume}$ = The mass flux of CH_4 measured within a spot sample (g CH_4/L); C_R = capture rate of emissions into collection pipe,; Δt = time period over which emissions are measured (1 second); $CH_{4\text{-}avg}$ = average concentrations during the measurement period (ppm); $CH_{4\text{-}bkgrnd}$ = background concentrations of CH_4 (ppm); Q_{air} = airflow rate during the measurement period (flow per unit time); and F_c = dimensional factor.

The effective usage of GF units to measure enteric CH_4 emissions has been controversial to some researchers (Velazco et al., 2016), with potential issues of GF technology's suitability to replace other measurement techniques like RC cited such as: Infrequent daily visitations or insufficiently long visitation events compromising useful datasets (Hammond et al., 2016a), alongside weather disturbances affecting animal willingness to visit units (Velazco et al., 2016), disruption of grazing due to milking times (Huhtanen et al., 2019) or animal bullying around units (Hammond et al., 2016a). However, the ability to measure M_p from animals *in-situ* and detect circadian rhythms (as opposed to the limitations of SF_6 experimental designs) places the GF system in a unique position of value within agricultural GHG measurements. GreenFeed systems have successfully been used in New Zealand's pastoral sector to estimate M_p from growing, dry and lactating cattle under differing pasture-based diets (Jonker et al., 2017; 2019), and even pasture-farmed deer (Bennett et al., 2021) among many other studies (Della rosa et al., 2021). Continued usage of GF units in pastoral agriculture could provide

a versatile and robust means to evaluate the emissions of ruminants under different conditions or treatments for many years to come (Jonker & Waghorn, 2020).

3. Materials & methods

3.1. Introduction & research objective

The methodology of the study reported in this thesis has sought to practicably determine the effect of increasing concentrate intake on pastoral dairy cows' methane emissions and milk production. This was firstly fulfilled by quantifying the average daily CH_4 production (M_p as g CH_4/d) from early-lactation, spring-calved grazing dairy cattle fed increasing rates of a concentrate pellet. Alongside M_p, methane yield (M_y as g CH_4/kg DM) is reported in this thesis using M_p and back calculated estimates of DMI ascertained using the SCA (1990) metabolisable energy requirement (ME_r) method. Likewise, methane intensity (M_i as g CH_4/kg FPCM) acts as the final reported metric of enteric CH_4 production in this thesis, and was calculated using M_p measurements milk production data corrected to fat and protein levels (FPCM as kg/d) stipulated in Subnel et al., (1994)

Reporting each of these metrics of enteric CH_4 production across treatments ensures the robust inferencing and evaluating of how the concentrate feeding affects CH_4 production in the rumen. As noted in section 2.9 *Supplementation & methanogenesis*, M_p may increase (or decrease) with supplement feeding, either due to increased voluntary feed intakes or from associated increases to digestibility. However, increases to M_p under concentrate feeding have been previously noted to be met with increases to DMI and/or milk production, thereby decreasing M_y and/or Mi and indicating an increase of animal efficiency per unit of CH_4 emitted (Jaio et al., 2014; Hatew et al., 2015; Hansen et al., 2022). Quantifying the changes to enteric CH_4 productivity in this range of metrics therefore holds great interest to many parties from researchers involved the field of agricultural emissions to pastoral dairy farmers to evaluate the gross (M_p) and marginal (M_y and M_y) emissions of dairy cattle under differing diets.

3.2. Experimental design

The thesis presents the results ascertained and analysed from a 63 day *in-situ* study staged in a random block design, which commenced on 16/09/22 and concluded on 18/11/22 at Massey University's Dairy No. 4 dairy farm (Massey Dairy 4; Palmerston North, Manawatu, New Zealand). This study utilised 72 experimental units (n = 72), which were stated as being mature dairy cattle that were milked twice daily. This research was approved by AgResearch's animal ethics committee on 12/09/2022 (Animal Ethics Application #0288) in agreement with key individuals at Massey University's Agriculture and Horticulture Enterprise group.

Research activities pertaining to these animals were staged across three successive periods. Firstly, all animals selected to participate in this study were allocated to a treatment group (see section 3.2.1 *Treatment Allocation*) before undergoing a 14-day diet transition. This diet transition altered animals' diets from their pre-study diet (which included pasture, pasture silage, maize silage, soya-hull based pellets and molasses under Massey Dairy 4's 2022 spring feeding planner) to their controlled treatment diets gradually, as to avoid potential nutritional complications (like ruminal acidosis from increased concentrate feeding). Following this transition period, animals were allocated a further 10 days to adapt to their full treatment diets and to start using GF units before beginning of the study's 33 day measurement period.

Data collected during this measurement period included animal CH_4 emissions, DMI of concentrates fed in the cowshed, pellets fed through GF units, samples of offered feeds for diet composition analyses and other animal metabolic data points (i.e., individual animal milk production and liveweight changes). These measured data points were then collated to provide the evidence required to measure M_p and estimate DMI at the animal level, which further enabled estimates of M_y and M_i to be made and tested for significance between treatment groups (see section 3.8 *Statistical analyses*).

Between processes of data collection, curation and analysis, significant reductions of data resolution were experienced in this study due to a range of factors. This was most pertinent with animals that become ill during the study, as these experimental units and all their collated datum were removed from all analysed datasets completely. As described below in section 3.2.2. Animal management, 72 animals were enrolled in the study, with 8 of those animals (2 per treatment acting as spare animals for statistical analyses. 13 animals were removed from the study and it's analyses due to illness or ill-thift (in accordance with AE application) and a further 17 animals' whole datasets were removed due to insufficient GF data collection for accurate CH_4 production modelling (as described in section 3.8.3 GreenFeed data modelling & alternative methane production estimates). The processes of data collection, curation and analysis are described in detail throughout section 3. Materials & methods, however a brief summary of data loss is noted below in Table 3.1.

Table 3.1 Overview of data collected and analysed across all animal and feed parameters in this study.

Group	Total datapoints collected	Datapoints analysed
Concentrate Refusals	2107	1853
Pasture samples	18[1]	18
Pasture silage samples	7[1]	7
Concentrate samples	5	2[2]
GreenFeed pellet samples	5	2[2]
BCS	591	378
LWT	591	378
Milk volumes	5953	3606
Milk composition samples	585	420
GreenFeed measurements	3196	2987

[1]: Represents the collected samples for NIRS + samples collected separately and submitted for wet chemistry. E.g. 15 pasture samples were collected as described in section 3.3.2 Forage Management, however 3 sub-samples from these original were also pooled and submitted for wet chemistry analyses.

[2]: Represents pooled samples of feedstuff (Days 0 -16 of measurement period; Days 17 - 32 of measurement period).

In its entirety, the research described within this thesis acted as a part of a wider series of studies performed by AgResearch in tandem with Massey University. The *in-situ* study at Massey Dairy 4 reported within this thesis was run in tandem with *in-vivo* CH_4 and DMI measurements at the New Zealand Agricultural Greenhouse Gas Measurement centre (AgResearch Grasslands, Palmerston North). This *in-vivo* study randomly selected 32 animals within the *in-situ* study's research animals (balanced across treatments) for 4 day measurements of precise CH_4 production and DMI measurements using RC at AgResearch Grasslands (not part of this thesis, data reported from RC measurement cited as Della Rosa et al., 2024). All RC measurements occurred within the *in-situ* study's 33-day measurement period and upon the conclusion of each RC session, study animals were brought back to Massey Dairy 4 to continue participating in the *in-situ* study until its conclusion.

3.2.1 Treatment allocation

Seventy-two spring calving dairy cows were selected to participate in this study across the following four treatment groups to receive concentrates at the following graded levels: 0 kg DM / day, Control (CON), 2 kg DM / day, Low (LOW), 4 kg DM / day, Medium (MED) and 6 kg DM / day, High (HIGH),

ahead of the study's 16/09/22 commencement date. Treatment groups themselves were strictly defined by the daily offerings of treatment feed (as kg DM of concentrate per day) and the full diet of each treatment group is visible in Table 3.2.

The treatment concentrate feed offered in this study was defined as being a pellet composed of 30% barley grain, 15% maize grain, 10% wheat broll, 25% palm kernel expeller, 8% soya hulls, 7% soyabean meal and 5% molasses (by wet weight) (Denver Stock Feeds Ltd, Palmerston North, NZ).

Table 3.2 Overview of treatment groups and feed allocations by treatment group.

Group	Treatment concentrate offered per day (kg DM)[1]	Daily Pasture offered	Daily Pasture silage offer (kg DM)	Daily GreenFeed Pellet offered (kg; wet weight)[2]
Control	0	*Ad-libitum*	~2	<0.8
Low	2	*Ad-libitum*	~2	<0.8
Medium	4	*Ad-libitum*	~2	<0.8
High	6	*Ad-libitum*	~2	<0.8

[1]All groups' treatment offerings were provided to animals within a ±5% co-efficient of variance (see section 3.3.5 *Treatment concentrate feed calibration & sampling).* [2]Daily offerings of GreenFeed attractant pellets were provided with a 0.8 kg DM / animal / day upper limit, which is discussed further in section *3.6.1 Study specific GreenFeed settings.*

Potential animals to participate in the study were firstly selected from Massey Dairy 4's commercial herd under the criterion of calving date, with a narrow calving window being essential to ensuring animals' lactation stage (week of lactation; WOL) were similar and comparable across treatments to remove systematic errors in milk production and liveweight change analyses. Secondary to this first criterion, all potential study animals were stipulated to be of mature age and in their second lactation or later. Ensuring all enrolled animals were of mixed age minimised the risk of hierarchal bullying within the mob's shared usage two GF units and tried to minimise animal to animal variation of maintenance energy requirements between groups. All enrolled animals were between three to seven years old and in their second to sixth lactation (see Table 3.3).

A large pool of these potential study animals (~120 cows) which met the WOL and age criterion were preliminarily examined for breed composition, live weight, body condition score (BCS; minimum 4.0) at commencement of study, breeding worth (BW) and production worth (PW) before being further considered for participation in the study. These considerations were made in an effort to ensure that study animals reflected a practicably normal distribution of genetic evaluation and makeup and were practicably reflective of the average New Zealand cow (Sneddon et al., 2019; DairyNZ 2023). Animals from this preliminary pool were also examined for ill-thrift, lameness and for

gamma-glutamyl transferase enzyme levels (to indicate current or previous liver damage or other historic health issues). A pre-study BCS examination for all candidate animals was also performed and only animals with a BCS of 4 or greater were included in the study.

Assuming all preliminary subjects within the study were healthy and represented a heterogenous sample of indicative New Zealand dairy cattle, 72 animals were selected. All animals selected to participate in the study calved within 36 days, between 23/07/22 and 28/08/22. The average breed composition of the study herd approximated F12J4 (~75% Friesian, using to Massey Dairy 4 lineage records) and ranged in breed composition from F15J1 to F5J11. Similarly, the average Breeding Worth (BW; as $ farm income per 5 tonnes DMI) and Production Worth (PW; as $ profit per lactation) (DairyNZ, 2020) values of animals within the study approximated $185 and $223 respectively, with BW values ranging from $32 to $274 and PW values ranging from $22 to $383.

All selected animals were blocked by lactation number (lactation 2, 3 and 4-7) and animals within each block were randomly allocated to one of four treatment groups. The average and standard deviation of each group's age, liveweight, breed, calving date, BW and PW were then checked to ensure each group's production parameters were similar. Table 3.3. shows a breakdown of animals' characteristics and the balancing of these selection parameters across treatments.

Table 3.3 Parameters of treatment allocations (as of study commencement, September 2022).

	Count of Ages			Pre-study Liveweight (kg)	Breed	Calving date	BW ($)	PW ($)
Group	3	4	5-7	Mean ± SD	Mean	Mean ± SD	Mean ± SD	Mean ± SD
Control	9	5	4	525 ± 37	13F3J	06/08/22 ± 10 days	194 ± 57	220.94 ± 91.66
Low	7	5	6	514 ± 52	12F4J	07/08/22 ± 11 days	189 ± 54	222.83 ± 112.62
Medium	7	5	6	517 ± 55	13F3J	06/08/22 ± 8 days	166 ± 59	207.61 ± 105.24
High	8	6	4	511 ± 53	12F4J	07/08/22 ± 10 days	188 ± 62	242.39 ± 129.37

3.2.2 Animal management

Upon the commencement of the study, animals were firstly marked with paint on each of their rumps to indicate which treatment group they were placed within e.g.: CON = green, LOW = blue, MED = orange, HIGH = pink. Paint was re-applied as needed to ensure animals were clearly and correctly marked throughout the study for mob monitoring. All study animals were run together in a single herd for simplistic grazing management, as treatment feeds could be offered individually during milkings (see section 3.3.4 *Treatment concentrate feed management*).

Animal wellbeing was monitored daily throughout the entire study by visual observations and by using an on-farm collar-based livestock management system provided by Waikato Milking Systems (Waikato milking systems; CowTRAQ^{TM} collar, Hamilton, New Zealand, 2023). Information collected from these collars informed both farm staff and research technicians of oestrus events and potential ill-thrift in study animals daily. Cows which demonstrated oestrus were artificially inseminated as required in the later weeks of the measurement period, however it is assumed this did not significantly affect animal DMI or CH_4 emissions within the study.

Animals which were identified as being ill during the study were seen by a veterinarian for assessment and treatment at the earliest possible opportunity. Thirteen cows experienced significant adverse health events during the entire study, all of which were promptly treated and removed from the study for rest and recovery as per Massey Dairy 4's standard operations. A summary of all research animals removed from the study due to ill-thrift are detailed in Table 3.4. Lameness was identified as the most prevalent health issue experienced by animals during the entire study timeline. However, no correlation between concentrate intakes and animal health issues across treatment groups were identified within this study and the incidence of lameness experienced by the study herd was comparable to that of Massey Dairy 4's commercial herd within the same period. While Table 3.4 shows the full extent of animal removals during the study's 63 day timeline, further animals were excluded from statistical analyses and a count of these final animals are included in Table 3.4 (full details of animal exclusions at time of statistical analyses are discussed throughout section 3.8 *Statistical analyses*).

Table 3.4 Overview of animal removals across all treatment groups throughout the study.

	Count of animals				Reasons for withdrawal from study
Group	Study Commencement	Measurement Period Start	Measurement Period Finish	Included in statistical analyses	(Count of Cases)
Control	18	17	16	11	Lameness (2)
Low	18	16	13	11	Lameness (3), Mastitis (1), Other[1] (1)
Medium	18	17	16	10	Systemic Infection (1), Other[1] (1)
High	18	17	14	10	Lameness (2) Pneumonia (1), Tyleria (1)

[1]Other withdrawals were non-health related and were due to animals receiving unverifiable amounts of incorrect treatment feed.

A power analysis was performed before the commencement of this study by a biometrician (as part of the animal ethics application) and it was denoted that a minimum of 16 experimental units (as animals) per treatment group were recommended to detect and declare 15% statistical differences

between treatments in all analyses. Two animals were added to each treatment group as spares. As sick animals removed from the study, these animals were not replaced and the study proceeded with unbalanced treatment group sizes throughout the study's measurement period.

Several other risks to animal health were identified for mitigation during the planning of the study. To minimise the risk of hypocalcaemia in early lactation, lime flour and magnesium oxide (along with other minerals for supporting early lactation) were mixed into the pasture silage fed to animals daily (see section 3.3.2 *Forage management*). This included a multivitamin containing cobalt, copper, iodine, selenium and zinc (AquaTrace® 5, Nutritech, fed at 5 g/cow/day), alongside fine magnesium oxide (87% MgO by weight, Ravensdown, fed at 20 g/cow /day), lime flour (90-95% $CaCo_3$ by weight, Ravensdown, fed at 200 g/cow/day) and coarse salt (100% pure, Ravensdown, fed at 40 g/cow/day).

In accordance with this study's animal ethics agreement, animals were monitored for signs of clinical bloat and ruminal acidosis regularly throughout the study, at approximately one hour after each milking. Constant monitoring of milk volumes and animal collar data throughout the study also ensured that sub-clinical acidosis would be detected promptly and remedied through vet intervention if required. A drench containing bloat oil (Bloat-eze DFA, FIL, New Zealand) was prepared and kept on hand throughout the study and no events of clinical bloat or acidosis were encountered during the entire study's timeline.

3.3. Feed management

As this thesis has sought to investigate the changes to M_p under differing levels of concentrate supplementation, all study animals would ideally have consumed an exclusive diet of pasture + treatment concentrates. However, pasture silage was offered as a partial substitute of fresh pasture due to the unusually cold spring and slow pasture growth experienced in the Manawatu within the early spring in the 2022 season (see Table 3.5). Pasture silage offerings also allowed relevant macronutrients and minerals to support early-lactation animals to be mixed into the diet (mentioned in section 3.2.2 *Animal management*). A strict pasture only diet was also unfeasible in this study as all animals could access GF units for CH_4 measurements at all times, consuming the attractant pellets dispensed by these devices in this process. However, the effect GF pellets would make on DMI and nutritive composition of animals' diets were minimised by ensuring GF pellets were of a similar composition to pasture and were consumed in small, limited volumes (see 3.4.1 *Study specific settings of GreenFeed units*) (Jonker & Waghorn, 2020).

3.3.1 Farm conditions, climate and pasture performance

Massey Dairy 4 is a 221 hectare (ha) research focussed dairy farm enterprise situated adjacent to Massey university's Manawatu campus in New Zealand's North Island, at approximately 80 meters above sea level. Situated on flat to rolling contours of the mid and upper alluvial terraces of the Manawatu region, the property is dominated by Ohakea and Tokomaru silt loams which are noted as being imperfectly drained and the property held average soil pH and Olsen P contents of 6.1 and 28 mg/g, respectively (last assessed in 2021). Approximately 45% of this study's measurement period was spent grazing the mid-terrace landscapes (dominated by Ohakea silt loam soils) of Massey Dairy 4, while the remaining 55% of this period was spent on high terraces of the farm property (dominated by Tokomaru silt loam soils). In total, 26.8 ha of Massey Dairy 4 was grazed by the study herd throughout the study's entire duration (Massey University, 2021).

Table 3.5. shows the mean monthly rainfall volumes (expected) and the volumes experienced during the 2022 spring in which this study was conducted. Expected and experienced daily temperature ranges for Palmerston North throughout the study's duration are also reported in Table 3.5. All expected and recorded weather data was ascertained from Palmerston North AWS, stationed ~10 km North of Massey Dairy 4 (Chappell, 2015; FAR, n.d.).

Table 3.5 Description of expected and actual temperatures and rainfalls for the study's duration.

Month	Average daily temperature range (max – min, C°)		Monthly average rainfall (mm)	
	Mean[1]	2022[2]	Mean[1]	2022[2]
August	8.5	8.2	69	129.8
September	8.5	8.2	85	77.7
October	8.6	8.0	84	49.7
November	9.1	9.0	75	128.9

[1]Mean data provided from Chappell (2015) using relevant climate data from 1981-2010; [2]2022 data provided from FAR (n.d., last updated 25/05/23).

This study commenced after an abnormally wet and cold winter, with Massey Dairy 4 experiencing 188% of mean expected rainfall volumes in the August prior to the study's commencement. Despite the excess rainfall and colder temperatures experienced throughout winter until November, pasture growth rates experienced on farm were noted as being within expectable ranges throughout the study's measurement period in late October and November. Observed pasture growth rates at Massey Dairy 4 throughout these months approximated the historically measured 39kg DM/ha/day and 41kg DM/ha/day monthly historic averages at this property (DairyNZ, 2023). Nitrogen fertilizer was only able to be applied on farm from 06/11/22 in this season due to ground

conditions and only one paddock received nitrogen fertilizer (at 23 kg N/ha) within the study timeline. This paddock received fertilizer on 06/11/22 and was grazed by the study herd from 16/11/22 to 18/11/22.

The pasture sward composition of areas grazed during the study approximated 80 : 20 perennial ryegrass : white clover, however clover availability was depressed in the pasture sward throughout the whole study due to cold soil conditions. Creeping buttercup, broad-leaf dock and chicory comprised the most prevalent weed species found within the pasture swards fed to these research animals. However, these weeds comprised a very small portion of the total sward and it is assumed that these plants had no effect on DMI or pasture quality (Massey University, 2021).

3.3.2 Forage management

Throughout the diet transition, adaptation and measurement periods of the study, pasture was offered in a rotational grazing system to targeted post-grazing residuals of 1750 kg DM/ha, via twelve-hour breaks. Animals were offered between 0.7 to 1.1 hectares per day and these high offers of pasture ensured all study animals were allocated ~22 kg DM of total feed / day, irrespective of treatment group (see Figure 3.1). Pre-grazing masses of breaks offered to the study herd in this measurement period ranged between 2916 and 4012 kg DM/ha, while post-grazing masses ranged between 2096 and 2964 kg DM/ha throughout the measurement period (see Figure 3.2). All pasture graphs (Figure 3.1, 3.2 and 4.1) are depicted with 95% confidence intervals around smoothed mean values in grey (95% CI).

