Evaluation of methodologies to quantify dry matter intake in grazing New Zealand dairy cattle

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Abstract

Dry matter intake (DMI) is a key driver of enteric methane emissions in cattle, therefore the accurate measurement of DMI in grazing dairy cattle is necessary for research in methane emission reduction. A gap in methodologies currently available in New Zealand (NZ) to accurately predict individual grazing cow DMI for use in greenhouse gas (GHG) emission research has been identified. Therefore, the objective of this thesis is to quantify the variation between individual intake of "grazing" dairy cattle in NZ predicted from five methodologies; two indigestible markers techniques being n-alkanes and titanium dioxide paired with indigestible neutral detergent fibre (iNDF) and three standard energetics back calculation equations, all compared to actual intake measured by the Calan gate disappearance method. To test the marker techniques, an experiment was designed to provide data on actual individual cow DMI to compare intake predicted by the n-alkane, titanium dioxide and iNDF methodologies. The experiment occurred from 26 October to 4 November 2022, using two 5-day measurement periods, within the DairyNZ Calan gate facility, so that actual individual cow intakes were measured and alternative methodologies could be compared against each other. Using 40-multiparous early-lactation Holstein Friesian cattle, in two treatment groups - Control (pasture only diet) or Supplement (pasture and supplement diet), with treatment groups balanced for age, days in milk, liveweight and milk production. The cattle were previously adapted to diet, treatment and indigestible markers used, over a 3-week period prior to the measurement period beginning. All the cattle were dosed with n-alkane C32 (377.6 mg) and titanium dioxide (5 g) twice a day, following faecal collection. Supplement, pasture and faecal samples were bulked by measurement period for each individual cow, alongside samples of n-alkane and titanium dioxide dosed, then analysed for n-alkanes C27 to C35, titanium dioxide and iNDF. The n-alkanes C29, C31, C33 and C35 were each paired with C32 to predict DMI, as was the pair titanium dioxide and iNDF. The n-alkane pair C32:C33 provided the most accurate prediction of pasture intake in relation to actual pasture intake, although there was an underestimation on average of 35 to 40%. Titanium dioxide and iNDF did not accurately predict total intake, with both over and underestimations of actual intake occurring. The back calculation methodologies included three energetic back calculation equations commonly used in NZ (NRC, MPI GHG Inventory and Nicol and Brookes). The ability of these methodologies to predict actual individual dairy cow DMI was tested across six datasets: five previous DairyNZ trials were used along with data from the experiment discussed above, all of which

have DMI measured from the Calan gate system. Liveweight change is a variable included in the Nicol and Brookes equation and within these analyses added large variation to the energetic requirements and calculated daily DMI for individual cows. Based on this, the equation from Nicol and Brookes was not included in the full analysis, consequently as the other two equations that were analysed in full were not suitable for use in non-lactating cattle, a trial was excluded due to the use of non-lactating cattle. At an individual cow level, a single equation did not consistently provide predictions within the same range of accuracy across the trials. The data indicated that of the equations analysed, the NRC equation provided the best DMI predictions for individual lactating dairy cows in terms of accuracy and ease of use. At the herd level, DMI predictions were in a range of accuracy for all equations, with the NRC equation providing the most consistent results.

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List of Abbreviations

ATP	Adenosine triphosphate
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
FCM	Fat corrected milk
GHG	Greenhouse gas
HR	Hour
iNDF	Indigestible neutral detergent fibre
iADF	Indigestible acid detergent fibre
KG	Kilogram
ME	Metabolisable energy
MPI	Ministry for Primary Industries
NADH	Nicotinamide adenine dinucleotide + hydrogen
NDF	Neutral detergent fibre
NRC	National Research Council
NZ	New Zealand
PA	Pasture allowance
RPM	Rising plate metre
SR	Stocking rate
TMR	Total mixed ration
VFA	Volatile fatty acid

Chapter 1: General Introduction

The dairy industry is a significant contributor to the NZ economy, with 95% of the milk produced in the country exported (Morris and Back 2021). The generally temperate climate in NZ supports pastoral-based farming with dairy farm systems typically being low input with on average 80% of the dairy cows' diet being pasture (Pinxterhuis et al. 2015; Ledgard et al. 2020).

As the concentration of GHG in the atmosphere has been increasing there has become a subsequent focus on reduction of emissions (Moss et al. 2000). Methane contributes 19% to global GHG emissions and although being a short-lived gas it has a high global warming potential (Olivier et al. 2017; Masson-Delmotte et al. 2021). Enteric methane emissions are produced by ruminant livestock and contribute 35.2% of NZ total GHG emissions and 73.1% of total agricultural emissions (Ministry for the Environment 2021). New Zealand dairy farmers are under an increasing amount of legislative and societal pressure to reduce methane emissions, with research on the reduction of methane emissions from ruminants a current focus of the agriculture sector, so that these legislative and societal pressures can be met (Foote et al. 2015; Leahy et al. 2019).

Within cattle, feed intake is a key driver of methane production with a strong linear relationship between DMI and enteric emissions (Knapp et al. 2014; Charmley et al. 2016). Therefore, for research on the reduction of methane emissions in dairy cattle, DMI must be measured accurately. Yet in dairy cattle predominately grazing pasture, this is a challenge as individual intakes are difficult to quantify. Whereas, in cattle that are housed or penned, intake can be quantified with relative ease, by weighing the difference between feed offered and refused, without interrupting normal behaviour (Lukuyu et al. 2014b). Housed dairy cattle can be fed grass, using the cut and carry method, but normal grazing behaviour cannot be exhibited. Therefore, although accurate DMI measurements can be obtained research in this manner cannot be considered a true representation of DMI for grazing dairy cattle.

A gap in methodologies currently available in NZ to accurately predict individual grazing cow DMI for use in GHG emission research has been identified in the literature. Methods currently available for estimating DMI of grazing cattle include; 1) RPM which cannot provide individual cow grazing intake with required accuracy; 2) indigestible markers which have not been used in NZ in recent studies due to previously reported inaccuracies, although certain markers have been reported as accurate in overseas research; 3) energetics back calculations

which are not a direct measurement of DMI; and 4) digital technologies and animal-based sensors which are costly and relatively new in their use. Therefore, the objective of this thesis is to quantify the variation between intake of "grazing" dairy cattle in NZ predicted from five methodologies; three standard back calculations and two indigestible marker techniques being n-alkanes and titanium dioxide paired with iNDF, all compared to actual intake measured by the Calan gate disappearance method. The aim of this thesis is to understand the variation in accuracy of the DMI predictions from these methodologies, and to potentially improve the accuracy of the most promising method.

Chapter 2: Literature Review

2.1 Introduction

In this chapter, I will summarise and discuss the scientific literature on methane emissions and pasture intake from grazing dairy cattle. I will focus primarily on the importance, challenges, and potential methodologies of quantifying DMI accurately from grazing dairy cattle as part of the research to develop methane mitigation strategies for NZ dairy farmers. I will begin by discussing the fundamentals of the NZ pastoral dairy farm system and provide an overview of enteric methane production from grazing dairy cattle. This will be followed by a section on the importance of accurately predicting DMI and methane emissions, in order to develop methane mitigation strategies for NZ farmers. Then I will introduce pasture intake and the significant influences that control pasture intake in dairy cattle, which emphasizes the complexity of the numerous interacting mechanisms, which influence and drive pasture intake. Expanding upon this, I will discuss the predominant techniques used for pasture intake estimation and highlight the gap(s) where these methodologies do not provide precise and accurate predictions of individual cow pasture intake. The final section introduces the methodologies that have potential for use within the NZ context, including the use of back calculations that are currently being used. This literature review is concluded with the research objectives and hypotheses of this study.

2.2 New Zealand pastoral dairy farm systems

In the 2020/21 season, there were approximately 4,904 million dairy cows in NZ (DairyNZ and LIC 2021). Dairy companies processed 21.7 billion litres of milk containing 1.95 billion kilograms of milksolids (DairyNZ and LIC 2021). With 95% of milk produced in NZ exported, the dairy industry is a significant contributor to the NZ economy (Morris and Back 2021).

NZ dairy farm systems are typically low input with grazed pasture making up over 80% of the dairy cattle diet on average, with perennial ryegrass and white clover the predominant pasture species (Pinxterhuis et al. 2015; Ledgard et al. 2020). The generally temperate climate and reliable rainfall of NZ supports pastoral-based farming, allowing for grazing all year round (Lee et al. 2013). However, pasture growth is variable throughout the season and in different

regions. Typically, peak pasture growth occurs in spring (September to November), while growth is slowest in winter (June to August) (Morris and Back 2021). In drier, hotter regions (e.g., Northland) pasture growth can be restricted during summer, while in colder, wetter regions (e.g., Southland), zero growth can occur for months during winter. Potential pasture production in Northland is predicted as 28 t DM/ha/year, whilst it is 19 t/DM/year in Southland, with this difference being strongly influenced by differences in light and temperature (Thom 2000). Consequently, pasture and milk production are seasonal, with calving typically occurring in early spring, so that lactation and peak nutrient demand coincide with peak pasture yield (Holmes et al. 1987). Dairy cattle are typically dried off in winter, so that lactation has ceased and nutrient demand is minimal, coinciding with the lowest pasture yield (Garcia and Holmes 1999).

In pastoral farming there is a focus on optimising pasture growth and intake throughout the season. This in part depends on the SR, being the number of cattle and therefore feed demand per hectare, that achieves high pasture utilisation (McCall and Clark 1999; Baudracco et al. 2010). As homegrown feed is utilised for the majority of milk production in comparison to feeding systems based on imported feed, costs are lower in grazing systems (Alqaisi Shawabkeh et al. 2011). This allows for profitable dairy farming, as production costs are kept low.

As a result of seasonal calving restrictions, diminishing pasture supply in late lactation (typically autumn/winter), and a national breeding objective that favours traits such as reproduction in conjunction with production NZ dairy cows have lower lactation persistency in comparison with other global dairy systems, (Zwald et al. 2001; Clark et al. 2007). In countries such as the United States that utilise intensive dairy farming systems, where supplementary feed makes up a larger percentage of the diet and high production genetics are favoured, the cows have significantly higher peak milk production, persistency of milk production and total lactation yield than in NZ (Zwald et al. 2001; Washburn and Mullen 2014).

2.3 Greenhouse gas production

2.3.1 Global and national context

The concentration of GHG (carbon dioxide, methane, and nitrous oxide) in the atmosphere has been increasing, resulting in greater temperatures of the lower layers of the atmosphere (Moss et al. 2000; Huang et al. 2016). The subsequent climate change has affected (and will affect) many aspects of life.

Methane (which makes up 19% of the global GHG emissions) is a short lived gas compared with carbon dioxide and nitrous oxide (11.8 vs 5-200 vs 109 years, respectively), but has a global warming potential 27 times greater than carbon dioxide (Olivier et al. 2017; Masson-Delmotte et al. 2021). Enteric methane is the predominant contributor to GHG emissions from livestock and is responsible for 40% of all global livestock emissions (Gerber et al. 2013; Myhre et al. 2014).

In NZ, enteric methane emissions contribute 35.2% of NZ's total GHG emissions and 73.1% of total agricultural emissions (Figure 2.1) (Ministry for the Environment 2021). As part of NZ's commitment to the Paris agreement, the government has pledged to reduce biogenic methane by 10% below 2017 levels by 2030, and by 24-47% below 2017 levels by 2050 (Ministry for the Environment 2021). In addition long lived GHG (Carbon dioxide and nitrous oxide) will be net zero by 2050 (Ministry for the Environment 2021). The split gas approach has benefits for the agriculture sector but means methane targets cannot be achieved by offsetting (e.g., planting trees, buying carbon credits) and thus absolute reductions in methane emissions are required. In addition, consumer demand is driving global companies (e.g., Nestle and Fonterra) to pledge environmental commitments, which in turn means NZ milk supply companies need to reduce GHG emissions, hence, additional on-farm reduction targets are being set for NZ dairy farmers (WRI & WBCSD 2011; Lazarus et al. 2021). Consequently, NZ dairy farmers are under increasing legislative and societal pressure to reduce methane emissions (Foote et al. 2015). Research on mitigation strategies to reduce methane emissions from ruminants is a current focus for the agricultural sector, so that farmers can meet legislative targets without significantly reducing production and profitability (Leahy et al. 2019).

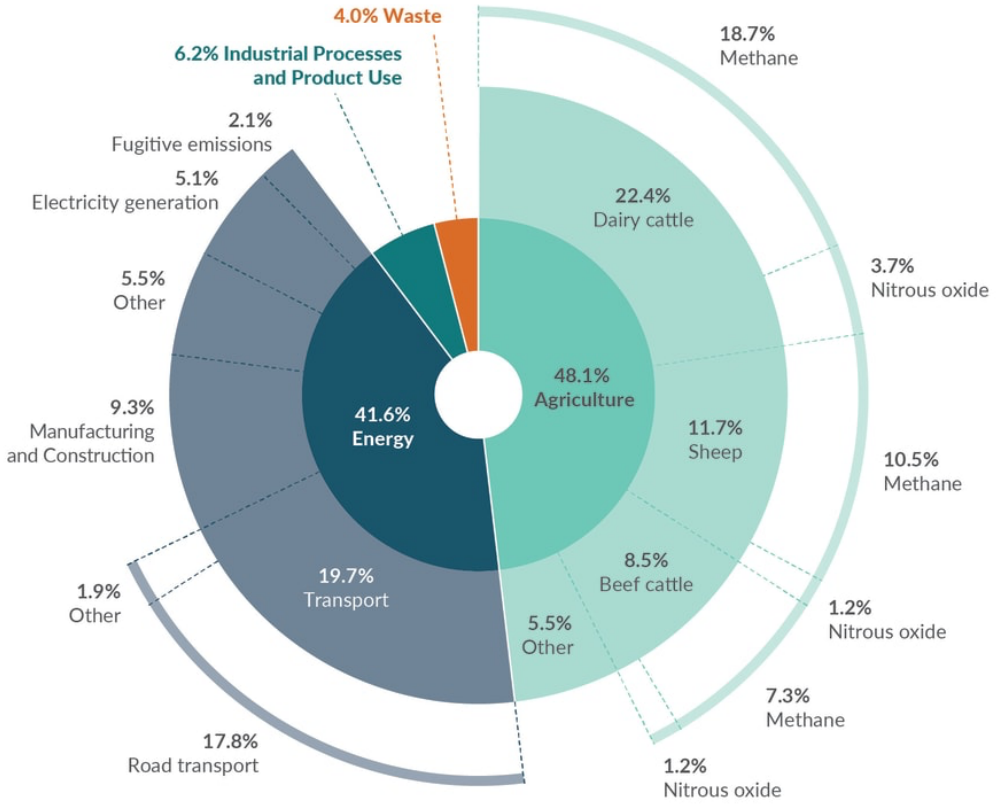

Figure 2.1: Gross greenhouse gas emissions by sector, sub-category and gas type in 2020 (Ministry for the Environment 2022)

2.3.2 Enteric methane production

Enteric methane is a by-product of digestion in ruminants where feed consumed is degraded from plant structural carbohydrates, proteins and other organic polymers by anaerobic bacteria to form monomer components (Figure 2.2) (Morgavi et al. 2010; Broucek 2014). Polysaccharides in the feed are hydrolysed into glucose, hexoses and pentoses, which are then rapidly fermented to produce VFA; mainly acetate, propionate and butyrate (Wilkinson 2012). Excess hydrogens are produced from acetate and butyrate production (Wilkinson 2012). Carbohydrate fermentation which produces hydrogen, VFA and carbon dioxide is supported by hydrogenases (Greening et al. 2019). This hydrogenase activity re-oxidises co-factors reduced in carbohydrate fermentation to form dihydrogen (Moss et al. 2000). The dihydrogen produced is transferred from bacteria, protozoa and fungi to methanogenic archaea which use dihydrogen to reduce carbon dioxide and other 1-carbon compounds to methane (Moss et al. 2000). Along with methane, one mole of water and three moles of ATP per mole of methane

are produced (Wilkinson 2012). Formate produced when pyruvate is converted to acetyl-coA may also be used as a hydrogen donor for methanogenesis by hydrogenotrophic methanogens (Hungate et al. 1970). Methane can also be produced through acetyl and methyl groups, although at a far lower frequency (Beauchemin et al. 2020).

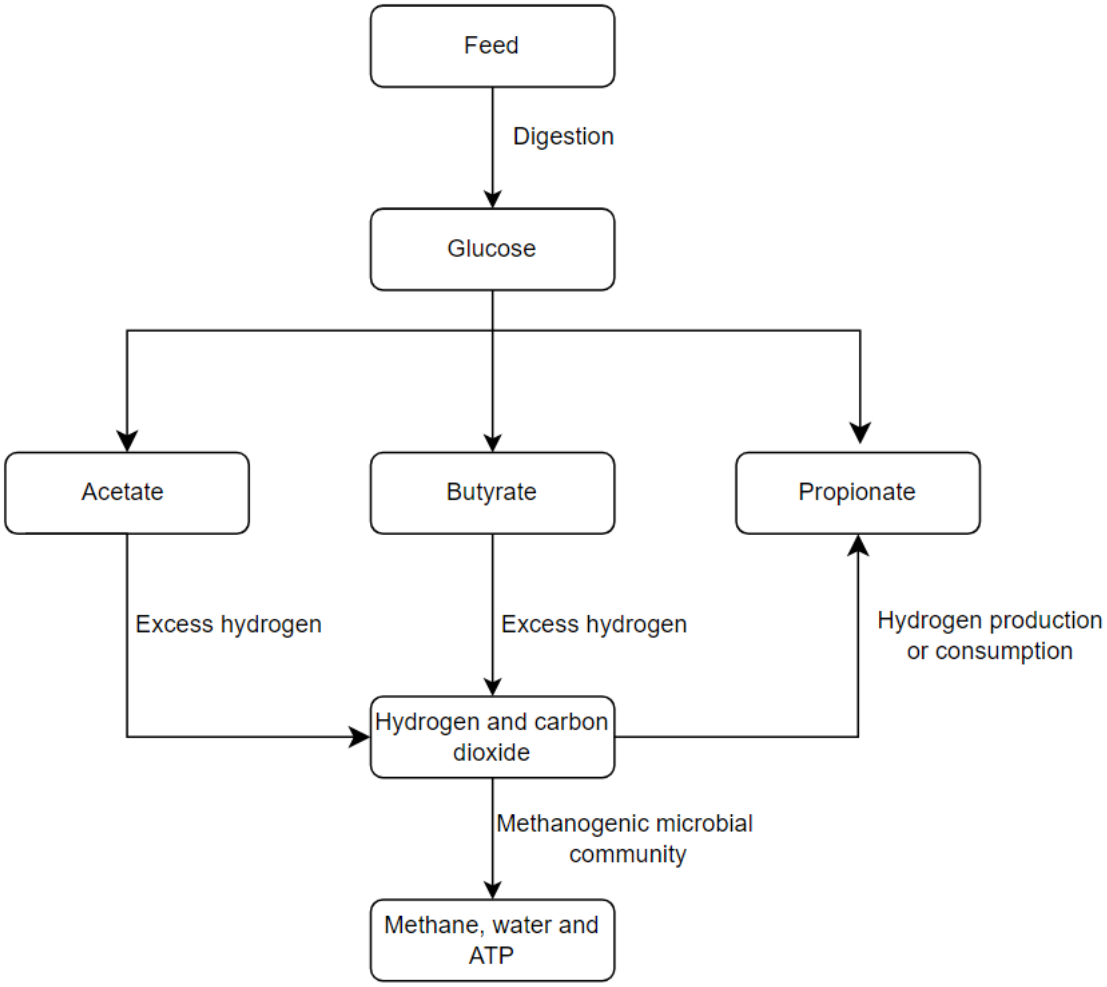

Figure 2.2: Process of methane production in the rumen adapted from Sejian et al. (2013)

The rumen is an anaerobic environment with an ecosystem of microbes which include fungi, protozoa, archaea and bacteria, these microbes aid in the production of methane through reducing the end products of fermentation (Wilkinson 2012; Knapp et al. 2014). Methane production in the rumen is the dominant hydrogen elimination process, hydrogen must be removed from the rumen, as dissolved hydrogen inhibits microbial activity causing NADH to accumulate (Morgavi et al. 2010). Consequently rumen fermentation pathways are supressed,

reducing carbohydrate degradation, microbial protein synthesis and microbial growth rates and decreasing the overall efficiency of the rumen (McAllister and Newbold 2008). Methane production within dairy cattle is influenced by many factors including, feed intake, feed type, pasture quality, physiological state, cattle age, cow genetics and supplementation quantity (Jonker et al. 2017; Lassen and Difford 2020).

2.3.3 Influence of feed intake on methane production

Feed intake is the key driver of methane production in cattle, with a strong linear relationship between DMI and enteric methane emissions (Knapp et al. 2014; Charmley et al. 2016). Total methane production is greater with higher intakes, as there is more feed being fermented and consequently greater dihydrogen production (Bannink et al. 2006). However, greater DMI above maintenance energy requirements is also associated with decreased digestibility, reduced rumen retention time, and consequently less methane production per kg of DMI (Lassey et al. 1997; Wilkinson 2012). This is because at a greater digestion rate the rate of VFAs produced exceeds the ruminant's ability to buffer and absorb the VFAs into the bloodstream, resulting in a decrease in the rumen pH (Okine et al. 1989). As a lower pH is unfavourable to the methanogenic microbial community, they are more likely to be outcompeted by propionate production, a pathway that competes against methanogenesis for hydrogen (Pereira et al. 2022). When cattle are fed *ad libitum*, approximately 52-64% of daily variation in methane production is due to variation in DMI (Knapp et al. 2014).

2.3.4 Influence of nutrient components on methane production

The nutrient composition of feed also influences methane production from cattle, with carbohydrate type being a significant factor. This is because the nature and fermentation rate of carbohydrates affect the proportions of butyrate, acetate and propionate formed and therefore methane produced (Boadi et al. 2004). Diets high in starch and low in fibre typically result in more propionate and less acetate produced in the rumen, which in turn results in less methane produced (Moss et al. 2000). In contrast, diets high in fibre and low in starch, result in more acetate and therefore more methane produced (Wilkinson 2012).

2.3.4.1 Pasture quality

Pasture quality, which depends on the maturity of the pasture and the growing environment, affects methane production. As pasture matures, the NDF concentration increases, whilst NDF digestibility decreases (Rinne et al. 1997). Greater NDF concentrations increase acetic acid production in the rumen, increasing methane production, while decreased NDF digestibility increases rumen retention time, also increasing methane production (Brask et al. 2013; Knapp et al. 2014). High-quality pasture has greater digestibility, as NDF concentrations are lower, resulting in decreased passage rates, decreasing methane production (Roche et al. 2009).

Pasture quality also changes throughout the season, dependent on temperature and rainfall. The NDF concentration is greatest in late spring and summer, as perennial ryegrass enters the reproductive phase, where the stem, which has the greatest content of NDF in the plant, elongates, decreasing the ratio of leaf to stem (Jacobs et al. 1999; Dalley and Geddes 2012; DairyNZ 2017). Along with this, low summer growth rates result in the accumulation of dead material, with dead material having a much greater NDF content (Moller et al. 1996). The NDF content is least in early spring and autumn as pasture is in an active growing phase and dead matter accumulation is low (DairyNZ 2017). Consequently, as methane emissions are influenced by NDF content, methane emissions of cows consuming pasture are likely to be lower in early spring and autumn than late spring and summer (Brask et al. 2013).

2.5 Factors controlling pasture intake

Forbes (2000) proposed that the physiological control of intake is where the cow, when fed *ad libitum*, regulates intake to meet energy demand. Under a pastoral grazing system *ad libitum* intake is unlikely to occur, thus limiting factors such as pasture composition and quality, along with pasture management will also affect pasture intake.

