Methane emissions from dairy heifers as affected by residual feed intake and breed

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Abstract

Reducing methane (CH_4) emissions without reducing milk production requires an improvement in feed conversion efficiency: that is an animal's efficiency in utilising feed for maintenance and production. Residual feed intake (RFI) is one measure of feed conversion efficiency; it can be defined as the difference between an animal's actual intake and its predicted intake based on its metabolic size and productivity. More efficient animals eat less than predicted (low RFI); inefficient animals eat more (high RFI).

Enteric CH_4 is an important source of digestible energy loss in ruminants, and research in beef cattle has reported a positive relationship between RFI and daily CH_4 production. Jersey (Jer) cows have also been reported to be more feed efficient than Holstein-Friesian (HF) cows. Thus, I hypothesized that high feed efficient (low RFI) animals would emit less CH_4 than the lower efficiency (high RFI) animals, and that Jer heifers would have lower CH_4 yield than HF heifers.

I measured the CH_4 emissions of 56 growing dairy heifers (20-22 mo old) in a 2 x 2 factorial arrangement: factors included two breeds (HF and Jer; n=28/breed) and two previously determined RFI categories (low RFI; -2.1 kg DM and high RFI; +2.0 kg DM; n=28/RFI category). All heifers were co-mingled and offered the same diet of dried lucerne cubes. Between RFI categories, heifers did not differ in body weight (BW) or BW gain (BWg); but low RFI heifers had 9.3% and 10.6% lower dry matter intake (DMI) and DMI/kg BW, respectively, than high RFI heifers. Similarly, RFI category did not affect CH_4/d or CH_4/kg BWg; but, CH_4/kg DMI was greater in low RFI heifers because of their lower DMI. These results might reflect more complete digestion of ingested feed in more efficient, low RFI heifers, consistent with previous reports of greater apparent digestibility of organic matter. Breed did not affect DMI/kg BW or BWg; Jersey heifers produced less CH_4/d, but not CH_4/kg DMI or CH_4/kg BWg. In conclusion, selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d), but may increase CH_4 yield (g/kg DMI).

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List of Abbreviations

ADF	Acid-detergent fibre
ATP	Adenosine triphosphate
BW	Body weight
$BW^{0.75}$	Metabolic body weight
BWg	Body weight gain
CH_4	Methane
CO_2	Carbon dioxide
CO_2-eq	Carbon dioxide equivalent
DM	Dry matter
DMI	Dry matter intake
FCE	Feed conversion efficiency
GHG	Greenhouse gas
HF	Holstein-Friesian
h^2	Heritability
IVGPT	In vitro gas production technique
Jer	Jersey
NDF	Neutral-detergent fibre
RFI	Residual feed intake
SF_6	Sulphur hexafluoride
VFA	volatile fatty acid
3NOP	3-nitrooxypropanol

Chapter 1 General Introduction

The world population increased by 3.2 billion people from 1970 to 2010 resulting in a 2.2% per year increase in the demand for agricultural products during that period (United Nations, 2009; Alexandratos and Bruinsma, 2012). In 2009, the world population was projected to increase by another 2.3 billion to reach 9.1 billion in 2050 (United Nations, 2009). Therefore, demand for agricultural products is expected to continue to increase.

The increases in demand associated with population growth are projected to be further enhanced by increases in per capita caloric consumption as well as by changes to diet composition in developing countries, as their populations move to include a greater proportion of higher quality, animal protein in their diet (see Figure 1.1). Overall, world consumption of agricultural products is expected to increase by 1.1% per year from 2005 until 2050 (Alexandratos and Bruinsma, 2012).

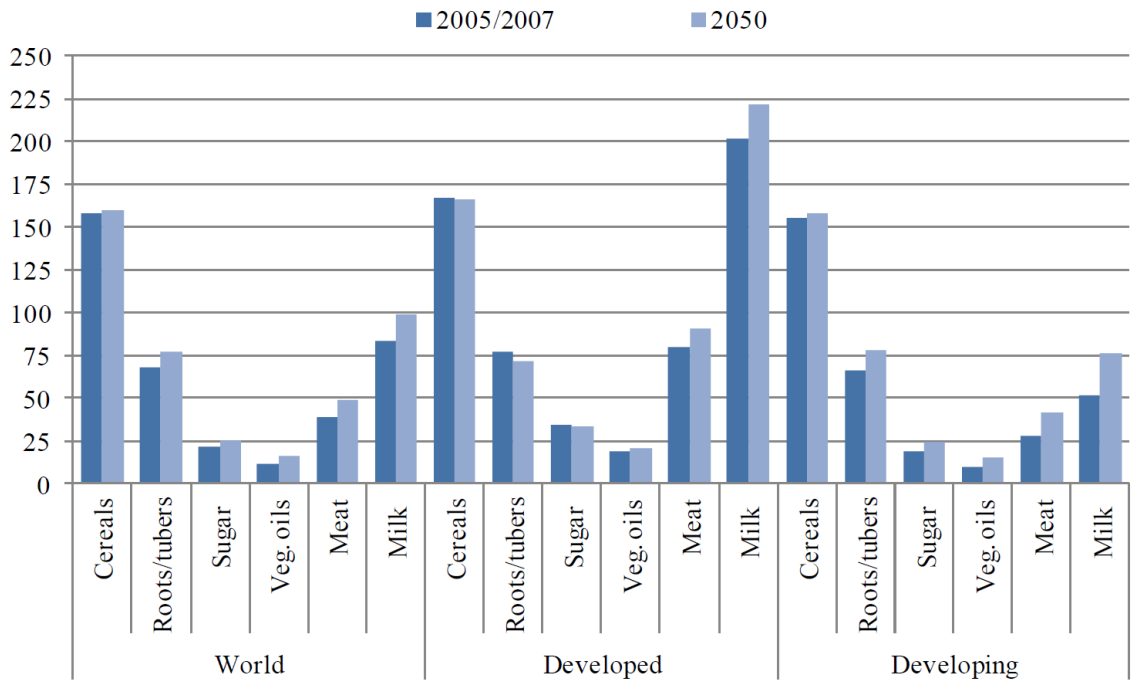


Figure 1.1. Food consumption per capita of major commodities (kg/person/year) comparatively in the developed vs. the developing world. Dark blue is actual values in 2005/2007. Light blue is projections for 2050 (Alexandratos and Bruinsma, 2012).

In line with this greater demand, there are significant opportunities for the New Zealand economy, which is, primarily, primary industry based. The dairy industry, on average, contributed $14.4 billion annually in export earnings over the past five years (New Zealand Institute of Economic Research, 2017). This corresponds to a $7.8 billion (3.5%) contribution to New Zealand's total gross domestic product (New Zealand Institute of Economic Research, 2017). In addition, the dairy sector plays an important role in supporting regional economic development, employing over 40,000 workers throughout New Zealand (New Zealand Institute of Economic Research, 2017). Dairy farmers also support numerous supply industries, spending more than $6.5 billion annually on agricultural services, fertilisers, forage crops, agricultural equipment, and financial services (Livestock Improvement Corporation and DairyNZ, 2017; New Zealand Institute of Economic Research, 2017; DairyNZ, 2018a).

However, the New Zealand dairy industry is being scrutinised for high greenhouse gas (GHG) emissions (Ministry for the Environment, 2018). Greenhouse gases are responsible for keeping the Earth's atmosphere warm enough for life; however, increasing concentrations of GHGs in the atmosphere are thought to be a primary cause of climate change (Forster et al., 2007; Ministry for the Environment, 2018). The three major GHGs are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide. To compare GHGs, emission data are presented in terms of CO_2 equivalents (CO_2-eq). Methane has a greater warming effect than CO_2; 1 kg CH_4 has a 100-year global warming potential of 25 kg CO_2-eq (Forster et al., 2007).

Agriculture and land use change (i.e. converting forest or permanent pasture to annual crops) are estimated to contribute 14 to 22% of global GHG emissions (Knapp et al., 2014). On a global scale, in 2007 the dairy sector emitted 1,970 million tonnes CO_2-eq, which accounted for 4% of total global anthropogenic GHG emissions (Gerber et al., 2010). However, in many developed countries the percent contribution of the dairy sector to GHG emissions sits below this 4% global average due to dilution of emissions from other sectors,

and high productivity of dairy cattle in developed countries compared with less developed countries (Hagemann et al., 2011; Knapp et al., 2014).