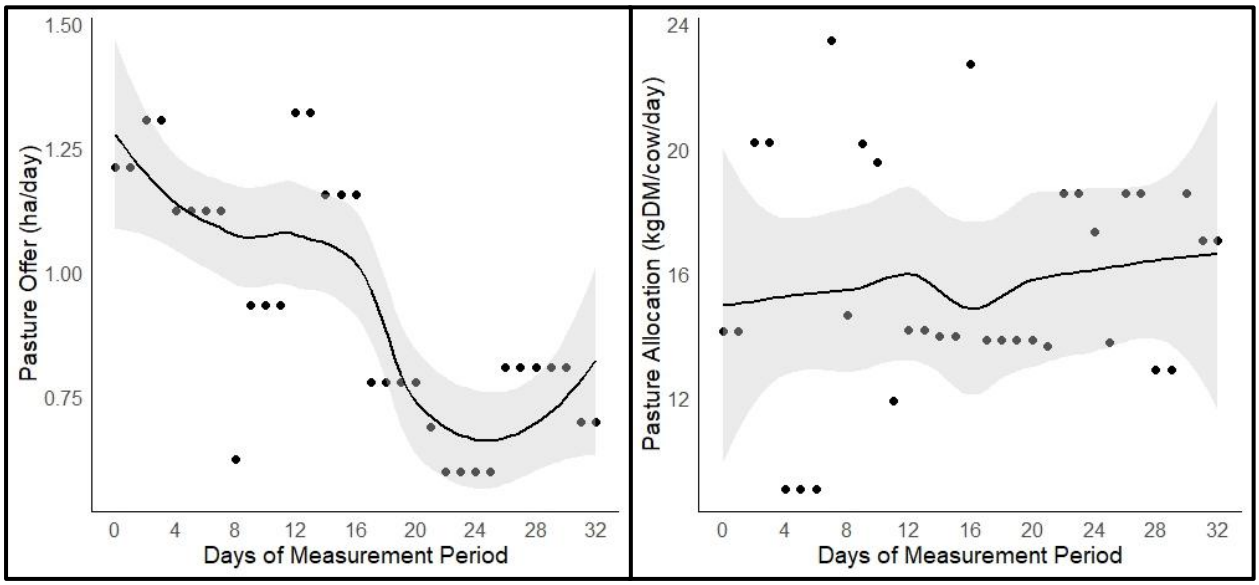

Figure 3.1 Pasture Offers (as area per day) and allocations (as kg DM per animal per day) throughout measurement period.

Pre-grazing pasture masses and daily pasture offers (as ha / day) varied significantly throughout the study, as pasture growth rates and pasture management strategies changed across the farm during the spring flush. Height recordings were performed three times each week (Monday, Wednesday and Friday) to determine the pre and post grazing masses of the study herd using a rising plate meter (F200 rising plate meter, FarmWorks, Fielding, New Zealand). These height measurements (as 0.5 centimetre clicks) were applied to the DairyNZ spring pasture measurement equations for October (average click height x 115 + 850) and November (average click height x 120 + 1000) accordingly, to measure pasture masses throughout the measurement period of the study (Farmworks, 2008). Pre-grazing and post-grazing masses of pasture are shown in Figure 3.2.

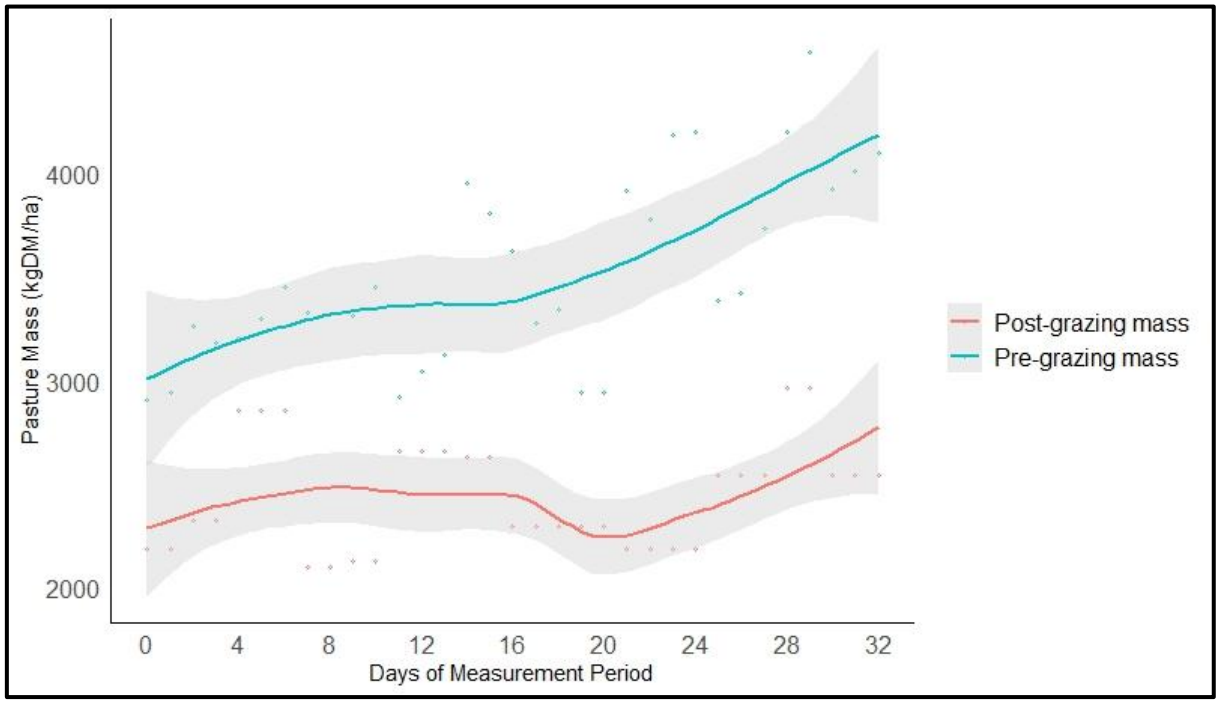

Figure 3.2 Pasture Mass Records throughout study measurement period.

Approximately 2 kg DM of autumn-cut and baled pasture silage, mixed with minerals (see section 3.2.2. *Animal management*), were offered to animals daily. Pasture silage was fed out to cows evenly across a single lane of the feedpad adjacent to the Massey Dairy 4 cowshed using a mixer wagon (feedpad and feeding lanes can be partially seen in Figure 3.3). Cows spent approximately 1 to 1.5 hours each day on this feedpad, immediately prior to afternoon milking. As the DM of individual bales fed throughout the study varied from 36% - 57% DM, pasture silage was always assumed to be 45% DM at each time of feeding to target this 2 kg DM / animal silage daily allocation. Silage added to the mixer wagon (recorded at time of feeding as kg wet weight), along with the total weight of minerals added to each mix, were recorded daily using calibrated scales within the mixer wagon (BvL

V-Mix 3.5, GmbH & Co., Germany; scale calibration performed by Webline Agriculture, New Zealand, in September, 2023). Sampling of pasture silage was conducted weekly (see section 3.3.3 *Forage sampling & measuring*) alongside these recorded daily offers of pasture silage (as wet weight) to estimate actual daily pasture silage offers (as kg DM). For metabolic equations, it was assumed that silage wastage rates approximated 10% across the measurement period (DairyNZ, 2023) and that all animals regardless of treatment ate the same kg DM of pasture silage on a given day (unless these animals were off farm at AgResearch Grasslands). Wasted pasture silage was cleaned from the feedpad bins weekly or fortnightly as required.

3.3.3 Forage sampling & measurements

Fresh cut pasture samples were taken three times weekly throughout the measurement period by cutting pasture to 6-8cm residual heights (approximating the 2000-2200 kg DM/ha grazing residuals being struck by animals using the October and November equations listed in section 3.3.2 *Forage management*). A total of 15 pasture samples were taken at random using five random quadrat cuts across the next upcoming grazing area at each sampling event. All pasture samples were taken between the hours of 7:00 a.m. – 12:00 p.m. to standardise the cutting conditions for DM% and soluble carbohydrate contents within pasture (Waghorn & Clark, 2004). Alongside pasture samples, a total of 5 pasture silage samples (taken weekly) were also collected from the mixer wagon after adding minerals and mixing the silage.

All fresh forage samples (pasture and pasture silage) were split into two sub-samples for DM% analyses and nutritive composition analyses. Fresh forage dry matter analyses were performed in triplicate using an oven set at 105° C for a minimum of 24 hours per sample at AgResearch Grasslands. Sub-samples for chemical composition testing were performed using near infrared spectroscopy analysis (NIRS) (Massey University Nutrition Laboratory, College of Sciences, Palmerston North). The subsamples required for NIRS were frozen at -20° C at their time of collection, before being freeze-dried and ground at their time of analysis. Freeze-dried pasture samples were tested for ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude fat (EE), soluble sugars plus starch (SSS), in-vitro digestible organic matter (OMD%) and metabolisable energy (ME) contents (as MJ ME / kg DM) as described in Corson et al., (1999). Pasture silage NIR analyses similarly included measurements for ash, CP, NDF, ADF, OMD and SSS (each as g / 100g DM) along with tests for pH measurements. Metabolisable energy contents of pasture silage ascertained from NIRS analyses were discarded and MJ ME / kg DM contents of pasture silage were assumed to equal 0.16*OMD% (SCA, 1990). Mineral analyses (wet chemistry) of phosphate, potassium, sulphur, calcium, magnesium,

sodium and chlorine were also analysed by Hills Laboratory (Hamilton) for both pasture and pasture silage analyses to calculate the dietary cation anion difference (DCAD) of the offered diet (NRC, 2001).

3.3.4 Treatment concentrate feed management

Concentrates were fed to all LOW, MED and HIGH treatment animals at each milking throughout the study via an in-shed feeding system. Controlled by a primary computer system (Jantec systems, Victoria, Australia), concentrate allocations were set for individual animals and feeding of concentrates only occurred after passing under an electronic identification tag (EID) reader, situated at the bale entry position of the Massey Dairy 4's 28-bale rotary cow shed. This EID reader relayed cow ID number back to the central Jantec system which ensured the correct treatment allocations were fed to each specific animal's bale during milking. Animals within LOW, MED and HIGH treatments received their treatment concentrate via a feed bin located at the cranial position of the milking bale soon after EID reading and these animals were able to consume their treatment feed during their time on the milking platform.

This feeding system ensured that animals could freely flow into the cowshed at any time during milkings and receive their correct treatment allocation and all milking events were conducted at a fourteen-minute rotation speed with a researcher placed at the bale walk off position. In turn, this gave animals consistent and sufficient time to consume their respective treatments and also allowed refused feed (if present) to be collected, measured and cleaned away before new animals walked onto the milking platform at the bale entry position.

3.3.5 Treatment concentrate feed calibration & sampling

As the in-shed feeding system delivered treatment on a per second basis and not a weight basis, electronically set calibrations were checked weekly to ensure a consistent correlation between weight of feed delivered per seconds of auger runtime In the Jantec system. Only a single calibration was able to be set for a given auger line and therefore a single calibration curve (g feed / s) was responsible for accurately delivering treatment amounts to the LOW, MED and HIGH treatment groups.

At each weekly calibration check, three repeats of LOW, MED and HIGH feeds were dispensed, collected, weighed and recorded in order to assess the current calibration's precision and accuracy. If the variation of weigh delivered per second for any LOW, MED or HIGH feeding rates exceeded a ±5% co-efficient of variation (CV), a new calibration was performed in the Jantec system by recording the

weight of concentrates delivered for 2 seconds of auger runtime. New calibrations were performed by recording a triplicate of 2s feed drops, weighing these drops to perform means and CV testing, entering a new calibration into the Jantec system and re-checking this new calibration against each of the LOW, MED and HIGH feeding rates again. A linear regression model of all calibration tests denoted that over 99% of variation of feed weight delivered by the Jantec system was explained by auger runtime (grams = 925.966s + 0; R^2 = 0.997; Standard error = 7.537).

Coinciding with weekly in-shed feeder calibration events, treatment samples for DM% and composition analyses were collected alongside pasture silage and pasture samples (as described in section 3.3.3 *Forage sampling & measuring*). The nutritional analysis of concentrates were however conducted by wet chemistry, rather than by NIRS, and included analysis of ash, CP, EE, NDF, ADF, water soluble carbohydrates, starch, and OMD (each as g / 100g DM) (with MJ ME / kg DM being estimated from OMD%) alongside mineral compositions. (Hill Laboratories, Hamilton). Samples of GF pellets were also collected weekly alongside treatment feed samples to be similarly analysed by wet chemistry. All samples of treatments and GF pellets were sub-sampled for independent DM% testing via 105 C° oven triplicate testing at AgResearch Grasslands. The results of all forage and concentrate composition analyses are presented in Table 4.1.

3.3.6 Treatment feed intakes

As treatments were fed to animals individually during milkings, animals had freedom to eat or refuse their treatment allocation twice daily, which in-turn could affect their daily DMI of treatments and potentially affect their pasture intakes. Recording refused treatment feed amounts during each milking was therefore conducted to assess the extent at which animals ate their allocated feed. Similarly, this process also prevented any treatment feed which was refused by a low, medium or high group animal from becoming available to other cows who may subsequently enter their bale that milking.

Refusal collections in the early transition period of the study (before DMI measurements were recorded) were performed once a day. Once a day refusal collections were imperfect at stopping animals who could potentially double dip into treatment feed which was not allocated to them at every milking. However, the action of shooing cows out of their milking bales for refusal collection was deemed a significant and stressful initial change to their routine behaviour during milkings. In-turn this transition period allowed the animals to adapt to researchers who collected refused treatments, reducing the risk of animal injury or lameness before the measurement period commenced. Twice a

day refusal collections began in the later GF adaptation period to ensure animals had transitioned to their allocated diets and to accurately record concentrate intakes of individual animals throughout the measurement period.

If treatment feed was refused by an animal, all the refused feed was collected into a measuring jug and recorded on a volumetric basis in a time efficient manner. A linear regression analysis was performed at the end of the study to correlate measured mls of refused treatments to g of refused treatments (g refused treatment = 0.5970 mls refused treatment ± 0.0060 standard deviation. Adjusted R^2 = 0.9946).

3.4. Milk records

3.4.1 Milking management

All animals were milked twice a day (TAD) throughout the study in Massey Dairy 4's 28-bale rotary cowshed at approximately 7:00 - 8:00 a.m. (morning milking) and 3:00 - 4:00 p.m. (afternoon milking) daily. Milking was performed by farm staff, with occasional assistance from research technicians. After each milking, animals were released to go back to their paddock as soon as possible, however on several occasions, animals were yarded after milking (for periods of up to 30 minutes) before being able to return to their paddock. Automatic cup removers (ECR Plus Automation, Waikato milking systems, New Zealand) removed milking cups from cows when milk flow rates through the milking clusters dropped below 0.2 L / minute. Likewise, an automatic teat-spraying unit (SmartSpray, Waikato milking systems, New Zealand) delivered 25ml of teat spray when milking cups were removed from animals. All animals were monitored for mastitis by farm staff daily and the study herd was checked multiple times for mastitis throughout the study.

3.4.2 Milk production measurement, sampling & analysis

Milk composition and milk production volumes were sampled and recorded through several means throughout the measurement period. Milk production at each milking (as L / animal / milking) was measured via in-line optical sensors and flow meters within the cowshed (Automation Yield Sense, LIC, New Zealand). All recorded in-line milk volumes were uploaded to a secondary computer system within the cowshed at the end of each day (Electronic milk meter, Waikato milking systems, New Zealand). Further milk sampling was then performed once a week, with samples collected at morning and afternoon milkings on Tuesdays during the measurement period, to analyse the composition of study animal's milk. In tandem with daily milk production, this was used to infer daily milk yield as

kilograms of milk (kg milk / day), FPCM (kg FPCM / day) and as milk solids per day (kg MS / day) for individual animals.

The five milk sampling days consisted of collecting 50ml milk samples from study animals at a.m. and p.m. milkings and up to 10 milk samples were collected for each animal across the measurement period. The milk meters which utilised for milk sampling (Waikato milking systems, New Zealand) modified the milk hose of each bales' milking cluster to continuously siphon a continuous (unverified) portion of each cows' milk away while milking cups were in use. Analyses of these collected milk samples were performed by AgResearch (Te Ohu campus, Palmerston North) using a FOSS FTIR analyser.

Some recorded milk volumes recorded in the measurement period were corrected from sampling issues experienced throughout the study which saw individual animals' milk yields mis-recorded or misrepresented. Any data individual animal milk volume records were removed if they exceeded 3 standard deviations from the mean of am and pm all animal's milk volumes, respectively. In total, 3663 individual milk volume data points were collected in the study, of which 3606 were considered normally distributed around the mean am and pm milk volume values. These collated datapoints ranging from 7.2 < 20.1 L in a.m. milkings and 3.9 < 12 L in p.m. milkings. Once processed, daily milk volumes were then averaged into weekly averaged values for individual animals, as to fit the weekly resolution of milk composition tests, for subsequent conversions of milk production to milk yields (as kg / day) using milk density values from milk composition tests.

All other milk composition data was checked for soundness through a brief co-efficient of variance (CV) analysis. These CV analyses were performed for each animals' weekly averaged values for milk volume (L / day), milk yields (using milk volumes and weekly density samples) (kg / day), lactose (%, as kg lactose / kg milk in a given day), fat (%), protein (%), total solids (fat + protein kg / day), FPCM (kg / day), milk urea (g / kg milk) at a tolerance level of ~20% % CV. Approximately 12% of weekly composition datapoints were missing due to animals' absence at AgResearch during chamber measurements and missing datapoints were replaced using an average of remaining data over the 5 week measurement period for each animal.

3.5. Liveweight & body condition score recording

Animal liveweights were recorded at the start, mid-point and end of the measurement period using calibrated static weigh scales (X300, Tru-test Ltd., New Zealand). Each of these records were

compiled from static weights made across three successive days, for a total of nine weighing days across the measurement period. All weighing sessions were held immediately after morning milkings in attempt to mitigate the effect of gut fill on liveweight to create a dataset of averaged animal liveweights throughout the measurement period. Table 3.6 reflects the average liveweight of animals within each treatment group at the start, mid and end points of the measurement period (Table 3.6 does not include the liveweights of animals removed from the study or analyses).

Table 3.6 Liveweight changes per treatment group across the measurement period.

	Pre-Measurement (Days -4 - -6)[1]	Measurement Mid-point (Days 9 - 11)[1]	Measurement conclusion (Days 30 - 32)[1]
Group	Average kg ± SD		
Control	488.6 ± 6.2	487.1 ± 5.9	476.9 ± 8.0
Low	534.5 ± 6.4	534.2 ± 5.2	518.2 ± 8.1
Medium	539.1 ± 7.0	542.0 ± 4.9	533.4 ± 8.5
High	538.2 ± 8.2	536.2 ± 5.5	525.9 ± 5.8

[1]: Body condition scores were also recorded alongside these liveweight records, with BCS assessments across three scoring days by the same independently certified research technician. The results of BCS and liveweight analyses are presented in Table 4.2.

3.6. Operations of GreenFeed units

An overview of the GF unit's functionality is provided in section 2.10.1 *Comparison of methane measurement techniques*, and the effective usage of GF systems to estimate *in-situ* emissions of grazing livestock has been well documented in previous literature (Jonker et al., 2019; Waghorn et al., 2020). As recommended by C-Lock Industries, a maximum of 40 animals should share a single GF unit and two trailer mounted GF units were utilised in this study to adequately facilitate the collection of CH_4 measurements from 72 experimental units. Upon the completion of every completed GF visit, records of: Time of day, visit duration, number of cup drops, animal EID, GF unit ID were recorded alongside mean values for CH_4 mass (as g CH_4 / day), and CO_2 mass (g CO_2 / day) were recorded and used for analyses in this study.

As selected animals had not previously participated in other CH_4 measuring studies, access to GF units was to be given at all practicable times throughout the GF adaptation period to try and increase early usage of the units, as encouraged in previous studies like Garnett et al., (2021). However, due to wet ground conditions which caused trailer mounted GF units to become stuck in paddocks, both GF units were placed on Massey Dairy 4's feed pad throughout the adaptation period and were only accessible to animals during pasture silage feeding times until the ground conditions improved (see in Figure 3.3).

Figure 3.3 Both GreenFeed units (serial no. #76 & #77) placed adjacent to the feedpad at Massey Dairy 4 during the study's adaptation period.

Extra feeding allowances and removal of both units' wings and the alleyways in front of each unit ensured that animals had no physical barriers limiting their usage of GF units early in their adaptation (see Figure 3.3). During the measurement period, animals had continuous access to units except milkings and weekly calibrations. However, significant technical difficulties experienced by the units during the study meant that unit #76 was not in use for the first 17 days of the measurement period (see Figure 5.2).

3.6.1 Study specific GreenFeed settings

In contrast to that seen in Figure 3.3, Figure 3.4 shows a GF in use in the paddock with the 1.8-meter alleyway attached to each unit while used *in-situ* during the measurement period. This ensured that only a single stalled animal could interact with each unit at any given time. Likewise, extended plywood 'wings' were fitted to the sidewalls of the hood to help minimise the effect of wind conditions from entering the hood of the unit and to ensure that other animals couldn't push an occupying animal

away from the unit. A photo of a GF unit in use with both the alleyway and wings attached is pictured in Figure 3.4.

Figure 3.4 GreenFeed unit #77 in use during the measurement period.

Throughout the earlier two weeks of the measurement period, both units were shifted every two days. However, daily shifting of units was anecdotally observed to increase GF visitations and daily shifting of units occurred as practicable during the final weeks of the study. Both units were monitored twice daily during animal bloat checks (see section 3.2.2. *Animal management*) to ensure that GreenFeed pellets were regularly topped up inside both units' feed hoppers and to ensure no intermittent issues were being experienced by either unit, such as feed blockages or internet connection issues.

Lucerne based alpaca pellets (Dunstan Nutrition, Hamilton, New Zealand) of approximately 4 mm diameter by 10 - 22 mm in length were used in this study to encourage animal visitation after having encouraged high visitation rates to the GF units in previous studies (Jonker & Waghorn, 2020). After being considered for their robustness and comparable nutritive composition to pasture (see Table 4.1), these alpaca pellets were successfully fed through GF systems without jamming the rotator

cup or blocking the particulate filters which protected gas analyser system. To increase the rates and duration of GF visitation events, both GF units were set to allow animals to receive up to seven cup drops of feed per visit. Eight total visits were allowed per day for each animal (one visit session allowed every 3 h) providing ~30 grams/cup drop (33.43 g / drop mean for GF # 76; 35.87 g / drop mean for GF # 77), offering a maximum of ~ 210 grams pellets per visit, or 800 grams per day maximum. Feed drops within a visit were limited to occur at 25 second intervals, as to ensure animals would be interested in remaining under the hood for over two minutes and therefore give a validated measurement.