2.5.1 Cow energy requirements

In pastoral systems cattle have access to fresh pasture for a given period, during which physical and physiological signals limit the amount of feed consumed by the cow. Physiological factors include energy requirements, which are determined by maintenance, lactation, body size and condition, growth, pregnancy, and activity.

These energy requirements change with lactation stage, such that in early lactation energy requirements are at their greatest, with energy required for milk production greater than intake capacity, resulting in a negative energy balance during this period, high producing dairy cows

mobilise body tissue to meet the extra energy requirements (Peyraud and Delagarde 2013). As lactation progresses (approximately 10 – 12 weeks) when peak intake occurs, energy balance becomes positive, and the cow will stop mobilising body tissue. The production potential of the cow affects the energy requirements, with higher producing cattle having greater energy requirements than lower producing cattle (Duranovich et al. 2021). Breed also has an impact on lactational energy requirements, with crossbred cattle having greater production energy efficiency than purebred Holstein-Friesian and Jersey counterparts (Schwager-Suter et al. 2001; Prendiville et al. 2009). As liveweight increases, energy requirements increase at a slower rate than intake capacity, therefore requirements of heavier cows are easier to meet for a specified potential milk production (Peyraud and Delagarde 2013). Gestation has a significant effect on energy requirements, with energy requirements of pregnancy increasing with foetal size and developmental stage, however, rumen displacement by the growing calf will depress feed intake in the last two weeks of pregnancy (Sniffen et al. 1993; Sguizzato et al. 2020).

2.5.2 Feed quality

The NZ dairy farm system is typically low input with a fundamentally forage-based diet, the dominant pasture species are perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), with little or no supplementary feed in the form of concentrates (Dong et al. 2015). Forages generally contain less ME per kg DM than concentrated feeds, thus cattle grazing forages must consume greater physical quantities to reach the same ME intake, as cattle fed concentrated diets (such as TMR) in alternative global dairy systems (Dong et al. 2015). Rate of digestion and movement of indigestible particles through the digestive tract are highly related to the quality of feed consumed, with the physical capacity of the digestive tract limiting forage intake (Roca and Gonzalez 2013).

The composition of the pasture offered affects the digestibility and retention time of the pasture in the digestive tract, which in turn affects intake. Fibre content influences digestibility, as the amount of crude fibre content increases, the energy availability and DMD decreases (Dillon 2007; Paquay et al. 2009). Whilst water soluble carbohydrate content of forage does not impact DMI (Taweel et al. 2005). The DM% and thus the internal water in grass can also restrict intake and consumption rate, with external water not having an effect., as external water is swallowed immediately when consumed, while internal water requires chewing to release (Estrada et al. 2003). Although this effect may also be in its relation to a reduction in palatability or greater dilution of DM% (Roca and Gonzalez 2013). It is not just specific nutrient components that

affect digestibility, but the interaction between nutritional components. For example, organic matter and NDF digestibility increase with increasing dietary crude protein concentration (Huhtanen et al. 2009). The nutritional composition of herbage varies between pasture species, height, age, proportions of leaf, stem and dead material, all impacting digestibility and therefore intake (Dillon 2007). Cattle will select pasture with nutritional components with increased digestibility if allowed to do so, due to selecting for increased digestibility, intake capacity is increased as there is less retention time within the digestive tract (Wales et al. 1998).

2.5.3 Grazing mechanics

Daily intake rate is controlled by bite mass and bite rate, although the nutrient composition of the pasture, PA, sward height and green leaf mass influence these (Alvarez-Hess et al. 2021). Moving from medium (40 kg DM/d) to low (20 kg DM/d) PA reduces cow intake, likely a consequence of reduced biting mass as available herbage is depleted, with bite rate remaining the same (Pérez-Prieto and Delagarde 2013). While the reverse is true when moving from medium (40 kg DM/d) to high (60 kg DM/d) PA, as intake will increase with bite rate increasing, while bite mass (which is anatomically regulated) remains the same (Pérez-Prieto and Delagarde 2013). Bite mass is also influenced by time spent grazing, with bite mass being greatest during the first hour of grazing pasture and as herbage is depleted, bite mass declines (Barrett et al. 2001; Alvarez-Hess et al. 2021). There is no effect of herbage mass on average daily bite mass, instead bite mass is significantly correlated with sward height and green leaf mass, with bite depth increasing linearly with increasing sward height (Gibb et al. 1999; Boval and Sauvant 2019; Alvarez-Hess et al. 2021).

2.5.4 Grazing time

In a rotational grazing management system, cattle under PA restrictions do not compensate by grazing for longer periods of time (Le Du et al. 1979). This is likely to be a result of preference as the restricted area forces the cows to graze lower in the sward profile. As the cow grazes down the sward profile, the nutritional composition differs and is less appealing to the cow (Pérez-Prieto and Delagarde 2013). Restricted grazing access (4 hr in Pérez-Ramírez et al. (2008) or two 4.5 or 3 hr periods in Kennedy et al. (2009)) to pasture results in longer periods of time grazing, in comparison with cattle with full time or greater pasture access (8 hr in Pérez-Ramírez et al. (2008) or 9 or 22 hr in Kennedy et al. (2009)). Pasture allowance was the same between time treatments, and cattle that spent full time (22 hr) on pasture had a lower post-grazing sward height (3.5 cm) than the other time periods (Kennedy et al. 2009; Pérez-Ramírez

et al. 2009). Therefore, this response may be due to reduced preference when cows have full time access to the pasture. The longer the cows have access, the more likely they will be to have grazed their pasture to a point in the sward profile where preference is reduced, thus they spend less time grazing.

2.5.5 Pasture intake through management

Pasture allowance, which is determined by pre- and post-grazing masses and area offered, are tools of grazing management used to allocate pasture (Roche et al. 2017). There is a strong positive relationship between PA and cow intake, with greater PA resulting in greater intake (Pérez-Prieto and Delagarde 2013). Cattle offered a greater PA have a greater average daily bite mass than those offered a lower PA (Alvarez-Hess et al. 2021). Time spent grazing is also greater as PA increases (Alvarez-Hess et al. 2021). Yet at increased PA, pasture utilisation decreases, thus greater PA results in greater intakes but reduced pasture utilisation (Pérez-Prieto and Delagarde 2013). For example: as PA increases from 20 to 30 kg DM per cow (50% increase in available pasture), there is a small (1.3 kg DM; 8%) increase in pasture intake, but a much larger (23%) decrease in pasture utilisation (Pérez-Prieto and Delagarde 2013) .

Decreased pasture utilisation results in wastage as good-quality pasture that could have been eaten is left in the paddock and losses quality before the next grazing. Pasture wastage is inefficient and not economically favourable. A balance between optimising pasture utilisation and cow intake is required to maximise pasture eaten throughout the season, as there is a strong positive correlation between pasture eaten and operating profit per ha (Neal and Roche 2020).

As the pre-grazing herbage mass increases so will the post-grazing residual herbage mass, and past a point, the rate post-grazing herbage mass is increasing will exceed herbage DM consumption (Wilkinson et al. 2019). Consequently, the efficiency of pasture utilisation decreases. Wilkinson et al. (2019) found a decrease of pasture utilisation efficiency from 80% at a pre-grazing herbage mass of 1,500 kg DM/ha and post-grazing herbage mass of 1,000 kg DM/ha, to 20% at a pre-grazing herbage mass of 9,500 kg DM/ha and post-grazing herbage mass of 2,000 kg DM/ha. This is likely due to decreasing pasture quality, the recommended pre-grazing pasture mass for lactating dairy cattle is between 2600 to 3200 kg DM/ha, as past this point quality begins to decline (Chapman et al. 2014). This is due to older leaves dying, as new leaves continue to emerge, and the plants fight for light, which results in more structure (lignin, fibre) in the stem and base of the sward. Thus, there is a higher proportion of material that is not appealing to the cow, causing increased refusal, less utilisation and greater residuals

(Baudracco et al. 2010). Grazing at a higher sward height does result in a higher daily DMI and rate of herbage intake as there is more grass available, up until a point where pasture quality declines as previously discussed (Pulido and Leaver 2003). This effect is more pronounced in a continuous grazing system than a rotational one, thought to be caused by cattle in a rotational system anticipating the time of electric fence moving, reducing time spent grazing by choosing to wait for the fence to move instead (Pulido and Leaver 2003). Post-grazing height has a negligible effect on daily intake, as it is a response of intake (Lee et al. 2008).

2.5.6 Effect of supplementary feed

Supplementation of a pasture diet with concentrates and conserved forages is used on many dairy farms in NZ, especially in winter and autumn when pasture growth is lower than feed demand. In addition, the use of supplementary feeds can provide the cow with nutrients and minerals that are not available through pasture alone (Kolver and Muller 1998). When supplementary feed is used, pasture DMI is typically reduced, known as substitution (Stockdale 2000). Substitution occurs as cow's energy requirements are partially fulfilled through the supplementary feed, resulting in reduced time spent grazing (Stockdale 2000). There is no effect of supplementary feed on biting rate or mass when grazing pasture (Bargo et al. 2003). On average the inclusion of concentrate supplementation increases milk production by 1 kg milk/kg concentrate, which is consistent with the 80 g MS/kg DM marginal milk production response reported by Neal et al. (2018) using 12 years of DairyBase data.

2.6 Methods predicting pasture intake

Accurately measuring pasture intake from cattle in grazing systems has been a challenge for decades. Pasture intake is a consequence of the physical and physiological drivers previously discussed. Feed consumed from cattle housed or penned can be quantified relatively easily by weighing the difference between feed offered and feed refused. Thus, feed intake can be calculated without disruption of normal cow behaviour (Lukuyu et al. 2014b). Current methodologies used to measure feed intake in grazing cattle include energetics calculations, internal and external indigestible markers such as n-alkanes, digital technologies, pasture disappearance via RPM, and animal-based sensors (Lukuyu et al. 2014b; Smith et al. 2021).

2.6.1 Rising plate meter

In pastoral systems, the RPM, a non-destructive method of estimating pasture intake, may be used to determine pasture disappearance and predict pasture intake at the herd level (Seymour et al. 2019). A weighted disc on the RPM compresses the pasture sward, with the compressed sward height measured, and then the sward height is converted into herbage mass (kg DM/ha) using a series of calibration equations (Bareth and Schellberg 2018; Klootwijk et al. 2019). Pasture intake is then estimated by obtaining mass measurements of a paddock before and after grazing, (using the RPM) and calculating the difference between the two. The stocking density of the area that has been grazed is then accounted for, giving an estimate of average DMI per cow for the herd or mob (Seymour et al. 2019).

The RPM is one of the easiest and lowest-cost methods of estimating feed intake in pasture systems and is therefore used by both researchers and farmers (Bareth and Schellberg 2018). As measurements are taken before and after grazing has occurred, natural grazing behaviour of the cow is not disrupted. Using an RPM is semi labour intensive, with labour cost being dependent on the size of area measured and number of repeats required (Bareth and Schellberg 2018).

With the RPM, the estimated pasture intake per cow is an average taken from the entire grazed the area, thus the RPM has a low accuracy and resolution for determining individual animal intake (Seymour et al. 2019). Herbage intake varies between individual animals, a result of the physiological and environmental factors that control intake, therefore averages are not a good representation of individual intake. Accuracy is also dependent on pasture and soil condition with sward height, pasture trampling, structure, pasture type and soil pugging influencing the sward compression, and thus the measured pasture height (Fehmi and Stevens 2009). Pasture DM content, pasture composition and season also influence pre-grazing and post-grazing measures using RPM, once again reflecting high variability and low accuracy (Stockdale 1984; Lukuyu et al. 2014b).

There is also human error and variability when operating an RPM which also decreases accuracy. Key reasons are the angle the RPM is placed at when measurements are taken, along with the amount of force exerted by the operator when placing the RPM down (Earle and McGowan 1979). The amount of force influences pasture and soil compression, with greater amounts of force, resulting in greater compression and under-estimation of herbage mass (Thomson et al. 2001). Variation within one operators measurements has not been found to be

significant, so human error can be avoided by using the same operator (Earle and McGowan 1979). However, depending on the trial requirements this may be impractical. Inconsistencies in the calibration methods used to convert sward height to pasture mass has also been identified (Thomson et al. 2001). Recommended calibration equations vary between RPM manufacturers, yet for accuracy, context specific calibration equations must be used, to be representative of the linear/curvilinear relationship between grass height and biomass (Klootwijk et al. 2019). This can be done through cutting and weighing pasture to produce a context specific calibration equation (Klootwijk et al. 2019). Although RPMs can be used to estimate pasture mass on-farm, they do not have sufficient accuracy to determine individual cow intake in a research context.

2.6.2 Calan gate system

The Calan Gate system accurately measures individual feed intake. Cattle are assigned to a specific bunker or feed bin, typically individually, although sometimes gates are shared, the cattle gain access to their feed through wearing an electronic collar that opens the corresponding gate (Motupalli et al. 2014). A weighed quantity of feed is put in the feed bin/bunker each day which the cow can access through the Calan gate. Feed that is not consumed is removed and weighed usually once every 24 hr, to determine total daily feed intake (Motupalli et al. 2014).

Calan gate systems are one of the most accurate forms of measuring feed intake, as all feed offered to the cow is accounted for, including wastage. Calan gate systems enable normal herd interactions whilst still measuring individual feed intake (Seymour et al. 2019). However, as feed is accessed through the gates, cattle must be housed or penned, and thus it cannot be used to measure intake from grazing cows (Seymour et al. 2019). Pasture can be cut and carried and fed to cattle, but requires harvesting prior to being fed, which may differ in nutritive composition from pasture consumed when grazing. As pasture continues to be metabolically active after being cut, with aerobic respiration of sugars continued to provide energy, reserve carbohydrates may also be utilised during this period (Muck et al. 2003). Consequently, perennial ryegrass sugar concentration declined by 9.0 ± 6.1 g/kg DM a day after cutting (Spoelstra and Hindle 1989). In contrast, Wylam (1953), reported that pasture sugar content did not decrease significantly by cut and carry methodology, although this will be dependent on how soon after being cut the pasture is consumed by the cow. Pasture that is relatively fresh or has been refrigerated will have reduced aerobic respiration after cutting (Wylam 1953).

Another disadvantage of the Calan gate system is feed access is restricted behind a gate, consequently modifying animal feeding behaviour, with cattle increasing intake rate to maintain feed intake (Ferris et al. 2006). Therefore, intake data collected from a Calan gate system may not be a true representative of intake for cattle in the general grazing population, although it can be considered a close indication.

Cattle require training to access feed through the Calan gate system, as it does demand the learning of new behaviour (Krawczel et al. 2012). Some cows will not respond to training, thus limiting the number of cows that can be used. Training requirements to ensure cows are sufficiently trained and accustomed to the Calan gate system results in greater labour and resource requirements increasing the cost and time required for trials using the Calan gate systems (Seymour et al, 2019). Additionally, the initial cost of purchasing and installing the system may be prohibitive. As only trained cows can be used, if a cow must be removed from the trial (e.g., due to illness, adverse event etc), a suitable replacement cow may not be available.

The Calan gate system is not suitable for measuring intake from grazing cows in a pastoral system. However, the system provides an accurate direct measurement of individual cow DMI, from housed cattle. Therefore, it is an adequate method to evaluate the accuracy of other techniques that indirectly measure DMI from grazing cattle.

2.6.3 Indigestible markers

In grazing ruminants, where DMI cannot be measured directly, indigestible markers are the most extensively used technique for estimating ruminant DMI (Velásquez et al. 2018). The technique involves an external marker that the animal is dosed with, and which is used to estimate faecal output, while an internal marker naturally present in feed is used to estimate DMD (Guinguina et al. 2019). The pairing of an internal and external marker is used to estimate DMI, with intake calculated through dividing faecal output by indigestibility (1 – DMD) (Velásquez et al. 2018).

An ideal marker for use in intake studies will fit the following criteria:

1. Be inert and have no toxic physiological effect on the animal.
2. Not be absorbable or metabolisable within the digestive tract.
3. Not interact or be influenced by any digestive processes.
4. Be physically similar or directly associated with dietary components.

5. Analysis has adequate specificity, sensitivity and does not interfere with other analysis.

(Faichney et al. 1975; Fahey and Jung 1983)

Although there are available markers sufficient for use in research, no current marker in ruminant digestion studies satisfies the full criteria discussed above (Merchen 1988; Velásquez et al. 2018).

2.6.3.1 N-alkanes

N-alkanes are mainly indigestible in ruminants, naturally derived from the cuticular wax of plant species. They typically contain hydrocarbons with odd chain lengths ranging from C21 to C37, with C29, C31 and C33 being the predominant types (Cottle 2013). To estimate feed intake the cow is dosed at least once daily with an even chain length n-alkane, typically C32, with a build-up period of five to seven days, followed by a three-to-eight-day adjustment period before the measurement period (Dove and Mayes 2006). In parallel, the cow is consuming an odd chain length n-alkane naturally occurring in herbage (Dove and Mayes 1991). During the measurement period, pasture samples, representative of pasture consumed are collected, and analysed for the concentration of n-alkanes and digestibility (Miguel et al. 2014). In addition, faecal samples are collected twice daily during the measurement period and analysed for n-alkanes. If plant and faecal n-alkane concentrations are determined at the same time analytical error and bias are reduced, increasing accuracy (Cottle 2013).

The ratio of plant odd chain n-alkanes to the dosed even chain n-alkane, not the absolute concentration of n-alkanes, is used to estimate pasture intake, therefore complete faecal samples are unnecessary (Dove and Mayes 2006). When using n-alkane pairs (odd and even chain) accuracy in estimating DMI is greater if pairs differ by one carbon atom, as faecal recovery rates are similar, correction for incomplete faecal recovery is not required (Wright et al. 2019). Pasture typically has a higher C33 concentration than temperate pasture legumes (Dove and Mayes 2006). N-alkane rate of recovery from the digestive tract increases curvilinear with carbon chain length, with carbon chain lengths above C33 having a near complete faecal recovery (Dove and Mayes 2006; Cottle 2013). C31, C32 and C33 have incomplete but similar faecal recoveries, hence their preferred use in the n-alkane method (Dove 2010).

Wright et al. (2019) used two different n-alkane pairs when evaluating the n-alkane method in dairy cattle. The pair C32:C33 was a more accurate estimation of pasture intake than C32:C31,

they found no effect of season and pre-harvest herbage mass on estimated DMI from n-alkanes (Wright et al. 2019). Although it is believed that effects may have been masked by differences between swards as there were differences in faecal recovery rates between summer and autumn, therefore requiring further investigation (Wright et al. 2019). If there are changes in botanical composition with season, this will affect n-alkane recovery rate and must be accounted for. A limitation of the n-alkane method is more research is required to investigate relationships and impacting factors which may influence intake estimations. Another study by Wright et al. (2020) estimated DMI through n-alkanes, in dairy cows fed low and high fresh herbage and partial mixed rations. Cows were housed so that DMI could be measured and compared to the n-alkane intake estimation and total faecal output could be collected (Wright et al. 2020). When n-alkane results were corrected for complete and incomplete faecal recovery, DMI was accurately estimated, although discrepancies between estimated and actual herbage DMI increased with amount of herbage offered (Wright et al. 2020). Whilst recovery rates of natural n-alkanes increased with amount of herbage consumed (Wright et al. 2020). When Dove and Mayes (1996) reviewed articles utilising n-alkanes to estimate feed intake in cattle and sheep, they found average differences between estimated and actual DMI at a group level ranged from -2.60 % to 2.57 %.

The n-alkane method is unique in that digestibility and intake are estimated at the same time, therefore allowing a true estimation of individual DMI (Dove and Mayes 1996). The method can be used in differing dairy systems, as recovery rates are not influenced by stage of lactation and feeding frequency (Dillon 1993). As the n-alkane feed intake methodology does not require direct external measures (similar to RPM and Calan gate systems), cow grazing behaviour is unaffected. Therefore, intake measurements are far more representative of a typical grazing cow in a pasture-based dairy farm system.

A point of difference to other indigestible markers is that n-alkanes are naturally present in plant species, therefore odd chain n-alkanes do not require dosing as well, decreasing cost. The n-alkane methodology can also be used to quantify diet selection (Dove and Mayes 2006).

For accurate predictions of pasture intake, the concentration of n-alkanes within consumed herbage is required (Wright et al. 2019). Thus estimations of intake using the n-alkane method are most accurate when the ruminant is consuming a herbage monoculture (Wright et al. 2020). Natural n-alkane profiles differ between plant species so when multiple herbage species are consumed, analysis can become complicated, decreasing accuracy (Wright et al. 2020). As it

can be difficult to collect representative samples of consumed pasture, as selective grazing will occur, making it challenging to determine which species will be consumed and to what grazing height. In a typical NZ dairy system, pasture species often vary within a paddock, with weed species, clover species and perennial ryegrass often present, therefore this method may not be as accurate or useful on dairy farms in NZ. For the n-alkane method to accurately predict feed intake, dosage and timing must be exact, although precise dosing can be controlled through methodology (Andriarimalala et al. 2020). There are high costs associated with the n-alkane method in comparison with other methods as a result of materials used and labour required, although these can be decreased through selection of a low-cost n-alkane dosing method (Seymour et al. 2019). The n-alkane method was developed, published, and evaluated in dairy cows over thirty years ago. Since then, pasture management has evolved, which has resulted in changes in the physical structure and quality of herbage which may affect accuracy of the method (Wright et al. 2019). The n-alkane method has not been widely used in NZ due to inaccurate results, in recent years, research from Ireland and Australia have used the n-alkane method with consistently accurate predictions of DMI (Wright et al. 2019; Hennessy et al. 2020; Wright et al. 2020; McClearn et al. 2021). It is believed this methodology could be investigated to better understand differences between the current NZ and Irish methodologies and outcomes.

2.6.3.2 Titanium dioxide

Titanium dioxide is an external indigestible marker that the cow is orally dosed with at least once a day, which can be used to estimate faecal output in dairy cattle (Velásquez et al. 2018). Titanium dioxide can be paired with an internal marker such as iNDF, iADF or cutin, with the pair used to estimate DMI (Velásquez et al. 2018). Although one of the most frequently used external markers, use of titanium dioxide has not been widely studied in ruminants (Titgemeyer et al. 2001; Scholljegerdes 2020). Titanium dioxide is found in both human and animal food, without limitations on its use, hence its popularity (Myers et al. 2004).

Use of Titanium dioxide is reliable in cattle, although faecal recovery rate has been seen to vary (Hellwing et al. 2015). A 99% faecal recovery rate in dairy cattle fed concentrate, corn silage and grass silage was reported by Hafez et al. (1988), whilst Titgemeyer et al. (2001) reported a 93% faecal recovery rate in steers consuming forage. Velásquez et al. (2021) reported significantly different results, finding faecal recovery rates varying between 130% to 157% in bulls fed concentrate and corn silage or Tifton-85 hay. Silva et al. (2021) reported underestimation with faecal recover rates ranging from 94% to 97% in grazing steers.

Variations in faecal recovery rates cause variability in faecal output estimates produced by titanium dioxide, resulting in either overestimation or underestimation of faecal output, this is of concern, as inaccuracies in intake predictions would occur (Velásquez et al. 2021). This can be mitigated through correcting estimated faecal output with real faecal output, although this does require the total faecal collection of at least one animal per treatment (Velásquez et al. 2021).

Use of titanium dioxide is subject to diurnal variation, with the faecal recovery rate affected by the time of day that faecal sampling occurs (Hafez et al. 1988). Hafez et al. (1988) reported that recovery from morning faecal samples were close to 100%, in comparison to 95% from afternoon samples. Titgemeyer et al. (2001) did not assess diurnal variation but observed significant differences in recovery of titanium dioxide among steers consuming forage. The effect of diurnal variation can be reduced through faecal sampling at least twice a day, at the same times each day (Hafez et al. 1988). When titanium dioxide is compared with chromium dioxide, a previously popular external marker, with reduced use since carcinogenic properties were reported, titanium dioxide has a faecal recovery rate closer to 100%, with less daily faecal concentration variation (Silva et al. 2021). This finding indicates titanium dioxide has greater homogeneity in ruminal digesta than chromium dioxide (Silva et al. 2021).