In countries such as New Zealand, however, where pastoral agriculture is a significant proportion of the economy, agriculture contributes a much larger portion of the total GHG emissions (Gerber et al., 2010; Hagemann et al., 2011; Knapp et al., 2014; Ministry for the Environment, 2018). In 2016, New Zealand produced 78,730 kt CO_2-eq of gross GHG emissions. Of this, the agriculture sector contributed the largest amount, producing 49% of New Zealand's gross emissions (Figure 1.2).

Within New Zealand's agriculture sector, the largest source of GHG emissions is enteric CH_4, accounting for 72% of the agricultural emissions in 2016 (Figure 1.2). Enteric fermentation is the largest contributor of CH_4 emissions, and dairy cattle were the source of 49% of enteric CH_4 emissions (Figure 1.2; Ministry for the Environment, 2018). Enteric CH_4 from dairy cattle alone contributed 13,620 kt CO_2-eq in 2016, which accounted for 17% of New Zealand's gross GHG emissions. Overall, these contributions are much greater proportions of the countries GHG footprint than other developed countries due to New Zealand's large contribution from the dairy sector to the economy, and thus lack of dilution of the emissions from other sectors (Knapp et al., 2014; Ministry for the Environment, 2018).

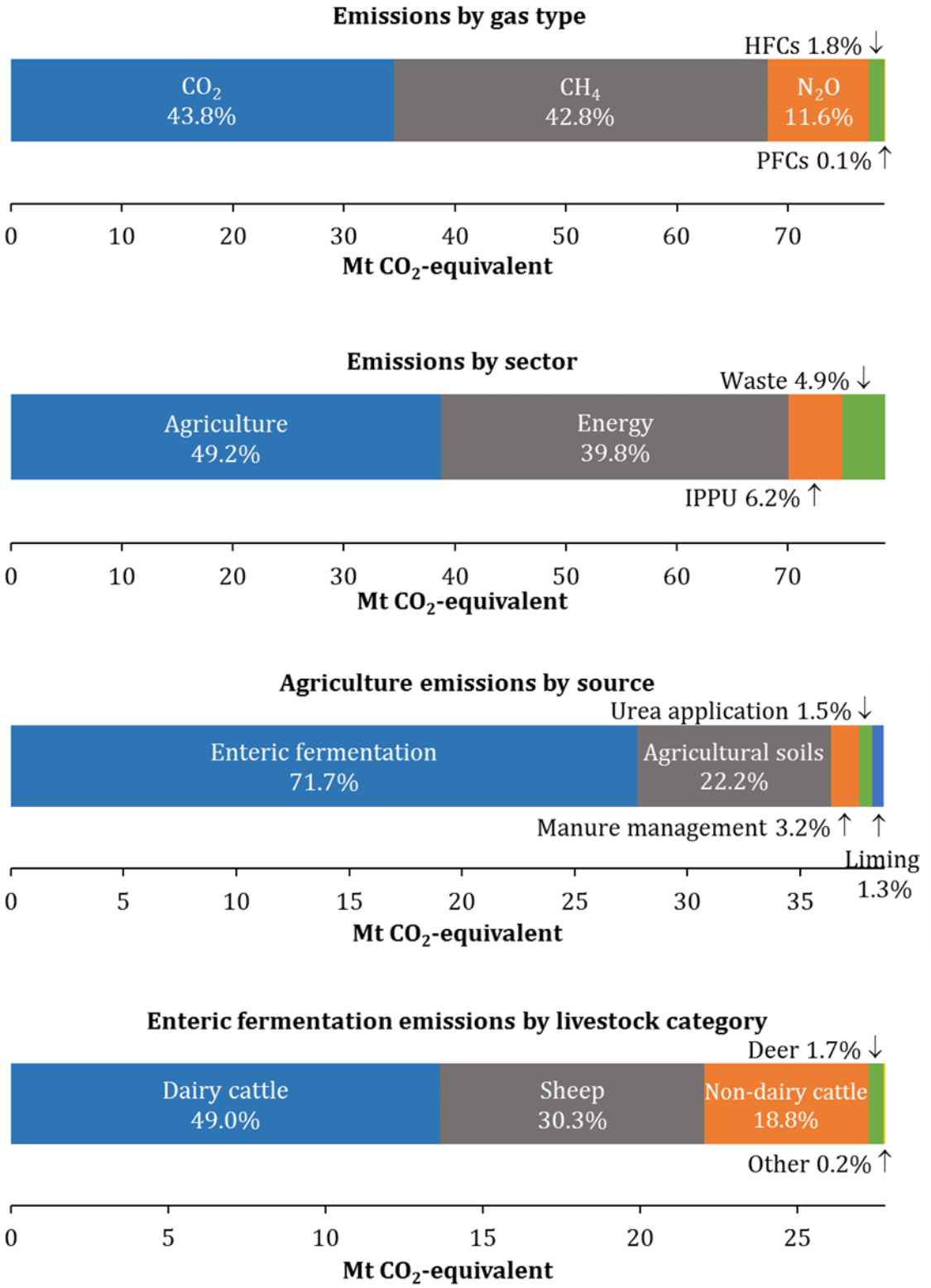


Figure 1.2. New Zealand's greenhouse gas emissions profile for 2016: gross emissions by gas type, gross emissions by sector, agriculture sector emissions by source, and enteric fermentation emissions by livestock category (Ministry for the Environment, 2018).

New Zealand's existing emissions target is to reduce total GHG emissions by 50% from 1990 levels by 2050 (New Zealand Institute of Economic Research, 2018). Achieving this target is expected to cost the country $28 billion and will require widespread innovation and changes in farming systems (New Zealand Institute of Economic Research, 2018). Because of the growing global population, it is unrealistic to reduce cow production to reduce CH_4 emissions.

Reducing CH_4 emissions without reducing milk production requires an improvement in feed conversion efficiency (FCE; Waghorn et al., 2012; Potts et al., 2015). There is a need to improve cattle feed efficiency to ensure that the demand for dairy and meat products by the ever-increasing global population can be met.

For these reasons, the primary objectives of this Masters thesis were to investigate the effect of both feed efficiency category, by the measure of residual feed intake (RFI), and dairy cattle breed on CH_4 production, yield, and intensity (Chapter 4). Residual feed intake is a measure of feed efficiency, which is estimated as the difference between an animal's actual dry matter intake (DMI) and its predicted DMI. A better understanding of the potential relationship between RFI category or breed and CH_4 emissions could open up the possibility for future genetic selection of environmentally-friendly and feed-efficient dairy cattle.

Chapter 2 Literature Review

2.1 Introduction

The purpose of this chapter is to review the scientific literature on the production of CH_4 in ruminant dairy cattle, and its relationship with RFI (a measure of feed efficiency) and cattle breed. The first section of the literature review introduces CH_4 in the New Zealand context and then details the digestion and metabolism of carbohydrates via enteric fermentation that leads to the production of CH_4. Particularly, I will highlight the major factors responsible for variation in CH_4 emissions, available CH_4 inhibitors, and the genetic correlations, heritability of CH_4 emissions, and differences in CH_4 emissions between dairy breeds.

The next section of the literature review introduces the concept of RFI, explains how it is estimated, the heritability of RFI, and the major factors known to contribute to variation in RFI. I then discuss the previous research surrounding CH_4 and RFI and any dominant trends apparent from this research.

I will conclude the literature review with my Masters research objectives.

2.2 New Zealand dairy farming system

New Zealand is in a unique position among OECD countries, in that grazed pasture is a major component of the diet for dairy cattle, and the nation's gross domestic product is heavily reliant on dairy exports' (Holmes et al., 2003; New Zealand Institute of Economic Research, 2017). The population of New Zealand was estimated at around 4.84 million at the end of 2017 (StatisticsNZ, 2018), and the dairy cattle population in New Zealand was estimated at 4.86 million in June 2017 (Figure 2.1; LIC and DairyNZ, 2017). In the 2016/17 season, New Zealand dairy companies processed 20.7 billion litres of milk containing 1.85 billion kilograms of milksolids (i.e., fat and protein; LIC and DairyNZ, 2017), of which more than

90% is exported (Burke and Verkerk, 2010). Because of the high proportion of exports, New Zealand is subject to a highly competitive and increasingly volatile world market milk price. Therefore, New Zealand milk producers must keep costs as low as possible to ensure that milk production is profitable (Holmes et al., 2003).