Programmed rules of the GF units stipulated that a new visit could only be created for any new animal entering a GF's hood so long as the animal retained their head under the hood for longer than two seconds to actuate the unit's proximity sensor. Starting a new measurement session was also conditional of the animal not having visited either GF unit in the last three hours or exceeding their daily limit of sessions to prevent dominant control of the units. Animals who successfully created a new measurement session, but walked away from their active session, were given five seconds to return to the unit's hood to continue their current session or the visit would be finished. Likewise, any animals detected to be interrupting the current measurement session of another animal would result in a temporary block of all feed from being dropped by the GF for two minutes.

3.6.2 GreenFeed gas calibrations, standard gas recoveries and feeder calibrations

Calibrations of the GF system's non-dispersive infrared sensors were performed weekly, to ensure that the NIR gas analyser within each device were accurately recording the emissions of livestock throughout the measurement period. Calibrations firstly recorded the atmospheric concentrations of CH_4 and CO_2 before 'Zero' and 'Span' gasses released into and are captured by the system via an automatic timed-release mechanism. Zero gases recommended for usage with GF devices typically contain 0% CH_4 and 0% CO_2, while span gases are recommended to contain between 500-1000ppm CH_4 and 5000-10000 ppm CO_2. Concentrations (as ppm) of zero gases used in this study contained 0 : 0 : 200,000 (CH_4 : CO_2 : O_2; ppm) and concentrations of span gases used contained 1002 : 10010 : 210500 (CH_4 : CO_2 : O_2; ppm).

Carbon dioxide gas recoveries were also performed in tandem with GF gas calibrations to ensure GF devices were accurately and precisely recording the flow (used to estimate and masses) of gases through the unit's whole system. Up to seven releases of CO_2 were performed at each gas

recovery occasion for each unit. Recorded gas recoveries ranged between 95.25%-105.46%, with a mean value of 100.10% and a standard deviation of 3.324.

3.7. GreenFeed data management

A total of 3,153 validated measurements were recorded from GF units #76 and #77 during the entirety of the study's measurement period (outlying values were screened and removed by C-Lock Industries prior to any analyses or data pre-processing within this study). Of these measurements, 2,777 across 42 animals passed the data quality requirements listed in section 3.6.1 *Study specific GreenFeed settings* and were used in further analysis. Curating GF datum points involved excluding 152 datapoints from 10 animals with low visitation records (animals with < 20 visits) and a further 3 animals' visits due to missing datasets within the estimate parameters of DMI. As mentioned in section 2.10.1 *Comparison of methane measurement techniques,* while some researchers have previously only analysed GF data from animals with 30 or more spot measurements each (Hristov et al., 2018), Della Rosa et al., (2023) found that analysing GF data with animals of 20 or more CH_4 spot measurements did not reduce the accuracy of predicted M_p. This allowed four animals with 20 < 29 GF measurements to therefore be kept for statistical analyses in this study. Figure 3.5 shows the mean CH_4 emission data output from these 42 animals (by hour of day) with CI set at 95%.

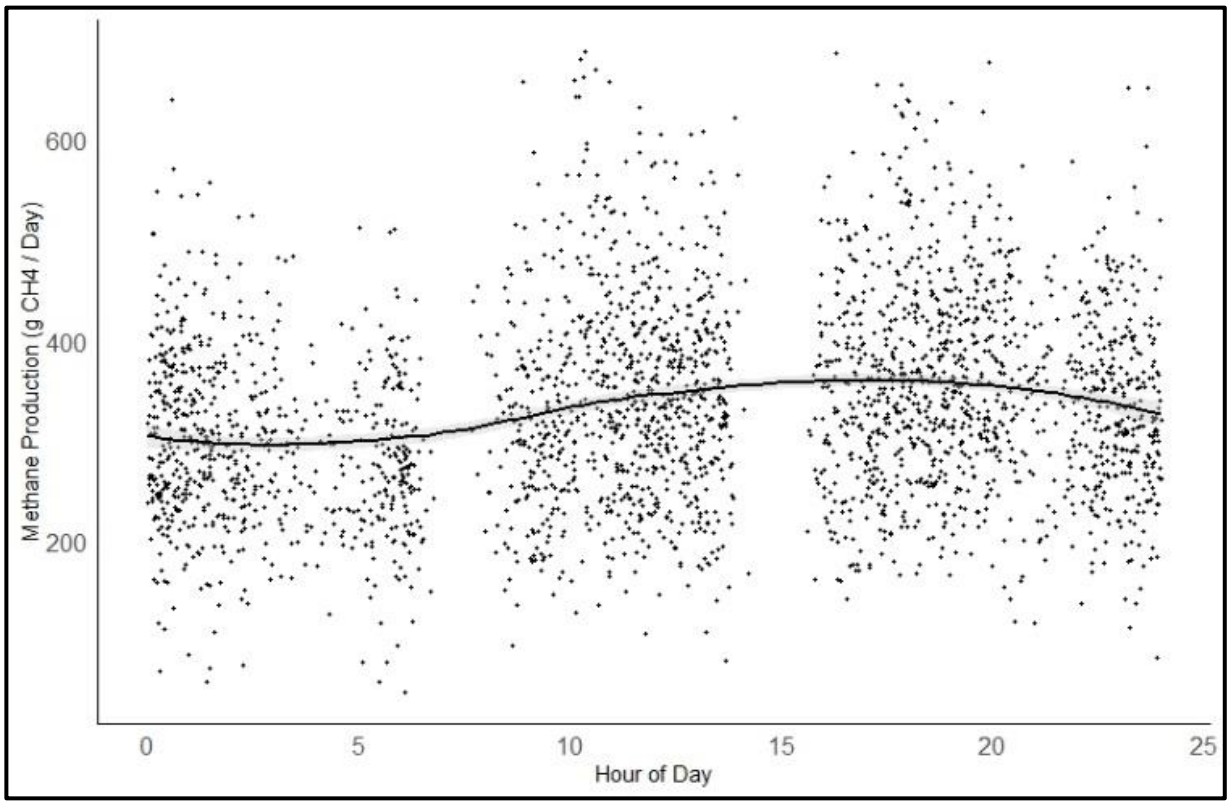

Figure 3.5 A non-parametric smoothed model of all animal emissions captured in the measurement period.

3.8. Statistical analyses

As noted in 3.7 *GreenFeed data management*, not all experimental units were analysed for M_p and DMI measurements and therefore the final treatment group numbers used for statistical analyses were unbalanced. For all statistical analyses, 11 animals were used in the CON and LOW treatment groups and 10 animals in both MED and HIGH treatment groups. A one-way analysis of variance (ANOVA) test was therefore used to detect significant differences of means between treatment groups using all modelled techniques. This statistical test was also chosen as not all experimental units were represented in every factor of the study, e.g. the pattern of GF visits presented in Figure 4.4 shows few visits to GF units around milking times (between the hours of 6:30 a.m. – 8:30 a.m. and 2:30 p.m. – 4:30 p.m.).

All ANOVA tests were adjusted by the Benjamini & Hochberg method (BH) (Benjamini & Hochberg, 1995). Levels of significance for all ANOVA tests were set to 95% and significant differences between treatment groups were declared when $P < 0.05$, with linear and quadratic effects also being reported for each ANOVA test where relevant. The normalcy and homoscedasticity of errors within each ANOVA test were evaluated by assessing plotted residuals against predicted values before reporting. Within each ANOVA test, standard error of differences (SED) between least square means are also reported in the results of this study.

3.8.1 Data and equations pertaining to energy use requirements of dairy cattle

In fulfilment of this study's research objective, DMI serves as an important research output to detect and quantify any marginal changes to the nutritional states and methanogenic states of animals consuming increasing amounts of concentrates. However, in this *in-situ* study, intake was not able to be accurately recorded for all offered feedstuffs and DMI is instead assumed from the metabolic requirements of animals involved in this study and known intakes of some feeds. This will be explored further in section 3.8.2 *Models & equations pertaining to DMI estimates*, however for the accurate estimation of DMI, the calculation of ME requirements (ME_r) was performed using the SCA (1990) method (which is also utilised for calculations of the New Zealand Greenhous Gas Inventory). The completion of equations from SCA (1990), alongside the conversion of known DMI recorded in the study (for GF pellets, concentrates and pasture silage) to ME intakes, provided the opportunity to estimate pasture intake through residual ME_r. A brief summation of this ME_r calculation method is as follows in Equation 3.1.

Equation 3.1 An overview of animal metabolisable energy requirements (SCA, 1990).

$$ME_r = ME_m + ME_l + ME_g + ME_c + ME_{graze}$$

Where:

ME_r = Sum of total ME requirements as estimate (MJ / animal / day)

ME_m = ME requirement for maintenance (MJ / animal / day)

ME_l = ME requirement of milk production (MJ / animal / day)

ME_g = ME requirement of change in body condition (BCS) (MJ / animal / day)

ME_c = ME requirement for gestation (MJ / animal / day) (if relevant)

ME_{graze} = An additional factor of energy expended in harvesting forages (MJ / animal / day)

The SCA (1990) method has historically been utilised in broader annual CH_4 accounting settings, and therefore factors like ME_c are considered void in this study which contained no pregnant animals. Likewise, external factors such as cold stress can also be included in the estimation of ME_r, however cold stress was not deemed significant to the climate and context of this study.

The values of ME_{graze} were provided from Rattray et al., (2007), as a +10% factor of animals' maintenance energy requirement, which is different from the worked equations from SCA (1990). For this study, only ME_m, ME_l and ME_g were therefore required for the calculation of ME_r.

3.8.1.1 Maintenance energy requirements

Maintenance energy describes the energy required for homeostasis. At a physical level, ME_m represents physical processes of respiration, organ function (factoring in energy usage during digestion) and cell turnover within the body (Rattray et al., 2007). Likewise, as a mathematical construct ME_m represents the basal level of feeding required for an animal to maintain a neutral energy balance with no net gain or loss of energy (CSIRO, 2007). Age and gender, along with basal liveweight, are the driving factors of ME_m and must be accounted for accordingly, with fasting metabolism decreasing by approximately 8% per year in ageing cattle (until six years of age) (CSIRO, 2007). Likewise, physiological states (lactating or non-lactating) also significantly affect animals' ME_m, as increasing productivity denotes greater chewing, digesting and cardiovascular functions while maintaining a productive state. Maintenance energy requirements for individual animals in this study were estimated using equation 3.2 (SCA, 1990):

Equation 3.2 The calculation of maintenance energy requirements (SCA, 1990).

$$ME_m = K \times S \times \frac{0.28LWT^{0.75} \times exp^{-0.03A}}{k_m} + 0.1ME_p$$

Where:

ME_m = ME requirement for maintenance (MJ / animal / day)

K = A coefficient of interspecific fasting heat production, equivalent to 1.4 in *Bos Taurus.*

S = A coefficient of gender specific fasting heat production, equivalent for 1.0 in mature females.

LWT = Animal liveweight (kg)

A = Age in years

k_m = Efficiency of the use of ME pertaining to maintenance

ME_p = ME required for production, in this instance $ME_p = ME_l + ME_g$

3.8.1.2 Milk production

Milk production is most significant component of ME_r in dairy cattle during peak lactation. The following equations to calculate ME_l drew data from daily in-line milk records and weekly milk composition tests. The energetic requirements for lactation (provided by SCA, 1990) are summarised in Equation 3.3, with Equations 3.4 – 3.11 providing supporting information to estimate the daily yields of milk produced by animals, the energy use efficiency involved in milk production and the average energy content of milk produced as well as the average ME content of the diets fed to animals.

Equation 3.3 The calculation of lactation energy requirements (SCA, 1990).

$$ME_l = \frac{Y \times Milk_{GE}}{k_l}$$

Where:

ME_l = Metabolisable energy required for milk production (MJ / day)

Y = Daily milk yield (kg / day)

$Milk_{GE}$ = Gross energy content of milk

k_l = Efficiency of use of ME for milk production

Equation 3.4 Milk yield calculation (SCA, 1990).

$$Y = \frac{V}{D}$$

Where:

Y = Daily milk yield (kg / day)

V = Daily milk volume[1] (L / day)

D = Density of milk[1] (kg / L)

[1]Missing data points were assumed from a smoothed average of other data points.

Equation 3.5 Gross energy of milk calculation (SCA, 1990).

$$Milk_{GE} = (0.376 \times F) + (0.209 \times P) + 0.948$$

Where:

$MILK_{GE}$ = Gross energy content of milk (MJ / kg)

F = fat content of milk, (g / 100 g milk).

P = protein content of milk, (g / 100 g milk).

The kinematics underpinning k_l (and k_g in Equation 3.12) are beyond the scope of this study and for the purposes of this thesis, the coefficient *k* represents a stepping stone of energy use efficient within the calculation of ME_r which is described further in CSIRO (2007).The calculation of k_l ensures that the animal variation of energy use efficiency for milk productivity (and liveweight change) is accounted for within the Least Square Means (LSM) of ME_l (and ME_g) when reported at the treatment level in Table 4.4.

Equation 3.6 Estimation of energy use efficiency for milk production (SCA, 1990).

$$k_l = 0.35 \times Q_m + 0.42$$

Where:

k_l = Efficiency of use of ME for milk production (typically ~0.6).

Q_m = The ratio of feed ME to GE within the diet.

Equation 3.7 The conversion efficiency of feeds' GE to ME contents (SCA, 1990).

$$Q_m = \frac{Feed_{ME}}{Feed_{GE}}$$

Where:

Q_m = The ratio of feed ME to GE within the diet.

$Feed_{ME}$ = Weighted average of ME content within animals' diets (as MJ ME / kg DM)

$Feed_{GE}$ = Weighted average of GE content within animals' diets (as GE ME/kg DM

The estimation of $Feed_{GE}$ and $Feed_{ME}$ were able to be readily estimated using feed composition analyses and Equation 3.8 and Equations 3.9 – 3.11, respectively. However, to complete a weighted average of $Feed_{GE}$ and $Feed_{ME}$ across each treatment's diets', both the DMI and the ratio of intakes of each feed stuff were required. Without known pasture intakes, this was unable to estimate and therefore the weighted average of each treatment groups' DMI (by feedstuff, expressed as a ratio) were completed using known DMI of animals while in RC. These ratios of assumed DMI by feedstuff for each treatment group are displayed in Table 3.7. As the ME and GE of each feedstuff were not numerically dissimilar, it was assumed that these approximate DMI ratios were sufficient for the calculation of $Feed_{ME}$ and $Feed_{GE}$ in this study as Q_m and k_l are fairly static in diets dominated by one feed type, such as pasture-based diets (CSIRO, 2007).

Table 3.7 The assumed ratios of DMI by feedstuff across treatment groups.

Group	Pasture	Pasture silage	GreenFeed Pellet	Treatment Concentrate
	Mean *± SD*			
Control	81.5% ± 1.7%	14.7% ± 1.4%	3.8% ± 0.9%	0% ± 0.0%
Low	74.5% ± 1.7%	13.9% ± 0.7%	2.9% ± 0.8%	8.6% ± 0.5%
Medium	65.9% ± 4.3%	13.7% ± 1.7%	2.7% ± 1.0%	17.7% ± 2.0%
High	56.2% ± 3.9%	14.0% ± 1.3%	2.4% ± 0.7%	27.4% ± 2.5%

Equation 3.8 Gross energy content of feed (Jentsch et al., 2003).

$$\mathrm{GE} = \sum[(CP \times 0.236) + (Lipid \times 0.398) + (NDF \times 0.189) + (NFC \times 0178)]$$

Where:

CP = Crude protein content of feed (g CP / 100g DM)

Lipid = Lipid content of feed (g Lipid / 100g DM)

NDF = Neutral detergent fibre content of feed (g NDF / 100g DM)

NFC = Non-fibre carbohydrate content of feed (g NFC / 100g DM)

Equation 3.9 Estimation of feed metabolisable energy (SCA, 1990).

$$ME = 0.16 \times DOMD$$

Where:

ME = Metabolisable energy content of feed (MJ ME / kg DM)

DOMD = Digestibility of organic dry matter, the portion of dried organic matter within a feedstuff that can be digested (g DOMD / 100g DM)

Equation 3.10 Estimation of digestibility of organic dry matter within feed (Freer et al., 1997).

$$DOMD = \frac{DOMD \times OM}{100}$$

Where:

DOMD = Digestibility of organic dry matter, the portion of dried organic matter within a feedstuff that can be digested (g DOMD / 100g DM)

OM = The organic matter within a feedstuff, with ash removed (g OM / 100g DM)

Equation 3.11 Estimation of non-fibre carbohydrates within feed (SCA, 1990).

$$\text{NFC} = \sum 100 - (Ash + CP + NFD + Lipid]$$

Where:

NFC = Non-fibre carbohydrate content of feed (g NFC / 100g DM)

Ash = Ash content of feed (g Ash / 100g DM)

CP = Crude protein content of feed (g CP / 100g DM)

Lipid = Lipid content of feed (g Lipid / 100g DM)

NDF = Neutral detergent fibre content of feed (g NDF / 100g DM)

3.8.1.3 Liveweights and body condition scores

As mentioned in 3.5 *Liveweight and body condition score recording,* liveweights and BCS were collected in tandem throughout the measurement period in three separate measurement events (Measurement start = Days -6, -5, -4; Measurement mid-point = Days 9, 10, 11; Measurement finish = Days 30, 31, 32). Extreme liveweights detected within any animals' measurements at the start, mid and end points (exceeding CV of 15% between any sets of three measurements within each animal's measurement point) were removed before each animal was assigned an averaged liveweight for the start, mid and end of the study's measurement period.

As the start of the measurement period (day 0) was did not coincide with the commencement of the measurement period (start liveweights were recorded at days -6 to -4), a linear regression was performed between average liveweight at day -6/-5/-4 and day 9/10/11, to establish an estimate of animal liveweights at day 0. The same process was conducted between the mid-point and endpoint liveweight measurements (regression of the average liveweight of animals at day 10 to day 32 of the study).

These processes together enabled liveweight changes (as Δ kg / day between day 0 and day 32 of measurement period) to be calculated for each animal and reported at the treatment level in Table 4.2. (This process was also repeated for BCS scores as part of the estimation in *necwl* in Equation 3.13, which required daily losses to BCS).

The model used to estimate ME_g was adapted from MPI (2013) in this study as opposed to (SCA, 1990). The justified usage of this alternative equation was highlighted in CSIRO (2007) indicating that changes within lactating dairy cow energy reserves are more accurately determined by BCS assessment, related to liveweight and standard liveweights of animals, rather than by assessment of liveweight change over time in isolation. The adapted calculation of ME_g is displayed in Equation 3.13.

Equation 3.12 The calculation of energy requirements associated with fat reserve changes (Bown et al., 2012).

$$ME_g = \frac{(neclw \times k_g \times LWG)}{k_l}$$

Where:

ME_g = ME requirement of change in body condition (BCS) (MJ/animal/d)

neclw = Net energy content of Liveweight as (10.1 + 2.47 × CS)

CS = Condition score change per day, scaled 1-8

k_g = The efficient use of ME for liveweight change in lactating animals. k_g = 1 in animals gaining weight, however k_g = 0.84 in animals losing weight, as to account for the efficiency of energy utilisation of body fat for milk secretion.

LWG = Liveweight change in kg/d

k_l = The efficiency of ME use for milk production.

3.8.2 Models & equations pertaining to estimating dry matter intake

As mentioned in 3.8.1 *Data & equations pertaining to energy use equations*, the primary DMI estimated made within this study was stated to be determined by SCA (1990) ME_r calculations. However, with the accurate capture of some feeds' intakes through this study (e.g. treatment concentrate and GF pellets), estimating DMI in this study can instead be summarised by Equation 3.13, which provided the values needed for the primary reporting of DMI in this study (see Table 4.4):

Equation 3.13 Dry matter intake estimate summary.

$$\text{DMI} = \sum DMI_{Silage} + DMI_{GF} + DMI_{Conc} + DMI_{Pasture}$$

Where:

DMI_{silage} = The assumed mean silage intake of animals (as kg DM / day) estimated at the daily level for each animal using pasture silage feeding records.

DMI_{GF} = The known mean intake of GreenFeed pellets (as kg DM / day) using collated GF data.

DMI_{Conc} = The known mean intake of treatment concentrate (as kg DM / day) using Jantec records and treatment refusal records.

$DMI_{Pasture}$ = The remainder of ME_r which is not fulfilled by the assumed and known consumptions of pasture silage, GF pellets and treatment concentrate (as MJ ME required/d converted to kg DM/d). Just as the other parameters of DMI were estimated at the daily level for each animal, $DMI_{Pasture}$ was estimated that the animal level for each day of the study for all analysed animals.

Intakes of both GF pellets and treatment concentrates were recorded at the animal level, each day of the measurement period in convertible metrics to DMI. For instance, GF raw data records could be summarised to count the number of feed cup rotations given to each animal on each day of the measurement period. Using the linear regression stated in section 3.6.1 *Study specific GreenFeed settings* in tandem with GF pellet composition data in Table 4.1; these records could be converted to DMI of GF pellets / animal for each day of the measurement period. This was further summarised to mean GF pellet DMI for each animal throughout the measurement period. Concentrate intakes could be similarly summarised to mean DMI / day values within the measurement period on an animal basis using concentrate delivery calibrations, feed composition data and refusal data mentioned throughout sections 3.3.4 *Treatment concentrate feed management* and 3.3.5 *Treatment concentrate feed calibrations*.