A limitation to the use of titanium dioxide as a marker in grazing dairy cattle is that titanium dioxide is present in soil. When grazing, soil may be voluntarily or involuntarily ingested, with it estimated that cattle consume 0.17 kg soil DM/day at a PA of 35 kg DM cow/day on ryegrass pasture (Jurjanz et al. 2012; Hellwing et al. 2015). Ingestion of soil that contains titanium dioxide will result in the cow consuming a greater amount of titanium dioxide than measured through dosing, likely to result in overestimation of faecal output (Hellwing et al. 2015). Along with this the analyses of titanium dioxide faecal concentration includes the use of a spectrophotometer, the wavelength used may affect the sensitivity gained for quantification of the titanium dioxide, resulting in inaccuracies of predictions (Guzman-Cedillo et al. 2017).

2.6.3.3 iNDF

iNDF is an indigestible cell wall component and the least digestible fibre constituent of herbage, as digestion of the plant cell wall is limited through the presence of lignin (Harper and McNeill 2015). As iNDF naturally occurs in pasture consumed by dairy cattle, it has been extensively used as an internal marker to estimate digestibility, when utilising indigestible markers to predict DMI (Lippke 2002).

Faecal recovery of iNDF is highly variable between feed and pasture type. This is likely a result of iNDF content variation between feed type and consequently differences in passage rate between feeds (Daniel et al. 2020; Velásquez et al. 2021). Faecal recovery of iNDF has been reported to range from 50% to 121% (Velásquez et al. 2018). When a hay-based diet was fed, faecal recovery of iNDF was near 100%, whilst cattle fed a corn-silage based diet had faecal recoveries ranging from 72 to 81% (Velásquez et al. 2021). If faecal recovery varies from 100%, faecal output will be over or underestimated, with a greater faecal recovery resulting in faecal output being underestimated (Velásquez et al. 2018). Therefore for accuracy it is important to determine real faecal recovery to correct the estimate of faecal recovery produced by iNDF (Velásquez et al. 2018).

In dairy cattle fed corn silage and alfalfa hay-based diets iNDF was found to have underestimated faecal output and overestimated digestibility, although the difference was negligible enough for iNDF to be considered a reliable internal marker (Lee and Hristov 2013). In sheep fed pasture, estimation of faecal output using iNDF was influenced by faecal collection procedure and amount of sampling days, subsequently resulting in unreliable and inaccurate faecal output estimations (da Costa et al. 2019). This is likely to be in relation to forage type and amount of concentrate fed (da Costa et al. 2019). In contrast iNDF consistently provided accurate estimations of DMD irrespective of the procedure for faecal sampling in cattle fed a hay or corn silage based diet (Velásquez et al. 2021). Consequently indicating that iNDF has sufficient adequacy for internal marker use when faecal recovery is corrected with real faecal output, otherwise overestimations or underestimations of DMD are probable (Velásquez et al. 2018).

When estimating faecal marker content there is the probability that long-term or short-term bias occurs (Langlands et al. 1963). Long-term bias is related to the ability of the marker to be totally recovered in faeces, it is assumed that any marker used for predicting intake is without long-term bias (Langlands et al. 1963). Whilst short-term bias is in relation to inconsistencies produced by faecal sampling procedure representing total faeces excreted, it has been reported that iNDF is free of short-term bias in the majority of faecal sampling procedures (Sampaio et al. 2011). To support this Velásquez et al. (2021) reported when using grab and bulk faecal sampling procedures iNDF produced DMI predictions similar in accuracy.

There is conflicting evidence on the accuracy of iNDF as an internal marker paired with the external marker of titanium dioxide to predict cattle intake. In dairy cattle fed herbage, accurate

results were produced with titanium dioxide paired with iNDF, with the average herbage intake prediction being only 1 kg lower than actual DMI (Hellwing et al. 2015). Similarly, Velásquez et al. (2021) stated that average DMI predictions in cattle fed corn-based silage were just 1.7% greater than average real DMI. In contrast when titanium dioxide was paired with iNDF only 72-h bulk sampling produced accurate DMI estimations (Velásquez et al. 2018).

The procedure used to analyse iNDF content influences digestibility estimations with both the incubation time of samples in the rumen of a cow, and pore size of the bag that samples are incubated in both reported to have an effect (Huhtanen et al. 1994; Berchielli et al. 2005). Research outcomes indicate that an incubation time of 288-hr, with the use of bags with a smaller pore size provides an accurate estimation (Huhtanen et al. 1994; Krizsan and Huhtanen 2013).

2.6.4 Energetics back calculation

Another method to estimate feed intake is the use of an equation to model intake, which does not require physical measurement of feed and is useful for measuring individual cow intake on a large scale (Halachmi et al. 2004). There are many equations available to estimate feed intake, with varying theoretical concepts behind them, the majority of models utilised currently are based on the physical/physiochemical theory of intake regulation (Galyean and Gunter 2016). This theory is based on intake of diets with low energy, and digestibility is controlled by physical factors including rumen fill and digestive passage rate, while intake of high energy and digestibility diets is controlled by energy requirements and metabolic factors (Galyean and Gunter 2016). Pittroff and Kothmann (2001) investigated the differences between seven equations for pasture grazing cows estimating intake during the first month of lactation, using a four-year-old, 580 kg cow with a 12 kg/day peak milk yield. Results between equations were highly variable, likely a result of different concepts, variables and databases used in development (Pittroff and Kothmann 2001). As the results are dependent on the equation used, accuracy is not high when comparing cow intake calculated with different equations.

The use of equations to estimate cow intake are more accurate in TMR systems, where feed is typically high energy, highly digestible and more consistent and controlled (Bargo et al. 2002). Consequently, cow intake is more likely to be controlled by energy requirements of the cow (Galyean and Gunter 2016). In contrast, feed in a pastoral-based system, is typically less digestible, lower in energy, and more variable. In addition, other aspects that regulate intake including, herbage mass, pasture composition, digestibility, time spent grazing and selective

grazing are for more likely to control intake than energy demand (Galyean and Gunter 2016). Thus, energy intake may vary each day as these influencing factors interact.

2.6.4.1 NRC

The equation created by the NRC to predict energy intake is frequently used, based on the theory that cow intake is controlled by individual energy requirements. With the equation for predicting DMI of a lactating Holstein Friesian being:

DMI (kg/d) = (0.372 x 4% FCM + 0.0968 x $BW^{0.75}$) x (1–- $e^{(-0.192 \text{ x } (WOL+3.67))}$)

4% FCM = 4% fat corrected milk

BW = liveweight (kg)

e = 2.71828 (e value adjusts for stage of lactation)

WOL = week of lactation

(NRC 2001)

The NRC equation only utilises animal factors that are measured with ease or known. Although this simplifies its use and makes it more accessible to all people, it does mean it is heavily reliant on assumptions, decreasing accuracy (NRC 2001). The accuracy of the equation is significantly dependent on the accuracy of the liveweight and milk production data used. Along with the assumption that the cow becomes pregnant at week 17 of lactation and gains liveweight accordingly (NRC 2001). The NRC equation requires milk volume to be adjusted to give a milk fat percentage of 4%, decreasing accuracy within cattle, within herds and within season with variable milk fat content, (Halachmi et al. 2004). In times of transition, such as early lactation and when cows cease lactation, the use of an equation to estimate feed intake is likely to be inaccurate, as in these times there is not a strong relationship between energy demand and feed intake (Halachmi et al. 2004). In early lactation cow intake cannot meet energy demands, and liveweight changes are a strong driver of energy intake. As lactation ceases, liveweight change again plays a big role in determining energy intake as the cow partitions energy to body condition gain. The NRC equation does not include dietary components as the majority of dairy cow feed globally (TMR) is formulated by first establishing cow feed requirements and DMI from this, before then selecting feed (Allen et al. 2019). This is not the same for pasture-based systems where feed type is not selected as pasture is predominantly consumed. This may affect the accuracy of the results as the effect of diet on

intake is not considered (Allen 2000). For example, NDF in pasture can impact on energy availability from the structural carbohydrates. It passes through the digestive tract at a decreased rate compared with other pasture components which in turn affects intake rate. As these dietary components are not considered, their influencing factors on intake are also not taken into account (Allen et al. 2019).

2.6.4.2 Nicol and Brookes

To estimate intake the ME requirements of the cow is calculated in the method by Nicol and Brookes (2007), through estimating energy requirements of maintenance, liveweight gain/loss, pregnancy, and milk production, which is then summed. The Australian CSIRO ME equations were used as a basis for the equations produced, with adaptations made for specific use in NZ livestock (Nicol and Brookes 2007).

Maintenance in this equation is defined as the amount of ME necessary for the animal to remain at a constant energy content, irrespective of milk production or gestation. With maintenance influenced by species, age, sex, topography of environment and physiological state of the animal. The maintenance calculation does not include grazing costs but accounts for cost of supporting productive tissues. The ME requirement for liveweight gain is influenced by the proportion of fat and protein gained, with a higher proportion of fat gained in mature animals. Lactation stage also influences liveweight gain, with lactating cattle requiring a greater amount of ME to gain. For gestation, ME is required for foetus and placenta growth, maintenance of foetus and preparation for lactation, with requirements increasing with gestation. ME requirements in lactation are determined by the daily milk yield and composition of fat and protein in the milk. (Nicol and Brookes 2007).

2.6.4.3 MPI GHG

The agricultural GHG emission inventory is published annually in NZ. To produce this report numerous calculations take place, including the calculation of livestock emissions, which is based on DMI. DMI is calculated through the ME requirements of the animal combined with the energy content of pasture (Figure 2.3). The estimation of DMI through ME requirements is based on the assumption that pasture intake satisfies energy requirements and no supplementary feed is used. There are four main factors, that are summed to produce total daily ME requirements for milking cows and heifers, being maintenance, milk production, additional energy expenditure associated with grazing and gestation, or growth of conceptus. Maintenance is defined as the amount of energy required to maintain the animal, accounting for fasting heat production, efficiency of ME use for maintenance and variations associated with liveweight, age, sex, and production. ME requirements for milk production are determined by daily milk yield, and fat and protein content of milk. For grazing the ME requirement is calculated through DMD, energy required for animal bodyweight maintenance and milk production, terrain of farm, green forage availability, efficiency of maintenance energy use and energy content of pasture. Gestation ME requirements are influenced by calf birth weight, maintenance of foetus, efficiency of ME use for foetus energy gain and gestation stage. As the equation is determined on an annual basis, liveweight gain is assumed to be zero for mature lactating cattle (Pickering et al. 2022).

Figure 2.3: Diagram summarising how energy requirements and DMI are calculated (Pickering et al. 2022)

2.7 Objectives

Following a review of the literature on methodologies used to predict DMI in dairy cattle, and the importance of these predictions in estimating GHG emissions, I have identified a gap in the methodologies available to accurately predict, individual DMI and GHG emissions of grazing cattle in NZ. Thus, there is a need to understand further the limitations of these available methodologies for grazing systems, and potentially improve the more promising methodologies including the n-alkane, titanium dioxide and iNDF techniques and energetic back calculation equations. To achieve this, the objectives of my Masters research are to:

1. Quantify the variation between intake of "grazing" dairy cattle in NZ predicted from five methodologies; three standard back calculations and two indigestible markers techniques, and actual intake measured by the Calan gate disappearance method.
2. Understand the variation in the accuracy of DMI predictions from different methodologies.

2.8 Hypotheses

Hypotheses are:

1. There will be variation in predicted intake from techniques (back calculations and indigestible markers) compared with actual intake of individual cows.

2. There will be variation between individual animals in the accuracy of estimations from different predictive techniques.

3. Methodology can be developed that provides a valid prediction of intake (within 10% variation from actual intake for individual cows).

Chapter 3: Indigestible Markers

3.1 Introduction

In NZ agriculture contributes 49.2% to GHG gross emissions, with methane from dairy cattle contributing 19.2% of these emissions (Ministry for the Environment 2022). Thus, reducing methane emissions from livestock has become a focus with government and industry targets (Moss et al. 2000; Ministry for the Environment 2021; Ministry for the Environment 2022). Feed intake is a key driver of methane emissions in cattle, with a strong linear relationship between DMI and enteric methane emissions (Knapp et al. 2014; Charmley et al. 2016). Within methane mitigation research it is important to measure animal performance, including pasture and total DMI, in conjunction with methane emissions. This will ensure animal performance is not compromised, and methane mitigation is accounted for accurately in on-farm and national inventories. Accurately measuring pasture DMI from grazing cattle is also very important for other areas of dairy research (Knapp et al. 2014; Charmley et al. 2016). It is now possible to measure methane emissions from individual grazing cows using Mobile GreenFeed units (C-Lock Inc., Rapid City, SD); however, measuring pasture intake from individual grazing cattle is challenging. An accurate indirect method may solve this challenge.

Indigestible markers are an indirect method of measuring DMI in grazing ruminants and are currently the most frequently used method in pastoral systems (Velásquez et al. 2018; de Sousa et al. 2019). In this experiment two common indigestible marker methodologies were tested, the first being the n-alkane method. The n-alkane method has previously been used in NZ but not in recent years, as a consequence of detected issues in the precision and accuracy of intake estimations (Kay et al. 2007). Yet other countries including Ireland and Australia with similar pastoral based dairy systems to NZ have been using the n-alkane method successfully (Wright et al. 2019; Wright et al. 2020; Moscovici Joubran et al. 2021; Liu et al. 2022). Therefore, we aimed to adopt the well-established n-alkane methodology used by the Irish research experiments, to determine if the new methodology would provide accurate estimates for DMI and have the potential to be used successfully in NZ grazing research.

The second indigestible marker methodology trialled was titanium dioxide as an external indigestible marker paired with iNDF as an internal indigestible marker. This methodology has been successfully used in both herbage and corn (maize) silage-fed dairy cattle overseas (Hellwing et al. 2015; Velásquez et al. 2021). Similarly, titanium dioxide paired with iADF

has been recently shown to produce accurate DMI estimations in NZ with red deer fed perennial ryegrass, and dairy cattle grazing perennial ryegrass (Garrett et al. 2020; Beck et al. 2022). Therefore, titanium dioxide paired with iNDF has the potential to be an indigestible marker method used in NZ to measure DMI in grazing dairy cattle, however, the accuracy has not yet been evaluated.

The delivery mechanism for most anti-methanogenic feed additives (For example: 3-nitrooxypropanol; 3-NOP, Asparagopsis, etc), will be via supplementary feed fed twice daily when cows are milked, or via slow-release bolus, Thus, it is important to be able to predict DMI from cows that are grazing pastures, or grazing pastures with supplementary feed fed twice daily.

Therefore, the purpose of this experiment was to evaluate the accuracy and precision with which two indigestible marker methodologies; n-alkanes and titanium dioxide paired with iNDF estimate DMI in individual dairy cattle fed a pasture diet with or without supplementary feed.

3.2 Materials and Methods

3.2.1 Experimental design

All animal manipulations were approved by the Ruakura Animal Ethics Committee (Hamilton, New Zealand) (Approval No. 0574) in accordance with the New Zealand Animal Welfare Act (1999). The experiment occurred during spring (19th September 2022 to 4th November 2022) at Lye Farm, DairyNZ, Hamilton, New Zealand.

Fifty multiparous, early lactation Holstein-Friesian cattle trained to use the Calan Gate Facility (American Calan, Northwood, NH) and GreenFeed system (C-Lock Inc., Rapid City, SD) were assigned to one of two treatments: Control (CTRL; pasture-only diet) or Supplement (SUPP; pasture and supplement diet) (n = 25 cows per treatment). Treatment groups were balanced for age, days in milk (DIM), liveweight and milk production (7-day milk total; kg)).

All cows underwent an adaptation period for three weeks (19th September to 9th October) prior to entering the Calan Gate facility. They were managed on perennial ryegrass/white clover-based pasture using rotational grazing decisions based on Macdonald and Penno (1998). The

cows grazed in their treatment groups (n = 25) with an 18 kg DM/cow/day allowance, as determined by RPM for pre and post-grazing masses.

During the adaptation period, cows in the SUPP treatment were transitioned onto a Denver Spring Pellet, (Denver Stock Feeds, Palmerston North). On day one (19th September) they were offered 1 kg DM of pellets, split between 2 feeds (0.5 kg DM of Pellets after the A.M. and P.M. milkings). The amount of supplementary feed offered after each milking was increased by 1 kg DM per day until cows were offered 4 kg DM per day, split over two feeds (2 kg DM per cow after the A.M. and P.M. milkings) on day four (22nd September). During the adaptation period, the supplementary feed was offered to cows as a group on a concrete pad near the milking shed. When all cows had finished eating the group was returned to their allocated paddock. Beginning on week two (3rd October), a week prior to entering the Calan gate facility, the supplementary feed was fed individually in a training barn facility with headlocks. The headlocks ensured each cow only had access to their allocated feed. The cattle were given a maximum of fifteen minutes to consume their supplementary feed, at which point they were returned to their allocated After the adaption period, 40 cows (20 per treatment) were selected to enter the Calan Gate facility (9th October). Cows selected all consistently consumed the supplementary feed in the allocated time, had no signs of lameness and were healthy. On 10th October, 40 multiparous, early lactation Holstein-Friesian cows entered the Calan gate facility and began a re-adaptation period for 7 days. On day 8, cows were dosed with the indigestible markers n-alkane C32 and titanium dioxide twice a day for 3 weeks. During the 1st week (18th October to 3rd November) there were no measurements taken to allow the dosed indigestible markers to reach a steady state. There were two measurement periods of five days, with period 1 from 25th October P.M. to 30th October A.M., and period 2 from 30th October P.M. to 4th November A.M.

Based on previous trials in the Calan Gate system, cows were allocated one gate, and subsequently, depending on the position of their gate divided into four cohorts by splitting each half of the barn in two with a metal gate (n = 10) (Figure 3.1). The Calan gate facility is free stall, allowing the cows in each cohort to interact with each other. All cows had *ad libitum* access to fresh water throughout the trial. Each cohort (n = 10) had free access to ten raised cubicle sand beds within their area. Pasture was accessed individually through a Calan Gate system (American Calan, Northwood, NH). The Calan gate electronic system functions by a gate at each feeding station, with a gate circuit board, transmitting an electronic signal. Each cow wears a collar containing a sensor key corresponding to the same frequency as the gate.

When the cow is in feeding position the signal unlocks the allocated gate, which the cow pushes open giving them access to their pasture. Consequently, each cow only had access to their individual pasture allocation.

	Cow tag number	Calan gate no.		Calan gate no.	Cow tag number	
Cohort 4	Gate not in use	42	Walkway	1	Gate not in use	Cohort 1
	6110	41		2	4669	
	7112	40		3	6638	
	8115	39		4	7137	
	8132	38		5	8216	
	8235	37		6	8255	
	8237	36		7	8358	
	8246	35		8	9117	
	9105	34		9	9128	
	9120	33		10	9131	
	9123	32		11	9132	
Dividing gate				Dividing gate		
Cohort 3	5656	31		12	4189	Cohort 2
	6121	30		13	4632	
	6166	29		14	7641	
	7146	28		15	8137	
	7161	27		16	8145	
	9121	26		17	8229	
	9127	25		18	8248	
	9130	24		19	9136	
	9138	23		20	9156	
	9149	22		21	9176	

Figure 3.4: Diagram of cow allocation and cohorts in Calan gate facility

3.2.2 Management

When housed in the Calan Gate facility the cattle were offered fresh pasture of predominantly perennial ryegrass (*Lolium perenne*), with small percentages (<5%) of white clover (*Trifolium repens L.*), other grasses (Poa species) and weeds (yarrow, dandelion, couch). The cows were offered approximately 90% of their individual voluntary pasture intake, to target no refusals, and encourage use of the GreenFeed units (C-Lock Inc., Rapid City, SD) to measure methane as part of another research project. The pasture was cut and carried twice a day no more than an hour prior to each feeding, with all cows fed half of their individual daily pasture allocation after the A.M. milking (8:30 am) and the other half after the P.M. milking at 15:30. Feed was pushed up again at 10am, 1pm and 8pm. Cows had unlimited access to their allocated gate while housed in the Calan gate facility. A Kuhn PZ 220 mower (Kuhn, Saverne, France) set at a cutting of 7 cm was used to mow the pasture, with a Bergman pickup wagon used to pick up cut pasture. Pasture was weighed prior to being forked into the allocated gates. After each milking, cows in the SUPP treatment were offered their supplementary feed (2 kg DM twice a day), fed in the training barn, with headlocks ensuring each cow only had access to their individual supplementary feed allocation. The cows were returned to the Calan Gate facility with free access to their allocated pasture 30 minutes after cows in the SUPP treatment had finished their pellets. This was designed to mimic on-farm practices, where dairy cows walk back to their allocated paddock after milking. Cows in the CTRL treatment were held on a concrete area during this 30-minute period.

3.2.3 Sampling and measurements

During measurement periods one and two (26th October to 4th November) any supplementary feed refused was recorded and weighed. If the refusal was 200 g or greater a 200 ± 5 g sample was taken, oven dried at $95^{o}C$ for 72 hours and weighed again to determine the DM. Prior to each feeding, a handful of supplementary feed (pellets) was collected from each bag to produce an 800 g bulk per measurement period. This bulk was then split into 3 tins of 200 ± 5 g, which was oven dried at $95^{o}C$ for 72 hours and weighed again to determine the DM. During measurement periods 1 and 2 an additional 200 g sample was collected from the 800 g bulk, the sample was frozen at $-20^{o}C$ then freeze-dried and ground to pass through 1mm sieve. For period 1 12.5 g of each sample and period 2 5.5 g of each supplement sample for that period was bulked to produce a 50 g bulk for each measurement period. From the bulks of period 1 and 25 g of each was subsampled for n-alkane analysis and 10 g for titanium dioxide analysis,

with two repetitions, with 0.5 g with six duplications of each period analysed for iNDF. Furthermore, 15g of each sample was bulked according to measurement period with this analysed for chemical composition using wet chemistry by Hill Laboratories (Hamilton, New Zealand).

In addition to the indigestible marker comparison, methane emissions were measured from all cows using GreenFeed systems (C-Lock Inc., Rapid City, SD), located within the Calan Gate facility (methane emission measurements were a part of another experiment and therefore not included in the scope of this thesis). All cattle had access to the GreenFeed units (n = 10 cows per GreenFeed unit) whilst housed in the Calan gate facility. Country Harvest alpaca pellets (Dunstan Nutrition, Cambridge, Waikato) were used as bait to attract visitations to the GreenFeed units, with a limit per cow of 6 visits per day. At each visit cows were offered 8 drops of pellets at 25 g pellets per drop, with 25 seconds between each drop. Cows were able to consume a maximum of 1.2 kg fresh weight per day of Country Harvest alpaca pellets (Dunstan Nutrition, Cambridge, Waikato), with pellet intake measured by the GreenFeed units (C-Lock Inc., Rapid City, SD). During measurement periods 1 and 2 a 200 g sample was taken from each bag of alpaca pellet used, frozen at $-20^{o}C$ then freeze-dried and ground to pass through 1mm sieve, a 50 g bulk was then produced for each measurement period with 4 g from each sample to form a bulk for period 1 and 6.25 g from each sample to form period two's bulk. From the bulks of period 1 and 2, 5 g of each was subsampled for n-alkane analysis and 10 g for titanium dioxide analysis, with two repetitions, 0.5 g with six duplications of each period was analysed for iNDF. Furthermore, 15 g of each sample was bulked according to measurement period with this analysed for chemical composition using wet chemistry by Hill Laboratories (Hamilton, New Zealand).