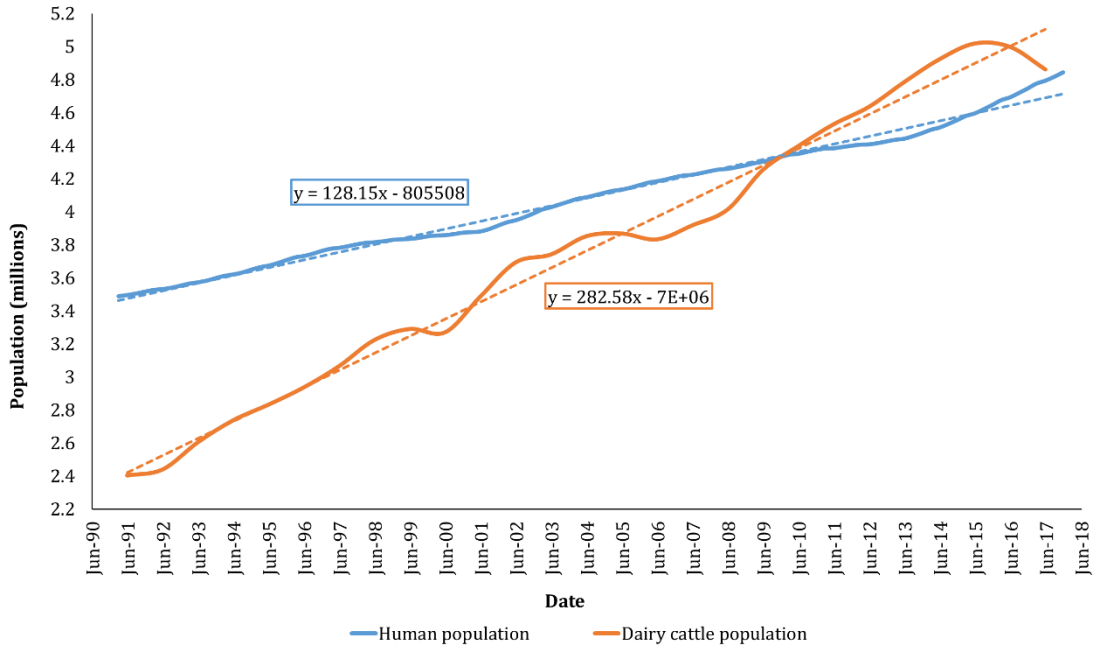


Figure 2.1. Human population and dairy cattle population of New Zealand from 1990-2017 (Livestock Improvement Corporation and DairyNZ, 2017; StatisticsNZ, 2018).

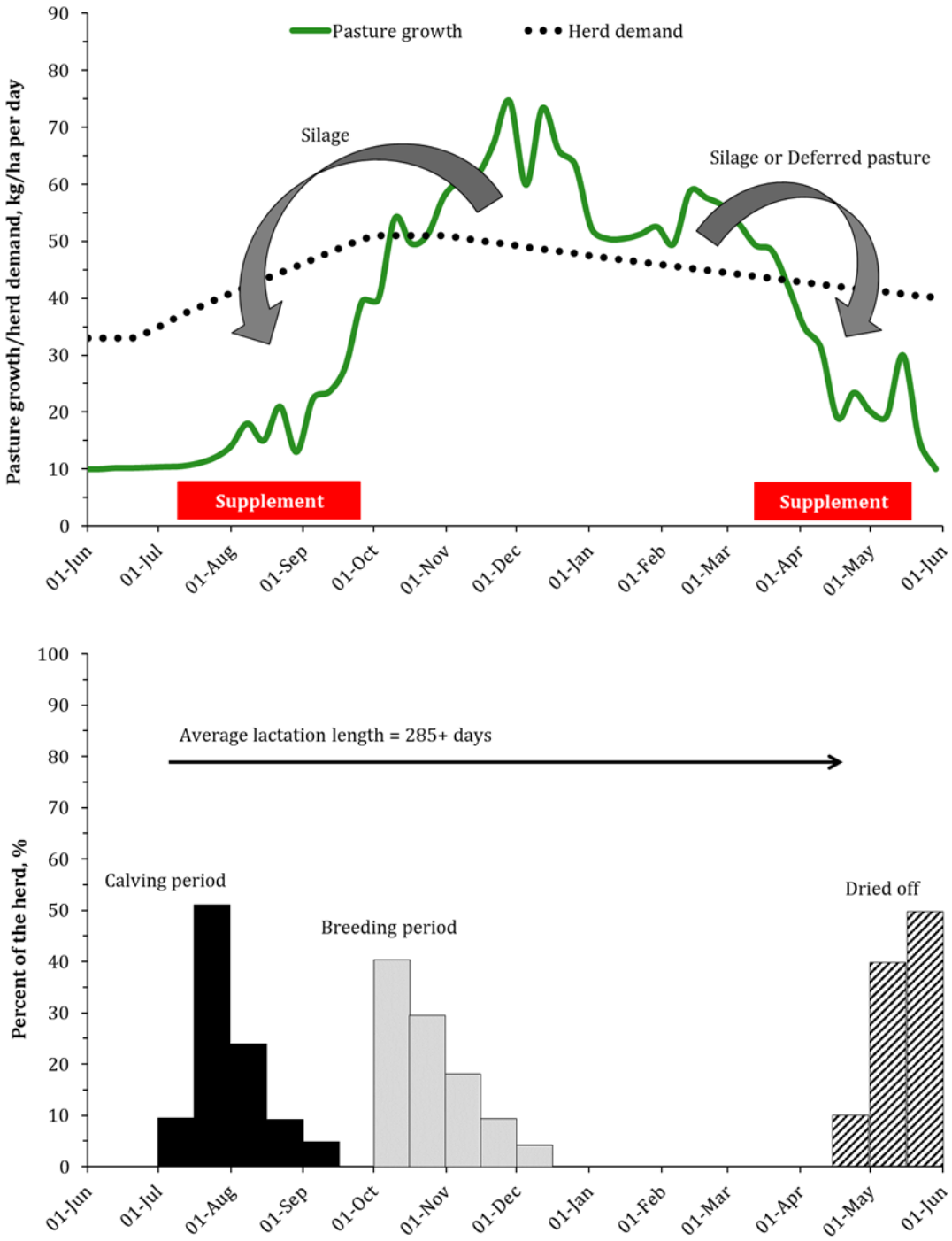
Due to the temperate climate and adequate rainfall in dairy regions of New Zealand, pasture growth rates and the environment are suitable to allow grazed pasture to make up the major component of the diet for dairy cattle. This is the case for only 10% of the world's cows (Holmes et al., 2003). As indicated in Figure 2.2, the average pasture growth rates in New Zealand are fastest over the spring months compared with any other time of the year. New Zealand's dairy system is based on seasonal production: the majority of cows calve in winter/early-spring, so that the peak milk production and hence highest feed demand by cows is during the spring where there is also an abundance of grass available (Holmes et al., 2003). Thus, this seasonal system synchronises herd feed demand with pasture growth (Figure 2.2). Pasture is a relatively cheap feed, especially when it is well managed (Roche et

al., 2017a) and, hence, most New Zealand farmers feed high amounts of pasture and lower amounts of non-pasture supplementary feeds.

2.2.1 New Zealand's unique greenhouse gas situation

In 2016, the agriculture sector was responsible for 49% of New Zealand's total GHG emissions (see Figure 1.2). In other developed countries, the agriculture sector typically contributes only around 12% of total emissions (Ministry for the Environment, 2018). The large proportion of GHG attributed to agriculture in New Zealand reflects the large contribution of pastoral agriculture to the economy, and thus lack of dilution of the emissions from other sectors (Knapp et al., 2014; Ministry for the Environment, 2018). In fact, because of our comparatively low population, New Zealand is the fifth highest emitter of GHGs per person globally (StatisticsNZ, 2017).

Due to the high populations of dairy cattle in New Zealand, the agriculture GHG emissions appear high per human capita. However, the high populations of dairy cattle also mean high production of dairy products. On average the emissions from the dairy industry on a global scale are estimated at 2.4 kg CO_2-eq/kg of fat and protein corrected milk (Gerber et al., 2010). In New Zealand, the animals are 'environmentally economic', with Oceania sitting below this rate at just over 1 kg CO_2-eq/kg of fat and protein corrected milk (Figure 2.3; Gerber et al., 2010).