As mentioned in *3.3.2 Forage management,* Pasture silage DMI could inferred using records of animal numbers in the study herd for each day of the measurement/RC period, daily pasture silage offers, feed composition data (see Table 4.1) and an assumed 90% utilisation rate of silage (DairyNZ, 2023). Once all other intakes of assumed pasture silage as well as known intakes of concentrates and GF pellets were accounted for, dry matter intakes of pasture were essentially estimated in this study as a residual of ME_r after these other feed intakes had been accounted for. Pasture intake can therefore be expressed as Equation 3.14.

Equation 3.14 Pasture intake estimation using known intakes and ME contents of feeds.

$$DMI_{Pasture} = \frac{ME_{\ r} - \sum[(DMI_{\ Silage} \times ME_{\ Silage}) + (DMI_{\ GF} \times ME_{\ GF}) + (DMI_{\ Conc} \times ME_{\ Conc})]}{ME_{Pasture}}$$

Where:

$DMI_{Pasture}$ = The estimated DMI of pasture as the residual of ME_r divided by the mean MJ ME of pasture.

DMI_{silage} = The assumed mean silage intake of animals daily (as kg DM / day) using pasture silage feeding records.

DMI_{GF} = The known mean intake of GreenFeed pellets (as kg DM / day) using collated GF data.

DMI_{Conc} = The known mean intake of treatment concentrate (as kg DM / day) using Jantec records and treatment refusal records.

ME_{silage} = The ME content of pasture silage (MJ ME / kg DM)

ME_{GF} = The ME content of GF pellets (MJ ME / kg DM)

ME_{Conc} = The ME content of concentrates (MJ ME / kg DM)

ME_{Conc} = The ME content of pasture (MJ ME / kg DM)

Within this model, feed analyses within each feedstuff was blocked to ensure that some variation of feed quality over the study's timeline was captured. These blocks were set at days 0 – 16 and days 17 – 32 of the study's measurement period. For pasture, the mean ME (as well as all other relevant feed qualities) of these feedstuffs for the first half of the measurement period were collated from 8 pasture samples collected between 17/10/2022 – 02/11/22. A similar practise was carried out to block the chemical analysis of two pasture silage, concentrate and GF pellet samples collected between 21/10/2022 – 28/10/2022. For the latter half of the measurement period, 7 pasture samples collected between 04/11/2022 – 18/11/2022 as well as three pasture silage, concentrate and GF pellet samples collected between 04/11/2022 – 18/11/2022 were used for these analyses. These blocked

feed compositions are not reported further in this thesis (see Table 4.1 for mean feed compositions; Figure 4.1 for changing pasture composition over the study's measurement period).

As DMI is very strongly correlated with M_p and as DMI is a major component to the calculation of M_y, alternative prediction equations of intake (DMI_e) were also provided in this thesis as a means to evaluate the accuracy and precision of calculated DMI using SCA (1990) metabolic calculations. Four prediction equations of DMI_e are reported in Table 4.4, using Equations 3.15 – 3.18:

Equation 3.15 Dry matter intake estimate$_1$ (DMI_1) (Fox et al., 1992).

$$\mathrm{DMI}_1 = 0.0185\mathrm{Liveweight} + 0.305FCM$$

Equation 3.16 Dry matter intake estimate$_2$ (DMI_2) (Vasquez & Smith, 2000).

$$\mathrm{DMI}_2 = 1.84 + 0.38FCM + 0.018Liveweight + 2.82\Delta Liveweight$$

Equation 3.17 Dry matter intake estimate$_3$ (DMI_3) (NRC, 2001).

$$\mathrm{DMI}_3 = 0.372FCM + 0.0968Liveweight^{0.75} + (1 - e^{(-0.192\times(WOL+3.67)}))$$

Equation 3.18 Dry matter intake estimate$_4$ (DMI_4) (Watt et al., 2015).

$$\mathrm{DMI}_4 = \frac{0.27CO_2 + 1.18Milk - 821.3}{126}$$

Where:

Milk = The average daily milk yield (kg / day) of each animal throughout the measurement period

FCM = The average daily fat corrected milk yield (kg / day) of each animal throughout the measurement period. See equation 3.19.

Liveweight = The average liveweight (kg) of each animal throughout the measurement period

ΔLiveweight = The average change in liveweight (kg / day) of each animal throughout the measurement period

WOL = The week of lactation for each animal utilised in statistical analyses, taken from the mid-point of the measurement period (02/11/22).

CO_2 = The average daily CO_2 production (g / day) of each animal throughout the measurement period

Equation 3.19 Fat corrected milk production (Hall, 2023).

$$\text{FCM} = 0.3994 \times \text{Milk Yield} + 15.0148 \times \text{kg fat}$$

Where:

kg fat = The average milk fat yield per day (kg / day)

Milk yield = The average milk produced by an animal per day, corrected for density. (kg / day)

3.8.3 GreenFeed data modelling & alternative methane production estimates

To fulfil the research objective of this study; two reported methodologies to calculate M_p were chosen. The primary methodology to express M_p in this study was chosen from Hristov et al., (2015) by summarising all validated CH_4 emissions recordings by hour of day at the animal to express hourly M_p for capturing diurnal variation of CH_4 emissions (as seen in Figure 3.5). A weighted average of emissions per day could then be collated from these hourly means before significance testing at the treatment level occurred. This method of CH_4 production modelling is expressed as an estimate of (M_p) throughout the results and discussion of this thesis. This same process was repeated to summarise all measurements of CO_2 production (as g CO_2 / day) for all animals within all treatment groups and is reported in Table 4.6 as a proxy of CH_4 emissions.

To ensure this weighted average method accurately and precisely modelled M_p at the treatment level, a mixed-model analysis of CH_4 production ($M_{p\text{-}mm}$), M_p from all 42 animals calculated using only GF measurements taken in first half of the trial ($M_{p\text{-}Period\ 1}$) and the latter half of the trial ($M_{p\text{-}Period\ 2}$), along with four M_p prediction equations (Mp_e) were provided in Tabel 4.6. A final baseline CH_4 production estimate ($M_{p\text{-}21.6}$) was also provided in Table 4.6 for further analysis and discussion of M_p, using calculated DMI multiplied by 21.6 g CH_4 / kg DM, the chosen MCR of New Zealand's GHG inventory. The mixed model analysis of $M_{p\text{-}mm}$ used within this study included fixed effects of animal ID nested within treatment, and hour nested within day along with GF ID number (FID) as a random effect. This mixed model analysis was performed in R studio version build 4.2.1 (R Studio Core Team, 2022) using the 'LME4' and 'predictmeans' (Luo et al., 2022) packages. A second modelling semi-parametric models using spline plots under 'predictmeans' (Luo et al., 2022) was also trialled in this thesis' analysis as a potential to best-fitting predicted M_p values over the measurement period's timeline. However, this model was discarded due to a high level of error. The three prediction equations of M_p were obtained from the review of Appuhamy et al., (2016) of the estimation of CH_4 emissions from cattle. These predictions equations of M_{pe} (listed in Equations 3.20 – Equation 3.22) and $M_{p\text{-}21.6}$ can therefore be used to interpolate whether any errors existed in the modelling or measuring of GF data.

Equation 3.20 M_p estimate$_1$ (M_{p-1}) (IPCC, 1997).

$$M_{p-1} = \frac{[0.060 \times GEI]}{0.05565}$$

Equation 3.21 M_p estimate$_2$ (M_{p-2}) (Yan et al., 2000).

$$M_{p-2} = \frac{[3.23 + 0.055 \times GEI]}{0.05565}$$

Equation 3.22 M_p estimate$_3$ (M_{p-3}) (Nielsen et al., 2013).

$$M_{p-3} = \frac{1.26 \times DMI}{0.05565}$$

Where:

GEI = Gross energy intake of the diet (GE consumed / day) (provided as estimate in this study using workings from Equation 3.7).

DMI = Dry Matter intake (kg / day) (provided as estimate in this study using workings form Equation 3.13).

4. Results

4.1. Feed composition

As a general trend, the ME content of pasture fed in this study were numerically constant and were between 10.2 MJ ME / kg DM to 10.5 MJ ME / kg DM for the duration of the 33-day measurement period (see Figure 4.1). Minor numeric changes to CP, NDF and NFC were observed across the measurement period. An ANOVA analysis of all feed sample components (e.g. DM%, MJ ME / kg DM, CP%, NDF% and NFC%) were performed in this study, however, no statistically significant trends in pasture composition or quality were observed throughout the timeline of this study, as indicated by 95% confidence interval (CI; shaded area around smooth trendline). Some numerical differences of pasture quality are observable over time throughout Figure 4.1., however insufficient power (through a lack of repeated measures) prevented significant changes to pasture quality throughout the study's measurement period to be declared.

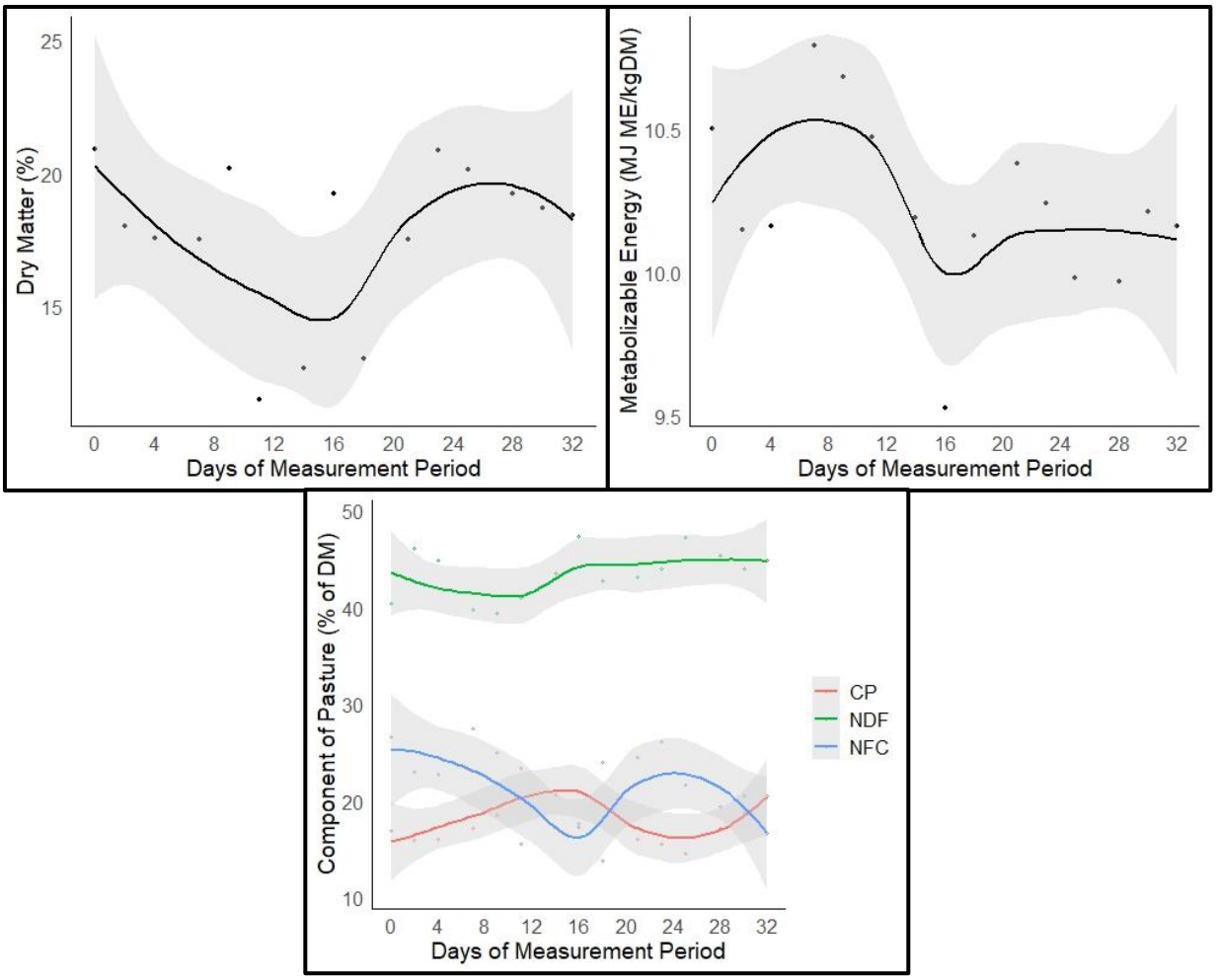

Figure 4.1 Changes to pasture composition throughout the measurement period.

Pasture silage was the most chemically variable feed offered in this study, with considerable numeric variation being observed across the DM, ash, ADF and OMD components of pasture silage throughout the study (Table 4.1). This captured variance may have been spatially or temporally derived e.g. different bales fed within the study may have been cut from areas of the farm, from different quality pastures at the time of cutting or may have been cut at different times of the autumn prior to the study.

Table 4.1 Mean ± standard deviation of chemical composition for all feeds used within this study's measurement period.

Chemical composition	Pasture[1] (n = 17)	Grass silage[2,3] (n = 5)	GreenFeed pellets[3] (n = 5)	Concentrate[3] (n = 5)
Dry matter (%)	17.1± 29.9	45.7 ± 9.4	88.2 ± 0.0	86.9 ± 0.0
Ash (% of DM)	12.4 ± 1.4	25.8 ± 2.7	9.6 ± 0.3	6.8 ± 3.0
Crude protein (% of DM)	18.2 ± 2.8	18.8 ± 1.7	13.0 ± 0.2	14.2 ± 3.8
Crude fat (% of DM)	4.7 ± 0.4	4.4 ± 0.8	1.6 ± 0.1	2.5 ± 0.4
Soluble sugars & starch (% of DM)	9.7 ± 1.3	10.8 ± 1.9	14.4 ± 0.2	39.4 ± 0.3
Acid detergent fibre (% of DM)	26.2 ± 2.3	25.8 ± 3.4	26.4 ± 0.7	16.4 ± 0.0
Neutral detergent fibre (% of DM)	43.6 ± 2.6	43.0 ± 1.4	46.2 ± 1.6	30.6 ± 1.8
Organic matter digestibility (% of DM)	72.0 ± 2.0	69.1 ± 4.3	75.7 ± 0.4	80.6 ± 3.8
Dietary cation anion difference[4]	192.6 ± 65.6	248.3 ± 133.6	195.7 ± 14.7	96.4 ± 3.2
Metabolisable energy (MJ ME/kg DM)	10.2 ± 0.3	9.1 ± 0.1	10.0 ± 0.0	11.1 ± 0.1

[1]Ryegrass based pasture, chemical composition measured across 15 individually collected samples using NIRS.

[2] Grass silage was mixed with mineral supplements.

[3] Average of two sample pools chemical composition (measured with wet chemistry).

[4] Calculated as: [(sodium (% DM) * 435) + (potassium (% DM) * 256)] – [(chlorine (% DM) * 256) + (sulphur (% DM) * 624)] (NRC, 2001).

4.2. Animal liveweight and body condition score change

Animals experienced minor, insignificant changes to liveweight (P = 0.322) with all analysed animals losing an average of 0.87% of their liveweight over the entire measurement period. This minor change of liveweight was seen across all treatment groups, with animals in the HIGH group numerically losing the most liveweight (-0.30 kg / day). Animals in the CON group experienced significantly lower initial and final liveweights than other treatment groups (P = 0.015), however this is co-incidental and an unintended consequence of mid-study animal exclusions (animals with high liveweight) as liveweight was effectively blocked across treatments in the study's design period (see Table 3.4).

Changes to BCS were poorly correlated to changes of liveweight across all treatment groups (Pearson's correlation coefficient (r) = -0.169; see Table 4.7) and the subjectivity of BCS measurements saw animals in the CON group experience decreasing liveweights while BCS within the treatment group numerically increased. The least square means of liveweights and BCS across all treatments are shown in Table 4.2 and mean liveweights of treatment groups (including 0.95 CI) across the measurement period are displayed in Figure 4.2.

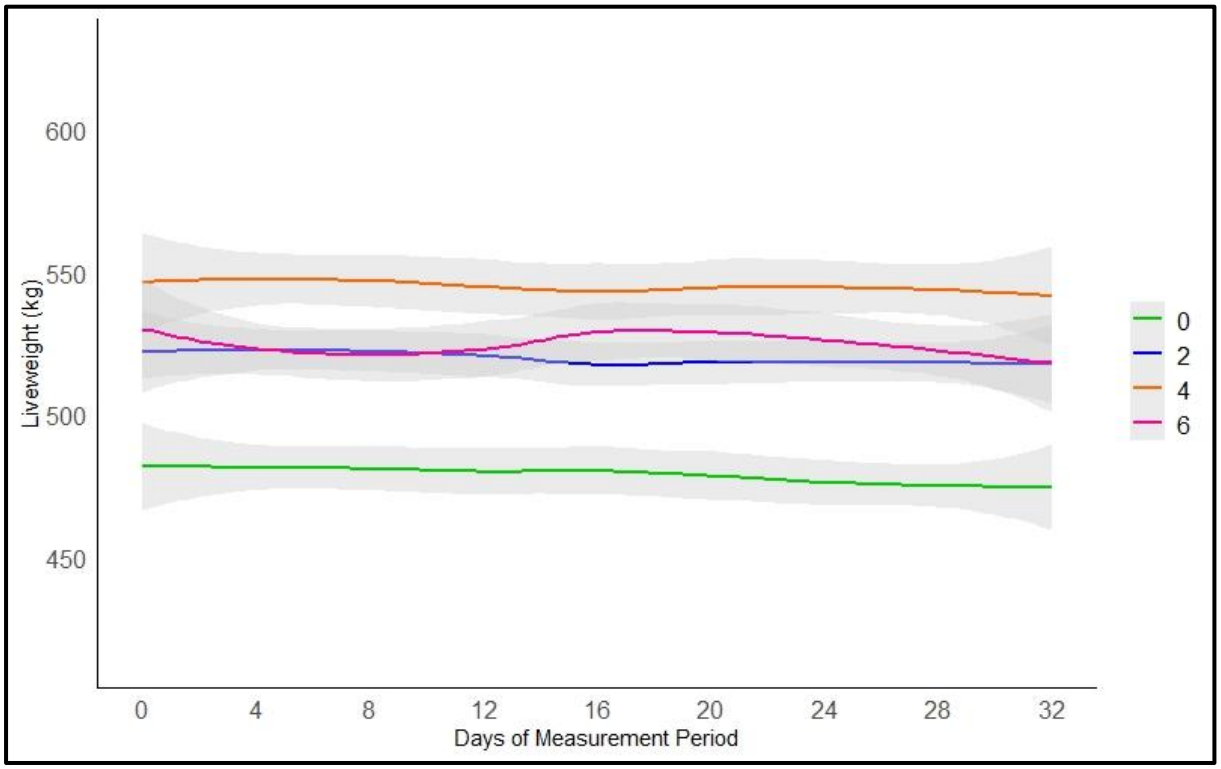

Figure 4.2 Liveweight change (kg) throughout the measurement period by treatment group (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6).

Table 4.2 Changes to liveweight and BCS of lactating dairy cows fed increasing amounts of high energy concentrates.

Liveweight and Body Condition Score	CON (n=11)	LOW (n=11)	MED (n=10)	HIGH (n=10)	SED	P value	L	Q
Liveweight (kg)								
Start liveweight	479.2^{a}	520.7^{ab}	544.9^{b}	529.3^{b}	19.67	0.015	0.008	0.045
Midpoint liveweight	477.4^{a}	519.4^{ab}	544.2^{b}	525.0^{ab}	19.10	0.010	0.007	0.035
Finish liveweight	474.6^{a}	517.9^{ab}	542.9^{b}	520.2^{ab}	19.57	0.010	0.016	0.021
Average daily weight loss	-0.15	-0.08	-0.06	-0.30	0.14	0.322	0.295	0.115
Body condition score (BCS) (1 – 10)								
Start BCS	4.1	4.1	4.1	4.2	0.14	0.770	0.531	0.568
Midpoint BCS	4.2	4.1	4.1	4.2	0.02	0.761	0.078	0.434
Finish BCS	4.2	4.1	4.0	4.1	0.21	0.694	0.442	0.467
Average daily change in BCS	0.003	-0.0002	-0.004	-0.01	0.005	0.265	0.045	0.921

CON: 0 kg of concentrate / day; LOW: 2 kg of concentrate / day; MED: 4 kg of concentrate / day; HIGH: 6 kg of concentrate / day; L: linear effect; Q: quadratic effect; ^{ab}: Different letters mean $P < 0.05$.

4.3. Animal milk production

Table 4.3 outlines the mean yields and composition of milk between treatment groups in this study's measurement period. Increasing rates of concentrate feeding were not found to significantly alter any factor of milk composition, despite some fat and protein yields showing a numeric trend of increasing with concentrate feeding rates. Milk composition was generally very consistent across the timeline of the study and no significant temporal differences to milk composition were detected across treatments (data not shown). Although daily milk volumes (as L / day) were found to be numerically different between groups, no significant changes to milk production were observed over time in the measurement period in Figure 4.3 (CI = 0.95).

Minor numeric trends were identified for increasing milk production, FPCM and FCM with increasing concentrate inclusion in the diet. Concentrate intakes were weakly correlated to increased FPCM yield ($r = 0.325$, $P < 0.05$; see Table 4.7) and fat yield (kg / day) was particularly influenced by concentrate feeding rates, with MED and HIGH animals numerically yielding 17% and 13% more milk fat daily than CON animals, respectively.

Table 4.3 Milk composition and milk production in lactating dairy cows fed increasing amounts of high energy concentrates.