Prior to measurement periods 1 and 2, when the cows were housed in the Calan gate facility (14^{th} October to 24^{th} October), a handful of pasture was taken from each individual cow pasture allocation when feeding in the A.M. and P.M. to produce a bulk sample. From this bulk sample, three tins of 150 ± 5 g were oven dried at $95^{o}C$ for 72 hours and weighed again to determine the DM percentage of that cut of pasture.

Prior to feeding, a quick dry matter analyses was performed using a microwave (Panasonic NNS782WF), this produced an estimate of the DM percentage of the mown grass which was then used to adjust daily pasture allocation provided to each cow. Two 50 g pasture samples were placed in glass bowels and microwaved for 8 minutes at 100% and 4 minutes at 40%,

then weighed, followed by 1 minute at 40% and weighed, with this being repeated until weight becomes stable. For calculations of individual intakes, the adjusted DM percentage (determined through oven drying) was used. During measurement periods 1 and 2, a handful of pasture was taken from each individual cow pasture allocation when feeding in the A.M. and P.M. to produce a bulk sample (pasture offered). A 50 g sample was also taken to determine botanical composition, with the A.M. and P.M. samples bulked together (100 g) with 40 g subsampled to dissect. In measurement periods 1and 2 an additional 200 g sample was taken from the bulk pasture sample, which was frozen at $-20^{o}C$, freeze dried and then ground to pass through a 1mm sieve with 5 g of each sample bulked according to measurement period. 5 g of each bulk was further subsampled for n-alkane analysis, 10 g for titanium dioxide analysis and 0.5 g with 4 duplications for iNDF analysis, with two repetitions for each period. Furthermore, 15 g of each sample was bulked according to measurement period with this analysed for chemical composition using wet chemistry by Hill Laboratories (Hamilton, New Zealand).

Prior to measurement periods 1 and 2 (11th October to 25th October) pasture refusals from each cow were weighed and recorded in the A.M. with a handful sample taken from each refusal and put into the corresponding bulk depending on weight 0-5 kg, 5-10 kg or 10+ kg. A 500 g sample was taken from each bulk with 3 tins of 150 $\pm$ 5 g oven dried at $95^{o}C$ for 72 hours, then weighed to determine the DM percentage. During measurement periods 1 and 2 (26th October to 4th November) each individual cow refusal was weighed and a 600 g sample was taken. Three tins of 150 $\pm$ 5g was oven dried at $95^{o}C$ for 72 hours, then weighed to determine the DM percentage of the refusal.

From the 26th October to 3rd November, pasture samples were collected pre-mowing A.M. and P.M. using snip cuts from the area to be mowed, 30 snips were taken in a W fashion (pasture SNIP). From the snip cut a 50 g sample was taken with the A.M. and P.M. sample bulked together, and a 40 g subsample taken from the bulk to dissect to determine botanical composition. A 200 g sample was also taken, frozen at $-20^{o}C$, freeze dried and then ground to pass through a 1mm sieve before 5 g of each sample was bulked according to measurement period. 5 g of each bulk was further subsampled for n-alkane analysis,10 g for titanium dioxide analysis, 0.5 g with 4 duplications for iNDF analysis, with two repetitions for each period.

For three weeks, (one week prior and during measurement periods 1 and 2) all cows were orally dosed twice a day before milking, in the herringbone vet race, with a paper bullet (Carl Roth GmBH, Karlsruhe, Germany) containing 377.6 mg of dotriacontane (C32) and gelatine capsule

(Torpac, Fairfield, NJ) containing 5 g of titanium dioxide, using a bolus gun. N-alkane paper bullets were prepared following methodology by Teagasc (2022). Three paper bullets containing 377.6 mg of C32 from each batch made and dosed were retained. These bullets were weighed prior and following freeze drying to determine DM. Following this they were ground to pass through a 1mm sieve with 0.60 g subsampled from each with three duplicates, which was analysed for C32 content. Three samples of 10 g of titanium dioxide dosed was analysed for purity.

Table 3.1: Nutritional composition of feed components; pasture, supplementary feed, and GreenFeed pellet offered during periods 1 and 2 (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.).

	Measurement Period	Crude protein (DM %)	Starch (DM %)	Ash (DM %)	ME (MJ/ kg DM)
Pasture offered	1	13.7	<0.5	9.3	12.1
Pasture offered	2	15.9	0.9	8.8	12.0
Pasture SNIP[1]	1	14.5	1.3	8.5	12.6
Pasture SNIP[1]	2	16.8	1.6	9.0	12.5
Supplementary feed	1	11.3	27.8	12.2	10.1
Supplementary feed	2	11.5	27.0	11.4	10.1
Greenfeed pellet	1	14.8	9.7	6.9	11.0
Greenfeed pellet	2	14.9	9.5	6.9	11.0

[1]Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion.

3.2.4 Faecal sampling

In measurement periods 1 and 2 (25th October to 4th November) faecal samples were collected from all cows in the experiment at 6:00 and 13:30 prior to the A.M. and P.M. milking. The collection started with a group of people quietly entering the right side of the barn (cohorts 1 and 2) and collecting faeces into buckets from any cows voluntary defecating. The cows were then brought into a herringbone vet race where any animals that had not defecated naturally were rectally palpated to produce a faecal sample. If no faeces were produced after two minutes of rectal palpation the cow would be left for several minutes before trying again, if defecation did not occur after this no faecal sample would be collected from that cow. This would then be

repeated on the left side of the barn with cohorts 3 and 4. From each bucket one 750 ml pottle was ¾ filled and frozen at −20°C. After the end of the trial the 750 ml pottles of faeces were thawed at room temperature overnight. Faeces were bulked on a fresh weight basis with 35 g of each sample collected from that cow in that measurement period added to the bulk to form a total weight of 350 g. If a sample was missed the weight per sample collected was adjusted so that the total weight of 350 g was still reached. The faeces were then oven dried at 60°C for 72 hours, ground to pass through a 1 mm screen, then subsampled with 10 g analysed for titanium dioxide, 2 g analysed for n-alkanes, with two repetitions and 0.5 g with two duplications analysed for iNDF. One aluminium pie tin was filled with faeces collected from individual cattle. This was then weighed, oven dried at 95°C for 48 hours and then cracked and flipped before being returned to the oven for a further 48 hours and weighed again, to determine the DM percentage.

3.2.5 Analysis of indigestible marker samples

The analysis of n-alkanes C27 through to C35 in faeces, pasture (offered - post mown and SNIP - pre mown), supplementary feed, GreenFeed pellets and paper bullets were analysed by AgResearch, Palmerston North, New Zealand, through methodology by Mayes et al. (1986) and Liu et al. (2022). The analysis of titanium dioxide in faeces, pasture (offered - post-mown and SNIP - pre mown), supplementary feed, GreenFeed pellets and form used in dosing was analysed by Massey University, Palmerston North, New Zealand through methodology by Jagger et al. (1992) and Short et al. (1996). The analysis of iNDF in faeces, pasture (offered – post mown and SNIP - pre mown), supplementary feed, GreenFeed pellets were incubated *in situ*, in the rumen of two cannulated cows grazing ryegrass based pasture using F57 filter bags (Ankom Technology) for 12 days by AgResearch, Palmerston North New Zealand. Following removal, the samples were analysed by Massey University, Palmerston North, New Zealand, methodology outlined in Della Rosa et al. (2022) was followed.

3.2.6 Calculations and statistical analysis

3.2.6.1 Calculations

Herbage intake estimated from n-alkane (C29, C31, C33 or C35) present naturally (i) and C32 dosed (j) was calculated as

$$\text{Herbage intake (kg DM/day)} = F_i/F_j \times \frac{D_j}{\left(H_i - \frac{F_i}{F_j} \times H_j\right)}$$

(Dove and Mayes 1991)

F_i and H_i, are the concentrations (mg/kg of DM) of the natural odd-chain n-alkanes in faeces and herbage, respectively. F_j, and H_j are the concentrations (mg/kg of DM) of the even-chain n-alkane in faeces, and herbage, respectively, D_j is the dose rate of alkane C32 dosed (mg/day).

Total intake from n-alkanes was calculated as

Total intake (kg DM/day) = Herbage intake (kg DM/day) + known pelleted supplement intake (kg DM/day) + known GreenFeed pellet intake (kg/DM/day)

Faecal output (FO) from titanium dioxide was calculated as

$$\text{FO (g of DM/d)} = \frac{\text{Ti dosed } \left(\frac{\text{g}}{\text{d}}\right)}{\text{Ti in faeces } \left(\frac{\text{g}}{\text{g of DM}}\right)}$$

(Velásquez et al. 2018)

iNDF content was calculated as

$$\text{iNDF, \% DM} = \frac{W3 - - (W1 \times C2)}{W2} \times 100$$

(Adams et al. 2020)

Where W1 is the initial bag weight (g), W2 is the initial sample weight (g), W3 is the weight of the dried bag and sample residue following ND wash (g) and C2 is the blank correction factor following ND wash being the average dry bag following ND wash weight divided by initial bag weight (g).

Total intake using titanium dioxide and iNDF was calculated from the following equations adapted from Kartchner (1980);

$$\text{Faecal output g/day} = \frac{\text{Titanium dioxide dosed g/day}}{\text{Titanium dioxide in faeces g/g}}$$

Faecal output from supplement g/day = supplement fed g/day × (1.0 – supplement digestibility)

Supplement digestibility has assumed to be 80% (NRC 2001)

Faecal output from forage g/day = faecal output g/day – faecal output from supplement g/day

iNDF in faeces g/day = faecal output g/day × iNDF in faeces %

iNDF in supplement g/day = supplement fed g/day × iNDF in supplement %

Faecal iNDF from forage g/day = iNDF in faeces g/day – iNDF in supplement g/day

$$\text{Faecal iNDF from forage \%} = \frac{\text{faecal iNDF from forage g/day}}{\text{faecal output from forage g/day}} \times 100$$

$$\text{Forage digestibility} = 1 - \left(\frac{\text{iNDF in forage \%}}{\text{faecal iNDF from forage \%}}\right)$$

$$\text{Forage intake g/day} = \frac{\text{faecal output from forage g/day}}{1 - \text{forage digestability}}$$

Total intake g/day = supplement fed g/day + forage intake g/day

3.2.6.2 Statistical analysis

Analyses were performed using R v4.2.1; R Core Team 2022 (R Foundation for Statistical Computing, Vienna, Austria). Four n-alkane pairings were used to predict pasture DMI being C32:C29, C32:C31, C32:C33 and C32:C35. A t-test was preformed to determine if there was a significant difference between n-alkane content within each measurement period from the two different pasture samples. One collected post-mown, from pasture allocated to the cow in the Calan Gate System (offered), and the other using SNIP cuts of the area in the paddock to be mown (SNIP). As there was no significant difference in the n-alkane content between the pasture analysed from the two sampling methods within the same measurement period the n-alkane content from the sample taken post-mown (offered) was used within the equations to predict intake.

Pasture and total intakes were predicted using the indigestible marker pair titanium dioxide and iNDF. As the two different pasture sampling methodologies (post-mown offered pasture, and SNIP cuts from the area in the paddock to be mown) yielded different iNDF concentrations, they were both used within the intake calculations.

To analyse the relationship between actual total and pasture DMI measured through the Calan Gate system and DMI predicted through the use of indigestible markers Pearson's correlation coefficient was used. Within the n-alkane correlation analysis, Pearson's correlation coefficient was performed to analyse the relationship between the pasture and total DMI predictions of each n-alkane pairing and actual pasture and total DMI, overall, between treatment groups and between measurement periods. For titanium dioxide and iNDF Pearson's correlation coefficient was performed to analyse the relationship between total and pasture DMI predictions using pasture offered and SNIP cut and actual total and pasture DMI, overall, between treatment groups and measurement periods. An ANOVA test was used to determine if there was a significant difference between means of each treatment group and measurement period for each n-alkane pairing and predictions using titanium dioxide and iNDF, along with actual and total DMI. An ANOVA test was also used to determine that there was no interaction between treatment group (CTRL and SUPP) and measurement period (Period 1 and 2) for the n-alkanes and titanium dioxide/iNDF. A linear graph of actual and predicted pasture intake for each treatment group (CTRL and SUPP) was also produced for n-alkane pairing C32:C33 and titanium dioxide and iNDF, a trendline with a fixed intercept of 0 was used to evaluate the mean underestimation of intake predicted by each indigestible marker within treatment group.

3.3 Results

3.3.1 N-alkane

The mean feed and faecal n-alkane content is detailed in Table 3.2A and 3.2B. Pasture in Period 2 had a greater n-alkane content than Period 1 and cattle in the CTRL treatment had greater faecal n-alkane content than the SUPP treatment.

Table 3.2A: Mean n-alkane content (mg/kg DM) of diet components and different pasture sampling methodologies for measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

	Measurement period	C27	C28	C29	C30	C31	C32	C33	C35
Pasture offered	1	24.28	6.84	109.69	11.73	183.62	9.62	114.92	11.68
Pasture offered	2	27.58	8.67	152.27	14.11	244.47	10.69	117.93	10.44
Pasture SNIP[1]	1	25.34	7.45	117.96	12.84	190.68	10.22	120.55	12.60
Pasture SNIP[1]	2	30.38	10.61	167.96	14.74	245.15	10.44	113.39	9.95
Pelleted supplement	1	2.24	0.00	4.08	0.00	5.48	3.36	0.00	0.00
Pelleted supplement	2	2.22	0.00	4.00	0.44	5.10	3.70	0.00	0.00
Greenfeed pellet	1	8.56	0.00	20.48	0.00	34.16	0.00	0.00	0.00
Greenfeed pellet	2	8.14	0.00	20.31	0.00	33.13	0.00	0.00	0.00

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion.

Table 3.2B: Mean n-alkane content (mg/kg DM) of cow faeces for treatment groups (CTRL; pasture-only diet and SUPP; pasture plus supplementary feed diet) and measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

Measurement period	Treatment group	C27	C28	C29	C30	C31	C32	C33	C35
1	CTRL	63.98	19.41	343.67	34.58	578.42	219.84	354.88	37.69
1	SUPP	47.31	13.91	259.86	25.55	432.95	188.18	267.33	28.59
2	CTRL	65.60	20.46	374.07	33.89	581.85	191.89	301.97	31.07
2	SUPP	50.61	15.49	290.35	26.18	456.65	175.13	236.91	23.75

Overall, the predicted pasture DMI for individual cows using the n-alkane technique was positively correlated with both actual pasture and total intake (R^2 = 0.73 to 0.81; Table 3.3, R^2 = 0.77 to 0.84; Table 3.4, respectively). Of the n-alkane pairings that were analysed, C32:C33, and C32:C35 had the greatest correlation with actual intakes (R^2 = 0.81 with pasture, 0.84 with total and 0.80 with pasture, and 0.79 with total, respectively), while the other two n-alkane pairings (C32:C29 and C32:C31) had a numerically lower R^2 value of 0.73 (pasture intake) and 0.77 and 0.78 respectively (total intake). When analysed at the treatment and measurement period level, the correlation between actual pasture DMI and pasture DMI predicted by the n-alkane technique ranged from R^2 0.60 to 0.89 and 0.60 to 0.88 for total DMI. Correlations were numerically greater in the SUPP treatment compared with the CTRL treatment, with C32:C33 producing a strong correlation in both treatments. Correlations between predicted and actual intakes were numerically greater in Period 2 compared with Period 1 for each n-alkane pairing, with C32:C33 producing the strongest correlation.

Table 3.3: Pearson's correlation (R^2) DMI (kg DM/d) and pasture DMI (kg DM/d) predicted by n-alkane pairings overall, between treatment groups (CTRL; pasture-only diet and SUPP; pasture plus supplementary feed diet) and measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

		Treatment group		Measurement period	
N-alkane pairings	**Overall correlation**	**CTRL**	**SUPP**	**1**	**2**
C32:C29	0.73	0.66	0.74	0.72	0.84
C32:C31	0.73	0.64	0.77	0.77	0.87
C32:C33	0.81	0.73	0.84	0.78	0.89
C32:C35	0.80	0.60	0.85	0.76	0.82

Table 3.4: (R^2) Pearson's correlation (between individual cow actual total DMI (kg DM/d) and DMI (kg DM/d) predicted by n-alkane pairings overall, between treatment groups (CTRL; pasture-only diet and SUPP; pasture plus supplementary feed diet) and measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

		Treatment group		Measurement period	
N-alkane pairings	**Overall correlation**	**CTRL**	**SUPP**	**1**	**2**
C32:C29	0.77	0.67	0.73	0.74	0.83
C32:C31	0.78	0.67	0.77	0.78	0.85
C32:C33	0.84	0.74	0.85	0.79	0.88
C32:C35	0.79	0.60	0.86	0.78	0.80

There were significant differences between the average pasture intake from cows in the SUPP compared with CTRL treatment, with cows in the SUPP treatment eating ~15% less pasture DMI (Table 3.5). There was also a significant difference between average total intake, with cows in the SUPP treatment eating ~ 7% more total DMI (Table 3.6). This difference also existed between the predicted pasture and total DMI using the n-alkane pairings, although the absolute numbers were numerically less. There was also a significant difference between pasture and total DMI from the measurement periods, with individual cows consuming less pasture and total feed (~5%, ~3%, respectively) in Period 2 (Table 3.7, Table 3.8). The DMI differences between Periods 1 and 2 were also reflected with the predicted DMI from all n-alkane pairings but C32:C35; however, the absolute values were less in the predicted DMI for both Periods, and the difference between Period 1 and 2 was greater with the predicted DMI.

Table 3.5: Pasture DMI prediction from n-alkane pairings and actual pasture DMI (kg DM/d) by treatment group (CTRL; pasture-only diet and SUPP; pasture and supplement diet)

Measurement variable	CTRL DMI (kg DM/d)	SUPP DMI (kg DM/d)	Standard error
Actual pasture intake	17.0[a]	14.4[b]	0.20
C32:C29	10.4[a]	8.83[b]	0.22
C32:C31	10.3[a]	8.75[b]	0.22
C32:C33	10.5[a]	9.03[b]	0.19
C32:C35	11.8[a]	10.0[b]	0.20

[a,b] Means with different subscripts are significantly different across rows at 5% confidence level.

Table 3.6: Total DMI prediction from n-alkane pairings and actual total DMI (kg DM/d) by treatment group (CTRL; pasture-only diet and SUPP; pasture and supplement diet)

Measurement variable	CTRL DMI (kg DM/d)	SUPP DMI (kg DM/d)	Standard error
Actual total intake	18.0[a]	19.3[b]	0.15
C32:C29	11.3[a]	13.5[b]	0.23
C32:C31	11.2[a]	13.5[b]	0.24
C32:C33	11.5[a]	13.7[b]	0.21
C32:C35	12.7[a]	14.7[b]	0.20

[a,b] Means with different subscripts are significantly different across rows at 5% confidence level.

Table 3.7: Pasture DMI prediction from n-alkane pairings and actual pasture DMI (kg DM/d) by measurement period (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

Measurement variable	Period 1 DMI (kg DM/d)	Period 2 DMI (kg DM/d)	Standard error
Actual pasture intake	16.1[a]	15.4[b]	0.20
C32:C29	10.8[a]	8.55[b]	0.22
C32:C31	10.8[a]	8.30[b]	0.22
C32:C33	10.6[a]	9.03[b]	0.19
C32:C35	11.2[a]	10.6[a]	0.20

[a,b] Means with different subscripts are significantly different at 5% confidence level across rows.

Table 3.8: Total DMI prediction from n-alkane pairings and actual total DMI (kg DM/d) by measurement period (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

Measurement variable	Period 1 DMI (kg DM/d)	Period 2 DMI (kg DM/d)	Standard error
Actual total intake	18.9[a]	18.3[b]	0.15
C32:C29	13.5[a]	11.4[b]	0.23
C32:C31	13.5[a]	11.2[b]	0.24
C32:C33	13.3[a]	11.9[b]	0.21
C32:C35	13.9[a]	13.5[a]	0.20

[a,b] Means with different subscripts are significantly different at 5% confidence level across rows.

Although there was a strong correlation between actual pasture DMI and predicted pasture DMI, absolute values for predicted pasture DMI were less than actual values (Figure 3.1 and 3.2). For every 1 kg DM/d of actual pasture DMI predicted pasture DMI from n-alkanes C32:C33, was 0.62 kg DM/d for the CTRL treatment and 0.64 kg DM/d for SUPP treatment. For example: if actual pasture intake measured 15 kg DM/d, the n-alkane predicted pasture intake was 9.3 and 9.6 kg DM/d for the CTRL and SUPP treatments, respectively (Figure 3.2 and 3.1). Subsequently, the average error (underestimation) in pasture DMI predicted by the n-alkane technique for the CTRL and SUPP treatments was 38% and 37% respectively.

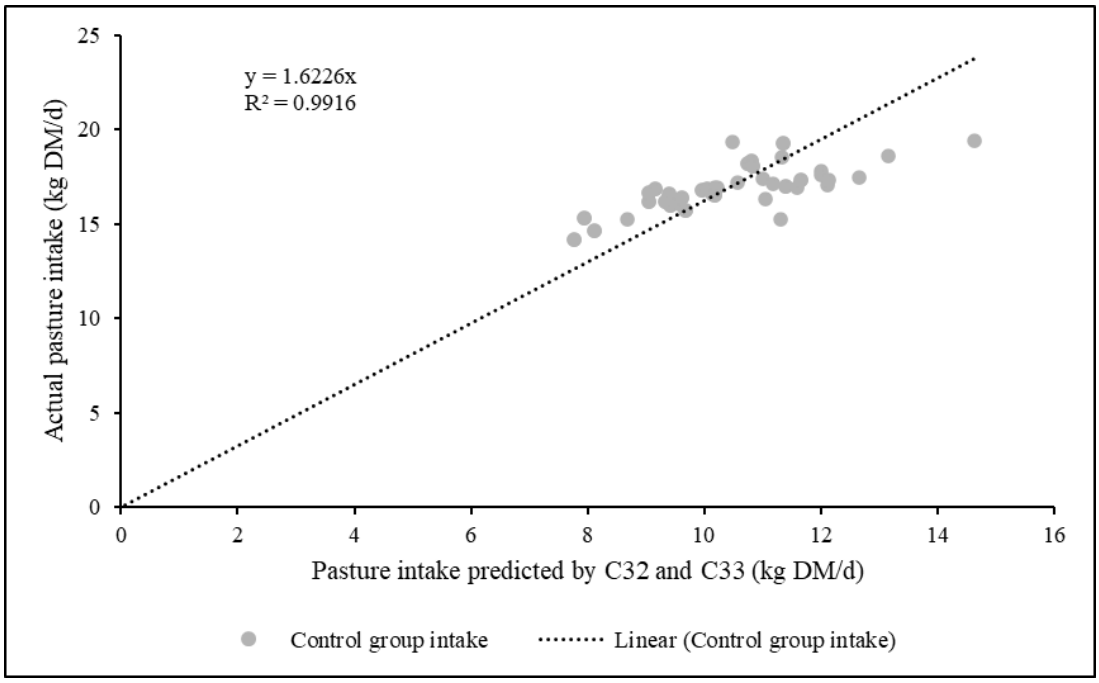

Figure 3.2: Linear model of actual pasture intake and pasture intake predicted by n-alkanes C32:C33 (kg DM/d) with a fixed intercept of 0 for the CTRL treatment group (CTRL; pasture-only diet).