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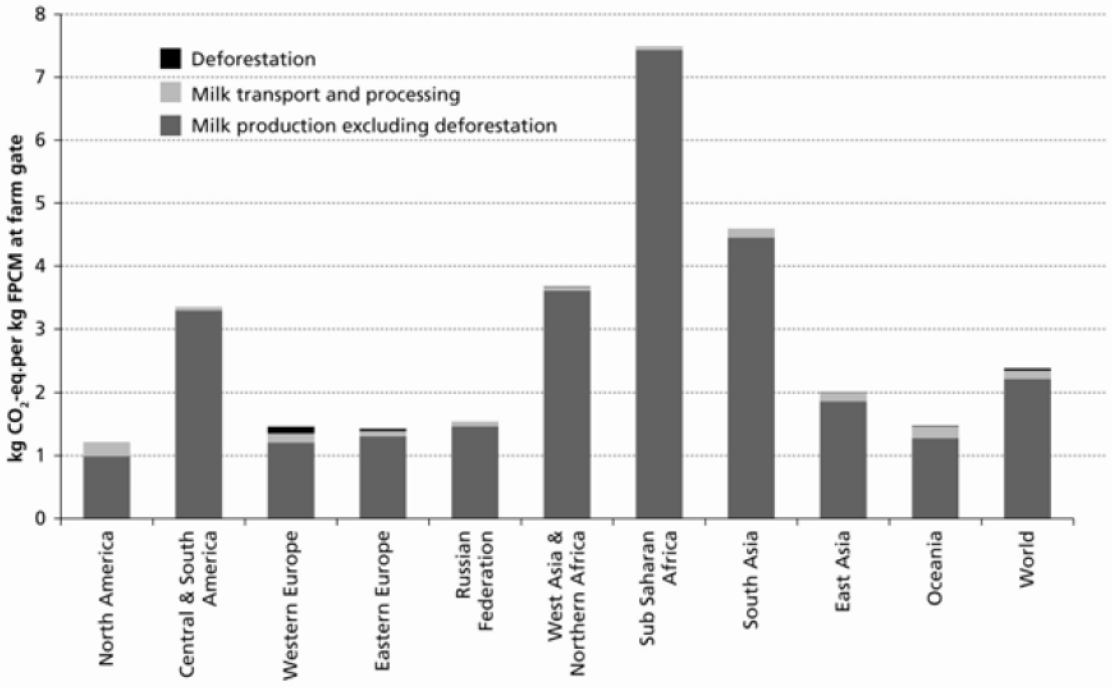


Figure 2.3. Estimated greenhouse gas emissions per kg of fat and protein corrected milk, averaged across the main regions of the world (Gerber et al., 2010).

In summary, New Zealand produces large amounts of GHGs per capita compared with other developed countries. However, this is due to a large cow population relative to the human population, and does not indicate an environmentally inefficient production system. Nevertheless, there is increasing pressure to reduce the industry's GHG footprint.

2.3 Methane

The New Zealand dairy industry is being scrutinised for high emissions of GHGs, particularly CH_4 (Ministry for the Environment, 2018). But CH_4 is a natural by-product of the unique digestive processes of foregut fermenters, like ruminant animals, and, so, is more than 50 million years in the evolutionary development of the species (van Soest, 1994).

Dairy cattle are ruminant livestock, a sub-order of mammals that digest feed initially via pre-gastric enteric fermentation. Enteric fermentation is the microbial breakdown of carbohydrates to facilitate growth, with products called volatile fatty acids (VFAs) that are absorbed by the animal and used for energy. Fermentation also results in the production of

microbial cells, which further nourish the animal with high quality protein, and waste products, such as the gases CH_4 and CO_2 (van Soest, 1994). Microbial fermentation occurs in the rumen (~90%) and large intestine (~10%; Murray et al., 1976; Lassey et al., 1997; de Haas et al., 2017). Enteric CH_4 is produced as a by-product and is released from the rumen into the atmosphere primarily through eructation (belching; McDonald et al., 2010).

Enteric fermentation of feed into VFAs is part of a reduction-oxidation (redox) cycle. The three main VFAs produced are acetate, propionate and butyrate. Producing acetate and butyrate from glucose creates excess reducing power (i.e., they are oxidative processes involving the loss of electrons/hydrogen). On the other hand, the production of propionate from glucose and production of CH_4 are reductive processes involving the gain of electrons/hydrogen; see Table 2.1. The redox balance (i.e. the excess hydrogen produced through the production of acetate and butyrate) is maintained partly through the production of propionate from glucose, but mainly through the production of CH_4 (Dijkstra et al., 2005). This means that as the acetate and butyrate-to-propionate ratio increases, the production of CH_4 will also increase, and vice versa (Table 2.1; van Soest, 1994). Variation in the fermentation pattern of VFAs lead to variation in the level of CH_4 emissions. These occur due to various mechanisms and will be discussed in Section 2.3.7.

Table 2.1. Theoretical stoichiometric carbon-hydrogen balance equations describing conversion of glucose in the rumen (van Soest, 1994).

Case 1
glucose $\rightarrow$ **2 acetate** + 2 CO_2 + **8 H**
glucose + **4 H** $\rightarrow$ **2 propionate**
glucose $\rightarrow$ **butyrate** + 2 CO_2 + **4 H**
CO_2 + **8 H** $\rightarrow$ **CH_4** + 2 H_2O
Net: 3 glucose $\rightarrow$ **2 acetate** + **2 propionate** + **butyrate** + 3 CO_2 + **CH_4** + 2 H_2O
Case 2: Acetate production increases threefold and propionate and butyrate are unchanged
3 glucose $\rightarrow$ **6 acetate** + 6 CO_2 + **24 H**
glucose + **4 H** $\rightarrow$ **2 propionate**
glucose $\rightarrow$ **butyrate** + 2 CO_2 + **4 H**
3 CO_2 + **24 H** $\rightarrow$ **3 CH_4** + 6 H_2O
Net: 5 glucose $\rightarrow$ **6 acetate** + **2 propionate** + **butyrate** + 5 CO_2 + **3 CH_4** + 6 H_2O

Note: In Case 1 the acetate-to-propionate ratio is 1:1 and the methane-to-glucose ratio is 1:3. In Case 2, acetate-to-propionate ratio is 3:1 and methane-to-glucose ratio is 3:5

2.3.1 Methane as an energy cost

Methane is not a usable energy for the animal, and is eructed as an unburned hydrocarbon (Lassey et al., 1997). The eructation of CH_4 represents approximately 7% of the animal's gross energy intake (van Soest, 1994; McDonald et al., 2010; Pacheco et al., 2014), and as such, can be regarded as an energy cost to the animal.

Global populations and subsequent demand for milk and meat are increasing. To meet this rising demand, animal productivity must continue to increase. Reducing CH_4 emissions from dairy cattle would increase energy efficiency through reducing energy losses. This means that energy efficient dairy cows would turn DM into milk more efficiently, thus increasing productivity and profitability (Waghorn and Hegarty, 2011; Pacheco et al., 2014).

In summary, it is important to reduce CH_4 emissions from enteric fermentation as this will reduce the amount of GHGs released into the atmosphere and, at least in theory, will

increase the productive efficiency of livestock (Pacheco et al., 2014), reducing pressure on other scarce resources, such as land and water.

2.3.2 Carbohydrates

Feed is comprised of water and dry matter (DM). The DM consists of both organic materials, such as carbohydrates, fats, proteins, and vitamins, and inorganic components (i.e., minerals; Figure 2.4). Ruminant livestock graze predominantly on plant tissue, which contains approximately 50-80% carbohydrate on a DM basis (van Soest, 1994).