Milk composition and production	CON (n=11)	LOW (n=11)	MED (n=10)	HIGH (n=10)	SED	P value	L	Q
Milk composition (g / 100 g milk)[1, 2]								
Fat	4.71	4.74	5.02	4.82	0.002	0.420	0.349	0.393
Protein	3.48	3.51	3.57	3.60	0.001	0.645	0.205	0.960
Casein	2.64	2.68	2.71	2.72	0.001	0.711	0.245	0.824
Lactose	4.79	4.77	4.85	4.81	0.0004	0.333	0.311	0.779
Urea (mg / 100 ml milk)	21.31	23.12	20.89	20.85	1.492	0.405	0.468	0.387
Milk components production (kg / d)[2]								
Milk	21.51	21.77	23.51	23.59	1.467	0.320	0.009	0.907
FCM	23.70	24.15	27.16	26.53	1.758	0.151	0.041	0.684
FPCM[3]	24.26	24.73	27.63	27.22	1.758	0.148	0.029	0.739
Fat	1.01	1.03	1.18	1.14	0.083	0.111	0.042	0.558
Protein	0.75	0.76	0.84	0.85	0.056	0.188	0.037	0.955
Milk Solids[4]	1.75	1.79	2.02	1.99	0.133	0.111	0.030	0.705

CON: 0 kg of concentrate / day; LOW: 2 kg of concentrate / day; MED: 4 kg of concentrate / day; HIGH: 6 kg of concentrate / day; L: linear effect; Q: quadratic effect; [ab]: Different letters mean $P < 0.05$.

[1]Milk composition calculated as the weighted average of the 10 milk composition sampling events (five morning and five afternoon milkings) collected across the measurement period.

[2]Items expressed in g/100 g milk or kg/d, except for milk urea.

[3]FPCM calculated as: [0.377 + (0.116 x Fat (%)) + (0.06 x Protein (%)) x Milk Yield (kg / day)] (Subnel et al., 1994)

[4]Milk solids calculated as: [(Fat g/100g x milk (kg/d)) + (Protein (g/100g) x milk (kg/d))]

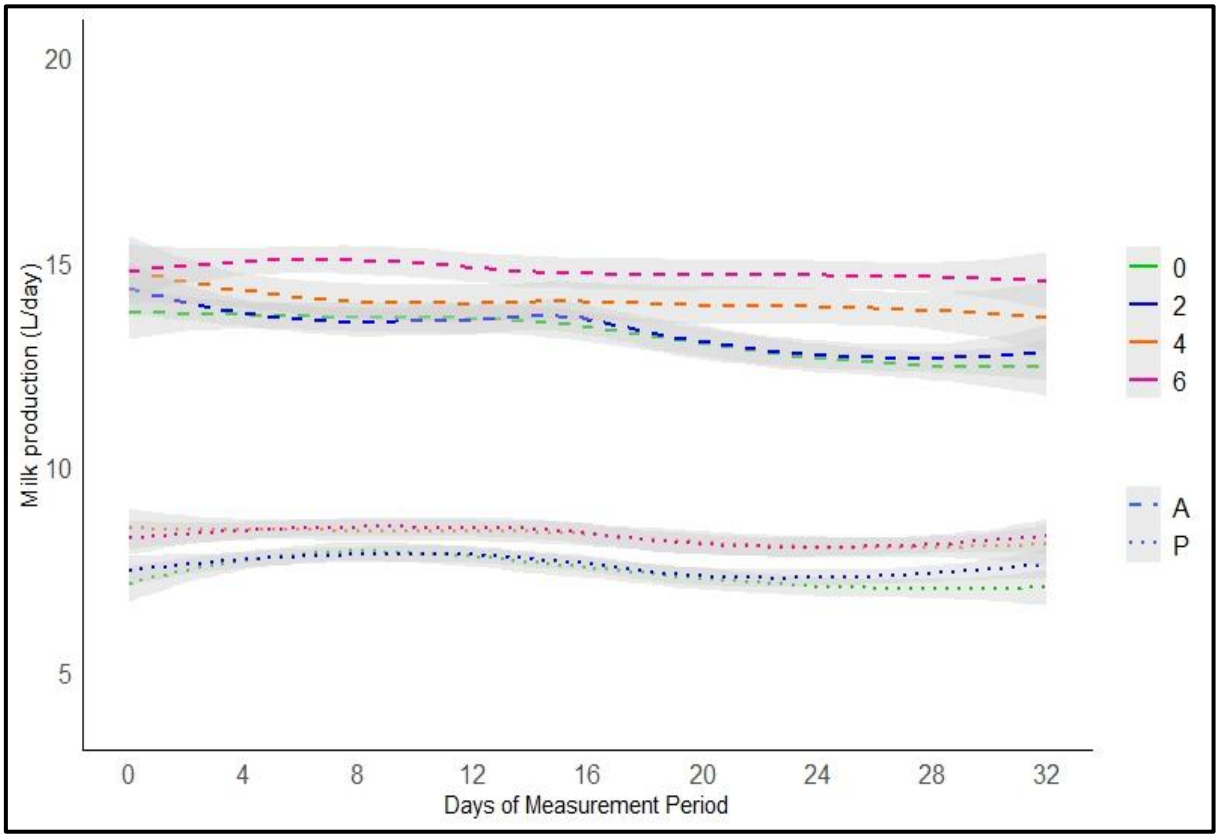

Figure 4.3 Mean milk yields (L / day) by treatment groups over study's measurement period. (A = a.m. milk volumes (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6).

4.4. Dry matter intakes & metabolisable energy requirements

Pasture intakes linearly decreased between treatment groups (P = 0.001), denoting a pronounced substitution of pasture from increasing concentrate intakes. Although the rates of liveweight change (see Table 4.2) and milk production (see Table 4.3) were found to insignificantly change with increasing concentrate supplementation; total ME requirements (see Table 4.4.) and DMI tended to numerically increase with increased concentrate feeding. Metabolizable energy requirements were found to increase linearly with the average MJ ME / kg DM of each treatment group's diet (P = 0.001), and this may partially explain the insignificant changes to DMI (calculated as ME_r / mean diet MJ ME / kg DM) across treatment groups (P = 0.168).

A statistical difference of ME_m was detected between groups (P = 0.007), this was due to the lower liveweight of CON animals (after exclusion of a heavy CON animals from the trial) and increasing milk energy requirements across LOW, MED and HIGH animals which is an important factor of ME_m.

Table 4.4 Dry matter intake (DMI) and metabolisable energy (MJ ME) requirement estimates of early lactation cows fed increasing amounts of high energy concentrates.

Parameters	CON (n=11)	LOW (n=11)	MED (n=10)	HIGH (n=10)	SED	P value	L	Q
Dry matter intake (mean kg DMI / day)								
Estimated total DMI[1]	18.6	19.0	20.6	19.9	0.935	0.168	0.077	0.389
Estimated Pasture DMI[1]	15.14[a]	13.80[a]	13.46[a]	10.86[b]	0.920	0.001	0.001	0.355
Estimated pasture silage DMI	2.72	2.71	2.74	2.74	0.028	0.596	0.212	0.847
GreenFeed pellet DMI	0.71[a]	0.57[ab]	0.54[av]	0.48[b]	0.072	0.021	0.003	0.408
Treatment concentrate DMI	-	1.93[a]	3.86[b]	5.77[c]	0.013	0.001	0.001	0.307
Substitution rate (kg DM pasture substituted / kg DM concentrate fed)[2]	-	0.70	0.44	0.74	0.282	0.523	0.859	0.241
Alternative Dry matter intake estimates (mean kg DMI / day)								
DMI_1	16.05[a]	16.97[ab]	18.35[b]	17.80[b]	0.679	0.001	0.006	0.133
DMI_2	16.17	16.92	18.46	18.54	0.915	0.039	0.010	0.615
DMI_3	17.75[a]	18.63[ab]	20.02[b]	19.51[b]	0.699	0.013	0.009	0.169
DMI_4	18.94	20.38	20.66	19.86	1.032	0.374	0.374	0.140
Daily metabolic energy (ME) intakes and requirements								
Weighted average ME intake (MJ ME / kg DM)	10.10[a]	10.19[b]	10.28[c]	10.36[d]	0.003	0.001	0.001	0.109
Total ME requirements (mean MJ ME / day)[1]	186.68	193.09	210.97	204.81	9.608	0.061	0.017	0.377
Maintenance requirements[1] (MJ ME / day)[1,3]	68.87[a]	72.00[ab]	75.42[b]	74.15[b]	1.750	0.007	0.001	0.098
Lactation requirements (MJ ME / day)[1]	120.05	122.06	136.25	133.77	8.731	0.172	0.052	0.734
Liveweight change requirements (MJ ME / day)	-2.25	-1.00	-0.68	-3.13	1.744	0.497	0.656	0.151

CON: 0 kg of concentrate / day; LOW: 2 kg of concentrate / day; MED: 4 kg of concentrate / day; HIGH: 6 kg of concentrate / day; L: linear effect; Q: quadratic effect; [ab]: Different letters mean $P < 0.05$.

[1]: Calculated using SCA method.

[2]: Calculated as: [(mean pasture DMI of CON – mean Pasture DMI of treatment) / mean concentrate DMI of treatment] (Bargo et al., 2003).

[3]: Maintenance energy requirements includes activity energy requirements.

Pasture intakes decreased significantly and linearly in this study (P linear = 0.001) with increasing concentrate intakes, however the rate of substitution varied considerably between LOW, MED and HIGH treatment groups and a statistically significant substitution effect of concentrate feeding was not detected in this study (P = 0.523). Animals in the LOW treatment group consumed 1.93 kg DM of concentrates / day and reduced pasture intakes (compared to CON) by 1.34 kg DM / day (70% substitution rate), MED animals ate 3.86 kg concentrate / day to reduce pasture intakes by

1.68 kg DM / day (44% substitution rate), and HIGH animals ate 5.77 kg DM concentrates / day with a reduction of pasture intake by 4.28 kg DM / day (74% substitution rate). Concentrate intakes were found to be moderately correlated against pasture intakes ($r = -0.577$, $P < 0.01$; see Table 4). MED animals in this study were found to consume 10.7% more kg DMI / day than CON animals and total DMI tended to increase with concentrate feeding rates ($r = 0.287$, $P > 0.10$; see table 4.7)

The correlations of DMI (calculated using the SCA method) and DMI_e are seen in Table 4.5. Calculated DMI, using methodology from SCA, (1990), and DMI_e predictions all showed a moderate to high level of statistically significant agreement. While DMI_4 was poorly to moderately correlated with all other DMI estimates, all DMI_e outputs demonstrated the same numeric rankings as DMI across treatment groups. Despite no significant differences being detected in calculated DMI across the treatment groups, DMI_1 and DMI_3 denoted significant and linear effects (P Linear = 0.006; P Linear = 0.009; seen in Table 4.4) of increasing intake under increased supplementation. Again, this can be largely explained by increasing milk production under additive supplementation. As seen in Table 4.5, calculated DMI using the SCA, 1990 method was in very strong agreement with $DMI_{e\text{-}1}$ and $DMI_{e\text{-}3}$ ($r = 0.882$, $P < 0.01$; $r = 0.908$, $P < 0.05$, respectively).

Table 4.5 Pearson's correlations between estimated dry matter intake (SCA, 1990) and DMI prediction equations of early lactation cows fed increasing amounts of high energy concentrates.

Parameters	DMI	DMI_1	DMI_2	DMI_3
DMI_1	0.882[a]			
DMI_2	0.804[b]	0.913[b]		
DMI_3	0.908[b]	0.983[b]	0.895[a]	
DMI_4	0.430[a]	0.608[a]	0.480[a]	0.607[a]

For all DMI equations, refer to section 3.8.2 *Models & equations pertaining to estimating dry matter intake.*
DMI: Equation 3.13; DMI_1: Equation 3.15; DMI_2: Equation 3.16; DMI_3: Equation 3.17; DMI_4: Equation 3.18
[a]: $P < 0.01$
[b]: $P < 0.05$

4.5. Methane production & GreenFeed visitations

Similarly to GF pellet intakes, visitation to GF units decreased linearly with increasing concentrate feeding (P = 0.008). Figure 4.4 demonstrates that sum of visitation in the CON group was significantly higher than in all other treatment groups, particularly after milkings (indicated by arrows).

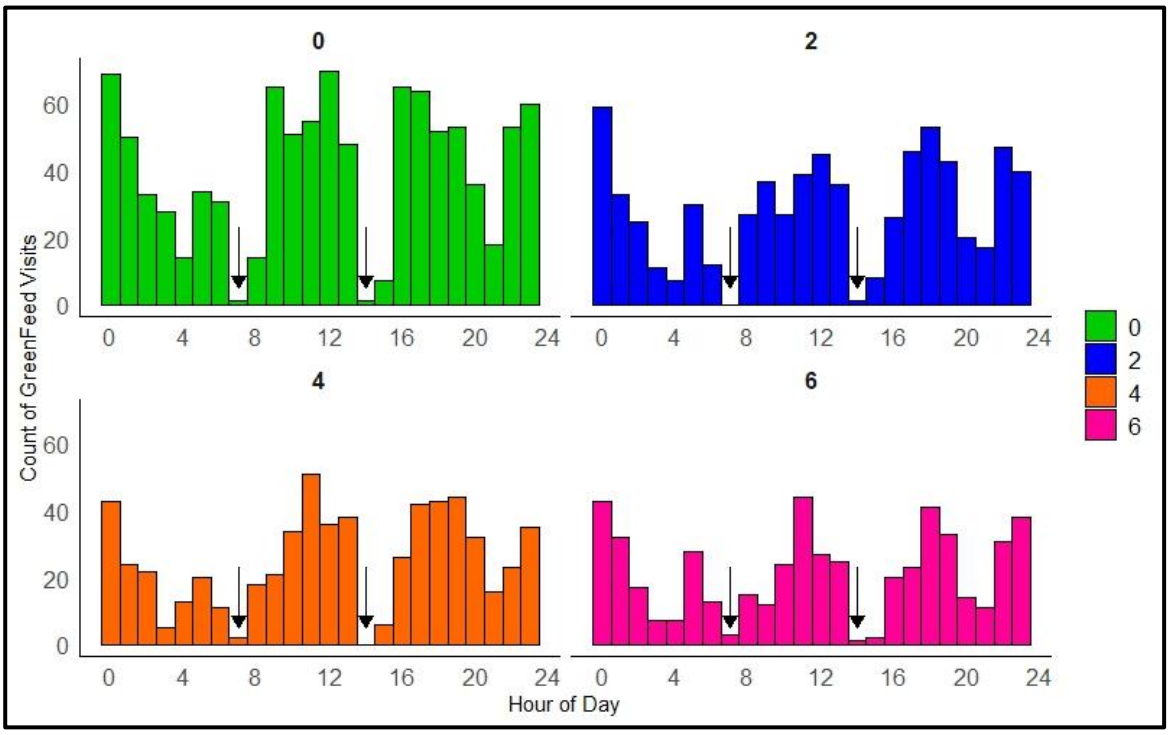

Figure 4.4 Visitation to GF units by hour of day for cows of each treatment (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6). Arrows indicate milking times.

Neither M_p nor $M_{p\text{-}mm}$ analyses, found statistical differences of CH_4 production between treatment groups (P = 0.185; P = 0.193, respectively; Table 4.6). Likewise, subsequent prediction equations of M_{pe} did not find statistically significant changes to CH_4 production with increased concentrate feeding. Figure 4.5 shows the diurnal weighted average of CH_4 emissions (as g CH_4/d) emitted by each treatment group by hour of day during the study (CI = 0.95).

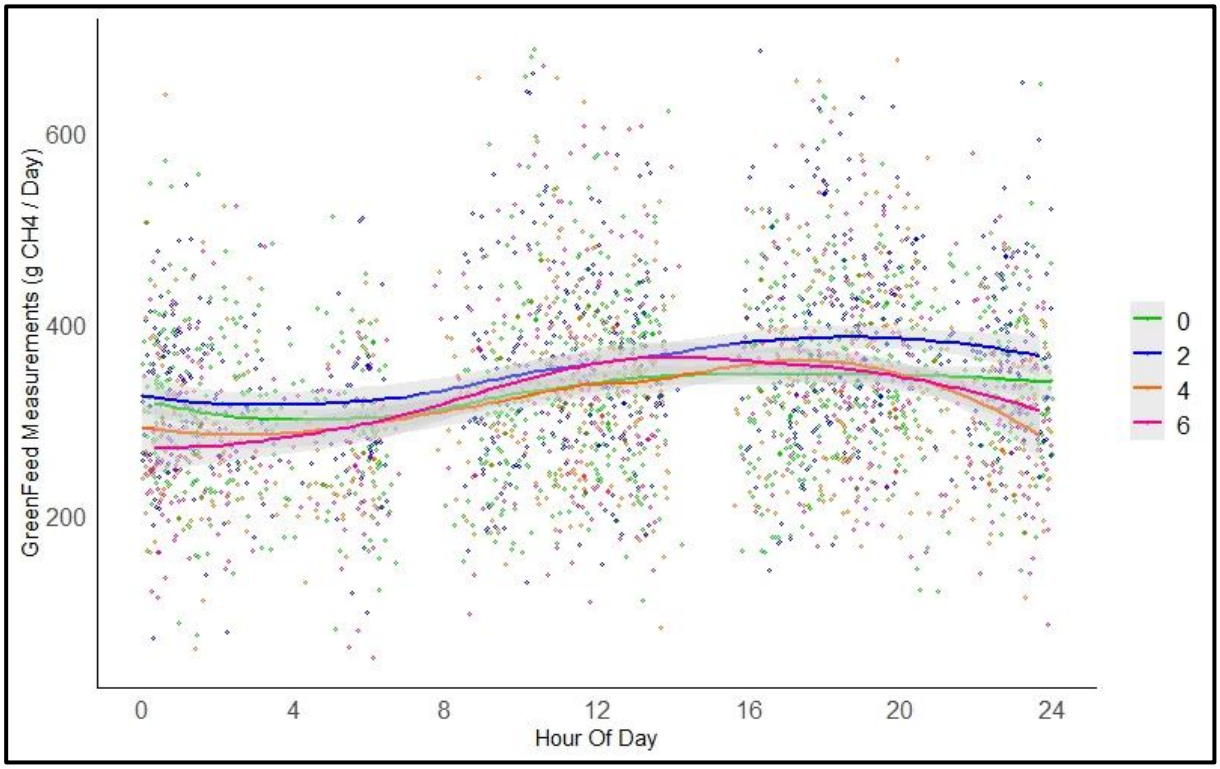

Figure 4.5 Average CH_4 production (g / day) in early-lactation dairy cattle fed increasing levels of concentrates (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6).

Despite finding that DMI and M_p did not significantly change between treatment groups, significant differences of M_y (P = 0.041), M_i (P = 0.022), g CH_4 / kg MS (P = 0.029) and CH_4: CO_2 ratios (P = 0.011) were however declared in this study. In particular, M_y M_i and CH_4 : CO_2 showed linear decreases (P linear = 0.025; 0.011; 0.006, respectively) with increased concentrate feeding. Methane yield showed a linear decrease (P linear = 0.025).

Table 4.6 Methane and carbon dioxide emissions in lactating dairy cows fed increasing amounts of high energy concentrates.

	CON (n=11)	LOW (n=11)	MED (n=10)	HIGH (n=10)	SED	P value	L	Q
GreenFeed visits/cow/day	2.82^{a}	2.03^{ab}	2.01^{ab}	1.68^{b}	0.383	0.025	0.008	0.377
CH_4 production measurements (g CH_4 / day)								
M_p	329.22	358.45	342.67	312.29	21.310	0.185	0.341	0.063
$M_{p\text{-}mm}$	328.56	356.83	340.89	311.30	21.017	0.193	0.335	0.058
CH_4 production measurements over time (g CH_4 / day)								
$M_{p\text{-}Period\ 1}$	289.33	316.52	315.77	267.30	25.585	0.174	0.413	0.041
$M_{p\text{-}Period\ 2}$	346.91	372.16	364.03	332.47	22.473	0.304	0.476	0.082
CH_4 production from prediction equations (g CH_4 / day)								
$M_{p\text{-}1}$	391.38	400.89	434.090	417.71	19.804	0.165	0.080	0.352
$M_{p\text{-}2}$	390.83	398.88	426.96	413.11	16.758	0.151	0.073	0.361
$M_{p\text{-}3}$	420.31	430.50	466.38	449.63	21.165	0.162	0.083	0.397
$M_{p\text{-}21.6}$	400.98	410.69	444.92	428.95	20.194	0.177	0.091	0.374
Gas emissions								
CO_2 (g CO_2 / day)	11785.4	12458.1	12581.8	12205.3	478.93	0.350	0.336	0.131
CH_4 : CO_2 (mol : mol)	0.0762^{a}	0.0786^{a}	0.0739^{ab}	0.0690^{b}	0.00276	0.011	0.006	0.084
CH_4 yield (g CH_4 / kg DM)	17.81^{ab}	19.01^{a}	16.82^{ab}	15.69^{b}	1.118	0.041	0.025	0.152
CO_2 intensity (g CO_2 / kg FPCM)	494.29	511.06	465.56	455.74	32.556	0.310	0.127	0.561
CH_4 intensity (g CH_4 / kg FPCM)	13.76^{ab}	14.73^{a}	12.70^{ab}	11.55^{b}	1.042	0.022	0.011	0.159
CH_4 / kg MS (g CH_4 / kg MS)	190.29^{ab}	203.80^{a}	174.07^{ab}	158.41^{b}	15.240	0.029	0.014	0.184

CON: 0 kg of concentrate / day; LOW: 2 kg of concentrate / day; MED: 4 kg of concentrate / day; HIGH: 6 kg of concentrate / day; L: linear effect; Q: quadratic effect; ^{abc}: Different letters mean $P < 0.05$.

To identify potential drivers of M_p under increased concentrate feeding, Pearson's correlations between M_p and key parameters of both DMI and animal production were performed (see Table 4.7). These correlations showed that M_p was poorly explained through concentrate feeding alone ($r = -0.141$, $P > 0.10$) and pasture intakes explained a greater amount of M_p variation across treatment groups ($r = 0.408$, $P < 0.01$). Dry matter intake was identified a weakly correlated explainer of M_p in this study ($r = 0.318$, $P < 0.10$) while FPCM was identified as a significant and moderate explainer of M_p ($r = 0.577$, $P < 0.01$).