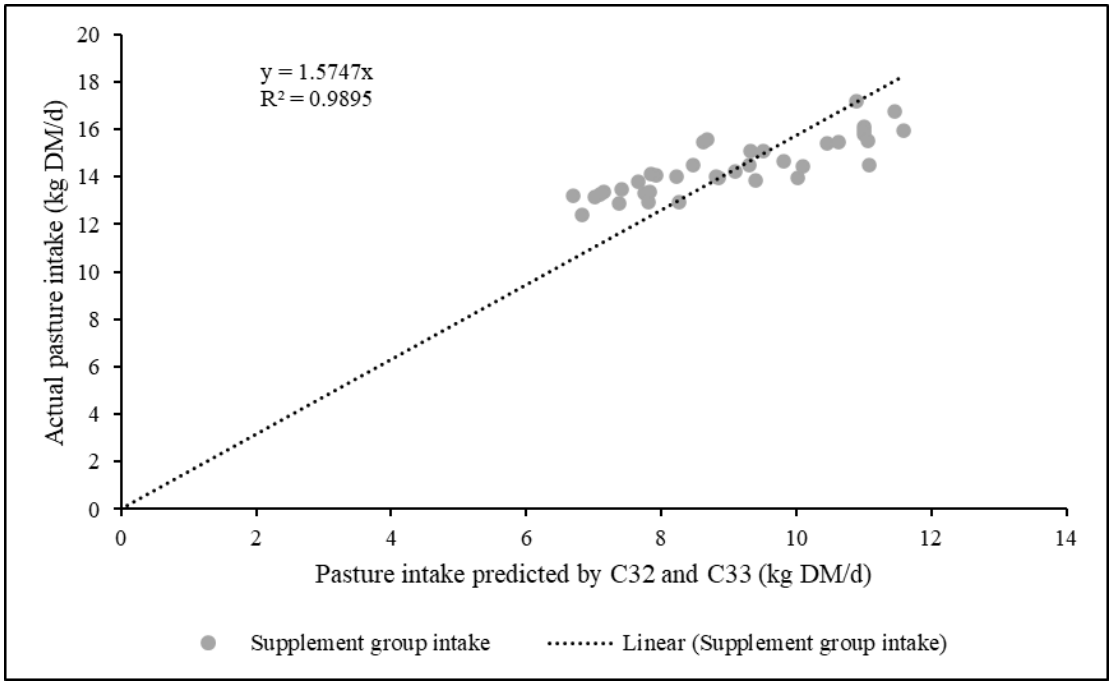

Figure 3.3: Linear model of actual pasture intake and predicted pasture intake by n-alkanes C32:C33 (kg DM/d) with a fixed intercept of 0 for the SUPP treatment group (pasture plus supplementary feed diet).

3.3.2 Titanium dioxide and iNDF

The mean feed and faecal titanium dioxide and iNDF content is detailed in Table 3.9A and 3.9B. Pasture iNDF content varied depending on pasture sampling method, whilst iNDF content in faeces was greater in Period 2 than Period 1 irrespective of treatment group.

Table 3.9A: Mean titanium and iNDF concentration of feed for each measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

	Measurement period	Titanium dioxide (%)	iNDF (%)
Pasture offered	1	< 0.1	5.92
Pasture offered	2	< 0.1	6.17
Pasture SNIP[1]	1	< 0.1	5.21
Pasture SNIP[1]	2	< 0.1	4.83
Pelleted supplement	1	< 0.1	12.17
Pelleted supplement	2	< 0.1	9.64
Greenfeed pellet	1	0.1	7.07
Greenfeed pellet	2	0.1	6.80

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion

Table 3.9B: Mean titanium and iNDF concentration of cow faeces for each treatment group (CTRL; pasture-only diet and SUPP; pasture and supplement diet) and measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

Measurement period	Treatment group	Titanium dioxide (%)	iNDF (%)
1	CTRL	0.34	28.71
1	SUPP	0.33	28.67
2	CTRL	0.33	32.79
2	SUPP	0.31	34.68

Overall, there was a moderate correlation between actual pasture DMI and predicted DMI using titanium dioxide and iNDF, whilst a weak correlation for actual total DMI and predicted DMI (Table 3.10; Table 3.11). There were differences in the iNDF content of pasture dependent on the sampling methodology, with the sample collected from the pasture offered to the cows in the Calan Gate System (post-mowing) having a greater iNDF content compared with the sample collected using SNIP cuts from the area to be mown (Table 3.9A). Using the sample from the offered pasture R^2 = 0.52 (pasture intake), 0.16 (total intake), compared with R^2 = 0.47 (pasture intake), 0.09 (total intake) when the SNIP cut samples were used (Table 3.10; Table 3.11). When analysed for treatment and measurement period, the correlation between actual total DMI and predicted DMI ranged from R^2 = 0.09 to 0.42 (Table 3.10) and ranged from 0.11 to 0.71 for actual pasture DMI and predicted (Table 3.11). Correlations between actual and predicted total and pasture DMI were numerically greater for the CTRL treatments compared with SUPP treatments. Correlations between pasture actual and predicted DMI were less in Period 2 compared with Period 1 for both pasture sampling methods (Table 3.10), whereas or total DMI Period 2 was numerically greater than period 1 (Table 3.11).

Table 3.10: Pearson's correlation (R^2) between individual cow actual pasture intake and pasture DMI predicted by titanium dioxide and iNDF (kg DM/d) through differing pasture sampling method, overall, between treatment groups CTRL; pasture-only diet and SUPP; pasture and supplement diet) and measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.

		Treatment group		**Measurement period**	
Pasture sampling method	**Overall correlation**	**CTRL**	**SUPP**	**1**	**2**
Pasture offered	0.52	0.42	0.14	0.71	0.57
Pasture SNIP[1]	0.47	0.34	0.11	0.71	0.57

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion

Table 3.11: Pearson's correlation (R^2) between individual cow actual total intake and total DMI predicted by titanium dioxide and iNDF (kg DM/d) through differing pasture sampling method, overall, between treatment groups CTRL; pasture-only diet and SUPP; pasture and supplement diet) and measurement periods (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.

		Treatment group		Measurement period	
Pasture sampling method	**Overall correlation**	**CTRL**	**SUPP**	**1**	**2**
Pasture offered	0.16	0.42	0.16	0.12	0.41
Pasture SNIP[1]	0.09	0.32	0.13	0.09	0.37

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion

Cows in the SUPP treatment had a lower pasture intake but a greater total intake (pasture plus GreenFeed Pellet plus supplementary feed) compared with cows in the CTRL treatment (Pasture plus GreenFeed Pellets; Table 3.12; Table 3.13, - 15% and + 7%, respectively). When titanium dioxide/iNDF methodology was used to predict DMIs, predicted pasture intake reflected the relationship between intakes for SUPP and CTRL cows (e.g., predicted pasture intake was less in SUPP cows); however, the magnitude of difference was greater (-35%) such that when total intake was predicted, (predicted pasture intake + measured supplement intake) the relationship between SUPP and CTRL cows was reversed (e.g., predicted total intake was less in SUPP cows). These relationships were independent of pasture sampling methodology.

Table 3.12: Mean of titanium dioxide and iNDF pair pasture DMI prediction (kg DM/d) and actual pasture DMI (kg DM/d) by treatment group (CTRL; pasture-only diet and SUPP; pasture and supplement diet)

Pasture sampling method used for prediction	CTRL predicted and actual DMI	SUPP predicted and actual DMI	Standard error
Actual pasture intake	17.0[a]	14.4[b]	0.20
Pasture offered	15.8[a]	10.4[a]	0.61
Pasture SNIP[1]	19.2[a]	12.8[b]	0.79

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion.
[a,b] Means with different subscripts between treatment group are significantly different at 5% confidence level across rows.

Table 3.13: Mean of titanium dioxide and iNDF pair total DMI prediction (kg DM/d) and actual total DMI (kg DM/d) by treatment group (CTRL; pasture-only diet and SUPP; pasture and supplement diet)

Pasture sampling method used for prediction	CTRL predicted and actual DMI	SUPP predicted and actual DMI	Standard error
Actual total intake	18.0[a]	19.3[b]	0.15
Pasture offered	16.9[a]	15.3[a]	0.40
Pasture SNIP[1]	20.3[a]	17.6[b]	0.53

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion.
[a,b] Means with different subscripts between treatment group are significantly different at 5% confidence level across rows.

Actual total and pasture intake were greater in Period 1 than in Period 2 (~ 3% and ~ 5% respectively) (Table 3.14; Table 3.15). However, the opposite relationship was predicted for both total and pasture intake with both pasture sampling methods.

Table 3.14: Mean of titanium dioxide and iNDF pair pasture DMI prediction (kg DM/d) and actual pasture DMI (kg DM/d) measurement period (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

Pasture sampling method used for prediction	Period 1 predicted and actual total DMI	Period 2 predicted and actual total DMI	Standard error
Actual pasture intake	16.1[a]	15.4[b]	0.20
Pasture offered	11.3[a]	15.0[b]	0.61
Pasture SNIP[1]	12.8[a]	19.2[b]	0.79

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion.
[a,b] Means with different subscripts between period are significantly different at 5% confidence level across rows.

Table 3.15: Mean of titanium dioxide and iNDF pair total DMI prediction (kg DM/d) and actual total DMI (kg DM/d) measurement period (Period 1; 25/10 A.M. – 30/10 P.M. and Period 2; 30/10 P.M. – 4/11 A.M.)

Pasture sampling method used for prediction	Period 1 predicted and actual total DMI	Period 2 predicted and actual total DMI	Standard error
Actual total intake	18.9[a]	18.3[b]	0.15
Pasture offered	14.2[a]	18.0[b]	0.40
Pasture SNIP[1]	15.7[a]	22.2[b]	0.53

[1] Pasture sample taken of the area to be mowed prior to mowing beginning, 30 snips were taken in a W fashion.
[a,b] Means with different subscripts between period are significantly different at 5% confidence level across rows.

Pasture DMI predicted by titanium dioxide and iNDF did not have a linear relationship with actual pasture DMI for both CTRL and SUPP treatments (Figure 3.4 and 3.5). Although for every 1kg DM/d of actual pasture DMI, predicted pasture DMI from titanium dioxide and iNDF, was 0.99 kg DM/d for the CTRL treatment and 0.89 kg DM/d for SUPP treatment. For example: if actual pasture intake measured 15 kg DM/d, the titanium dioxide/iNDF predicted pasture intake was 14.85 and 13.35 kg DM/d for the CTRL and SUPP treatments,

respectively (Figure 3.4 and 3.5). Subsequently, the average error (underestimation) in pasture DMI predicted by the titanium dioxide and iNDF technique for the CTRL and SUPP treatments was 1% and 11% respectively. But evidently both Figure 3.4 and Figure 3.5 show that the distribution range of actual pasture intake is smaller than the predicted pasture intake range than the predicted pasture intake range, which has greater variability.

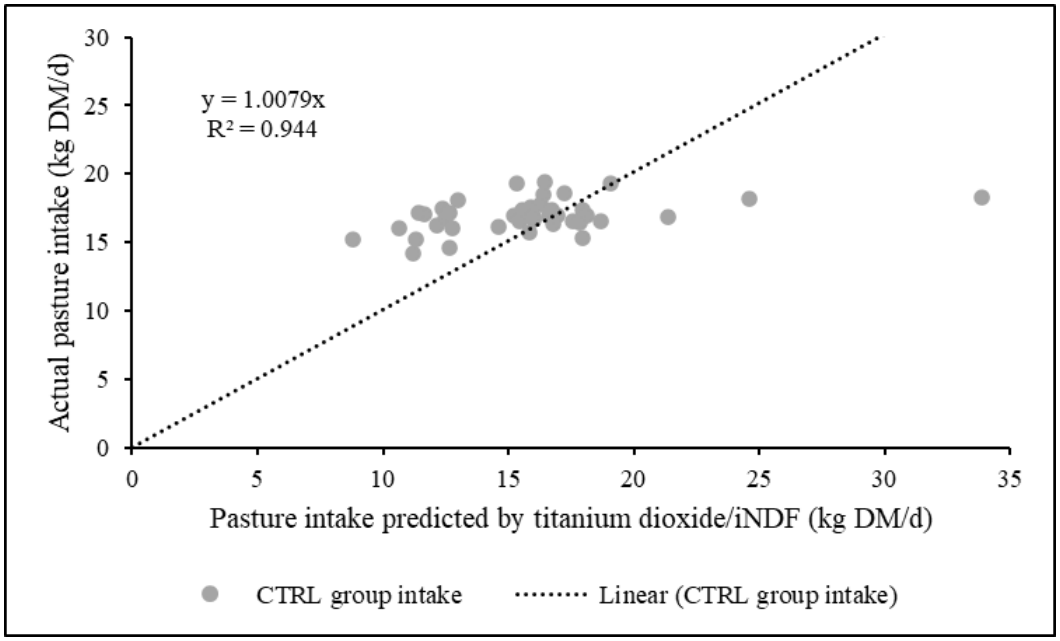

Figure 3.4: Linear model of actual pasture intake and pasture intake predicted by titanium dioxide and iNDF (kg DM/d) with a fixed intercept of 0 for the CTRL treatment group (CTRL; pasture-only diet).

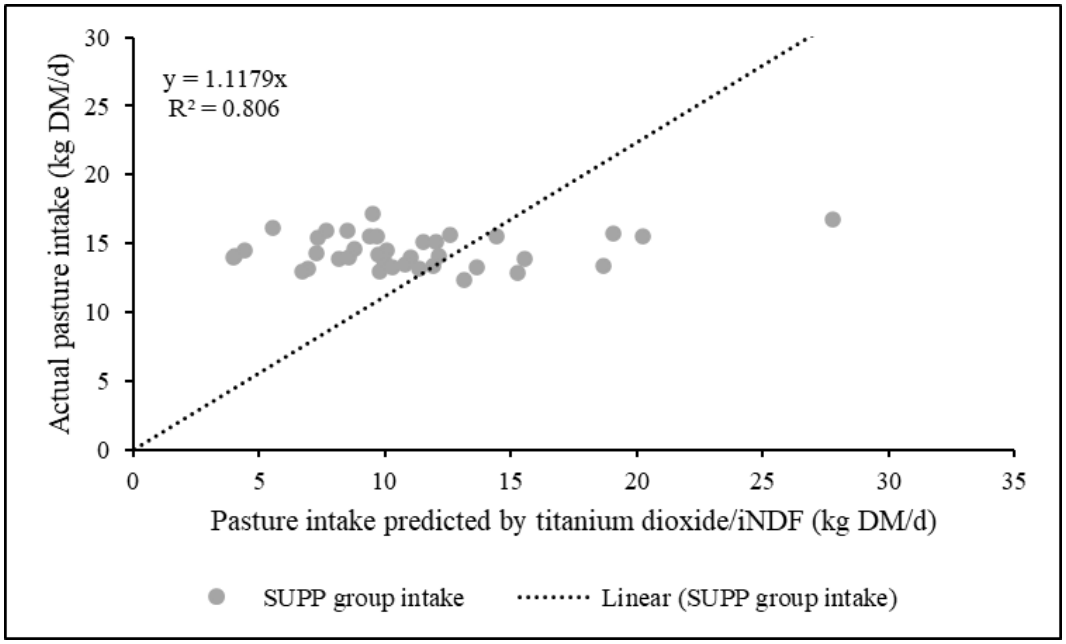

Figure 3.5: Linear model of actual pasture intake and predicted pasture intake by titanium dioxide and iNDF (kg DM/d) with a fixed intercept of 0 for the SUPP treatment group (pasture plus supplementary feed diet

3.4 Discussion

3.4.1 N-alkane

The following section focuses on the indigestible marker method of n-alkanes, discussing the predictions provided by the n-alkane pairings analysed, the variation between actual and predicted DMI, along with potential causes of this variation. The effect of treatment group and measurement period is discussed, in addition to possible sources of this variation between cows and trial period. Finally potential sources of error for the use of the n-alkane method in this thesis are highlighted.

The pairing of n-alkanes C33 and C35 to C32 predicted DMI with greater accuracy than when C31 or C29 was paired with C32, in terms of having a greater numerical correlation and less of a discrepancy between predicted and actual DMI. This is in agreement with literature that have reported more accurate intake predictions with increased carbon chain length, as faecal recovery of odd chain herbage n-alkanes increases with carbon chain length (Mayes et al. 1984; Mayes et al. 1986; Stakelum and Dillon 1990; Dove and Mayes 1991). In addition, when consecutive n-alkanes with a longer carbon chain length are used there is less discrepancy between recovery rates of the n-alkane pair, and thus less under/over prediction (Dove and Mayes 1991). Of the n-alkane pairs analysed in the current experiment, C32:C33 provided the most accurate pasture and total intake predictions. C32:C33 are the n-alkane pair most frequently used to provide accurate DMI predictions in cattle, with discrepancies between actual DMI and predicted ranging from 0.07 kg/d to 0.31 kg/d (Dove and Mayes 1996; Andriarimalala et al. 2020). In the current experiment discrepancies between actual and predicted pasture DMI were greater, ranging from 3.42 kg DM/d to 8.87 kg DM/d, with a consistent underestimation of pasture intake for each individual cow. Discrepancies between actual and predicted total DMI range from 3.61 kg DM/d to 9.05 kg DM/d.

The n-alkane content of pasture from this experiment is within the range stated by Dove and Mayes (1991) for perennial ryegrass (*Lolium perenne*), therefore it is unlikely that the discrepancy in accuracy of intake prediction is a result of the n-alkane content measured in the pasture. The underestimation of pasture and total intake is more likely a result of discrepancy between the faecal recovery of the natural and dosed n-alkane. Olivan et al. (2007) reported intake of beef cattle was underestimated by C31 and C33 paired with C32 when the faecal recovery of C31 and C33 were lower than that of C32.

Surprisingly the overall accuracy of pasture DMI prediction using the n-alkane pairing C32:C35 was similar to that of C33 and C31 paired with C32. As n-alkane pairs consisting of an odd and even n-alkane have greater DMI prediction accuracy when differing by one carbon atom as faecal recovery rates are similar (Wright et al. 2019). C32 and C35 differ by two carbon atoms, so it would be assumed that the intake predictions would have a lower level of accuracy then C33:C32 and C31:C32, which both differ by one (Andriarimalala et al. 2020). Along with this most pasture species do not have a high enough concentration of C35 to allow for use in predicting intake and therefore the accuracy of DMI predictions by C35:C32 found are unexpected (Dove and Mayes 1991).

3.4.1.1 Treatment group

There was lower pasture intake but greater total intake from cows in the SUPP compared with CTRL treatment. This is consistent with the literature and is due to pasture substitution, where DMI of pasture is typically reduced when supplementary feed is consumed by grazing dairy cows (Holmes and Roche 2007; Baudracco et al. 2010). Cows typically graze for 12 minutes less for every 1 kg DM of supplementary feed consumed (Bargo et al. 2003). In the present experiment, substitution rates were approximately 66%, such that for every 1 kg DM supplementary feed offered, cows substituted 0.66 kg DM pasture.

Correlations between predicted and actual intakes were numerically greater for the SUPP treatment compared with the CTRL treatment; however, the difference between predicted (using C32:C33 alkane pairs) and actual intakes were similar for the SUPP and CTRL treatments (37% and 30% vs 38% and 36% difference for pasture and total intakes, respectively). Oliván et al. (2007) conducted two consecutive experiments with beef cattle fed lucerne hay at a low feeding (1.06 kg DM/100 kg body weight) then high feeding (1.72 kg DM/100 kg body weight) level. Reporting a greater correlation between individual cow intake observed and predicted by n-alkane pairings C31:C32 at a low feeding level. Although for n-alkane C33:C32 there was a greater correlation at a higher feeding level (Oliván et al. 2007). The findings of a greater correlation at a greater intake for C32:C33 are consistent with the current experiment, although the difference of DMI between high and low feeding level used in Oliván et al. (2007) is greater than the difference of DMI between CTRL and SUPP treatments. It is believed that the lower correlation at a lower intake in Oliván et al. (2007) is from lower faceal recoveries, as n-alkane absorption in the digestive tract is greater

due to feed rate of passage being lower at a low intake compared to a high. Unal and Garnsworthy (1999) also reported lower faecal recoveries of C32, C33 and C36 in dairy cattle consuming 20kg/day of silage than those consuming 25 kg/day and 30 kg/day, but the difference in faecal recoveries was not significant. To the contrary Ohajuruka and Palmquist (1991) found C31 faecal recovery decreased with increasing intake in dairy cattle. With the cause of C31 loss increasing with intake being unknown (Ohajuruka and Palmquist 1991). Lower faecal n-alkane concentrations from dairy cows fed supplement in addition to pasture compared to just pasture was also reported by Malossini et al. (1994). This may be a consequence of the supplement fed containing less n-alkanes than the pasture resulting in a dilution effect (Malossini et al. 1994). Evidently there is conflicting evidence on the influence of intake level and feed type on n-alkane faecal recovery and subsequent intake predictions. In the current experiment faecal concentration of n-alkanes was lower for the SUPP treatment, although intakes were greater. It is probable the difference between SUPP and CTRL is in relation to the difference in intake and addition of supplement.

In contrast, Mayes et al. (1986) reported that in grazing sheep fed differing levels of pasture and supplement, faecal recoveries of n-alkanes were unaffected by diet or feeding level and subsequently the accuracy of herbage intake predictions were unaffected. N-alkane recovery in sheep has been reported to be greater and less variable in comparison to cattle (Dove and Mayes 1991; Andriarimalala et al. 2020). Although both sheep and cattle are ruminants, they differ in digestive physiology being DMI capacity, digesta retention time, digestion and fermentation characteristics which may cause the differences in n-alkane recovery and variability (van Gastelen et al. 2019).

3.4.1.2 Measurement period

Unexpectedly there were differences in the correlations between n-alkane predictions and actual intakes in the two measurement periods. There was a small difference in actual pasture and total intakes between the two measurement periods with a lower pasture and total intake in Period 2, compared with Period 1 (difference of 0.7kg DM (4%) and 0.6 kg DM (3%) for pasture and total DMI respectively). The difference in intake between the two periods was predicted by all alkane pairs except C32:C35. In Period 2 there was a greater correlation between actual and predicted pasture and total intake, compared with Period 1 for all n-alkane pairings. This may be a result of the greater n-alkane concentration in pasture in Period 2, compared with Period 1, producing a more accurate representation of actual intake

in its predictions. Although pasture composition between the periods was similar with no difference in ME, there was a greater crude protein concentration in Period 2 than Period 1. Dillon (1993) reported a greater n-alkane concentration in high quality pasture, being pasture with greater ME and crude protein, with lower fibre concentrations, as the high-quality pasture had a higher proportion of leaf to stem. Wright et al. (2019) has found to the contrary with lower n-alkane concentrations in pasture with a higher crude protein concentration and lower fibre, but this difference was minimal. The concentration of n-alkanes in pasture were greater in Period 2 than Period 1 but, the faecal concentration of n-alkanes C32, C33 and C35 were lower in Period 2 than Period 1, indicating variable n-alkane recovery.

As the trial periods occurred consecutively it is unlikely that there would have been a great difference in the composition of pasture fed to the cattle. Additionally, although there are significant differences between the actual intakes of Periods 1 and 2, the numerical difference on average is 3 to 5%. Therefore, the difference in the performance of the n-alkane method between measurement periods is unexpected and the underlying cause should be explored.

3.4.1.3 Sources of error

Underlying sources of error that may cause inaccuracies of DMI predictions using the n-alkane method include dosage of the even chain n-alkane, analysis of pasture for n-alkanes, the collection of representative pasture samples and discrepancies in faecal recovery.

It is likely the underestimation of intake through the n-alkane method is in relation to the cattle not being dosed with a sufficient amount of C32. The Irish methodology for preparing and dosing the cows with C32 was followed for this experiment, with oven dried paper bullets pipetted with solution containing C32 before being oven dried. Following this method 377.6 mg of C32 per paper bullet was meant to be present, with the cattle being dosed with 755 mg/d. After analysing the n-alkane content from a sample of each batch of paper bullet dosed it was determined that on average each paper bullet contained 328.5 mg of C32, with the cattle dosed with 657 mg/d, this difference of 98 mg/d is likely to have meant the cows were being underdosed, therefore resulting in the underestimation. It is unknown why the paper bullets contained less C32 than intended, but it is harder to ensure that the contents of C32 remain within a paper bullet than a capsule.