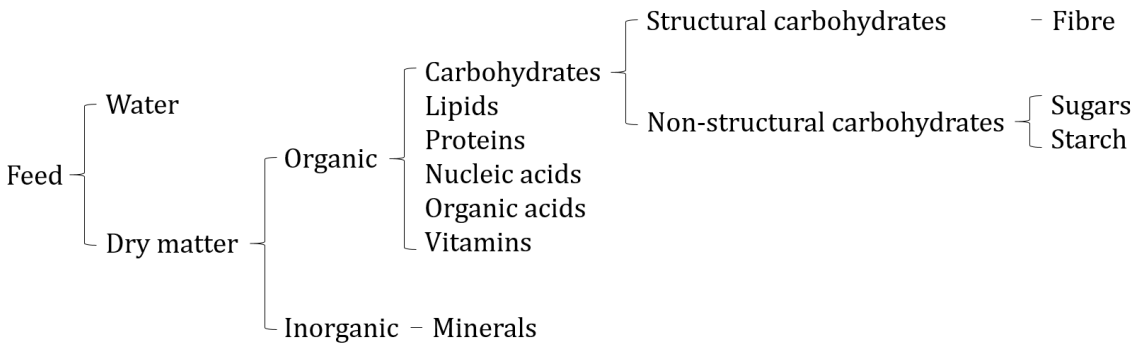


Figure 2.4. The various components of feed (adapted from McDonald et al. 2010).

The carbohydrate in plants consists of structural and non-structural carbohydrates. The non-structural carbohydrates are made up of the plant tissue cell contents, whereas the structural carbohydrates are made up of the cell wall components (McDonald et al., 2010). The non-structural carbohydrates are simple or monosaccharide sugars and starch (van Soest, 1994). The structural carbohydrates are fibre (see Figure 2.4). Fibre consists of cellulose, hemicellulose, and lignin. Polysaccharides are non-sugar carbohydrates, which are polymers of monosaccharide units. Starch, cellulose and hemicellulose are all polysaccharides of glucose (McDonald et al., 2010).

2.3.2.1 Non-structural carbohydrates

Non-structural carbohydrates are simple sugars and starch. Simple sugars are monosaccharides such as glucose and fructose. Also present are oligosaccharides, which are linked units of monosaccharides, such as sucrose, lactose and cellobiose. Starch is a

polysaccharide of glucose linked by α-glycosidic bonds (McDonald et al., 2010). Digestive enzymes, (i.e. amylase, sucrase, maltase and lactase) in the animal's gastrointestinal tract can enzymatically hydrolyse these α-glycosidic bonds, and thus the simple sugars and starch are readily digested into glucose (van Soest, 1994).

2.3.2.2 Fibre

Fibre consists of cellulose, hemicellulose, and lignin. Cellulose is the most abundant carbohydrate in the world, accounting for 20-40% of plant DM (van Soest, 1994). In contrast to starch, cellulose is a β-linked polysaccharide of glucose. Hemicellulose is a polysaccharide of glucose and other sugars, but with shorter chains than cellulose. The β-(1,4)-glycosidic bonds in fibre cannot be broken down by mammalian enzymes. Ruminants have adapted a digestive system that involves pregastric microbial fermentation to break these bonds. Therefore, the ruminant digestive system can break down fibre efficiently, whereas the monogastric digestive system cannot (McDonald et al., 2010).

The evolution of the ruminant system to digest fibre is an important tool for the utilisation of large quantities of otherwise indigestible feed (van Soest, 1994).

2.3.3 Ruminant digestive system

Ruminant animals have a four-chambered stomach, consisting of the rumen, reticulum, omasum, and abomasum (Figure 2.5). The rumen is the first and largest compartment, and is the site of microbial fermentation. Bacteria are present in the rumen, with populations of around 10^9-10^{10} bacteria per mL of rumen contents. Over 200 species of rumen bacteria have been identified (McDonald et al., 2010). Protozoa and fungi are also present in the rumen but in much smaller numbers.

Figure 2.5. Diagram of the ruminant four-chambered stomach. Arrows indicate direction of flow of digesta (McDonald et al., 2010).

Feed entering the rumen is partially fermented by these microorganisms, through the cleavage of the glycosidic bonds. Rhythmic contractions of the rumen walls assist this chemical breakdown to mix the microorganisms through the digesta.

The digesta also undergoes physical breakdown through the process of rumination (chewing of the cud). The digesta at the anterior end of the rumen are contracted through the oesophagus into the mouth where it can be thoroughly chewed and swallowed again. This helps to reduce particle size and extract soluble content for fermentation (van Soest, 1994; Clauss et al., 2010). Clauss et al. (2010) reported that ruminants produce finer faecal particles than hindgut fermenters and non-ruminant foregut-fermenters of comparable body size, as indicated by Figure 2.6. The amount of time spent ruminating varies depending on diet. In grazing cattle, the time spent ruminating is about equal to the time spent grazing (around 7-9 hours per day; McDonald et al., 2010; Sheahan et al., 2011). Undigested feed and microbial cells continue through the digestive tract.

The omasum is primarily responsible for water absorption (Moran, 2005). The abomasum and small intestines are equivalent to the stomach and small intestine of monogastric animals, where material is digested via enzymatic action and products are absorbed. The large intestine is the site for post-gastric microbial fermentation. Any undigested material leaving the large intestine is excreted in the faeces.

Figure 2.6. Mean particle sizes of faeces in mammalian hindgut fermenters, non-ruminant foregut fermenters, and ruminants of varying body size (Clauss et al., 2010).

2.3.4 Carbohydrate digestion and metabolism

The majority of a ruminant's energy (around 70%) is provided by carbohydrates (Moran, 2005). The process of carbohydrate digestion produces usable energy, in the form of VFAs, along with the gases CO_2 and CH_4. The VFAs produced are mainly acetate, propionate and butyrate, with isobutyrate, valerate, isovalerate, 2-methylbutyrate and others present in small amounts (Dijkstra, 1994). Some of the CO_2 produced can be used to maintain bicarbonate levels in saliva, but CH_4 cannot be used by the cow in any way. These gases are lost by eructation (belching). Therefore, the production of these gases is a by-product of enteric fermentation and is a source of energy loss from the system.

Carbohydrate digestion occurs in two stages, which are illustrated by the pathways in Figure 2.7. The first stage is the decomposition of complex carbohydrates to simple sugars (McDonald et al., 2010). This is the breakdown of the polysaccharides and oligosaccharides into monosaccharides or simple sugars, occurring in the rumen. For example, cellulose is decomposed into cellobiose through the action of β-1,4-glucodiases cleaving the β-1,4-glycosidic bonds. The cellobiose is then converted to glucose or glucose-1-phospate by a phosphorylase (McDonald et al., 2010).

The second stage of carbohydrate digestion is converting the products into energy. The simple sugars produced (i.e. glucose, fructose, maltose) are immediately converted to pyruvate by glycolysis. This is known as the Embden-Meyerhof pathway (Moss et al., 2000; Dijkstra et al., 2005; McDonald et al., 2010). One mole of glucose forms two moles of pyruvate, as presented in equation 1 below (McDonald et al., 2010).

$$\text{Glucose} \rightarrow 2\,\textbf{pyruvate} + 4\,\text{H} \quad (1)$$

Pyruvate is an intermediate product in carbohydrate metabolism. Pyruvate can be metabolised to form any of the VFAs, which can be readily absorbed through the rumen wall. The major VFAs and CH_4 are produced through chemical equations 2-5 (van Soest, 1994; Moss et al., 2000; Dijkstra et al., 2005).

$$\text{Pyruvate} + H_2O \rightarrow \textbf{Acetate} + CO_2 + 2\,\text{H} \quad (2)$$

$$\text{Pyruvate} + 4\,\text{H} \rightarrow \textbf{Propionate} + H_2O \quad (3)$$

$$2\ \text{pyruvate} \rightarrow \textbf{Butyrate} + 2\,CO_2 \quad (4)$$

$$CO_2 + 8\,\text{H} \rightarrow \textbf{CH}_\textbf{4} + 2\,H_2O \quad (5)$$

Figure 2.7. Pathways of carbohydrate metabolism in the rumen (van Soest, 1994).

Acetate is the main VFA produced in carbohydrate digestion. Both acetate and butyrate are formed through the tricarboxylic acid cycle (Dijkstra et al., 2005). This is through the conversion of pyruvate to acetyl-Coenzyme A, then through to acetate and butyrate. Propionate can be formed in several ways, as illustrated in Figure 2.7. The first is through

the formation of succinate, which is used more during the digestion of fibrous diets. The second pathway, through lactate and acrylate, is used more during the digestion of concentrate diets (McDonald et al., 2010).

The VFAs produced are absorbed through the rumen wall and transported to the liver or other tissues in the bloodstream where they are converted to adenosine triphosphate (ATP) and provide energy for the animal, and the gases are eructed via the mouth.