Table 4.7 Pearson's correlation of M_p, DMI and reported animal production parameters.

Parameters	Concentrate intake	M_p	DMI	Pasture intake	Δ Liveweight	Δ BCS	Fat (%)	Protein (%)
M_p	-0.141							
DMI	0.287[c]	0.318[b]						
Pasture intake	-0.577[a]	0.408[a]	0.615[a]					
Δ Liveweight	0.152	-0.100	-0.009	-0.136				
Δ BCS	-0.309[c]	0.064	-0.021	0.245	-0.179			
Fat (%)	0.154	0.044	0.338[b]	0.161	-0.269[c]	-0.245		
Protein (%)	0.205	-0.173	0.178	-0.029	0.030	-0.381[b]	0.439[a]	
FPCM	0.325[b]	0.557[c]	0.972[a]	0.557[a]	0.147	-0.106	0.304[c]	0.232

Concentrate intake: As kg DM treatment eaten / animal / day; M_p: Weighted average modelled GF data across hour of day; DMI: Dry matter intake (Equation 3.13); Pasture intake: As kg DM pasture eaten / animal / day (estimated using Equation 3.14); Δ Liveweight: Liveweight change (As kg / day); Δ BCS: (As change / day); Fat (%): Fat content of milk (g / 100 g); Protein (%): Protein content of milk (g / 100 g); FPCM: Fat and protein corrected milk (kg / day).

[a]: $P < 0.01$

[b]: $P < 0.05$

[c]: $P > 0.10$

From examining Table 4.6, it is noted that the accuracy and the precision of M_p and $M_{p\text{-}mm}$ were numerically similar. Both these modelled outputs were very strongly correlated ($r = 0.938$, $P < 0.01$; see Table 4.8, which demonstrates that the fixed and random effects applied within this mixed model did not significantly affect the accuracy and precision of predicting M_p using GF data in the current study. All three M_{pe} outputs, while numerically similar, estimated CH_4 emissions from all treatment groups to be much greater than both modelled M_p and $M_{p\text{-}mm}$ outputs' (Table 4.6). Pearson's correlations between M_p and M_{pe} (Table 4.8) found that modelled methods of M_p were in high agreement with each other, but not with any M_{pe} outputs ($r = 0.318$ - 0.321, $P < 0.05$). Likewise, it was found that M_p was poorly correlated with $M_{p\text{-}21.6}$ ($r = 0.321$; $P < 0.05$). Carbon dioxide emissions, which were estimated using the same methodology as M_p, were strongly correlated with M_p ($r = 0.801$, $P < 0.01$) to provide a further confidence in the ability for GF to accurately capture and measure all gas emissions from animals in the study.

Table 4.8 Pearson's correlations of modelled (based on GF data) and predicted CH_4 emissions.

	M_p	$M_{p\text{-}MM}$	$M_{p\text{-}1}$	$M_{p\text{-}2}$	$M_{p\text{-}3}$	$M_{p\text{-}21.6}$
$M_{p\text{-}MM}$	0.938[a]					
$M_{p\text{-}1}$	0.321[b]	0.343[b]				
$M_{p\text{-}2}$	0.321[b]	0.343[b]	0.999[a]			
$M_{p\text{-}3}$	0.318[b]	0.343[b]	0.999[a]	0.999[a]		
$M_{p\text{-}21.6}$	0.318[b]	0.343[b]	0.999[a]	0.999[a]	0.999[a]	
CO_2	0.801[a]	0.792[a]	0.423[a]	0.422[a]	0.421[a]	0.421[a]

For all methane production (M_p) and methane production estimate (M_{pe}) methodologies, refer to section 3.8.3 *GreenFeed data modelling & alternative methane production estimates.*

M_p: Weighted average of CH_4 production modelled using GF data across hour of day; $M_{p\text{-}mm}$: Mixed model of GF data using animal ID nested within treatment, and hour nested within day as fixed effects as well as GF ID as a random effect; $M_{p\text{-}1}$: Equation 3.20; $M_{p\text{-}2}$: Equation 3.21; $M_{p\text{-}3}$: Equation 3.22; $M_{p\text{-}21.6}$: As 21.6 g CH_4 / kg DM (MFE, 2021); CO_2: Weighed average of CO_2 production modelled using GF data across hour of day.

[a]: $P < 0.01$

[b]: $P < 0.05$

5. Discussion

The results presented in this thesis offer several insights into the trends of CH_4 production and animal performance under increasing levels of concentrate supplementation in dairy cows grazing *ad libitum* pasture as well as into the effective usage of GF devices for *in-situ* CH_4 measurement. While the average MJ ME / kg DM of animals' diets significantly increased with concentrate feeding rates, minor increases of DMI experienced across treatment groups were observed alongside insignificant numeric increases to milk production (i.e. kg MS production increased by 2 – 15%, P = 0.111) without significantly affecting liveweight or BCS. Changes to M_p using GF measurements across concentrate feeding levels were not statistically significant. However, numeric changes to M_p were statistically supported by linear decreases to the ratio of CH_4/CO_2 expired by the animal (P linear = 0.006), as well as linear decreases of M_y (P linear = 0.011) and M_i (P linear = 0.014) with increasing concentrate feeding levels.

5.1. Effect of concentrate feeding on animal performance and methane production

5.1.1 Changes to liveweight & body condition score

A broad assessment of BCS and liveweight change within this study indicated that concentrate supplementation did not significantly influence animal fat reserves in these early lactation dairy cows. Animals in the HIGH treatment group experienced greater liveweight loss than all other treatments, however, changes to liveweight in early lactation are expected with DairyNZ (2021b) reporting that BCS losses of 0.5 - 1.0 can be expected in the first 50 - 100 days of lactation. Likewise, Holmes et al., (2002) reported that BCS loss post-parturition is mostly explained by factors of BCS at time of calving and feeding rates in early lactation, rather than diet composition. A lack of BCS change across both time and treatment group in this study could therefore be explained by the similar BCS of animals at the study's commencement (BCS 4.0 > 5.0 for all animals at study commencement). The unrestrictive voluntary feed intakes experienced by all study animals in the measurement period, as demonstrated by the pasture post-grazing residuals which all exceeded 2000 kg DM/ha (as shown in Figure 3.2), may have similarly explained the lack of BCS change across treatment groups.

5.1.2 Changes to milk production

Bargo et al., (2003) predicted that for every kg DM supplement fed to early lactation cattle, milk yield could be expected to increase by 1.03 kg on average. In this study, increasing concentrate feeding rates did not significantly increase milk yield, with LOW, MED and HIGH animals seeing milk yield responses of 0.13, 0.28 and 0.36 kg milk per kg DM concentrate fed. While FPCM was strongly

correlated with DMI in the current study ($r = 0.972$, $P < 0.01$),the inconclusive trend between increasing DMI with increasing concentrate feeding rates ($r = 0.287$, $P > 0.10$) may have therefore explain why concentrate feeding only explained a portion of FPCM increases across treatment groups in this study ($r = 0.325$, $P > 0.05$). Insignificant linear increases of MS yield were also observed between treatment groups in this study, with MS increasing by up to 15% in MED animals when compared to CON animals. However, the detected increases of MS production found in this study may be conservative and/or inconclusive when compared Jiao et al., (2014), which confidently demonstrated a 30% increase of MS yield ($P < 0.001$) as concentrate supplementation increased from 2.0 kg DM / day to 8.0 kg DM / day in the diets of pasture-based dairy cattle.

Hatew et al., (2015) examined the effects of feeding diets containing 60 : 40 ratios of roughage (pasture silage) and high or low starch containing concentrates. The high starch concentrate (containing corn by-products) diet was found to be associated with increased rumen microbial biosynthesis, increased rumen microbe washout and increased uptake of CP and amino acids for milk protein synthesis. Likewise, increased C_3 production from high starch diets have been associated with increased protein and lactose concentrations in milk, alongside decreases of milk fat (%) (Liu et al., 2023). This is in agreement with the findings of Aguerre et al., (2011) who identified a linear increase of protein (%) in milk composition ($P < 0.001$) alongside linearly decreasing milk fat (%) ($P = 0.04$) which is consistent with literature on supplementary feeds' interaction on milk component concentrations (Holmes et al., 2002). Likewise, Jaio et al., (2014) noted statistically significant ($P < 0.001$) and linear decreases of milk fat yields by up to 20% with increased concentrate feeding rates. Decreasing milk fat concentrations or yields are generally associated with BCS loss, as mobilised fat reserves readily yield high levels of milk fat precursors (Holmes et al., 2002). A decrease of milk fat concentrations were observed across all treatment groups within Jaio et al., (2014) as minor liveweight losses were noted over time ($P = 0.085$) and as concentrate feeding rates increased ($P < 0.001$). However, in the current study both milk fat (%) and protein (%) tended to increase ($P > 0.10$) with increased concentrate feeding ($r = 0.154$ and 0.205, respectively).

Despite dairy cattle's efficient conversion of feed to milk in early lactation, mean responses of milk production from supplement feeding generally do not exceed 1 kg milk per kg of supplement fed. Responses of milk production are generally greatest in animals which are in early lactation, with both high BCS and PW and are fed supplements which are of significantly greater feed value than offered pasture (as to encourage an additive effect on DMI) (Holmes et al., 2002). In this study, all analysed animals fulfilled these animal-based criteria. However, very high pasture allocations alongside the

moderate increase in feed value between pasture and concentrates fed in the study (10.24 mean MJ ME/kg DM in pasture; 11.12 mean MJ ME/kg DM in concentrates) may have contributed to the high levels of pasture substitution across treatment groups and associated poor milk yield responses across the treatment groups in this study. Milk yield increased by 0.26 kg/d (0.13 kg milk/kg concentrate) for LOW animals, 2.00 kg/d (0.53 kg milk/kg concentrate) for MED animals and 2.08 kg/d (0.36 kg milk/kg concentrate) for HIGH animals, which was substantially less than the 0.56 – 0.80 kg milk / kg concentrate noted by McEnvoy et al. (2008) which included similar levels of concentrate offers to pasture fed early lactation dairy cows.

5.2. Dry matter intake

Total DMI in this study tended to increase numerically with increasing levels of concentrate feeding ($r = 0.287$, $P > 0.10$). Dry matter intakes are generally considered to be governed and restricted by animal physiological states, as well as the digestibility, harvestability and the bulk of ingesta (Holmes et al., 2002). In this study, the ability to harvest feed was not considered to be a limiting factor of DMI, with high allocations of pasture as well as some refusals of pasture silage and concentrates being experienced across all treatment groups. Changes to DMI in this study were therefore only moderately correlated with pasture intakes ($r = 0.615$, $P < 0.01$) as opposed to being strongly correlated with animal production (particularly milk production, as mentioned in section 5.1.2 *Changes to milk production*).

Within Table 4.5, all prediction equations of DMI_e, with exception to DMI_4, were found to be positively and strongly correlated with estimated DMI using the SCA (1990) method, with DMI_3 being very strongly correlated with DMI ($r = 0.908$, $P < 0.01$). These prediction equations, however, are all dependant of animal production metrics such as changes in liveweight or milk production, which act as proxies for animal energy inputs and outputs (Smith et al., 2021). Therefore, the statistically significant correlations between predicted DMI_e and DMI estimated using SCA (1990) could be expected in this study and this correlation may not indicate whether estimated DMI using SCA (1990) was any more or less accurate than any estimation of DMI_e.

The poor correlation between measured DMI and DMI_4 ($r = 0.430$, $P < 0.01$) could be tied to the methodology Watt et al., (2015) used to develop DMI_4. This study used predominantly North American type Holstein-Friesian animals, all of which were in mid-lactation and were fed 3.57 – 6.07 kg DM concentrates / day alongside *ad-libitum* pasture. Each of these factors may have affected the accurate application of DMI_4, particularly to CON animals which did not receive concentrates daily.

Watt et al., (2015) also reported much greater average milk yields from mid-lactation dairy cattle (as opposed to early-lactation animals in the current study), at 29.39 ± 11.61 kg milk / day ($P < 0.001$). Despite this, Watt et al., (2015) also reported a range of mean DMI from 18.44 – 23.59 kg DM / day ($P < 0.001$), which were similar to the reported DMI estimates in the current study. This may indicate that DMI estimation using SCA (1990) method may have overestimated intakes in the current study (relative to measured milk production) and that predicted DMI_1 or DMI_3 may have offered more accurate estimates of feed intake in light of this context.

The measured DMI of animals transported to AgResearch Grasslands for RC measurements within this study were reported to be 17.2% lower in CON animals, 6.8% lower in LOW animals, 15.0% lower in MED animals and 6.5% lower in HIGH animals when compared to measured DMI from this *in-situ* study. A statistically significant, linear effect of increasing DMI with increased concentrate feeding levels was detected in RC measurements (SED = 0.900, P linear = 0.007) with these decreases in recorded DMI also being associated with increased M_p (when compared to GF data collected in the current *in-situ* study) (Della Rosa et al., 2024). This may again indicate that the applied SCA (1990) method of DMI estimation was over-estimative and further questions the accuracy of M_y values provided in Table 4.6. In particular, the M_y of CON animals highlights the impact of DMI over-estimation, with CON animals being found to produce 17.5% less than the 21.6 g CH_4 / kg DM expected from New Zealand's GHG inventory. If DMI_1 was used in place of estimated DMI, as the closest approximate of animal intakes found within the RC study, M_y predicted using GF data could also have been more aligned with the results presented in Della Rosa et al., (2024). In this scenario, M_y could have averaged 20.58 g CH_4 / kg DM in CON animals, which linearly decreased (P linear = 0.002, data not presented) with increasing concentrate feeding levels to yields of17.47 g CH_4 / kg DM in HIGH animals.

5.2.1. Substitution effect

The substitution effect of supplement feeding is largely denoted by daily herbage allowance and total DMI feeding levels, with underfed animals generally experiencing greater increase intake alongside benefits to animal performance (McEnvoy et al., 2008). In this study, a moderate and significant correlation between increasing concentrate intakes and decreasing pasture intakes was identified ($r = -0.577$, $P < 0.05$). A significant level of substitution in this instance reinforces the findings in McEnvoy et al., (2008) and it is likely that the high pasture allocations offered to all animals within the study mob attributed to a moderate level of substitution in this study.

From a review of 33 studies, Rattray et al., (2007) suggested that substitution the rates of commonly fed supplements to dairy cattle can be expected to range from -0.3 to 0.9 kg DM pasture forgone per kg DM supplement fed, with a mean expected substitution rate of 0.31 kg DM pasture. Animals within LOW and HIGH treatment groups experienced substitution rates of 0.70 and 0.74 kg DM pasture forgone per kg DM concentrate fed, respectively. Animals in the MED group experienced the lowest level of substitution in the study, consuming the same amount of pasture as animals in LOW while still consuming a mean of 3.86 kg DM of concentrate daily. Regardless, this study showed a linear decrease of pasture intake with increasing levels of concentrate feeding (P linear = 0.001), similar to that found by Jaio et al., (2014). Animals in the MED group may have experienced decreased substitution rates due to their greater ME_P requirements (210.97 MJ ME / day) with the highest mean liveweight and highest level of FPCM production in this study.

As animals in the HIGH group did not experience higher DMI or FPCM production levels when compared with animals in MED, this may be indicative of a tipping point of treatment diet on satiety and voluntary feed intakes (Hassanat et al., 2014). As noted in section 2.7.2 *The relationship between digestibility and methanogenesis*, high intake levels of high NSC feeds, such as the starch-rich concentrates fed in this study, are associated with a rapid release of C_3 from the rumen. Several markers suggest that animals within the HIGH treatment group may have experienced increased C_3 yields in this study, including increases of protein and lactose in HIGH animals' milk composition (Olijhoek et al., 2018; Rathert-Williams et al., 2023) as well as decreasing CH_4 : CO_2 emissions with increasing concentrate feeding(see section 5.3 *Effect of concentrate supplementation on methane emissions*). As observed in Figure 4.4, animals within the MED and HIGH treatment groups visited the GF units less frequently immediately after milkings. This could be a further indication of increased satiety after concentrate feeding, with these animals preferring to rest or ruminate immediately after milkings as opposed to more active foraging behaviour potentially elicited by CON and LOW animals.

Assuming grazing conditions, harvestability, grazing targets, as well as pasture quality were more indicative of that in a more normal farming system/season (see section 5.6.2 *Key limitations of study operations*); this study could have expected to see low levels of response from concentrate supplementation in early lactation and therefore expect high levels of substitution as concentrate feeding rates increased, assuming pasture offers were adequate to support peak lactation and pasture NSC and NDF levels were similar to that of the treatment feed (Homles et al., 2002). In a normal pasture-based system, the milk yield and DMI response to concentrate feeding could be expected to increase as lactation progresses into mid and late lactation, and subsequently further studies could

expect to see a reduction of M_i alongside an additive DMI effect with increasing concentrate feeding throughout mid to late lactation (Rattray et al., 2007).

5.3. Effect of concentrate supplementation on methane emissions

Methane production, as M_p or M_{pe}, did not significantly change as concentrate inclusion rates increased in the diets of early lactation dairy cows. This is in agreement with the generalisation stated by Olijhoek et al., (2018), which reported that concentrate inclusions under 35% of total DMI generally did not significantly affect CH_4 emissions (HIGH animals within the current study consumed 29.0% of their mean DMI as concentrates). Likewise, this finding is in agreement with many other studies investigating nutritional enteric CH_4 mitigation strategies, with Arndt et al., (2022) concluding that many studies which focus on nutritionally manipulating enteric CH_4 emissions do not significantly change M_p. Van Wyngaard et al., (2018) and Jaio et al., (2014), both assessed the effect of feeding increasing levels of concentrate supplements on CH_4 emissions in pasture-based dairy cattle and found that increasing concentrates up to 8 kg DM (53.6% and 51.9% of total DMI at these studies highest inclusion levels, respectively) did not significantly affect M_p. However, increasing levels of supplementation did significantly alter M_y ($P = 0.025$ and 0.005, respectively) and M_i ($P < 0.001$ and < 0.001, respectively) in both of these studies, which were also demonstrated in this study.

A quadratic decrease of M_p with increasing inclusions of a high NSC feed were also noted by Lee et al., (2004) which examined how increasing white clover content in pasture affected M_p. This study reported that graded increases of white clover content within pasture (0%, 15%, 30% or 60% of total sward DM) significantly increased DMI ($P < 0.001$) and M_p ($P = 0.022$), particularly at 15% of DMI and 60% of DMI inclusion rates. The study also reported that increasing DMD and NSC contents of forage diets were strongly associated with significant decreases to M_y. Similar results can be seen in the current study, with significant linear decreases to M_y being observed as concentrate feeding increased (P linear = 0.025). In tandem, minor trending increases of DMI were observed as concentrate inclusion rates increased in the current study ($r = 0.287$, $P > 0.10$), further emphasising the importance of DMI as a more significant driver of M_p ($r = 0.318$, $P < 0.05$) (Lee et al., 2004; O'Neill et al., 2011).

While M_p did not significant change under increasing levels of concentrate feeding, numeric increases of M_p were experienced in both LOW and MED groups, before M_p was observed to decrease in HIGH animals, which may indicate that HIGH concentrate inclusion rates were close to supporting a significant decrease of CH_4 emissions in early lactation dairy cows. This potential trend of changing M_p under supplementation is also supported by linear decreases to the $CH_4 : CO_2$ ratio as concentrate

feeding increased (P linear = 0.006). At the HIGH treatment level, the ratio of CH_4 : CO_2 emitted by animals numerically decreased by -10.0% when compared to CON animals, despite HIGH and CON animals consuming similar DMI (19.9 kg DM / day and 18.6 kg DM / day, respectively). This statistically significant decrease in CH_4 : CO_2 emitted from HIGH animals may suggest that some H_2 was sunk in alternative metabolism pathways within the rumen (such as increased C_3 production). It is also possible that limited H_2 availability may have also resulted from increased ingesta flow rates, decreased NDF fermentation and/or decreases in rumen pH in HIGH animals (Moss et al., 2010; Olijhoek et al., 2018; Wang et al., 2020).

Despite finding no statistically significant differences of M_p between treatment groups, some effect of diet on CH_4 emissions are entirely plausible within this study, which is evidenced in to decrease by 10.3% and 13.8% decreases to mean M_y and M_i between CON and HIGH animals. Lee et al., (2004) theorised that changes to M_y under increased white clover feeding were resultant of a repartitioning of GE from CH_4 production into ME for animal production. This rationale is also echoed by Beauchemin et al., (2020), and supports the decreases to M_i under increasing FPCM observed across treatment groups in this study. In contrast, Jaio et al., (2014) found that feeding 2, 4, 6 and 8 kg DM of concentrate to pasture fed dairy cattle insignificantly decreased Mp with linearly increasing DMI, which was attributed to decreasing rumen pH under the increase in concentrate feeding. Decreasing rumen pH under increased concentrate feeding rates were also observed by Wyngaard et al., (2018), and pH change was identified as a key mechanism of M_y reduction across these studies. Potential increases of ruminal C_3 production in MED and HIGH groups within this study are supported by increased FPCM, milk protein production and decreasing CH_4 : CO_2 (Hammond et al., 2016b; Aguerre et al., 2011; (Hatew et al., 2015)

5.4. Accuracy & precision of methane measurements

Within Table 4.6, the estimates of M_y, along with those of M_p and M_i, do not necessarily agree with contemporary research in New Zealand's pastoral sector. Again, this is most clearly evidenced by the M_y of CON animals, which was estimated to be 17.6% lower than the MCR of the New Zealand GHG inventory (21.6 g CH_4 / kg DM) (MfE, 2021). As milk production ascertained in this study indicated comparable precision (using SED) to contemporary studies, like Jaio et al., (2014) (SED of milk yield = 0.87, as opposed to 1.467 in the current study) and Jonker et al., (2017) (SED of FPCM = 1.50, as opposed to 1.758 in the current study). Methane intensity, as a function of milk production and M_p, is therefore thought to be more precise than estimates of M_y in the current study, as well as being free of some of the accuracy and repeatability issues surrounding DMI estimates listed throughout section

5.2 *Dry matter intake* and section 5.6.2 *Key limitations of study operations*. In this study therefore, an inaccurate estimation of M_y could be driven by not only imperfect estimates of DMI (as discussed in section 5.2 *Dry matter intake*), but also potentially inaccurate estimates of M_p.