In the present experiment, all pasture species present in samples were analysed together for n-alkanes. After the experiment, verbal conversations with Irish researchers revealed that they

identify and separate pasture species from the sample, then determine the proportion of each species in the pasture and analyse these separately for n-alkanes. Then they calculate the total n-alkane content by multiplying the proportion of each species by their n-alkane content. This difference in methodology for analysing and determining n-alkane content may have contributed to the lower accuracy of intake predictions in the current experiment and requires further investigation as to the differences in pasture n-alkane content from these two methodologies.

The pasture fed in this experiment consisted predominantly of perennial ryegrass (*Lolium perenne*), but there were small percentages (<5%) of white clover (*Trifolium repens L.*), other grasses (Poa species) and weeds (yarrow, dandelion, couch) present. For accurate predictions of pasture intake, the concentration of n-alkanes in consumed pasture must be known (Wright et al. 2020). As n-alkane profiles differ between plant species, when numerous plant species are being consumed, it becomes difficult to collect samples representative of that consumed and the complexity of analysis increases, reducing accuracy of predictions (Wright et al. 2020). So as the pasture in the current experiment is not a monoculture this may have been an additional source of error. An opportunity to test this hypothesis would be to sow monocultures and determine if n-alkane recovery and intake predictions are more accurate with these pastures.

3.4.2 Titanium dioxide and iNDF

The method of titanium dioxide paired with iNDF to predict DMI is focussed on in the following section. The individual cow DMI predictions provided by this method, the variation between actual and predicted DMI, along with potential causes of this variation are discussed. Along with the effect of pasture sampling method, treatment group and measurement period, in addition to possible sources of this variation between cows and trial period.

3.4.2.1 Faecal recovery

Faecal output of titanium dioxide on average was 3.44 kg/day, which agrees with Silva et al. (2021) who reported faecal outputs ranging from 2.71 to 3.18 kg/day. Real faecal output was not known for this experiment therefore the faecal recovery of titanium dioxide could not be determined. Discrepancies between estimated faecal output and real faecal output have been reported, Velasquez et al. (2018) reported an average difference of 1.8 kg of DM/day between real and estimated faecal output, with estimated faecal output being underestimated. After correcting faecal recovery with real faecal output Velasquaz et al. (2021) reported accurate dry matter digestibility and faecal outputs were produced by titanium dioxide and iNDF, producing accurate DMI predictions. Therefore, real faecal output must be known so that correction can occur, to increase the accuracy of DMI predictions. As the accuracy of DMI predictions are highly variable in the current experiment, with discrepancies between actual total and pasture DMI and that predicted from offered pasture ranging from 0.02 to 15.55 kg DM/d and from SNIP 0.03 to 24.98 kg DM/d, it is likely that faecal recovery is incomplete. For accurate DMI predictions a faecal recovery close to 100% is required, a greater faecal recovery will result in a lower faecal output estimate and vice versa (Velásquez et al. 2018; Silva et al. 2021). Velasquez et al. (2018) reported DMI estimates were on average 28% less than actual DMI in dairy cattle fed a corn silage-based diet, a consequence of greater than expected faecal recovery of titanium dioxide and underestimated dry matter digestibility from iNDF. Titgemeyer et al. (2001) also reported inaccurate DMI estimates, with the main cause being differences in faecal recovery from 100%. For this study pasture and total DMI predictions were on average 18% greater than actual DMI, although there were both under and over estimations. Incomplete faecal recovery of titanium dioxide can be a consequence of among animal variation, not correcting for real faecal output, chemical analysis, and the precision of titanium dioxide dosing, therefore it is probable within this

experiment that the most significant cause of error is not correcting for real faecal output (Titgemeyer et al. 2001; Schaafstra et al. 2018; Silva et al. 2021). Faecal recovery greater than 100% is a consequence of titanium dioxide being consumed from alternative sources (Velásquez et al. 2018). Although the pasture was cut and carried in this experiment, there were days where a greater amount of soil then average was present in the pasture fed to the cattle, although this would have been present in pasture samples, the soil is a potential alternative source of titanium dioxide resulting in greater faecal recover (Hellwing et al. 2015).

3.4.2.2 Sampling method

Using the "pasture offered" sampling method provided a more accurate prediction of total intake than using the pasture SNIP cuts. Total and pasture DMI predicted from pasture SNIP cuts were greater than those predicted by pasture offered but were more variable. On average predictions from pasture offered were underestimated, whilst predictions from SNIP cuts were overestimated for Period 2 and the CTRL treatment, whilst underestimated for Period 1 and the SUPP treatment, although under and overestimations of DMI were produced by both sampling methods. The pasture samples from SNIP cuts had lower iNDF content than the pasture offered samples, which may have influenced the intake predictions. This indicates, utilising a sampling method that is representative of pasture consumed by the cow is important. In this experiment, the sampling method of pasture offered provided a more representative sample and a more accurate prediction; however, in a grazing (in paddock) trial SNIP cuts would be the pasture sampling method use. Therefore it would be recommended to take more than the 30 SNIP cuts used in the present experiment, to create a more representative sample and potentially improve the accuracy of the DMI prediction.

3.4.2.3 Treatment group

There was a greater correlation between actual and predicted total and pasture DMI for cows in the CTRL compared with the SUPP treatment; however, there was no difference between faecal recovery of titanium dioxide between treatment. Predicted total intakes on average for the CTRL treatment were less than for the SUPP treatment, with the reverse true for actual intake, regardless of pasture sampling method. Whilst irrespective of pasture sampling method, predicted pasture intakes on average were greater for the CTRL treatment than SUPP, following the relationship that occurred for actual pasture intake. There was no difference between faecal recovery of titanium dioxide between treatments groups. This is in

agreement with Titgemeyer et al. (2001) who reported that faecal recovery was unaffected in steers fed prairie grass with or without supplementary feed. Although inaccuracy of DMI estimations were reported with the main cause being faecal recovery of titanium dioxide, digestibility was also underestimated by titanium dioxide, with underestimation being greater in forage based diets as they had lower digestibility (Titgemeyer et al. 2001). Both diets in the current experiment were forage based, with the SUPP treatment having a diet of greater digestibility, yet inaccuracies were greater for this group and when supplement was accounted for within intake predictions, in contrast with these findings. The lower total intake of cows in the CTRL treatment may be the reason for the greater correlation, as at a lower DMI, passage rate through the digestive tract is slower. Resulting in the faeces composition being more homogenous and as the concentration of iNDF and titanium dioxide is uniform throughout the faeces, representative faecal samples can be collected and analysed, allowing for DMI predictions with greater accuracy. In contrast, at a greater total intake, passage rate is faster and there is greater variation in faeces composition within the day (Owens and Hanson 1992; Velásquez et al. 2021).

3.4.2.4 Measurement period

There was a stronger correlation between actual and predicted total DMI in Period 2, compared with Period 1. Comparatively the reverse occurred for pasture DMI, with a weaker correlation between actual and predicted in Period 2 compared with Period 1. Actual total and pasture DMI was less in Period 2, compared with Period 1; however, predictions from titanium dioxide/iNDF were the opposite, with greater total and pasture intake predicted for Period 2 on average. This indicates that intake was overestimated in Period 2, whilst underestimated in Period 1, which may be a result of iNDF content of pasture offered and faeces being greater in Period 2 than Period 1. Measurement period and the difference occurring when only pasture intake is predicted in comparison to pasture and supplement does require further research to understand the underlying cause of these effects.

3.5 Limitations

There are limitations to be considered with this experiment and reported results, the first being that as there is no methodology available within NZ to accurately measure DMI of grazing cattle in paddock, the Calan gate system was used to measure intake so that indigestible marker predictions could be compared to an accurate DMI measurement. The Calan gate system, although it allows for pasture to be fed, the cattle must be housed and therefore are not grazing. As cattle are not in paddock grazing, grazing behaviours and subsequent intakes may be modified so results cannot be considered an exact representation of grazing dairy cattle, although as pasture is still being consumed it is a close representation.

Upon analysis of the paper bullets containing C32 used to dose the cattle it was found that on average dosage was 657 mg/d, this was 98 mg/d less than intended and may have resulted in lower accuracy of the results. A meta-analysis of n-alkane studies has reported for good accuracy of feed intake estimates in cattle a dosage exceeding 700 to 800 mg/d of C32 is required (Andriarimalala et al. 2020). Although this is not a large difference (43 mg/d) it still may have influenced results.

The indigestible marker methodologies used within my Master's research needed to be suitable for use in grazing cattle, therefore total faecal output of a cow was not measured as this would not have been feasible in paddock. The methodology of titanium dioxide and iNDF was utilised without a correction factor to determine if it could be used accurately without. It was determined that the correction of faecal output is required for this method reflected in the low accuracy of the results. Not knowing actual total faecal output also meant that the actual faecal recovery of the internal and external marker could not be determined. So we were not able to specify if the cause of inaccuracy is from the over or underestimation of faecal recovery of titanium dioxide or iNDF.

The pasture fed in this experiment was spring pasture, with the predominant species being perennial ryegrass. As the nutritive characteristics of perennial ryegrass changes between seasons, with this including iNDF content, n-alkane concentrations in pasture may also vary between seasons (Jacobs et al. 1999; Wright et al. 2019). Accuracy of results from the indigestible markers may subsequently differ in other seasons outside of spring.

3.6 Suggested further research

Due to the limitations stated in the section above, which include the housing of cattle and dosage of n-alkanes, to further the understanding and accuracy of the use of indigestible markers n-alkanes and titanium dioxide and iNDF to predict DMI in grazing dairy cattle the following further research is recommended.

To determine if dose of n-alkane C32 influences the accuracy of DMI predictions, an experiment where cattle are dosed with an increased amount of C32 (700 – 800 mg daily) could be conducted. An alternative dosing apparatus may be necessary to do this, as with the paper bullets it was difficult to ensure that all is the solution was absorbed and therefore dosed into the cow.

It would also be valuable to compare the methodologies for determining the n-alkane content in pasture consumed, whether accuracy is increased if the proportion of each pasture species is determined and analysed separately for n-alkanes, in comparison to assuming the sample collected is representative of that fed and analysing the pasture sample as one.

Another source of difference between NZ and Ireland that may influence the accuracy of n-alkanes, is the monoculture nature of herbage swards in Ireland in comparison to the multi species nature of NZ's. By cultivating a monoculture perennial ryegrass sward in NZ to use as feed for an experiment using n-alkanes, the influence of this difference could be determined.

To gain a better understanding on the potential of the pair titanium dioxide and iNDF to predict DMI it is necessary to complete further research where intake is measured through a Calan gate system and by the use of these indigestible markers. The inclusion of at least one additional cow that total faecal output is collected from would allow for the correction of estimated faecal output. Along with being able determine the faecal recovery of the indigestible markers and whether the incomplete faecal recovery of titanium dioxide or iNDF are a source of error. Within this the use of both pasture and pasture and supplement diets to develop further understanding on the effect diet has on pasture and total DMI predictions would have additional benefit.

3.7 Conclusion

Although currently the n-alkane method is not within a range of accuracy to be used in NZ grazing dairy cattle scientific research, there is evidence to support that with further research to determine the cause of discrepancies between the faecal recovery of C33:C32, which would allow for methodology improvements, there is potential for this methodology. The DMI predictions by titanium dioxide and iNDF appear to be highly variable, a likely result of variation in faecal recovery. There may be potential if faecal recovery is corrected for accurate estimation, but total faecal collection is not as suitable for pasture trials. Therefore, as there are alternative indigestible markers such as n-alkanes that have more potential to be used with accuracy and are better suited to grazing dairy cattle, further research should be focused on the improvement of the n-alkane pair C32:C33.

Chapter 4: Energetics Back Calculations

4.1 Introduction

Another indirect methodology for estimating feed intake in grazing dairy cattle is using a series of equations to calculate the ME requirements for the different biological processes of the cow and deriving a DMI estimation from this. Back calculating using energetic requirements is often used in scientific research on grazing cattle, as it utilises commonly measured animal factors such as milk production and liveweight, which are easier to obtain on farm than direct feed intake in a pasture-based system (NRC 2001). There are many equations available, formulated with differing theoretical concepts which can result in varying estimates of DMI when using the same factors (Galyean and Gunter 2016). The purpose of this chapter is to evaluate the accuracy and variation between three equations frequently used in NZ dairy cattle scientific research to estimate DMI, in comparison to actual intake. Along with whether the accuracy of intake estimates varies between animals, and what effect more frequent measurements of milk composition and liveweight have on the accuracy of estimated intakes.

4.2 Materials and Methods

To evaluate the different energetic equations in their ability to predict actual intakes previous DairyNZ trials were combined into a single dataset. These trials measured individual animal intakes using the Calan gate system (Lye Farm, DairyNZ, Hamilton, New Zealand) and fulfilled the following criterium, where the following was known:

1) Actual individual cow intake (multiple intake measures per cow)
2) Liveweight
3) Calving date
4) Age
5) Milk yield
6) Milk protein
7) Milk fat
8) Breed
9) Feed type
10) Experimental treatments
11) Did not contain confidential information.

This resulted in a dataset of five previous Calan gate trials, along with data from the Calan gate trial that occurred in Chapter 3. Liveweight measurements and herd tests occurred at different frequencies in each trial, which allowed for the effect of measurement frequency to be analysed. A trial with non-lactating cattle was included allowing the comparison between DMI predictions for lactating and non-lactating cattle. Feed type also varied between trials which allowed for the accuracy of DMI predictions from each equation between feed types to be analysed (see Appendix A for further detail on each trial).

The following assumptions were made to produce the dataset;

- Where liveweight, milk fat, and protein data were not provided on a daily basis then data from the nearest date measured was used.
- When condition score was not known it was assumed to be 4 out of 10 (Macdonald et al. 2008).
- Days pregnant used in the MPI GHG equation was assumed as per Pickering et al. (2022) (Table 4.1).
- Calf birthweight was assumed as 9% of the adult cow's liveweight in the last month prior to birth when using the GHG MPI equation (AFRC Council 1993).
- Availability of green forage (tonnes/ha) was assumed to be 3.5 tonnes of DM per hectare (Pickering et al. 2022).
- Any cattle over the age of 6 were assumed to be 6 years old, as a maximum value of 6 is used in the formulation of the MPI GHG equations and liveweight gain/loss was assumed to be 0 in adult lactating cattle (Pickering et al. 2022).
- Monthly digestibility of feed used in the MPI GHG equation was assumed as per Table 4.1 (Pickering et al. 2022).
- Milksolids (%) was assumed as fat (%) plus true protein (%) when using the Nicol and Brookes (2007) equation.
- Pregnancy requirements for the Nicol and Brookes (2007) equation were calculated based on the assumptions of calf birthweight being 40 kg, the month of April being 12 weeks prior to calving and the month of May as 8 weeks prior to calving.
- Liveweight gain/loss was calculated in two different ways to compare the difference between accuracy of DMI predictions between more frequent or less frequent liveweight measurements. For the more frequent model all liveweight measurements taken in the trial period were included, for the less frequent only the first and last

liveweight measurements in the trial period were included. Liveweight gain/loss was assumed in both cases as the liveweight change between two dates divided by the number of days between the dates to produce a gain or loss average over each day.

Table 4.1: Monthly feed digestibility and cumulative days pregnant (Pickering et al. 2022)

Month	Monthly feed digestibility (kg/kg)	Days pregnant
July	0.794	269
August	0.811	0
September	0.824	0
October	0.820	0
November	0.770	26
December	0.752	57
January	0.741	88
February	0.711	116
March	0.697	147
April	0.737	177
May	0.753	208
June	0.783	238

4.2.1 Producing the dataset and calculations

A summary of the past DairyNZ trials used are presented in the Appendix A (Table 5.0). Cattle in trial AABE were non-lactating therefore NRC and MPI GHG equations which are formulated for lactating cattle could not be used to calculate DMI predictions resulting in five trials being utilised in the dataset for these equations (NRC 2001; Pickering et al. 2022). As liveweight change was a variable used in the Nicol and Brookes (2007) equation liveweight gain/loss which had been calculated in two ways, for reasons previously discussed in assumptions, was used to calculate DMI predictions twice, one with the more frequent liveweight measurements and the other with the less frequent. This is with the exception of trial B22X where liveweight measurements only occurred twice within the trial period, therefore predictions remained the same between the two. The trial AABE had non-lactating cattle and was included in the dataset of all trials when calculating correlations, whilst a dataset

with only lactating cattle was also produced and analysed. It was determined that there was not a large difference in correlations with the inclusion of the dry cattle dataset therefore it was excluded from any further analysis.

4.2.2 Calculations and statistical analysis

4.2.2.1 DMI using equations from NRC (2001)

DMI predicted for lactating Holstein-Friesians using the NRC (2001) equation was calculated as:

$$\text{DMI (kg/d)} = (0.372 \times 4\%\ \text{FCM} + 0.0968 \times \text{BW}^{0.75}) \times (1 - e^{(-0.192 \times (\text{WOL}+3.67))})$$

Where 4% FCM is 4% fat corrected milk, BW is bodyweight (kg), e is 2.71828 (e value adjusts for stage of lactation) and WOL is week of lactation.

4% fat corrected milk was calculated using the equation by Gaines (1928):

$$4\%\ \text{FCM} = (0.4 \times \text{kg milk}) + (0.15 \times \text{kg milk} \times \text{fat \%})$$

4.2.2.2 DMI using equations from MPI GHG (Pickering et al. 2022)

DMI for an average dairy milking cow and heifer using the MPI GHG equation was calculated as:

$$\text{DMI} = \frac{\text{ME}_{\text{total}}}{\text{Feed}_{\text{ME}}}$$

Where ME_{total} is the total ME requirements of the average animal per day (MJ/d) and Feed_{ME} is the energy content of feed consumed or ME per kg of DM for pasture (MJ/kg).

Total ME required per day (MJ/d) was calculated as:

$$\text{ME}_{\text{total}} = \text{ME}_{\text{m}} + \text{ME}_{\text{l}} + \text{ME}_{\text{c}} + \text{ME}_{\text{g}} + \text{ME}_{\text{graze}}$$

Where ME_{m} is ME required for maintenance (MJ/d), ME_{l} is ME required for milk production (MJ/d), ME_{c} is ME required for gestation or growth of conceptus (MJ/d), ME_{g} is ME required for liveweight gain (MJ/d) and ME_{graze} is ME expenditure of grazing (MJ/d).

ME required for maintenance (ME_m) was calculated as:

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

Where K is a coefficient accounting for differences in fasting heat production assumed to be 1.4 in cattle, S is the coefficient accounting for differences in basal metabolic rate between male and female, assumed to be 1.0 in females, LW is liveweight of the animal, A is age in years (up to a maximum value of 6). k_m is efficiency of ME utilisation for maintenance, ME_p is ME required for production (MJ/d).

The efficiency of ME utilisation of maintenance ($k_{m)}$ was calculated as:

$$k_m = 0.35 \times Q_m + 0.503$$

Where Q_m is the ratio of feed ME to gross energy concentration of pasture

The ratio of feed ME to gross energy concentration of pasture was calculated as:

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

Where $Feed_{ME}$ is ME content of feed and $Feed_{GE}$ is the gross energy per kg of DM for feed, assumed to be 18.45 MJ per kg of DM (MJ/kg).

ME requirements for milk production was calculated as:

$$ME_i = \frac{Y \times MILK_{GE}}{k_i}$$

Where Y is daily milk yield per milking cow (kg/d), $MILK_{GE}$ is the gross energy content of milk (MJ/kg) and k_i is the ME efficiency of utilisation for milk production.

Gross energy content of milk ($MILK_{GE}$) was calculated as:

$$MILK_{GE} = 0.376 \times F + 0.209 \times P + 0.948$$

Where F is the fat content of milk (%) and P is the protein content of milk (%)

The ME efficiency of utilisation for milk production (k_i) was calculated as:

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

Where Q_m is the ratio of pasture ME concentration to gross energy concentration of pasture

ME requirements for liveweight gain in growing lactating animals was calculated as:

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

Where neclw is the net energy content of liveweight (MJ), LWG is liveweight gain (kg/day) and k_g is net efficiency of dietary ME conversion to liveweight gain.

Net energy content of liveweight (neclw) was calculated as:

$$neclw = 10.1 + 2.47 \times cs$$

Where cs is body condition score.

ME requirements for gestation was calculated as

$$ME_c = 0.025 \times LW_c \times \frac{0.0201E_t \times e^{-0.0000576prg}}{k_c}$$

Where LW_c is calf birth weight (kg) which is assumed to be 9% of the adult cow's liveweight, E_t is energy required for gravid uterus in utero (MJ/d), prg is the number of days the cow has been pregnant and k_c is the ME efficiency of utilisation of conceptus energy gain assumed to be 0.133.

Energy required for gravid uterus in utero (E_t) was calculated as:

$$E_t = 10^{151.665 - 151.64e^{-0.0000576prg}}$$

Where prg is the number of days the cow has been pregnant.

ME required for grazing was calculated as:

$$ME_{graze} = \frac{\left(\left(C \times (0.9 - DMD) \times \left(ME_m + ME_p - Z_1\right)\right) + 0.05 \times \left(\frac{T}{GF + 3}\right) \times Feed_{me}\right) W}{k_m \times Feed_{me} - C \times W \times (0.9 - DMD)}$$

Where C is the additional energy for eating required for grazing animals assumed to be 0.006, DMD is dry matter digestibility, ME_m is the ME required to maintain animal weight (MJ/d). Z_1 us the amount of energy received from milk (MJ/d), ME_p is the ME required for production (MJ/d). T is the terrain factor assumed to be 1, GF is availability of green forage assumed to be 3.5 tonnes DM/ha, W is animal liveweight (kg), k_m is efficiency of utilisation for maintenance and $Feed_{me}$ is the energy content of feed intake or ME per kg DM of pasture.

4.2.2.3 DMI using equations from Nicol and Brookes (2007)

DMI for a dairy cow using the Nicol and Brookes (2007) equation was calculated as:

$$DMI = \frac{ME_r}{Feed_{ME}}$$

Where ME_r is the total ME requirements of the cow per day (MJ/d) and $Feed_{ME}$ is the energy content of feed consumed or ME per kg of (MJ/kg).