The conversion of acetate to ATP is as follows: acetate is first converted to acetyl-coenzyme A in the presence of acetyl-coenzyme A synthetase, then the acetyl-coenzyme A is metabolised via the tricarboxylic acid cycle, releasing energy in the form of ATP. Butyrate varies slightly in that it is first converted to β-hydroxybutyrate in the rumen before being absorbed across the rumen wall; from there it follows the same conversion process to ATP as acetate. Acetate and butyrate are used as energy by a wide variety of tissues, with butyrate especially important in delivering energy to skeletal and heart muscle (van Soest, 1994). The net energy yields for acetate and butyrate are 8 moles ATP per mole of acetate and 20 moles ATP per mole of butyrate (McDonald et al., 2010).

The majority of absorbed propionate is converted to glucose in the liver via gluconeogenesis. The propionate is first converted to succinyl-coenzyme A, which then enters the tricarboxylic acid cycle to form malate. Malate is converted to oxaloacetate then phosphoenolpyruvate, which is then converted to glucose. The glucose is used to provide the energy, and the net energy gain is 13.5 moles ATP per mole of propionate (McDonald et al., 2010). So, from 1 mole of pyruvate, the rumen can produce either 8 moles of ATP from acetate, 10 moles of ATP from butyrate, or 13.5 moles of ATP from propionate.

The energy produced is used for maintenance, activity, lactation, body condition, pregnancy and growth (Moran, 2005).

2.3.5 Methane production

As previously outlined, the production of VFAs are part of a reduction-oxidation (redox) cycle. Producing acetate and butyrate from glucose creates excess reducing power (i.e., the loss of electrons). The redox balance is maintained partly through the production of propionate from glucose, but mainly through the production of CH_4 (Dijkstra et al., 2005).

Methane production, or methanogenesis, is undertaken in the rumen by bacteria known collectively as methanogens, and is essentially the reduction of CO_2 to CH_4 by hydrogen; however, the reaction occurs via a complicated pathway (McAllister et al., 1996; Boadi et al., 2004; McDonald et al., 2010).

Methanogenesis occurs via four reductive intermediates; formyl, methenyl, methylenyl, and methyl. It has been hypothesised that these compounds are not present as free intermediates, but are instead bound to a series of coenzymes throughout the process (McAllister et al., 1996). There are seven steps in the reduction process, which to date is said to include six coenzymes (Figure 2.8).

- In step 1, CO_2 is fixed with methanofuran (MFR) to produce formyl-MFR.
- Next, the formyl group is transferred to tetrahydromethanopterin (H_4MPT) to form 5-formyl-H_4MPT (step 2).
- Over steps 3-5, 5-formyl-H_4MPT is reduced to methenyl-H_4MPT, then to methylene-H_4MPT, and finally to methyl-H_4MPT. These steps are assisted by coenzyme F420.
- From methyl-H_4MPT, the methyl group is transferred to coenzyme M (HS-CoM) to produce methyl-coenzyme M (step 6).
- In step 7 the methyl-coenzyme M is reduced to CH_4 by methyl-coenzyme M reductase. This reaction completes the cycle by formation of CH_4, and also encourages the formation of formyl-MFR to produce more CH_4 (Rouviere and Wolfe, 1988; McAllister et al., 1996).

Figure 2.8. Proposed mechanism for methanogenesis – the reduction of CO_2 to CH_4 by methanogens (Rouviere and Wolfe, 1988).

2.3.6 Methane phenotypic units

Methane emissions can be evaluated by several phenotypic units such as daily CH_4 production (g/d per cow), CH_4 yield (g CH_4/kg DMI), or CH_4 intensity (g CH_4/unit of production). This section will discuss the strengths and weaknesses of each CH_4 phenotype (Table 2.2), and the correlations between the phenotypes (de Haas et al., 2017).

2.3.6.1 Daily methane production

Daily CH_4 production measured in grams or litres per cow per day is the absolute value of the CH_4 being released into the atmosphere and is the end trait that must be improved in order to reduce climate change effects (Robertson and Waghorn, 2002; de Haas et al., 2017). However, daily CH_4 production is highly correlated with DMI (Lassey et al., 1997) and, thus, selection for low CH_4 production is likely to mean selection for low animal production. With increasing human population and subsequent demand for animal products, there is a

substantial argument against the logic of reducing animal production; thus there is a need to consider alternative CH_4 phenotypes.

In grazing systems, there is a further consideration with reducing CH_4 production per animal. Although this strategy may reduce the daily CH_4 output per animal, farmers may increase their stocking rate to maintain milk output per hectare to counteract the assumed reduction in genetic merit for milk production in the low CH_4 emitting cows. Therefore, there will be more cows (and replacement animals) per ha, all of which have basal metabolism requirements. This would likely result in more total CH_4 per farm, even though the daily CH_4 per animal is less.

2.3.6.2 Methane yield and intensity

Methane yield measured per unit of input or feed intake, or CH_4 intensity measured per unit of output as body weight gain or milk production take into consideration the animals' 'return' of valuable product for the CH_4 produced (de Haas et al., 2017).

Although these phenotypes are not what regulators desire, they are of more interest to farmers, in that they consider the production of (detrimental) CH_4 per (positive) output. However, changes in an animals' DMI or production will considerably change these ratios and hence the phenotypic measure; for example, a higher production animal will, by default, produce less CH_4/kg milk because:

a. The CH_4 produced in association with maintenance is diluted by the additional products
b. The increase in DMI results in faster passage rate and a reduction in CH_4 production/kg DMI

However, these phenotypes do not measure the total CH_4 emitted into the atmosphere. Cows that produce less CH_4 per input or per production, but have higher intakes and production, may produce higher CH_4 overall. As an absolute measure, this means that they

are 'worse' for the environment and, although this is offset by higher production, they are still releasing more GHG into the atmosphere.

Table 2.2. Several CH_4 phenotypes with their definitions, units, strengths, and weaknesses (adapted from de Haas et al. 2017).

Trait	Definition	Unit	Strength	Weakness
Methane production	CH_4 production per day	g CH_4/d or l CH_4/d	Absolute value that needs improving	High correlation with intake and production
Methane yield	CH_4 per unit of intake	g CH_4/kg DMI	Phenotype of interest for the user	Ratio trait. Not an 'absolute' measure of CH_4
Methane intensity	CH_4 per unit of output	g CH_4/kg BWg or g CH_4/kg MS	Phenotype of interest for the user	Ratio trait. Not an 'absolute' measure of CH_4

2.3.7 Variation in methane emissions

The level of CH_4 emissions in cattle is affected by a range of factors (Waghorn and Hegarty, 2011). Methane emissions vary based on an animals' DMI level, diet composition, the quality and processing of the diet, the rumen microbial populations, and an animals age (Beauchemin et al., 2008; Grandl et al., 2016). There is also a level of genetic variation between animals which causes variation in CH_4 emissions (de Haas et al., 2011), which will be discussed in Section 2.3.9.

Variation in the fermentation pattern of VFAs lead to variation in CH_4 emissions. Moss et al. (2000) reported Equation 6 for estimating the moles of CH_4 produced from the molar percentages of acetate, propionate, and butyrate. This indicates that increasing acetate or butyrate production will increase CH_4 production, whereas increasing propionate production will decrease CH_4 production (Moss et al., 2000). Changes in the fermentation pattern arise predominantly through the diet and are discussed throughout Section 2.3.7.1.

$$\text{mol } CH_4 = 0.45 \text{ mol Acetate} - 0.275 \text{ mol Propionate} + 0.40 \text{ mol Butyrate} \quad (6)$$

The acetate-to-propionate ratio can vary from around 0.9-4.0, meaning that CH_4 production and energy losses vary greatly also (between 2-12% of digestible energy; Johnson and Johnson, 1995). At very low acetate-to-propionate ratios (i.e. 0.5), CH_4 production is 0% of digestible energy, as the hydrogen released during the production of acetate are used in the production of propionate. On the other hand, if acetate-to-propionate ratios are very high (i.e. if all feed is digested to acetate and no propionate is produced) CH_4 production could be up to 33% of digestible energy.

2.3.7.1 Diet

The main dietary factors affecting CH_4 emissions are: DMI, diet composition, quality and processing of feed, and the rumen microbial population.