In a broader analysis of nutritional CH_4 mitigatory studies, the replication of M_y across repeated studies has been noted as inconsistent (Beauchemin et al., 2008). Pacheco et al., (2014) found the mean M_y of sheep fed pasture-only varied by as much as 14.2% across differing *in-vivo* RC experiments studies, without changing feeding levels, in a wider analysis of RC experiments. Similar to the current study, Waghorn et al., (2016) also found unexpectedly reduced M_p (when compared to an estimate of $M_{p\text{-}21.6}$ made within this study) in early lactation dairy cattle fed pasture-only. This study found these animals emitted 18.1 g CH_4 / kg DM and 325 g CH_4 / day while yielding 26.8 kg milk / day and eating 17.93 kg DM / day, which are comparable metrics of animal intake, M_p and production to the CON animals within the current study. Lastly, in a wider context, there has even been extensive debate around the inherent accuracy of the current MCR within New Zealand's GHG inventory within recent years, with Australia's adoption of a M_y of 20.7 g CH_4 / kg DM for dairy cattle and a potentially higher M_y of 23.43 g CH_4 / kg DM also being suggested for New Zealand's inventory (Waghorn, 2021).

However, despite the subjectivity of accurate M_p in current literature, when examining the RC measurements of the animals used in this study, GF measurements and RC measurements were found to be numerically different (Della Rosa et al., 2024). In addition, the poor agreement of M_p to all M_{pe} across treatments ($r = 0.318$ - 0.321, $P < 0.05$ for all interactions between M_p and M_{pe} or M_p to $M_{p\text{-}21.6}$), alongside the large residual of $M_p - M_{pe}$ for CON animals, and an anecdotal increase of M_p across all treatment groups' in RC measurements (despite decreased DMI while in RC); indicates that GF measurements may not have accurately represented CH_4 emissions data in this study. This is not in agreement with Hristov et al., (2018) which found that CH_4 emissions measured using GF devices were comparably accurate and precise to measurements made by RC in a review comparing six studies ($R^2 = 0.92$). However, this finding is in agreement with (Hristov et al., 2018)'s observation that quantifying an agreed relationship of M_p with M_y within *in-situ* or *in-vivo* studies can be done with much greater confidence and precision using CH_4 measurements from RC as opposed to GF data.

To test the precision of M_p and assess the relative accuracy of M_p, the residuals of treatment groups' mean CH_4 emissions across the measurement period, as the differences between measured M_p and M_{pe} (and M_p - $M_{p\text{-}21.6}$), are plotted in Figure 5.1 to assess the level of agreement between M_p and different M_{pe} outputs chosen for this thesis (and between M_p and $M_{p\text{-}21.6}$). Across each plot within

Figure 5.1, there are very poor levels of agreement between M_p and modelled outputs of M_{pe}, as the 95% CI of mean M_p does not overlap the baseline (0) of mean M_{pe} (or $M_{p\text{-}21.6}$) in any plot. In turn this is confirmational that mean M_{pe} and mean $M_{p\text{-}21.6}$ are systematically greater than mean M_p in this study. The lack of slope bias across these plots indicates that no interaction of treatment on residual CH_4 was obvious in any M_{pe} methodology. However, it can be noted the difference between M_p and any M_{pe} tended to increase with increasing concentrate feeding, as seen in Table 4.6. The residual of M_p - M_{p2} demonstrated a significant and linear effect of decreasing residual CH_4 across treatment groups (P linear = 0.025) with the least square mean of M_p in CON animals being 62.61 g CH_4 / day less than M_{p2} estimates of CON animals, and likewise mean of M_p in HIGH animals being 100.81 g CH_4 / day less than M_{p2} estimates of HIGH animals.

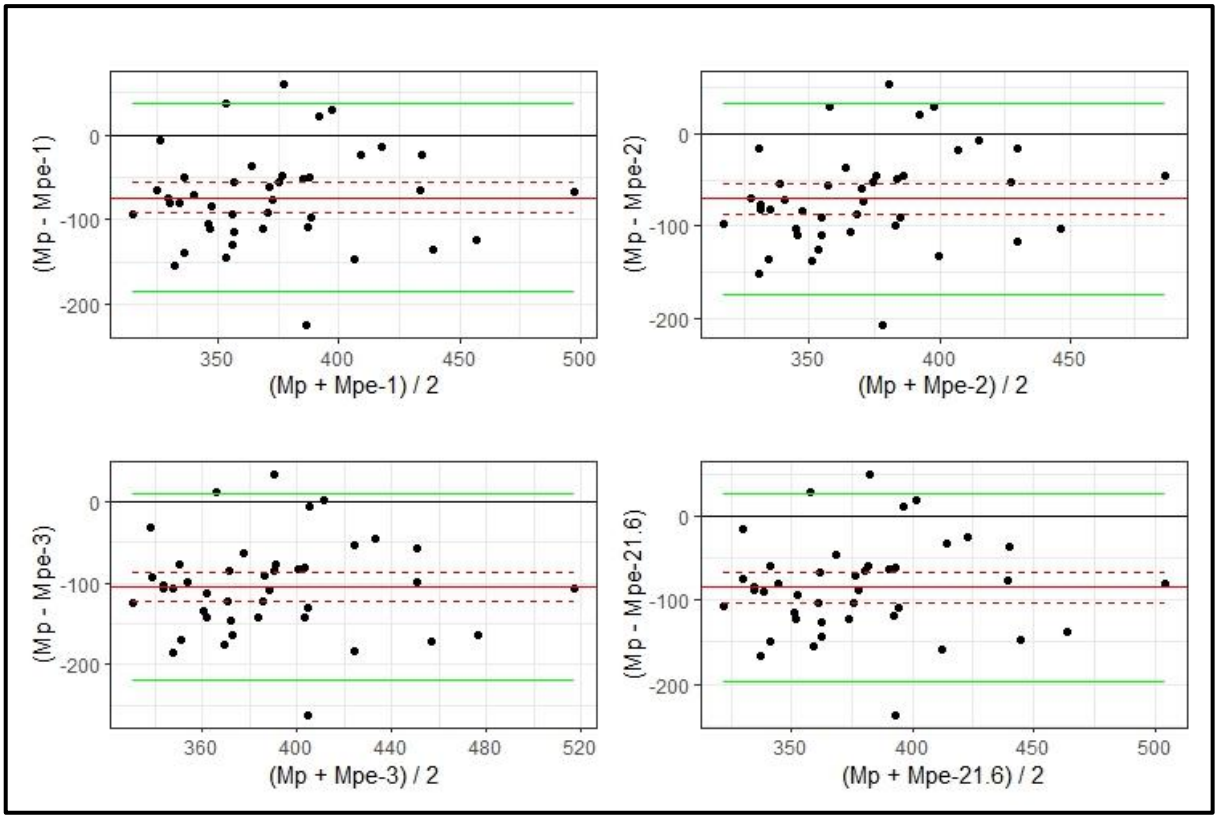

Figure 5.1 Bland-Altman plots of the residuals between M_p and predictions of M_{pe} (including $M_{p\text{-}21.6}$). In these plots, green lines represent the limits of agreement (standard error x 1.96), red lines represent the mean bias of the two variables and the red dotted line represents the 95% confidence interval for the mean bias.

This particular linear decrease of M_p – M_{p2} may indicate that M_{p2} (and presumably other M_{pe} predictions due to the very high Pearson's correlations between all M_{pe} empirical models) were not able to accurately measure CH_4 emissions across treatment groups in this study and may not be suitable for detecting the effect of concentrate feeding rates of M_p. This may be tied to these

prediction equations' reliance on similar measures of GEI or DMI in isolation as predictor variables, (Appuhamy et al., 2016) which cannot interpolate any potential physiological effects (e.g. changes to ingesta flow rates, C_3 production or rumen pH) to animals across treatments. Hristov et al., (2018) noted the use of empirical models to estimate M_p may be limited without a large sample set and/or when attempting to detect treatment effects to M_p, including that of feed quality. Therefore, these empirical models may not be reliable for predicted CH_4 emissions in this study or similarly designed *in-situ* studies assessing the impact of concentrates on M_p.

To further reflect on the potential inaccuracies within estimated M_p in this thesis, experimental power and effective study duration have been isolated by Hammond et al., (2016a) and Jonker et al., (2020) as essential factors to M_p estimation and significance testing using emissions data from GF devices. Similarly, Waghorn et al., (2016) emphasised the importance of an even distribution of GF visits throughout the hours of each day to improve the accuracy of modelled emissions estimates at the animal or treatment level. Likewise, Hristov et al., (2018) also noted that importance of balanced visitation across hour of day and was a potential source for error in GF studies and suggested that 30 observations per experimental unit over a 3 to 5 week study were optimal for establishing reliable CH_4 production data at the animal level. Manifizer et al., (2017) was similarly able to demonstrate that 20 spot samples per animal over a 7 – 14 day period could consistently produce representative CH_4 and CO_2 emissions data of individual animals. The effects of GF visit distribution (by hour of day) and the rates of visitation per day are further explored in section 5.5 *Trends of GreenFeed usage and effect on methane production* using $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$ within Table 4.6 to illustrate these effects on GF data.

Figure 5.2 shows the changes to mean M_p over time throughout the study's measurement period. As M_p is a strongly correlated with DMI, increasing M_p values over time could be expected to be associated with an increase of DMI (or ME_r in this study). A sharp increase of CH_4 emissions across all treatment groups involved in this study, as seen in the second half the measurement period in Figure 5.2, is therefore not in agreement with this notion. Figure 4.1 showed that no significant changes to the composition of pasture occurred throughout the entire study (minor changes to NSC% or CP% in pasture were related with changes to DM%) and likewise Figures 4.2 and 4.3 demonstrate that neither liveweight nor milk production significantly changed across the measurement period to drive increases of DMI over time in the study's duration. Although Figures 3.3 and 3.4 emphasise pasture offers increased across the study, pasture refusals in tandem, also suggesting that pasture intakes were not restricted at any point in the measurement period and are therefore unlikely to have

changed. Regardless, notable increases of CH_4 emissions over the measurement period's entirely timeline (as hours), across all treatment groups, are shown in Figure 5.2.

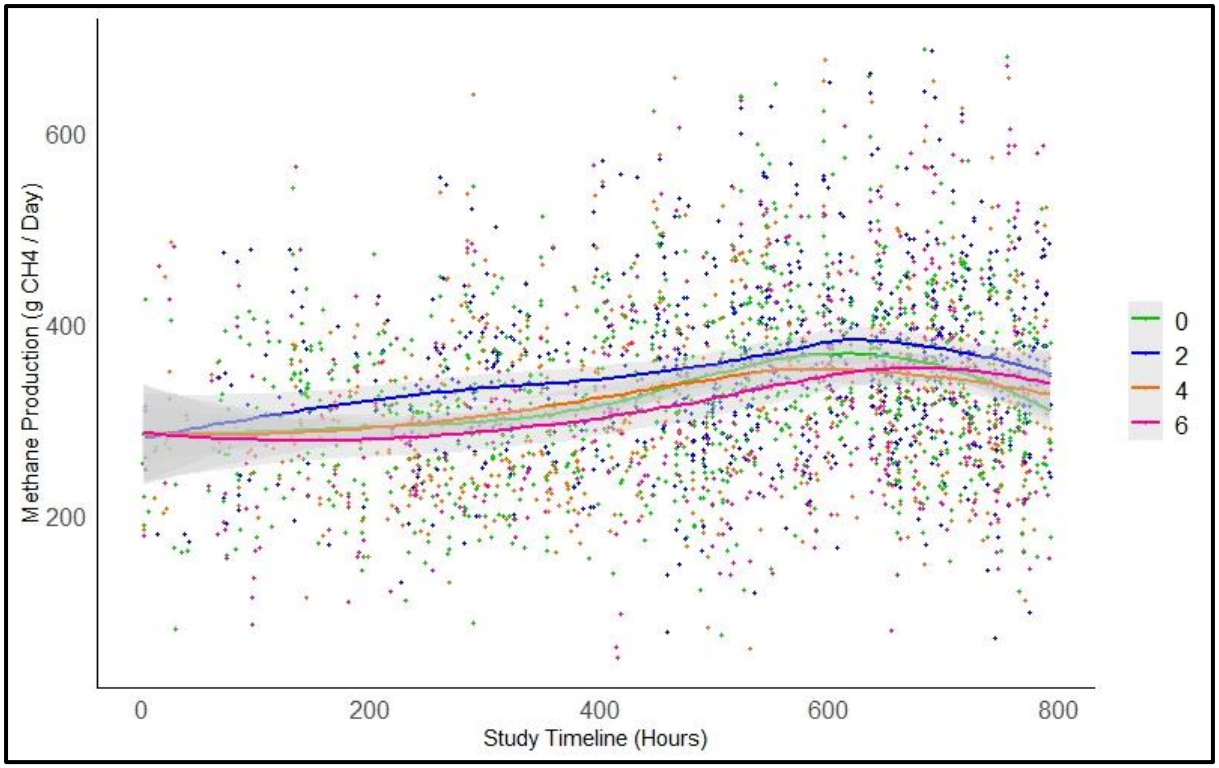

Figure 5.2 Increasing CH_4 emissions (g / day) over measurement period timeline across all treatments (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6).

To contextualise these changes of CH_4 emissions over time, a separate Pearson's correlation (data not shown) showed $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$ estimates to be strongly and linearly correlated ($r = 0.877$, $P < 0.01$). This can be observed between the consistent differences between $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$ least square means in Table 4.6, with $M_{p\text{-period }2}$ values being between 48.26 – 65.17 greater than those of $M_{p\text{-period }1}$ least square means for each treatment. As this linear increase of M_p between the first and second half the measurement period occurred without significant increases ME_r or other factors of voluntary feed intakes, M_p may have changed over time due time due to changing GF data accuracies. A narrowing of SED between $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$ M_p data (from 25.585 to 22.473, respectively) also suggests that a factor which was external to DMI influenced the precision of modelled M_p over time, which may be reflected in the trends of GF visitation over the study's measurement period.

5.5. Trends of GreenFeed usage and effect on methane emissions

To investigate this potential error within estimated M_p and the increasing CH_4 emissions noted between $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$, the distribution of GF visits across the measurement period may provide some inference into how and why CH_4 emissions changed over time. When viewed alongside Figure 5.2, Figure 5.3 demonstrates an increase of GF visitation in the latter half of the measurement period (as average CH_4 emissions were increasing), which may partially explain the observed numeric increases to $_{Mp\text{-period }2}$ in Table 4.6.

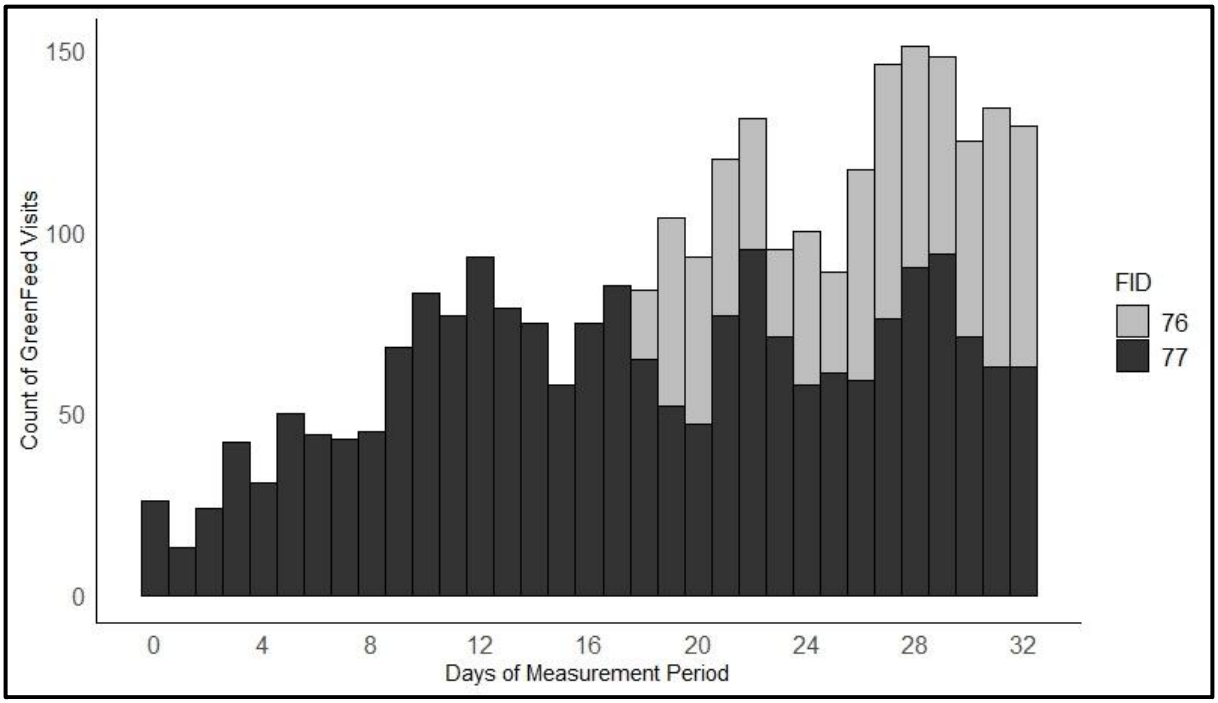

Figure 5.3 Counts of daily GF visitations showing an increase of GF visits over the measurement period (76: GF unit ID #76; 77: GF unit ID #77).

Increased GF visitation in the latter half of the measurement period can largely be attributed to the withdrawal of GF #76 from the study between day 0 – 17 of the measurement period, due to intermittent technical issues experienced by the unit. While increased GF visitation has been associated with increased accuracy of M_p modelling (Hristov et al., 2018), the methodology underpinning M_p (as well that which underpinned $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$ estimates for animals and treatment groups) used a weighted arithmetic mean of CH_4 emissions captured across hour of day for each animal over time. Therefore, increasing GF visitation may not necessarily affect M_p, provided a representative and valid CH_4 spot measurement is recorded for each animal at each hour of day. To examine the effect of GF visitation on M_p therefore, changes to hourly visitation rates (as shown in Figure 5.4) and as well as the effect of increasing hourly emissions on M_p are required.

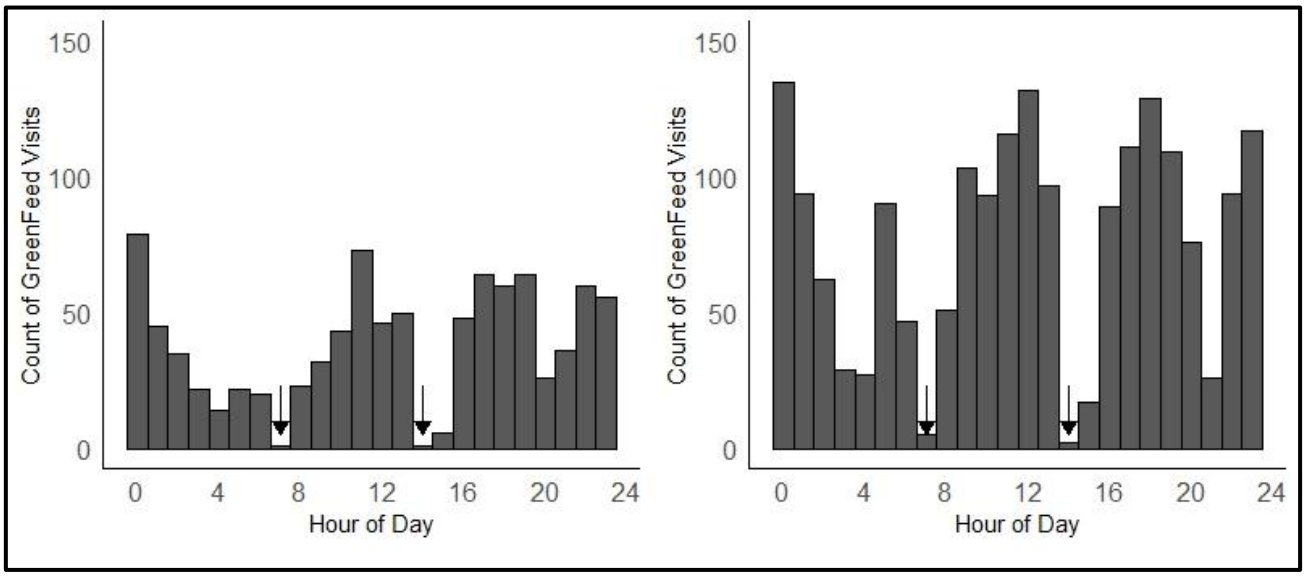

Figure 5.4 Increasing counts of GF visitation by hour of day between during period 1 (Days 0 – 16 of measurements) and period 2 (Days 17 – 32 of measurements). Arrows indicate milking times.

While Figure 5.3 demonstrated that total visitation increased dramatically once GF #77 was introduced into the study, Figure 5.4 shows that the timings and distribution of vistation greatly changed during as animals further adapted to GF units and as GF availbility increased. In the first half of this study, analysed animals had only visited GF units across 12 hours of the day, on average. By contrast, in the latter half of the measurement period, study animals had visited GF units in 18 hours of the day on average (including the hours in which milking occurred). More hourly values of CH_4 production were therefore available for potentially more accurate estimates of CH_4 production in $M_{p\text{-period }2}$, as opposed to the comparitvely limited dataset used in $M_{p\text{-period }1}$.