ME requirements (ME_r) using the equation from Nicol and Brookes (2007) was calculated as:

$$ME_r = ME_m \pm ME_{lw} + ME_P + ME_l$$

Where ME_m is ME for maintenance (MJ/day), ME_{lw} is ME for liveweight gain/loss (MJ ME), ME_P is ME for pregnancy (MJ ME) and ME_l is ME for lactation (MJ ME/ Litre)

ME for maintenance (ME_m) is calculated as:

$ME_m \left(\frac{MJ}{day}\right) = 0.56MJ \times kg^{0.75} \pm 5\%$ (per MJ ME for diets below/above 11.0 MJ ME/kg DM)

Where kg is liveweight (kg)

ME for lactation (ME_l) is calculated as:

Table 4.1: ME requirement for lactation from Nicol and Brookes (2007)

Milk composition						
Milk fat (%)	4.0	4.5	5.0	5.5	6.0	6.5
Milk protein (%)	3.2	3.5	3.7	3.9	4.2	4.4
Milksolids (%)	7.2	8.0	8.7	9.4	10.2	10.9
ME requirement						
ME (MJ ME/litre)	5.7	6.2	6.6	7.0	7.5	7.9
ME (MJ ME/kg milksolids)	80	77	76	75	73	72

± 8% per MJ ME for diets below/above 11 MJ ME/ kg DM

Liveweight gain (ME_{lw}) in lactating adult cattle is calculated as:

$ME_{lw} = 48$ MJ ME $\times$ kg gain $\pm$ 5% per MJ ME for diets below/above 11 MJ ME/kg DM

Liveweight gain (ME_{lw}) in non-lactating adult cattle is calculated as

$ME_{lw} = 60$ MJ ME $\times$ kg gain $\pm$ 12% per MJ ME for diets below/above 11 MJ ME/kg DM

Liveweight loss (ME_{lw}) in lactating cattle is calculated as:

$ME_{lw} = 35$ MJ ME $\times$ kg gain $\pm$ 8% per MJ ME for diets below/above 11 MJ ME/kg DM

ME for pregnancy (MJ ME) (ME_P) is calculated as:

Table 4.2: ME requirements for pregnancy from Nicol and Brookes (2007)

Calf birth weight (kg)	**Weeks before calving**					
	-12	**-8**	**-6**	**-4**	**-2**	**0**
25	5	9	12	16	21	28
30	6	11	15	19	25	33
35	7	13	17	23	30	39
40	9	15	20	26	34	45

± 5% per MJ ME for diets below/above 11 MJ ME/ kg DM

Litres of milk produced per cow was calculated as:

$$\text{Milk litres} = \frac{\text{kg milk}}{1.037}$$ (Parmar et al. 2020)

4.2.2.4 Statistical analysis

On investigation of the spread of individual cow intakes using the Nicol and Brookes equation it was decided that the accuracy of the equation was not sufficient to take through to further data analysis (Figure 4.1). It was then decided to investigate the outcome if an averaging approach was taken to produce liveweight loss/gain by using the liveweight change over the course of each trail period (from first liveweight measurement to last) averaged over the trial length. However, this was still found to be insufficient to correct for the limited liveweight data and reduce the error induced by its inclusion. As such, the Nicol and Brooks equation has been removed from the analysis from this point of the chapter forward. Consequently as the Nicol and Brookes equation was the only equation suitable for use in non-lactating cattle the lactating cattle dataset was used for analysis which did not include trial AABE.

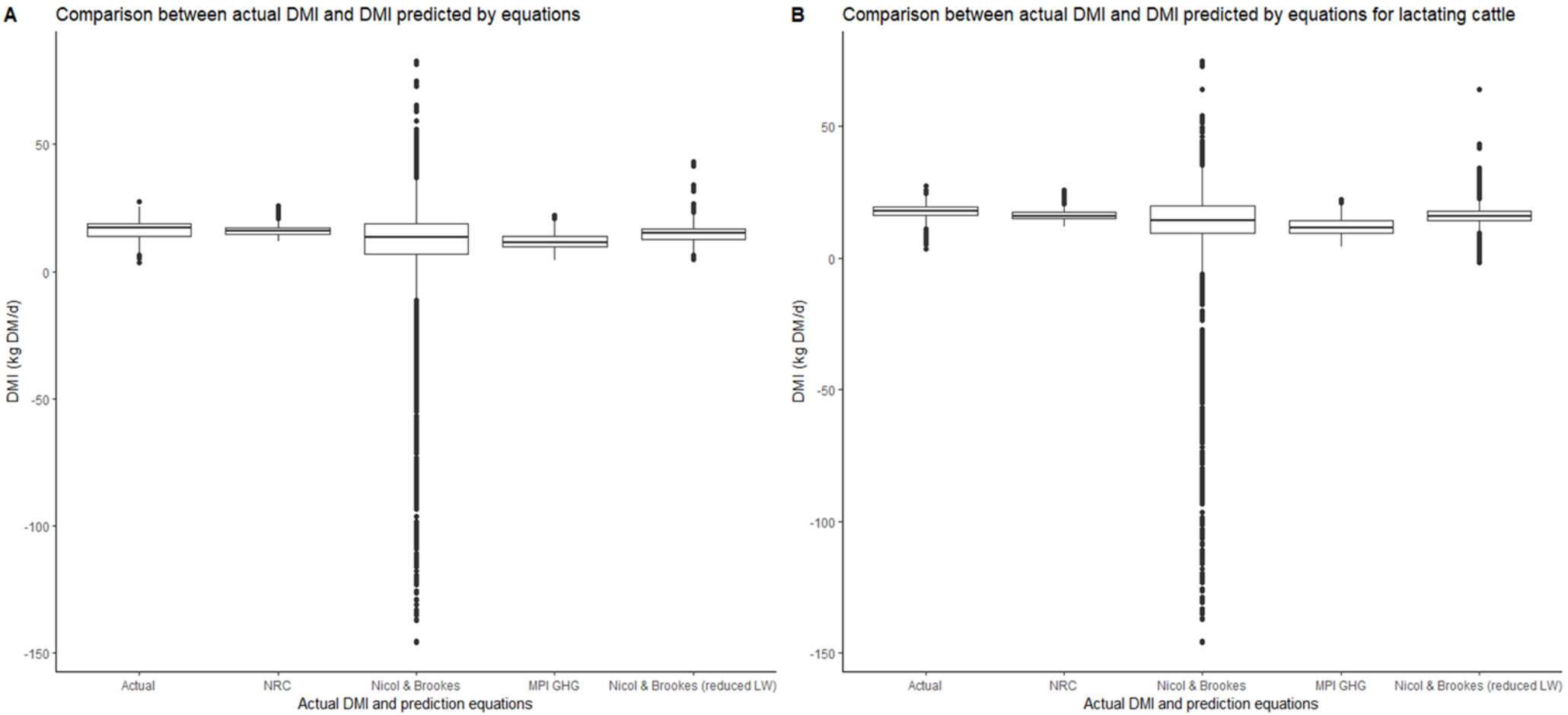

Figure 4.1: Mean actual and predicted from energetic back calculation individual cow DMI (kg DM/d), A being the full dataset and B being the lactating dataset. Results are from DairyNZ Calan gate studies using Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

Data were analysed using Rv4.2.1; R Core Team 2022 (R Foundation for Statistical Computing, Vienna, Austria). To analyse the relationship between actual DMI measured through the use of Calan gates and DMI predicted by energetics calculations, along with the relationship between equation DMI predictions, overall and in individual trials, Pearson's correlation coefficient was used.

A multiple linear regression model was used to investigate variables used in all the energetics equations. In this model the dependent variable of actual DMI measured by the Calan gate method was used with the fixed effects of cow number, feed type, age, week of lactation, and days pregnant, feed digestibility, milk protein (%), milk fat (%), 4% fat corrected milk, and liveweight. An all-possible regression analysis combination was then performed on this model, to analyse the relationship between DMI and these variables.

A multiple linear regression model for each energetics equation was also used with actual DMI measured by the Calan gates as the dependent variable and the variables in each equation as the independent variables, to investigate the variables used in each equation. For the NRC equation the independent variables included were liveweight, week of lactation, 4% fat corrected milk and cow number. For the MPI GHG equation the independent variables were feed ME, milk fat (%), milk protein (%), condition score, liveweight, age, days pregnant, digestibility and cow number. Calf weight was excluded from the MPI GHG equation model as it is an assumption calculated as 9% of cow liveweight and as cow liveweight was already included in the model it was deemed to be confounding. An all-possible stepwise regression was then used an all models to analyse the relationship between variables. Significance has been declared at $P < 0.05$. As the dataset being analysed is large with many variables, a principal component analysis was used to investigate the numerical components of the energetics equations to determine the relationship between the variables and identify the most important variables influencing accuracy of the DMI predictions. The principal component analysis was performed using the following R packages; ggcorrplot (v0.1.4; Kassambara A 2022), FactoMineR (Sebastien Le, Julie Josse, Francois Husson 2008) and factoextra (v1.0.7; Kassambara A, Mundt F 2020).

4.3 Results

4.3.1 Individual cow DMI

The predicted DMI by the energetics back calculation equations explained within a range of 50 to 60% of the variance in actual lactating individual cow DMI (Table 4.4). There is a diminishing return when increasing in complexity of models in regard to the measurement parameters to predict DMI.

Table 4.4: Proportion of variation for actual individual lactating cow DMI (kg DM/d) explained by measurement variables used in energetics back calculation equations. Results are from five DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

	Model	R^2
All variable model	DMI ~ 4% FCM + Liveweight + Week of lactation + Feed ME + Fat (%) + Protein (%) + Digestibility (kg/kg) + Days pregnant + Age + Condition score + Feed type + Cow number	0.5721
All variable simplified model	DMI ~ 4% FCM + Liveweight + Week of lactation + Feed ME + Fat (%) + Protein (%) + Digestibility (kg/kg) + Days pregnant + Age + Feed type + Cow number	0.5721
NRC all variable and simplified model	DMI ~ 4% FCM + Liveweight + Week of lactation + Cow number	0.5448
MPI GHG all variable model	DMI ~ Feed ME + Milk fat (%) + Milk protein (%) + Condition score + Liveweight + Age + Days pregnant + Digestibility (kg/kg) + Cow number	0.5604
MPI GHG simplified model	DMI ~ Feed ME + Milk fat (%) + Milk protein (%) + Liveweight + Age + Days pregnant + Digestibility (kg/kg) + Cow number	0.5604

In lactating Holstein Friesian dairy cattle under a range of management conditions (refer to Appendix A – Table 5 for further detail) the all variable model and all variable simplified model explained the greatest amount of variation between actual individual cow DMI measured through the Calan gate system and DMI predicted (57%). The GHG MPI equation model explained the greatest amount of variation between actual individual cow DMI and DMI predicted by the equation (56%), with or without condition score. The NRC equation model explained a similar amount of variation (54%) whilst being the simplest model.

Within the all variable model condition score and feed type have a P. value greater than 0.05 and are therefore not considered significant and are not required in the model to produce accurate DMI predictions (refer to Appendix B – Table 5.1). The significance of the individual cow when using these equations does vary depending on the cow itself with P-values for individual cows varying from 1.68 e-3 at the minimum estimate to 7.3 e-12 at the maximum estimate, 86% (158 of 184 cows) were found to be statistically significant ($P < 0.05$) (Appendix B – Table 5.2). Therefore, accounting for repeat measures of the measurement variables on each cow is highly important for the accuracy of DMI predictions.

The NRC equation has the greatest correlation between predicted and actual DMI for both the individual trials and lactating cattle dataset (Table 4.5).

Table 4.5: Pearson's correlation coefficient of actual individual lactating cow DMI (kg DM/d) with individual cow DMI (kg DM/d) predicted from energetics back calculation equations. Results are from DairyNZ Calan gate studies using Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

Pearson's correlation coefficient with actual intake			
		GHG MPI	**NRC**
Lactating cow dataset		0.15	0.33
Lactating cow trials	B23N[1]	0.37	0.35
	B22X[2]	0.38	0.44
	AADO[3]	0.24	0.38
	AABN[4]	0.49	0.62
	AABA[5]	0.36	0.42

[1] Trial B23N used 40 early lactation cattle with a mean liveweight of 483 kg over a measurement period of 25 days.
[2] Trial B22X used 36 late lactation cattle with a mean liveweight of 486 kg over a measurement period of 18 days.
[3] Trial AADO used 40 late lactation cattle with a mean liveweight of 532 kg over a measurement period of 67 days.
[4] Trial AABN used 40 early lactation cattle with a mean liveweight of 436 kg over a measurement period of 20 days.
[5] Trial AABA used 42 late lactation cattle with a mean liveweight of 491 kg over a measurement period of 31 days.

Table 4.5 shows the Pearson's correlation coefficient between actual lactating cow individual DMI (kg DM/d) measured through the Calan gate system and predicted DMI (kg DM/d) by the energetic back calculation equations used, using the different trials (see Appendix A for further detail on these). There was a moderate correlation using the NRC equation and low correlation using the GHG MPI equation. On a trial level the GHG MPI equation had a moderate

correlation with all trials, but AADO which had a low correlation. The NRC equation had a moderate correlation with all trials, other than AABN which had a strong correlation.

Pearson's correlation coefficient between the individual lactating cow DMI predictions by the energetic back calculation equations used was performed. There was a strong correlation between DMI predictions by the NRC and GHG MPI equations (73%).

4.3.2 Herd level DMI

At a herd level accuracy of DMI predictions compared to actual DMI for the NRC equation ranged from 85% to 110% and for the MPI GHG equation accuracy ranged from 45% to 100%.

Table 4.6: Mean actual DMI (kg DM/d) of individual trials compared to DMI (kg DM/d) predicted from energetics back calculation equations calculated through the means of animal measurements by trial. Results are from 5 DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

Trial	Actual DMI (kg DM/d)	NRC equation (kg DM/d)	Predicted DMI (kg DM/d) as a percentage of actual DMI (kg DM/d)	MPI GHG equation (kg DM/d)	Predicted DMI (kg DM/d) as a percentage of actual DMI (kg DM/d)
B23N[1]	18.39	19.63	107%	14.40	78%
B22X[2]	17.18	16.68	97%	14.13	82%
AADO[3]	18.28	15.46	85%	8.6	47%
AABN[4]	17.02	16.20	95%	16.57	97%
AABA[5]	14.23	15.36	108%	10.18	72%

[1] Trial B23N used 40 early lactation cattle with a mean liveweight of 483 kg over a measurement period of 25 days.
[2] Trial B22X used 36 late lactation cattle with a mean liveweight of 486 kg over a measurement period of 18 days.
[3] Trial AADO used 40 late lactation cattle with a mean liveweight of 532 kg over a measurement period of 67 days.
[4] Trial AABN used 40 early lactation cattle with a mean liveweight of 436 kg over a measurement period of 20 days.
[5] Trial AABA used 42 late lactation cattle with a mean liveweight of 491 kg over a measurement period of 31 days.

At a herd level on average the NRC equation provides the best intake predicton at 98% of actual DMI (kg DM/d), with the MPI GHG equation providing an average prediction at 75% of actual DMI (kg DM/d). The NRC equation at a herd level provided a 15% underestimation

of intake for trial AADO. The MPI GHG equation DMI predictions underestimated actual intake for all trials, with the most significant underestimation being for trial AADO.

The spread of actual DMI is greater than the spread of DMI predictions by the NRC and GHG MPI equations (Figure 4.2). When comparing the means of individual cow DMI there is not a great variation shown between actual and predictions produced by equations, as this would be considered at a herd level where variation in measurements and therefore predictions is reduced.

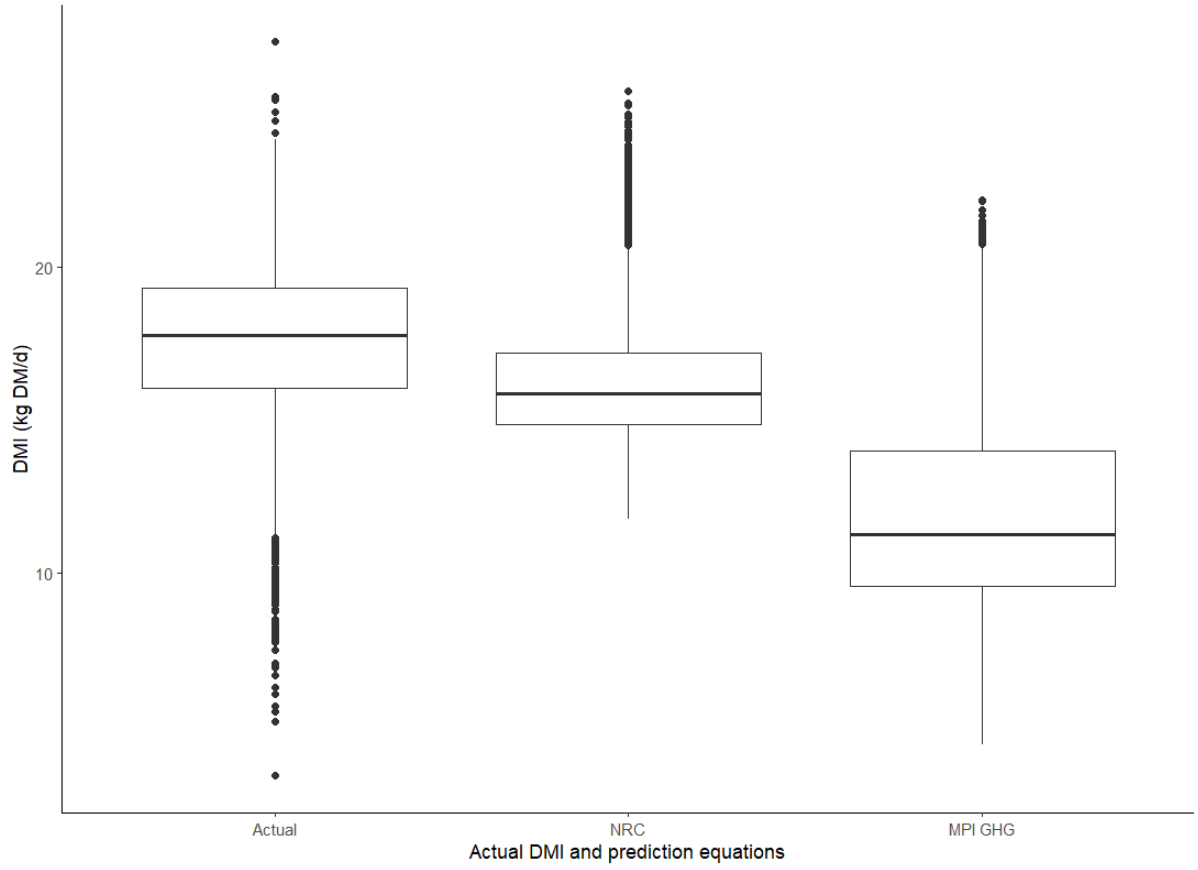

Figure 4.2*:* Mean actual and predicted from energetic back calculation individual cow DMI (kg DM/d). Results are from DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies)

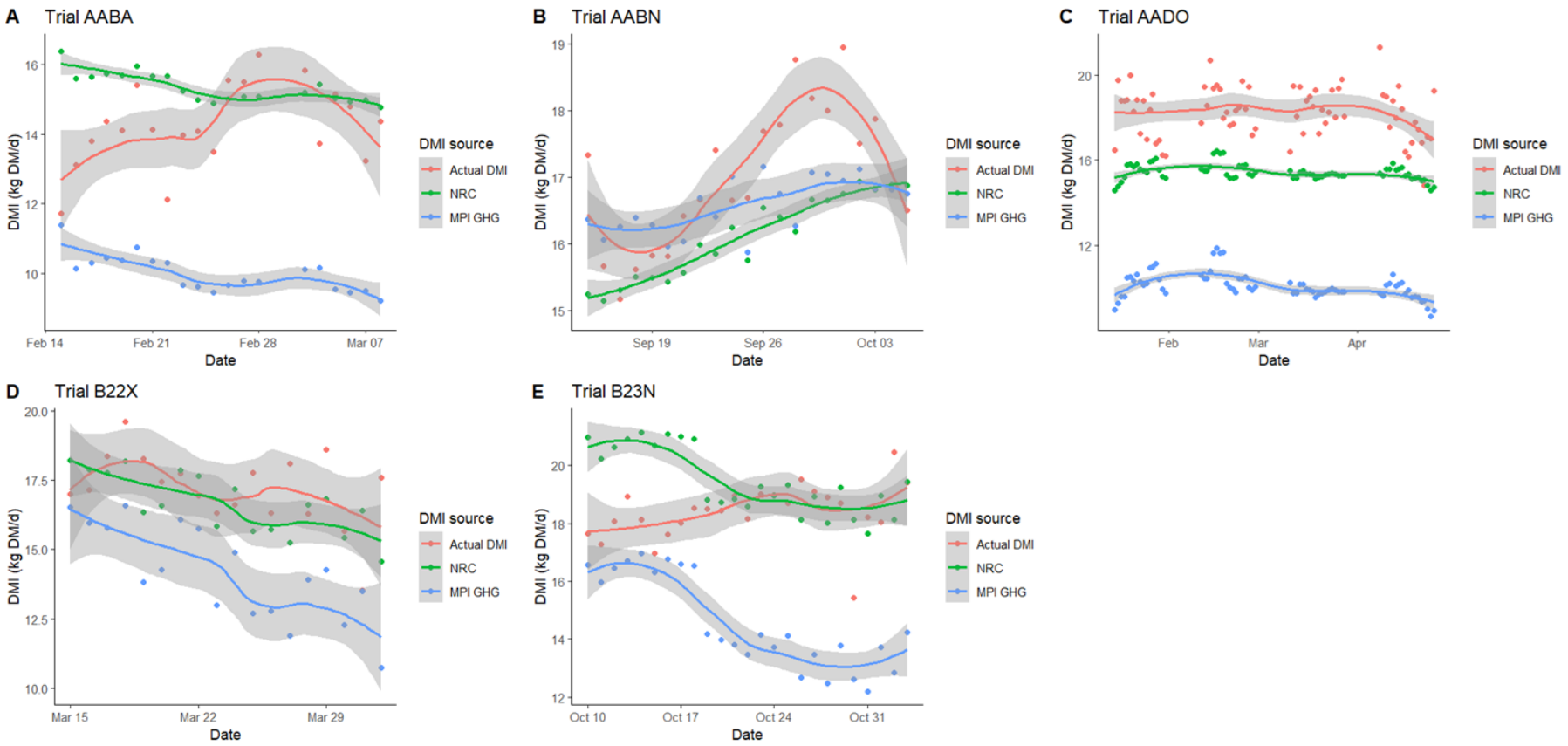

Figure 4.3: Individual trial mean actual and predicted from energetic back calculation individual cow DMI (kg DM/d) over trial period (shaded area is 95% confidence interval). Results are from DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies.