2.3.7.1.1 Dry matter intake

Dry matter intake is a major driver of CH_4 emission levels (Storm et al., 2012). Increased DMI increases total CH_4 production, but, generally, reduces digestibility and CH_4 emissions per kg DMI (Lassey et al., 1997). Increased DMI results in faster passage rates and a more rapid turnover of rumen contents. Increased passage rates of digesta create a time restriction for methanogenesis. This means that CH_4 production decreases per kg DMI (van Soest, 1994).

2.3.7.1.2 Diet composition

Methane emission levels are also affected by diet composition and type of carbohydrate (Sejian et al., 2011). Enteric CH_4 is produced during microbial fermentation of carbohydrates. Carbohydrates include cellulose, hemicellulose, starch, and sugars, as described in Section 2.3.2. Different types of carbohydrates are digested by different microbes into different end-products. The type of carbohydrate ingested can change rumen microorganism populations and ruminal pH.

- High fibre diets promote the production of bacteria that digest cellulose, such as *Ruminococcus flavafaciens* and *Fibrobacter succinogenes*.

- High starch and sugar diets encourage the growth of bacteria that digest these substrates, i.e. *Streptococcus bovis* and *Bacteroides ruminicola* (van Soest, 1994).
- Concentrate diets promoting high protozoal biomass increase protozoa (i.e. *Entodiniomorphs spp*.) populations in the rumen (Dijkstra et al., 2005).

As presented in Table 2.3, the type of carbohydrate digested affects the relative molar proportions of VFAs produced. Highly fibrous diets (i.e. cellulose) produce high proportions of acetate and butyrate-to-propionate. This would be expected to increase the reducing power and hence lead to high emissions of CH_4. On the other hand, diets of predominantly non-structural carbohydrates (i.e. starch and sugars) produce relatively high propionate, and low butyrate, which would decreases CH_4 emissions (Dijkstra, 1994; Knapp et al., 2014).

Table 2.3. Stoichiometric parameters for the fermentation of substrate in the rumen (moles VFA produced per mole substrate fermented) for roughage diets (>60% roughage) (Dijkstra, 1994).

	Acetate	Propionate	Butyrate	Valerate
Cellulose	1.32	0.17	0.23	0.03
Hemicellulose	1.13	0.36	0.21	0.05
Protein	0.40	0.13	0.08	0.33
Starch	1.19	0.28	0.20	0.06
Sugars	1.38	0.41	0.10	0.00

2.3.7.1.3 Quality and processing of feed

The quality of feed and processing of feed affect the passage rate of feed (van Soest, 1994; Knapp et al., 2014). Higher quality feed is typically more digestible and usually contains less fibre, subsequently increasing the passage rate of feed (Pacheco et al., 2014). The processing of feed such as pelleting or grinding decreases the rumination frequency required and can increase feed intake, again leading to increased passage rates. Therefore, higher quality or pelleted/ground feed will also reduce CH_4 emissions per kg DMI.

However, in New Zealand, high quality pasture is highly digestible yet contains a high quantity of fibre (Roche et al., 2009). The temporal trends for the concentrations of neutral-detergent fibre or NDF (■; cellulose, hemicellulose and lignin) and acid-detergent fibre or ADF (◆; cellulose, lignin and silica) are presented in Figure 2.9. The difference between NDF and ADF is an estimate of the hemicellulose concentration (van Soest, 1994). The figure also indicates hemicellulose as a percentage of NDF (▲). As far as fibre goes, hemicellulose is highly digestible and rapidly fermented, cellulose is digestible, but more slowly digested, and lignin is indigestible (van Soest, 1994; Roche et al., 2009).

Over the period of the year, the pasture nutritive value is lowest over the summer months, when NDF is at its peak, and the hemicellulose as a percentage of NDF is at its lowest. This also coincided with the lowest organic matter digestibility of 70-75%. Conversely, the nutritive value of pasture is highest over the spring period when NDF is lowest and hemicellulose as a percentage of NDF is at its highest. This also coincided with the peak organic matter digestibility of 80-85% (Roche et al., 2009).

Therefore, due to higher proportions of hemicellulose, high quality New Zealand spring pastures are highly digestible, but contain high fibre. Robertson and Waghorn (2002) investigated the seasonal variation in CH_4 emission from dairy cows in New Zealand, and reported that CH_4 yield (g/kg DMI) was lowest in the spring season when pasture was most digestible (18.0 g/kg DMI in September, compared with 22.2 and 23.8 g/kg DMI in December and March, respectively). The CH_4 intensity results followed a similar trend, with a low of 11.7 g/kg milk in September, compared with 19.4 and 24.3 g/kg DMI in December and March, respectively (Robertson and Waghorn, 2002).

Figure 2.9. Temporal trends in the concentrations of NDF (■) and ADF (◆), and hemicellulose as a percentage of NDF (▲) in pasture. Least square means for each variable are depicted without connecting lines, while the cosine functions are included in the figure within connecting lines among data points (Roche et al., 2009).

2.3.7.1.4 Rumen microbial population

Another determining factor for variation in CH_4 emissions is the variation in the rumen microbial population. The diet composition and rumen pH are the major determining factors for the number and type of microorganisms populating the rumen (Dijkstra et al., 2005). The rumen microbe populations influence the VFA proportions produced and the CH_4 production rates. For example, lower ruminal pH is associated with reduced CH_4 emission rates (Sejian et al., 2011).

Rumen microorganisms are selective digesters. This means that different microbes ferment specific substrates into different end products, as presented in Table 2.4. The fermentation pattern varies within the same diet depending on rumen microbial populations (Dijkstra, 1994). For example, Ruminococcus flavafaciens and *Butyrivibrio fibrisolvens* are both microorganisms that digest fibrous substrates such as cellulose. Both microbes produce

acetate as an end product; however, only *B. fibrisolvens* produces butyrate (van Soest, 1994). This means that larger populations of *B. fibrisolvens* compared with *R. flavafaciens* would increase butyrate and decrease acetate production for the same fibrous diet, leading to reduced CH_4 production. Another example is through the soluble carbohydrate (starch) fermenters *Bacteroides ruminicola* and *B. amylophilus*. Both microorganisms produce formate, acetate and succinate, but only *B. ruminicola* produces propionate. This means that for a predominantly starch diet, animals with high rumen populations of *B. ruminicola* would produce higher amounts of propionate and, by association, reduced CH_4, than those with rumen populations of *B. amylophilus*.

Methanogenic bacteria populations have a direct influence on CH_4 production rates. Methanogens are sensitive to the rumen environment and so changes to diet and rumen pH can affect methanogen populations. Diets that require less rumination time and have a faster passage rate reduce CH_4 production because the digesta pass from the rumen too quickly for methanogenesis to take place. Low ruminal pH is also an unfavourable condition for methanogens (van Soest, 1994). As methanogens are responsible for the production of CH_4, unfavourable conditions directly decrease the CH_4 production rate. This also shifts the redox balance toward propionate production (van Soest, 1994).

Ruminal protozoa tend to produce acetate and butyrate over propionate (Dijkstra et al., 2005). An association between rumen ciliate protozoa and methanogens has been reported (Dijkstra, 1994; Johnson and Johnson, 1995). The results suggest that methanogens attach to the protozoa, which enables a rapid hydrogen transfer. The rapid hydrogen transfer allows more acetate to be produced by protozoa. This means that the same substrate digested by protozoa yields more acetate and butyrate, and less propionate, than it would if digested by bacteria (Dijkstra, 1994; van Soest, 1994). As a result, CH_4 emissions are reportedly reduced by an average of 13% in the absence of ruminal protozoa (Sejian et al., 2011).

Table 2.4. Major species of rumen microorganisms and their substrates, products, and requirements (van Soest, 1994).