As discussed in section 2.5.1 *Patterns of methanogenesis* Crompton et al., (2011) reported that CH_4 emissions peaked 45 – 140 minutes after feeding, and likewise M_p were lowest immediately prior to feeding. In this study, concentrates were consumed during milkings and new pasture breaks were offered to the study herd after milkings. This likely meant that if LOW, MED and HIGH animals did not visit GF units soon after returning to their paddock from milking, their peak CH_4 emissions on that day may have been missed. The timing of GF visits therefore has therefore been deemed a crucial factor for the successful capturing of peak (and minimum) CH_4 emissions throughout the each day and increased visits during peak emissions times (approximately 8:00 am – 11:00 a.m. and 3:00 pm – 6:00 p.m. see Figure 5.6) may have strongly influenced the differences between $M_{p\text{-period }1}$ and $M_{p\text{-period }2}$. The average GF visitation rate (average count of visits by hour, per day) for each treatment group is presented in Figure 5.5.

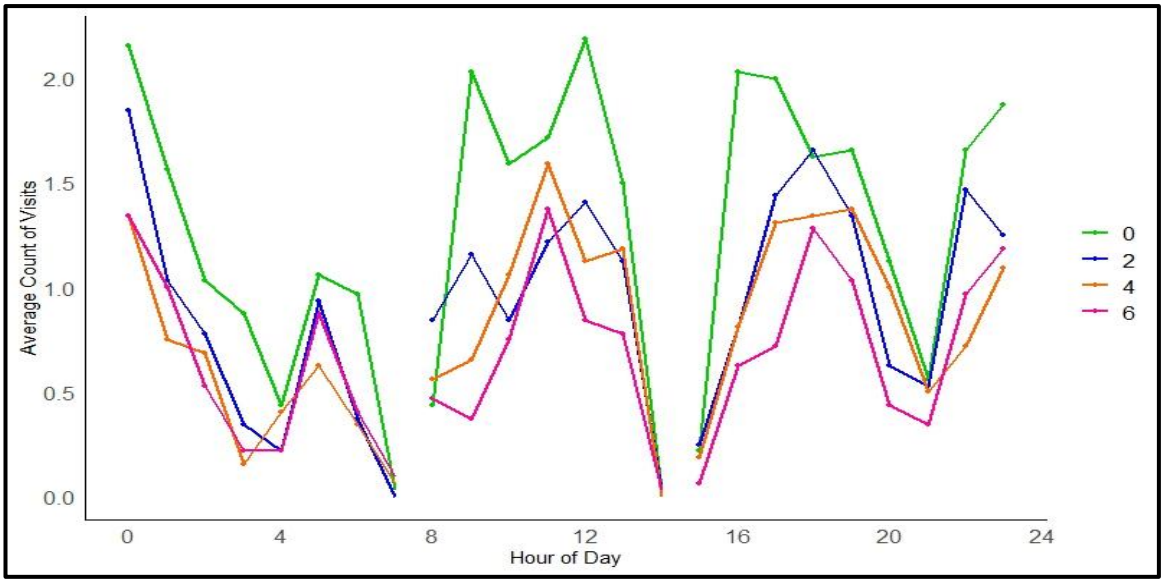

Figure 5.5 Average GreenFeed visits by hour of day for each treatment group over the measurement period. Visit data collected at normal milking times removed (between 7:00 – 8:00 a.m. and 2:00 – 3:00 p.m.) (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6).

Upon returning to their paddock after milking, CON visited GF units significantly (P = 0.025) more than other animals and GF visitation was shown to linearly decrease with increased concentrate feeding (Table 4.6, P linear = 0.08). The eagerness of CON animals to visit GF units after milking is thought to be due to these animals not receiving feed during milking and being less satiated than other animals after milking. In tandem, lessened access to GF units in the first half of the measurement period, with only one unit available for animals in this time, may have further impaired LOW, MED and HIGH animals' competitive access to the GF unit(s) against CON animals, particularly through during peak emissions hours. This frequent losses of peak emissions data from LOW, MED and HIGH animals in the earlier half of the measurement period may have especially explained why $M_{p\text{-period }2}$ was numerically greater than $M_{p\text{-period }1}$.

Figure 5.6 presents the circadian rhythm of mean CH_4 emissions across the hours of day for all treatment groups. While the total emissions per day did not significantly change between treatment groups, MED and HIGH groups demonstrated an intense peak of CH_4 emissions which is consistent with previous studies (Crompton et al., 2011). Animals within the LOW group experienced the greatest average peak of CH_4 across the hour of day within the study, which is consistent with their numeric ranking of M_p compared to other treatments in Table 4.6.

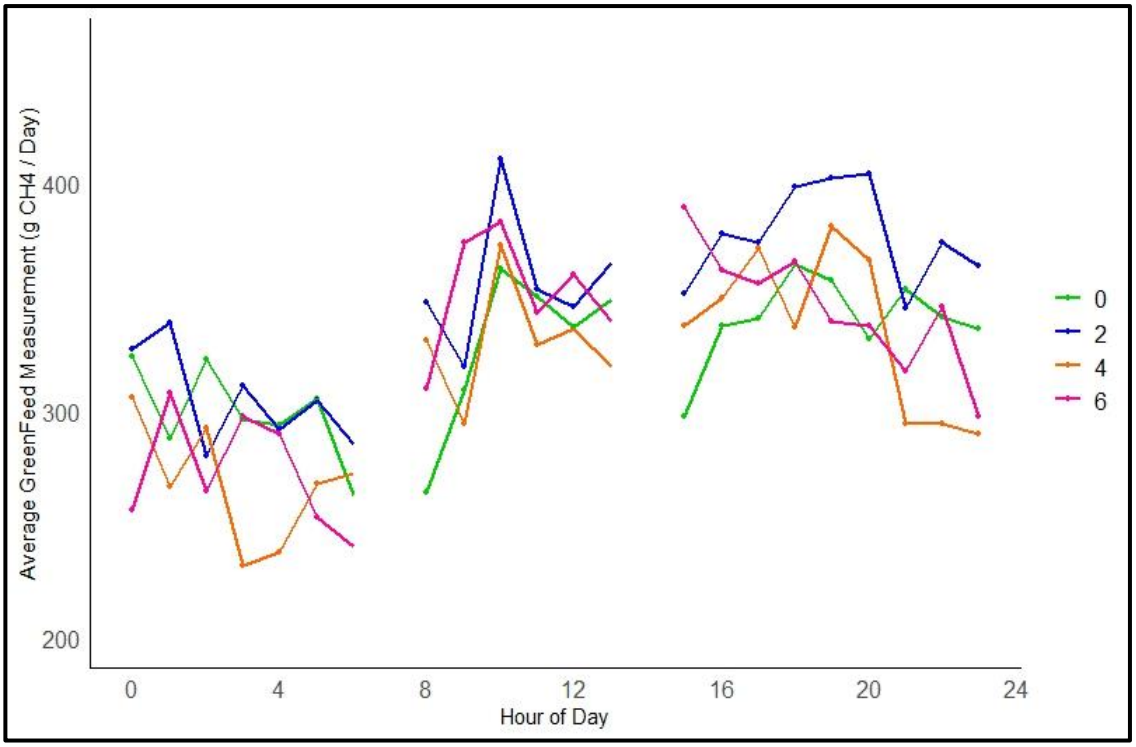

Figure 5.6 Average CH_4 measurements by hour of day for each treatment group over the measurement period. Emissions data collected at normal milking times removed (between 7:00 – 8:00 a.m. and 2:00 – 3:00 p.m.) (as kg DM concentrates / cow / day; Green: 0; Blue: 2, Orange: 4; Purple: 6).

While M_p was potentially underrepresented for all treatment groups within this study, the circadian rhythm of these animals' CH_4 emissions is in alignment with those previously reported in literature (Crompton et al., 2011; Waghorn et al., 2016; Hristov et al., 2018). As the trends of M_p over hour of day within this study are in alignment with those expected from previous research, using both RC and GF to measure animals' emissions, GF units in this study were able to demonstrate some precision in this study. It is hoped that with greater GF visitation, greater training to GF units in the earlier periods of the study and/or greater experimental units, this study could have ascertained more representative measurements of CH_4 emissions at the treatment level for even more confident estimations of M_p (Jiao et al., 2014; Hatew et al., 2015).

5.6. Key limitations of thesis

The major limitations of this thesis centre around the levels of uncertainty within estimates of M_p and DMI, further impacting the accurate assessment of M_y and M_i in the results reported across Tables 4.4 and 4.5. A major source of error in both the estimations of DMI and M_p in this thesis stem from the removal of experimental units and reduction of statistical power used in all analyses. In

section 3.2.2. *Animal Management*, it was mentioned that a preliminary power analysis conducted before the study's commencement estimated that 16 animals per treatment would be optimal to estimate significant differences of M_p and DMI between treatment groups. The final count of animals used for all data analyses (n = 42) therefore provides the greatest key limitation of this thesis, with significant design and operational limitations driving this loss of statistical power. Animal exclusions from lameness and other health related issues (see Table 3.3), as well as poor GF data (animals with <20 visits being excluded from all analyses) resulted in the exclusion of 30 experimental units alone from this study. However, further analysis into study design and operations can reveal other factors which may have further affected the accuracy of reported results within this thesis.

5.6.1 Key limitations of study design

The removal of 11 animals due to ill-thrift, particularly from lameness, acted as a significant and potentially preventable limitation to the statistical power and subsequently reported results of this thesis. Significant changes to animal management may have included the consultation of animal health records before the commencement of the study to ensure selected study animals did not have a history of lameness. Likewise, a greater emphasis on the selection of animals with pigmented hooves (Chesterton et al., 1989) and a reduction of animal time on concrete (e.g. such as animals being held on yards after milking) (Westwood et al., 2003) may have further reduced the incidence of lameness during the study. While ruminal acidosis and ketosis have been previously linked to increased lameness incidence (Chesterton et al., 1989), the lack of clinical acidosis observed on-farm in this study, no clinical acidosis being reported by rumen samples retrieved by Della Rosa et al., (2024), as well as the lacking relationship between lameness frequency and treatment group, suggests that nutritional factors were not a significant factor of lameness in this study. The removal of experimental units due to lameness and the implication of statistical power across treatment groups may have also affected the balancing of animals across treatments. In particular, the pre-study balancing of animal liveweight and genetic productivity potentials (BW and PW) were key selection criteria (see Table 3.3) to ensure the accurate estimation of both DMI and M_p in this thesis which animal removals may have incidentally skewed.

To ensure consistent replication of pastures and pasture silages consumed by all animals (irrespective of treatments) and to ensure that variation between GF units' measurements could be analysed as a fixed effect between treatment groups the daily level; all animals in this study could only be managed in a single grazing mob. This constraint was also necessary due to limited GF device availability at the timing of this study. Therefore, this study's findings simplistically assume that

changes to total DMI across treatment groups were seen directly through changes to pasture DMI, without considering the potential impact of concentrate feeding rate's impact on animals' grazing behaviour(s). Increasing concentrate feeding rates may have affected how and when animals grazed throughout hour of day (and throughout each pasture break), however the cutting height of pasture samples were taken to pasture residual height to assume that all animals ate the same quality pasture throughout each break to mitigate this potential grazing/time effect between treatments (see section 3.3.3. *Forage sampling & measurements).* Some random error is inevitably to be expected within this study's estimation of grazing animals' DMI, as the indirect estimation of pasture DMI used in this thesis did also not account for spatial day to day variation of forage intake at the discretion of individual animals'. In lieu of direct and individual measurements of forage intakes, the use of digestibility markers or *in-vitro* assays may have therefore further improved all estimations of total DMI and pasture DMI in this study (Smith et al., 2021).

Accurate measurements or estimates of pasture DMI at the animal level were also further complicated by several study requirements, such as the provision of pasture silage at two kg DM per animal, per day. This additional conserved forage was fed to study animals to reduce grazing pressure on pasture and to mitigate soil pugging in the notably wet and cold spring conditions experienced at Massey Dairy 4 in the 2022-2023 season. Providing pasture silage also allowed for essential minerals to be provided in the animals' daily ration, as mentioned in section 3.2.2 *Animal Management*. During the study's initial planning stages, pasture was to comprise the only forage offered to study animals, and pasture silage would have been preferably removed from the diet or offered to individual animals (which was not feasible at Massey Dairy 4). To attempt to practically mitigate this complication, individual intakes of each diet component (pasture, pasture silage, GF pellet and concentrates) were analysed over animals' four day period at AgResearch Grasslands during RC measurements. These results are presented separately within Della-Rosa et al., (2024), however it is important to note that this study utilized cut pasture (as part of offering pasture to animals in RC) and therefore these results may still not reflect the true pasture DMI of these grazing animals *in-situ*. Likewise, DMI can be expected to significantly decrease between *in-situ* and *in-vivo* study designs in the field of ruminant nutrition (Della Rosa et al., 2021) and the DMI reported in this study may still be fairly accurate of these animals' true *in-situ* intakes.

As highlighted in section 3.1 *Introduction & research objective*, all reported calculations of M_p, M_y and M_i (as least square means; see Table 4.5) at the treatment level are strictly estimates only. It is the author's prerogative to declare that this thesis acts as only one small contribution

towards many New Zealand researchers' wider investigations into changing CH_4 emissions under changing animal management conditions. Replication of accurate M_p has been the subject of significant debate for many years using respiration chambers as highlighted by Pacheco et al., (2014). Likewise, other external effects of animal breeding and previous farm/feeding management may also have affected the DMI and CH_4 production of these animals. Therefore, the changes to CH_4 emissions under increasing levels of supplementation detected from this study should be most keenly analysed for the confident ranking of results before gold standard quantification of these trends may be more rigorously tested (e.g. by Della-Rosa et al., 2024).

5.6.2 Key limitations of study operations

While section 5.6.2 *Key limitations of study design* identified the key limitations of this study's design, several further limitations to this thesis' records and reported results may have stemmed from complications felt during this study's operations. Some of these issues may have been uncontrollable by the study co-ordinators, such as the cold spring felt on-farm in this season which may have affected pasture utilization rates and pasture quality. This environmental limitation had further implications on study design or operations, such as the introduction of pasture silage to these animals' diets (which could not be fully controlled), or through poor pasture utilization which further complicated DMI estimates.

Poor weather conditions continued to present several other key issues to this study's operations. For instance, soil pugging limited the GF adaptation period in the earlier periods of the study and therefore may have affected overall visitation to GF units throughout the study as much of the GF adaptation period saw animals interacting with GF units only on the farm's feedpad for one hour per day. Likewise, the removal of GF #76 for the first half of the measurement period likely further impacted all study animals' adaptation to GF units and it is suspected that this particularly reduced the visitation and the spatial distribution of visits from LOW, MED and HIGH animals across hour of day in earlier days of the study's measurement period. This, coupled with the CON animals' affinity for lucerne pellets in-lieu of concentrates from the milking shed may have driven the suspected underrepresentation of M_p from animals within these treatment groups.

Similarly, saturated soils and a cold spring affected the spring pasture flush, impacting the quality of pasture offered to animals within this study. Figure 4.1 demonstrated that pasture offered in the study's measurement period contained approximately 10.0 – 10.5 MJ ME / kg DM, which was anecdotally noted as being lesser than the feed quality expected within the perennial ryegrass based pasture swards on Dairy 4 during October and November. This lesser quality pasture may not have

been indicative of the average diet of early lactation dairy cattle in New Zealand and using the SCA (1990) method to estimate DMI using MJ ME / kg DM values may have led to an overestimation of DMI in animals fed lesser quantities of concentrates. In turn, this may have potentially skewed M_i estimates to favourably decline with increasing concentrate supplementation. Additionally, capturing any significant changes to pasture quality may have been helpful in inferring the milk yield response from increasing concentrate feeding, which was not captured with the sampling resolution of pasture offered in this study. In this thesis, CP levels in pasture during the measurement period varied from 13% - 25% (of DM), a numerically large change, which in this instance could not be declared a statistically significant change due to low power. Greater sampling frequency at the daily level during the measurement period, may have allowed greater insight into the effect of feed quality on animal production and may have increased the accuracy of DMI estimations and M_y/M_i estimations in turn.

Wet conditions may have further contributed to poor pasture utilization rates, as observed in pasture residual heights in Figure 3.2. This meant that all animals were offered an *ad-libitum* diet, regardless of treatment group, and when considered in conjunction with the feeding of pasture silage, this feeding practise may have affected the actual substitution rates experienced by supplemented animals. This study could in future be replicated on free-draining soils in a year with a mild or more predictable spring to ensure that a spring flush would be experienced. This could further enable grazing rotations to strike more appropriate pasture residual targets (e.g. 1500 – 1700 kg DM / ha) of pasture-based New Zealand dairy farmers in the spring period. This in turn could provide a contextually relevant CON diet (Holmes et al., 2002) from which true supplementation would be experienced as greater allocations of concentrates were offered in LOW, MED and HIGH animals.

Finally, GF visitation would need to have been increased in this study alongside the mitigation of animal removals to ensure that GF data could be accurately summarised into an estimate of M_p at the treatment level. Comparing the variance of $M_{p\text{-Period }1}$ and $M_{p\text{-Period }2}$ effectively demonstrated this effect in section 5.4 *Accuracy and precision of methane measurements* and while the values of $M_{p\text{-Period }2}$ were greater than those of $M_{p\text{-Period }1}$, both demonstrated greater variance at the treatment level than the values provided by M_p. In turn, to improve the accuracy of reported CH_4 emissions data from this thesis, greater data collection (more GF visits per animal) as well as greater spatial representation of GF data (across hour of day for individual animals) may have been required.

6. Conclusion

The production of CH_4 (g / day) remained similar in early lactation dairy cattle fed increasing levels of concentrate supplements. Statistically significant decreases of M_y, M_i and CH_4 : CO_2 were however detected in this study as concentrate feeding rates increased, suggesting that the increasing CH_4 production that can be expected with increasing DMI with concentrate supplementation was offset by a reduction in CH_4 per unit of DMI and milk production. In particular of M_y, the accurate estimation of DMI between treatment groups, and particularly the pasture intakes of animals fed increasing levels of concentrates, has been previously discussed as being inherently difficult to quantify by Smith et al., (2021)

The M_y of LOW, MED and HIGH animals linearly decreased with increasing concentrate feeding levels. This suggests that the 21.6 g CH_4 / kg DM methane conversion rate for dairy cattle within New Zealand's GHG inventory may not accurately reflect the emissions of animals across all common feeding systems used in New Zealand's dairy sector. However, the lack of agreement between CON animals' M_y and this baseline M_y within the GHG inventory, alongside poor agreement of M_p and M_{pe} prediction models suggests that M_y may have been under-predicted in this study.

Therefore, the extent to which concentrate supplements affect the CH_4 emissions of New Zealand dairy cattle may require further research, both *in-situ* and *in-vivo,* to better quantify the emissions of pasture-based dairy cattle fed increasing levels of supplements. Future research to expand the findings of this study could also include an investigate how concentrates containing differing levels of starch and fibre contents could affect the CH_4 emissions and milk production of dairy cattle.

7. General recommendations

For the New Zealand pastoral dairy sector, this study adds information on whether dairy farmers can sustainably employ alternative feeds to reduce their on-farm emissions. This study did find that increasing concentrate feeding rates could significantly decrease M_y and M_i on farm. However, some of this benefit is offset by increased GHG emissions for the production of concentrate feed. Mazzetto et al., (2021) reviewed that emissions from fuel use and crop feeding can comprise well over 10% of the carbon footprint of milk and emissions tied to planting, harvesting, transporting grains within concentrates feed production need to be considered further. Further debating on the environmental impacts of high starch concentrate feeds, including wider impacts like soil nitrogen losses and eutrophication in cereal grain growing, are also to be considered before recommending GHG mitigatory diets for widespread on-farm adoption.

Similarly, part of a sustainability approach to on farm CH_4 mitigation also includes the cost : benefit analysis of any mitigatory tool. Feeding concentrates to pasture-based dairy cattle during early lactation has long been criticised as being potentially cost-ineffective (Penno et al., 1996). In the instance of attempting to reduce M_y and M_i on farm, concentrate feeds may be less cost effective than other feeds, like corn silage (Hassanat et al., 2014) or fodder beet (Jonker et al., 2017) which have also been shown to significantly decrease M_p and M_y in forage-based diets of dairy cattle. Similarly, the low milk yield responses per kg DM of supplements fed in this study may mean that concentrate feeding in early lactation (when pasture is available in abundance) may not be a cost-efficient part of the season to target CH_4 reductions using nutritional strategies (Rattray et al., 2007). Further research, both quantitative and qualitative will be therefore required before specific diets can be recommended for on-farm adoption in New Zealand.

For the field of ruminant nutrition, study specific recommendations include the training of animals to use GF units thoroughly within *in-situ* studies as to encourage high visitation early in a study (this was not possible in this study due to wet soil conditions and breakdowns of GF units). A high level of visitation and a wide distribution of visits across the hour of day were found to be essential for the accurate and precise estimation of M_p in this grazing study. Encouraging higher levels of GF visitation also may have minimized the experimental unit removals from this study and increased the statistical power in subsequent analyses. Repeated animal usage across GF studies (if and where possible) is recommendable in future GF studies.

Greater responses to milk yield, DMI and M_p from increased concentrate feeding may have been observed in this study if pasture allocations were not *ad-libitum*. Similarly, tightly controlling or removing pasture silage allocations from these animals' diet may have reduced the random error of estimated pasture intakes in this *in-situ* for more accurate DMI estimation. Finally, it is recommendable that extra data sources to extrapolate changes to C_3 production in the animal are factored into similarly designed studies. Data such as rumen fluid or faecal samples to infer whole tract digestibility, changes to rumen pH or C_3 production, or changes to methanogen populations under increasing supplementation could be useful to better quantify the changes within the rumen as concentrate feeding levels increased.

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