A. Trial AABA used 42 late lactation cattle with a mean liveweight of 491 kg over a measurement period of 31 days. B. Trial AABN used 40 early lactation cattle with a mean liveweight of 436 kg over a measurement period of 20 days. C. Trial AADO used 40 late lactation cattle with a mean liveweight of 532 kg over a measurement period of 67 days. D. Trial B22X used 36 late lactation cattle with a mean liveweight of 486 kg over a measurement period of 18 days. E. Trial B23N used 40 early lactation cattle with a mean liveweight of 483 kg over a measurement period of 25 days

None of the equation predictions follow a simiar trend to actual mean daily intake in trail AABN. The MPI GHG equation mean daily intake predictions are lower than actual intake for trials AABA, B22X and significantly lower for AADO, where all predictions from the other equations are also lower, there are are also many outliers for this trial in comparison to the others

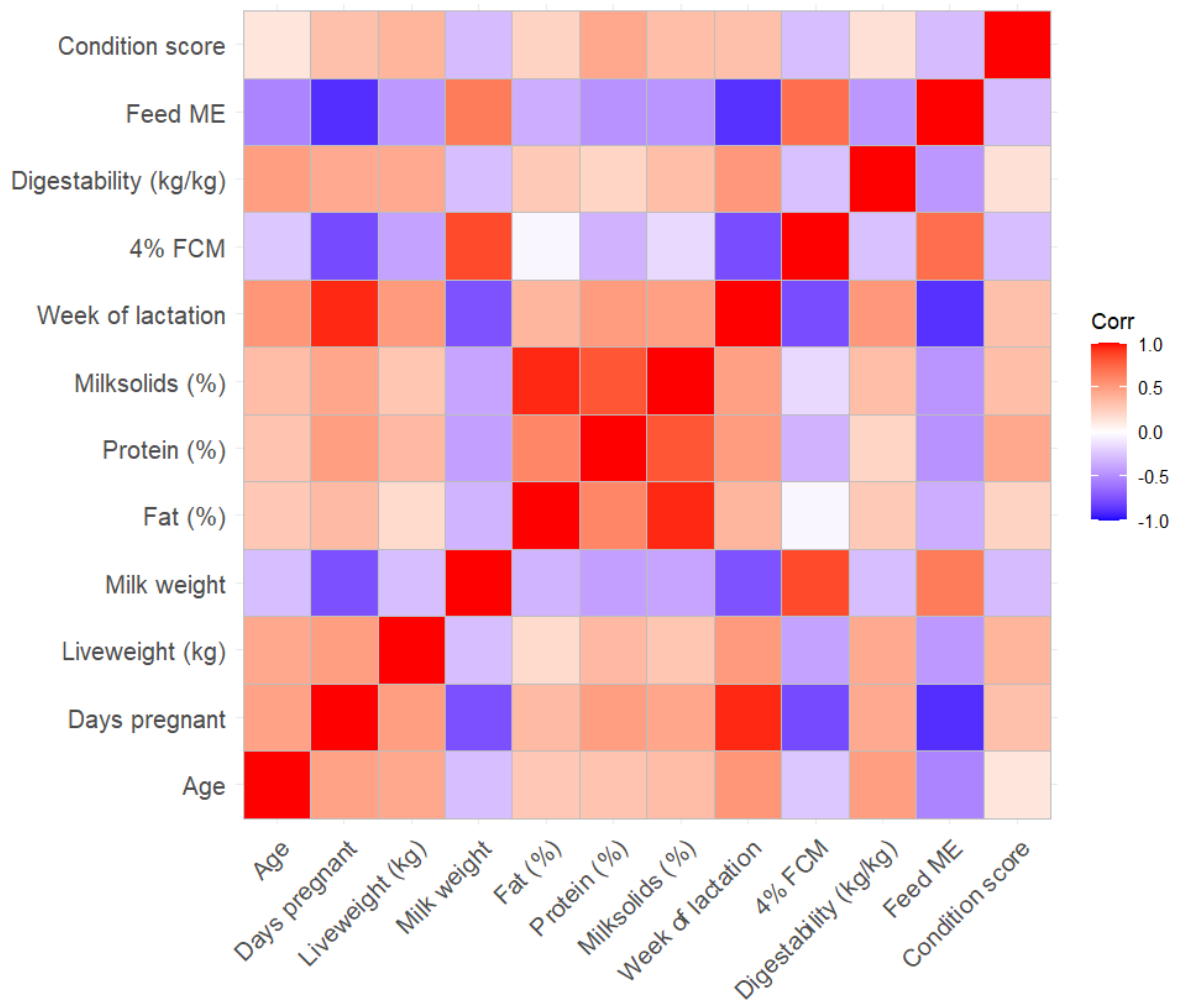

Figure 4.4: Correlation matrix of variables used in energetics back calculation equations to predict individual cow DMI (kg DM/day). Results are from a principal component analysis on five DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

A principal component analysis was performed to investigate the variables used in energetic back calculation equations to predict individual cow DMI (kg DM/d), variables used were age, days pregnant, liveweight, milk weight, milk fat (%), milk protein (%), milksolids, week of lactation, 4% FCM, feed digestibility, feed ME and condition score.

Condition score had a weak correlation with all variables (Figure 4.4). Feed ME, milk weight and 4% FCM had a moderate positive correlation with each other but no or negative correlation with all other variables, these variables all had a strong negative correlation with week of lactation and days pregnant (Figure 4.4). Milk variables; milksolids, fat and protein (%) were all strong to moderately positively correlated with each other (Figure 4.4).

93.4% of total variation was explained by component 1 (86.6 %) and 2 (6.8%) (Figure 4.4B), with week of lactation, days pregnant, feed ME, 4% FCM and milk weight being major contributors to components 1 and 2 (Figure 4.4C). Milk protein and fat (%), milksolids (%), 4% FCM, feed ME, milk weight and week of lactation are located in the negative quadrants (Figure 4.4A), with condition score contributing little to component 1 (Figure 4.4C). All other variables are in the positive quadrant and related to component 1 Figure 4.4A and C).

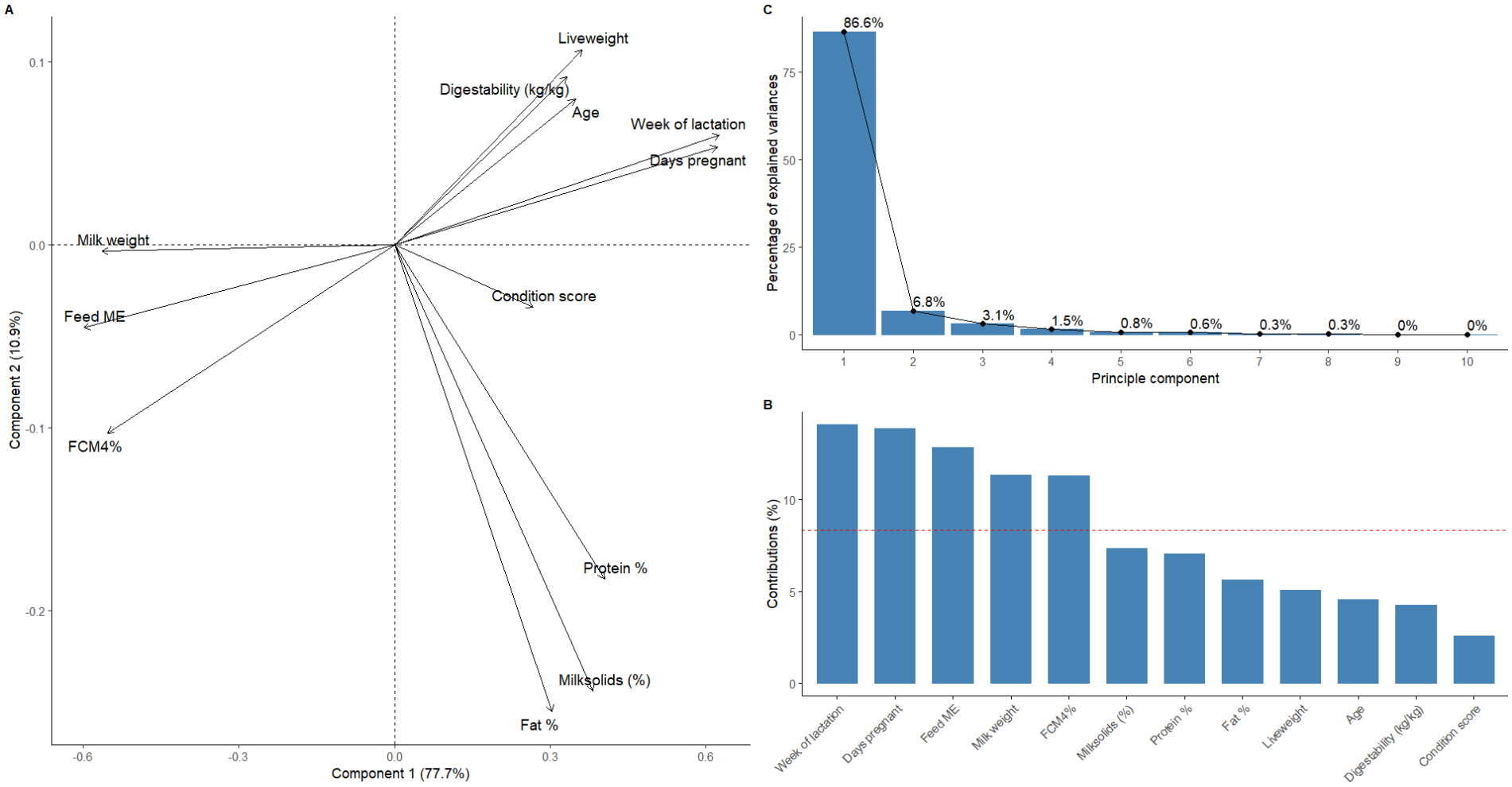

Figure 4.4: PCA analysis of variables used in energetics back calculation equations to predict individual cow DMI (kg DM/day). A. Biplot of variables used in energetics back calculation equations to predict individual cow DMI (kg DM/day). B. Percentage of explained variance by each principal component using variables used in energetics back calculation equations to predict individual cow DMI (kg DM/day). C. Percent contribution of each variable variables used in energetics back calculation equations to predict individual cow DMI (kg DM/day) to components 1 and 2, the red dotted line indicates expected value if contribution was uniform. All results are from five DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

4.4 Discussion

4.4.1 Individual cow DMI

Using the current dataset, when considering the number of measurement variables required to produce a prediction by the equation, the amount of variance explained by this and the correlation between actual and predicted DMI the use of the NRC equation is preferable if an energetics back calculation equation was to be used. It is the most basic of the equation analysed, only requiring the measurement of 4% FCM, liveweight and week of lactation to be known, therefore ease of use is high to achieve accuracy similar or greater to the other equations.

To improve the accuracy of intake predictions by the energetic back calculation equation, multiple individual cow measurements of liveweight (kg), 4% FCM and protein (%) are important, along with multiple measurements of feed ME. Numerous measurements ensure that these important variables within the equation are a true representation of that individual cow, allowing for better predictions. The most important animal measurement variables in the equations used to predict DMI are lactation variables (week of lactation, 4% FCM, milk weight and milksolids (%)). Of energy requirements, lactation has the greatest metabolisable energy requirement each day (Van de Haar and St-Pierre 2006). The large energy requirement for lactation has been accounted for in each equation analysed and as it has such a large contribution to energy requirements in these equations, the lactation variables have a significant influence on the DMI predictions. Therefore, when taking animal measurements for use in energetic back calculation equations an emphasis on the accuracy of these lactation variable measurements and the frequency these measurements are taken at should be a primary focus.

Within this context none of the equations analysed are consistent or close enough to accuracy at an individual cow level to be used to predict individual grazing dairy cow daily DMI to be used in scientific research. A consequence of ME requirements within these equations differing from actual cow ME requirements. It is unknown why ME requirements are greater than what is used in the equations, but it is hypothesised that it may be in relation to error in determining feed ME, differing ME requirements between genetic strains and differing efficiencies between feeds and the use of ME for physiological processes (Mandok et al. 2013). Additionally, mechanisms controlling energy intake and partitioning are affected by both diet and physiological state, with lactation stage being a major influence and the effect

of feed characteristics differing with lactation stage (Allen 2014). Therefore, it is likely keeping the feed effect consistent across the lactation stages within the MPI GHG equations is a source of error.

4.4.1.1 NRC

Using the NRC (2001) equation trials with an *ad libitum* feed allowance have a greater correlation between actual and predicted individual cow DMI (kg DM/d) than trials with a targeted 90% of voluntary intake feed allowance. This is as expected as the daily DMI of the cattle consuming 90% of their voluntary intake are unable to consume as much as they would at *ad libitum*, therefore are more likely to have an overestimation of their actual DMI by that predicted. Trial AABN had the greatest correlation (62%), whilst trial B23N had the lowest (35%), these trials both used early lactation cows, along with similar feed ME, feed type, trial length and number of cows. Only differing in that AABN had the lightest mean liveweight of all trials (436 kg), B23N being 483kg, and differing feed allowances being *ad libitum* for AABN and 90% voluntary intake for B23N. This indicates that feed allowance and liveweight may be key influences in regard to the accuracy of the NRC equation.

The NRC equation does not account for diet characteristics, primarily focussing on animal characteristics, as DMI is also limited by the physical capacity of the digestive tract, with digestion rate and movement of indigestible particles through the digestive tract being highly related to feed quality and consequently feed type this may be a source of variation or error (Roca and Gonzalez 2013). Equations with both animal and diet effects included provide better DMI predictions than those with only animal effect included, when dairy cattle are fed TMR, as cattle grazing pasture must consume greater physical quantities to reach the same ME intake as those fed TMR, along with pasture having greater nutritional variation than that manufactured it is likely that the same effect if not greater would be seen in grazing dairy cattle (Dong et al. 2015; Jensen et al. 2015).

Another likely source of error, influencing the accuracy of the NRC equation, is the difference between cattle used to develop the NRC equation and those used within this study, along with differences in feed type. The NRC (2001) equation was developed using data from Holsten Friesian dairy cattle in North America over a 10-year period from 1988 to 1997. The majority were fed a TMR diet, with corn silage and alfalfa being the forage source used in

most experiments (NRC 2001). As a consequence of differing feed sources and genetic strains from different selection criteria, North American Holstein Friesian cattle have greater liveweights, and milk production but a lower milkfat content than NZ Holstein Friesians (Kolver et al. 2000; Harris and Kolver 2001; Patton et al. 2008). Although using this logic it would be expected that cattle with greater liveweights would have greater DMI prediction accuracy using the NRC equation, instead the current study has found trials using cattle with a lower mean liveweight have greater DMI prediction accuracy than those with a greater mean liveweight. In addition, cattle used to develop the NRC equation (2001) had a mean observed DMI of 22.3kg DM/d, whilst the current studies trials used for the lactating cow dataset have mean actual DMIs ranging from 14.23 to 18.39 kg DM/d. As previously discussed, cattle consuming TMR are able to consume less feed for the same ME than cattle consuming pasture, as TMR has a greater digestion rate and is less bulky than pasture.

4.4.1.2 MPI GHG

The correlation between actual DMI (kg DM/d) and that predicted by the MPI GHG equation on a trial basis, appears to be influenced by the liveweight of the cattle in the trail. AABN has the greatest correlation (49%) along with the lightest mean liveweight (436 kg), whilst trials B22X, B23N and AABA all have moderate correlations along with similar mean cow liveweights, with no other major similar aspects between these trials. The cow liveweight used in the MPI GHG equation is calculated as a weighted average of cow weight from three main dairy cow breeds from NZ dairy statistics, the cow weight used in the equation varies depending on year but has been no greater than 463.61 kg (Pickering et al. 2022). This may be why cattle in trials with a lighter mean liveweight have a greater prediction accuracy than those with a heavier mean liveweight. Trial AADO has a low correlation and has the heaviest mean liveweight (532 kg), along with the trial period being longer than the other trials (67 days). An assumption of the MPI GHG equation is that only pasture is consumed, with no supplementary or other forage types therefore the greater accuracy for trial AABN, the only trial where only pasture is consumed could be a consequence of this (Pickering et al. 2022).

The MPI GHG equation is based on the Australian Feed Standard's algorithms developed from free grazing ruminants, to reflect that the majority of livestock in NZ graze pasture, instead of the default IPCC algorithms based on grain fed cattle, farmed under very different circumstances than the ones in NZ (Pickering et al. 2022). The purpose of the equation is to

generate population level data on DMI for dairy cattle, so averaged animal production and feed data is used, therefore it is understandable that at an individual cow level accuracy of predictions are insufficient, although it is only liveweight that differs between my trial datasets and numbers used in the equation. With liveweight varying per year but being no greater than 463.609 kg and mean liveweight on a trial basis varying from 436 kg to 532 kg, all other variables used when calculating DMI are either assumptions derived from those used by MPI GHG or do not largely vary from those used (Pickering et al. 2022). Therefore, it is likely the lack of accuracy is deriving from the variation of animal factors that is at an individual cow level or there are errors within the assumptions used when developing the equations. As there is variance within how cattle are farmed in New Zealand simplifying to a population level would vastly decrease accuracy and the applicability of the MPI GHG equation.

4.4.2 Herd level

At a herd level all equations that were analysed produced DMI (kg DM/d) predictions closer to accuracy, than when used to predict individual cow DMI. This result is unsurprising, with as seen in Table 5.2 (Appendix B) the effect of individual cow is significant when energetic back calculation equations are used to predict individual cow DMI, therefore a source of unaccounted for variation within these equations when used at an individual level. The significance of individual cattle is likely a result of differing feed efficiencies, with a cow with a greater feed efficiency having a lower DMI than the average predicted from physiology and production, whilst an inefficient cow consumes a greater DMI (Waghorn and Hegarty 2011). In comparison this increase in overall accuracy of DMI predictions for the different trials when comparing individual cow with herd level is likely a result of animal characteristic averages being used, reducing variation that is present between measurements of individual cows and between days within cow. Along with the equations used being developed with herd level cow data. At this herd level the NRC equation appears to provide the most consistent close to accuracy DMI predictions across the trials, in comparison to the other equations. The increased accuracy of the NRC (2001) equation at a herd level in comparison to at an individual cow level may be a result of mean production data (days in milk, number of lactations, liveweight, milk and composition) being used to develop the model, not individual cow. Within the MPI GHG equation the trial AADO has a significant underestimation (47% difference between actual and predicted).

The Nicol and Brookes equation has previously been reported to be accurate at a herd or group level, there is, however, limited scientific publications on the usage of this equation for individual dairy cattle with limited liveweight change measurement. In the current study although this equation did not have continued use within analysis due to the large variation in energy requirements at an individual cow level with the inclusion of liveweight change, at a herd level there was a 78%-109% between actual and predicted DMI from the Nicol and Brookes equation, suggesting that its use at a herd level is appropriate. Future studies should be undertaken to investigate the use of this equation and the influence frequency of liveweight measurement has on prediction accuracy, to increase the applicability of the equation for intake and methane production estimation.

4.5 Limitations

The limitations of the results and analysis preformed must also be considered. Numerous assumptions were made to produce the datasets, to be either in accordance with the assumptions of a specific equation or as there was not data available for that measurement variable. Dependent on whether the assumption made was close to the real value, if data was unavailable may influence the accuracy of the prediction.

The equations used were formulated for the purpose of predicting intake at a herd level rather than at an individual cow level, hence the difference of accuracy in DMI predictions found for individual compared to herd averages. Due to limitations of time and the scope of the thesis, when analysis occurred at a herd level, this was done by considering the cows within an experiment as a herd. There will be further research with the datasets used, analysing the predictions of intake where herd is considered as a treatment group within an experiment. The accuracy of these intake predictions is likely to be high, as cows within treatment groups are evidently treated under the same conditions, but the effect of individual cow measurement variability is still minimised.

Only two energetic back calculation equations were analysed in full as there is a limited number of equations available suitable for use in pastoral systems, with even fewer suitable for use at an individual cow level. Neither of the equations used were applicable for non-lactating dairy cattle, as such only lactating cows were analysed.

The accuracy of individual cow DMI predictions that were found in this chapter are based on an analysis using correlations, linear models and a principal component analysis, through calculations using both assumptions and measured animal variables. It is recognised that results on accuracy may differ using different assumptions. An alternative analysis may also provide differing accuracy results.

Finally, the number of experiments and cows within the dataset is also a limitation. A greater number of trials would introduce a greater amount of variation between cows, along with potentially allowing for more data on cattle under the same or similar conditions. This would have allowed for stronger conclusions to be made, but due to confidentially, or trials not meeting the criteria for inclusion this was not done.

4.6 Suggested further research

Further analyses that were outside the scope of this thesis within the timeframe, but I believe would be highly beneficial include separating out treatment groups for each trial within the dataset, as this would likely provide clarification on which equation provides accurate DMI predictions for certain feed and cow characteristics. Along with analysing DMI predictions at a herd level and treatment herd level and whether the accuracy of these predictions are within a suitable range for scientific research. It is far more likely that herd level DMI predictions will have sufficient accuracy than at an individual level, as the equations used were created at a herd level, so validation of this with a large NZ dataset and multiple farming conditions would be highly beneficial.

Further research on the improvement of an equation so that there is a NZ specific energetic back calculation equation with sufficient accuracy for individual grazing dairy cow DMI prediction should be a primary focus. I personally believe that the NRC (2001) equation is best suited to this and using a large NZ individual cow dataset where lactating dairy cattle are fed pasture and small quantities of supplement to adapt the assumptions based on farming condition and cows in North America to NZ specific, would allow for an equation that produces DMI predictions with greater accuracy.

4.7 Conclusion

In conclusion the Nicol and Brookes, NRC and MPI GHG energetic back calculation equations do not satisfy the requirements for use in predicting DMI in scientific research at an individual cow level using the current analysis methodology, although there is potential to come into the range of accuracy when used at a herd level. At an individual cow level a single equation does not consistently provide predictions within the same range of accuracy across the trials and therefore differing feed, and animal characteristics including lactation stage and liveweight. This suggests that each equation is better to suited to a particular cow and feed, depending on the assumptions used to develop the equation. The accurate measurement of lactation variables should be a focus when collecting data that will be used with an energetic back calculation, as it has the most influence on prediction accuracy, in comparison to other variables used with the equations. Energetic back calculation equations used to predict DMI are an easier and cheaper method to predict DMI than other alternative methods used in research, as measurement variables used are often already being measured in a research context, therefore an accurate equation to predict individual cow DMI would be highly beneficial and should be a research priority.

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Appendices

Appendix A

Table 5.0: Summary of each DairyNZ experiment used within Chapter 4

Trial			Cows				Milk					Feed		
Trial name	Measurement period	Number of treatments	Number of cattle	Age	Liveweight (kg)	Days pregnant	Lactation stage	Milk weight (kg/day)	Fat (%)	Protein (%)	4% Fat corrected milk	Forage type	Supplement type	Allowance
Calan pasture intake (B23N)	10/10/2022-3/11/2022	2	40	4.3	483	3	Early lactation	17.17	4.75	3.47	28.39	Perennial ryegrass	Alpaca pellet	90% voluntary intake
Once daily milking (AADO)	15/01/201-25/04/2013	2	40	4.8	532	130	Late lactation	11.11	5.06	3.90	12.80	Perennial ryegrass and perennial ryegrass silage	NA	90% voluntary intake

Plantain research (B22X)	15/03/2021-1/04/2021	3	36	5.5	486	149	Late lactation	10.25	5.62	4.03	17.92	Perennial ryegrass and plantain	NA	*Ad libitum*
Body condition score supplement (AABE)	4/04/2011-9/05/2011	10	45	4.9	511	185	Non-lactating	NA	NA	NA	NA	Perennial ryegrass, perennial ryegrass silage and maize silage	Maize grain and PKE	6.5 kg DM/cow base rate perennial ryegrass, 2.5 kg DM/day or 5 kg DM/day supplement and silage
Summer Calan herb (AABA)	15/02/2011-8/03/2011	5	42	4.6	491	125	Late lactation	9.02	4.88	3.43	14.21	Pasture (Perennial ryegrass, white clover), plantain	NA	*Ad libitum*

												and chicory		
Feed conversion efficiency Calan (AABN)	15/09/2011/ - 5/10/2011	2	40	3.2	436	0	Early lactation	11.96	4.14	3.41	24.38	Perennial ryegrass	N/A	*Ad libitum*

Appendix B

Table 5.1: Parameter estimates and standard error for each variable in the all variable model (Table 4.1). Results are from five DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

Term	Estimate	Standard error	P value
Intercept	40.80	20.70	0.05
Liveweight (kg)	0.02	0.00	5.56 e-21
Age	4.72	0.77	1.02 e-9
Week of lactation	-0.14	0.02	1.62 e-9
Digestibility (kg/kg)	-5.92	1.10	8.69 e-8
4% fat corrected milk	0.06	0.01	2.47 e-8
Protein (%)	-0.59	0.13	5.12 e-6
Fat (%)	-0.10	0.05	0.03
Feed ME	-4.22	1.83	0.02
Days pregnant	0.01	0.00	0.04
Condition score	0.01	0.12	0.94
Mean individual cow	0.27	0.72	0.70
Feed type	-0.11	2.04	0.42

P. value is the significance of the measurement variable parameter in the all variable model (Table 4.1)

Table 5.2: Distribution of parameter estimates for individual cow effects from the all variable model (Table 4.4) and the corresponding SE and P. values for those estimates. Results are from five DairyNZ Calan gate studies using lactating Holstein-Friesian dairy cattle under a range of management conditions (see Appendix A for further detail on individual studies).

Statistic	Estimate	Standard error	P. value
Minimum	-13.50	4.30	1.68 e-3
First quartile	-6.61	2.60	0.05
Median	2.76	0.58	2.81 e-6
Mean	0.27	0.72	0.70
Third quartile	5.58	1.02	5.91 e-8
Maximum	11.42	1.66	7.3 e-12

P. value is the significance of the measurement variable parameter in the all variable model (Table 4.1)