	Substrate										
Species	C	Hm	Pectin	Starch	Sugars	Lipids	Protein	Acids	H_2	Products	Requirements
Structural CHO fermenters											
Ruminococcus albus	H F C	F X								1,2,Et,H_2,CO_2	NH_3,CO_2,Br,V,2±
R. flavafaciens	H F C	F X								1,2,Su,H_2,CO_2	NH_3,CO_2,Br,Sta
Fibrobacter succinogenes	H F C	H Hm		F Dx						1,2,Su	NH_3,CO_2,Br,2,5,V,Sta
Butyrivibrio fibrisolvens	H F C	F X					F Pr			1,2,4,Et,La,H_2,CO_2	NH_3,CO_2,Br,V,Sta
Eubacterium cellosolvens	H F C	F X					F Pp			1,2,4,La,CO_2	
Pectinolytic species											
Succinivibrio dextrinosolvens		F Pn	F Pc							1,2,Su,La	Sta
Lachnospira multiparus	F Cb		F Pc							1,2,Et,La,CO_2,H_2	2,V,Sta
Nonstructural CHO fermenters											
Bacteroides ruminicola	F Cb	F X	F Pc	F S	F Hx		F Pr			1,2,3,Su	
B. amylophilus				F S			H Pr			1,2,Su	NH_3,CO_2
Selenomonas ruminantium	F Cb	F Pn		F S	F Hx	F Gl	F Pr			2,3,4,Su,La,H_2	2,CO_2±
Streptococcus bovis	F Cb		H Pc	F S	F Hx		F Pr			1,2,Et,La	
Succinomonos amylolytica				F S	F G					2,4,5,Su,H_2	
Eubacterium limosum	F Cb	F Pn	F Me		F G Fr			F La	U H_2	2,4	
Megasphaera elsdenii				F Ml	F Su	F Gl	F Pp	F La		2,3,4,5,6,H_2,CO_2	
Lipolytic species											
Anaerovibrio lipolytica					F Fr	F Tg	A	F La		2,3,Su,CO_2,H_2	A,V
Proteolytic species											
Peptostreptococci sp.					Fr		F Pr A			2,4,Br,NH_3,CO_2	
Clostridia sp.	F Cb	F X±	(F Pc)	F S	F Sc Fr		F Pr A			1,2,4,Br,Et,La,H_2,NH_3,CO_2	
Organic acid fermenters											
Megasphaera elsdenii				F S	F Ml	F Gl	F Pp	F La		2,3,4,5,6,H_2,CO_2	
Veillonella alcalescens								F La	U H_2	2,3,H_2,CO_2	
Hydrogen utilisers											
Methanobacterium ruminatium									U H_2	CH_4	2,CO_2,Br,He,NH_3,V
Vibrio succinogenes									U H_2	Et,CO_2	

A = amino acids
Br = branched-chain fatty acids
C = cellulose
Cb = cellobiose
Cf = cellulosic fragments
CO_2 = carbon dioxide
Dx = dextrins
Et = ethanol
F = ferments and utilises substrate
Fu = fumarate
Fr = fructose
G = glucose
Gl = glycerol
H = hydrolyses substrate but does not use products
H_2 = hydrogen
He = heme
Hm = hemicellulose
Hx = hexose
La = lactate
Ma = Malate
Me = methanol
Ml = maltose
Pc = pectin
Pn = Pentose
Pp = peptides
Pr = Protein
S = starch
Sc = sucrose
Sta = stimulated by amino acids
Su = succinate
Tg = triglycerides
U = utilises
V = vitamins
X = xylan
1 = formate
2 = acetate
3 = propionae
4 = butyrate
5 = valerate
6 = caproate
± = only in some strains

2.3.7.2 Age

Grandl et al., (2016) reported that CH_4 production, CH_4 yield and CH_4 intensity were significantly correlated with age, increasing up to around 2000 days of age (lactation 3), followed by a decline. The decline in CH_4 emissions in older cows may result from a reduction in fibre digestibility, as both results tended to show a parallel pattern of change with age (Grandl et al., 2016). This led to stable CH_4 emissions per unit of digestible fibre across all ages (Grandl et al., 2016). The reduction in fibre digestibility in older animals is

likely to reduce the total VFA produced by enteric fermentation, and hence reduce the total CH_4 emissions.

2.3.8 Methane mitigation

There are a variety of methods being trialled to suppress or inhibit methanogenesis. However, there are still challenges to finding a method that is successful, persistent, affordable, and without unfavourable effects to other aspects of the environment, animal, or the resultant feed. The theory behind inhibiting methanogenesis is to channel the carbon and hydrogen usually available for methanogenesis into propionate production (van Soest, 1994).

2.3.8.1 Ionophores

Ionophores, such as Monensin, are antibiotics that reduce CH_4 production (van Soest, 1994; Johnson and Johnson, 1995; Smith et al., 2008). An ionophore is a chemical compound that interferes with cation transport, thus decreasing the hydrogen available for methanogenesis. Ionophores inhibit hydrogen production by binding to sodium, potassium and other cations and limiting the counterflow of these ions through microbial membranes (van Soest, 1994; McDonald et al., 2010).

The suppression of hydrogen available for methanogenesis channels the carbon used for acetate and butyrate production, toward propionate production instead. Therefore, ionophores can increase propionate production, and decrease CH_4 production. However, the decrease in CH_4 is neither consistent nor persistent, and emissions return to initial levels within about 2 weeks, as rumen microorganisms adapt to the ionophore (Johnson and Johnson, 1995). Furthermore, ionophores are antibiotics, and are, therefore, undesirable in milk or meat production due to rising antibiotic resistance in human populations (Boadi et al., 2004). Overall, ionophores do not persistently decrease CH_4 and are thus not a potential CH_4 inhibitor.

2.3.8.2 Antimethanogenic drugs

Antimethanogenic drugs mainly consist of halogenated methanes or halogenated methyl derivatives, such as bromochloromethane, 2-bromoethane sulfonate, and chloroform (Hristov et al., 2013, 2015b). These compounds work either by affecting methanogens directly, or by affecting syntrophic groups on which the methanogens depend. Halogenated methyl compounds inhibit methanogens directly, whereas other compounds disrupt the oxygen balance and are generally inhibitory (van Soest, 1994). Studies have indicated that these compounds can reduce CH_4 emissions by up to 60% in vivo in ruminant animals (Hristov et al., 2013, 2015b).

However, these compounds are environmentally adverse due to their effects on depleting the ozone layer (bromochloromethane) and are banned in many countries, or are known carcinogens (chloroform; Hristov et al., 2013, 2015; Kinley et al., 2016). Research has also suggested that feeding cattle a red macroalgae seaweed (*Asparagopsis taxiformis*) acts as a potent antimethanogenic drug. However, this action is due to the presence of bromoform which is chemically similar to bromochloromethane (Kinley et al., 2016) and, hence, the feeding of this seaweed to cattle may be worse for the environment than the CH_4 itself.

Overall, the viability of these antimethanogenic drugs as CH_4 inhibitors is limited due to concerns about the impact of the drugs on the environment and on animal health (Hristov et al., 2015b).

2.3.8.3 Feed additives

Other feed additives that have been considered to reduce CH_4 emissions include certain organic acids, fats and oils (Pacheco et al., 2014). However, these supplements may also reduce animal productivity. The organic acids malate and fumarate were considered as propionate precursors to reduce CH_4 production by adding alternative hydrogen acceptors to maintain the redox balance (Kolver et al., 2004; Smith et al., 2008). However, high doses of malate and fumarate were required to reduce CH_4, making this an expensive method and

not always successful (Smith et al., 2008; Hristov et al., 2013). The addition of malic acid is also associated with reduced DMI, which will reduce animal productivity (Sejian et al., 2011).

Another potential feed additive to mitigate CH_4 was garlic oil. In vitro trials indicated that garlic oil was very effective in reducing CH_4 emissions from simulated rumen fermentation samples (Soliva et al., 2011; Hristov et al., 2015b). However, when garlic oil was tested in vivo in sheep, it did not result in a reduction in CH_4 emissions (Klevenhusen et al., 2011).

2.3.8.4 Methane inhibitors

One of the more promising CH_4 inhibitors being investigated to date is 3-nitrooxypropanol (3NOP), which is the result of a small molecule inhibitor development program at DSM Nutritional Products (Basel, Switzerland). Hristov et al. (2015) investigated the use of 3NOP as a feed additive to inhibit CH_4 production. It works by targeting methyl-coenzyme M reductase, thereby inhibiting the last step of methanogenesis (Duin et al., 2016). Hristov et al. (2015) reported a 30% decrease in CH_4 emissions using 3NOP compared with the control. This applied to total CH_4 emissions, emissions per unit of DMI, and emissions per unit of energy-corrected milk. Alongside the reduced CH_4 emissions, the 3NOP cows had an 80% increase in body weight gain compared with control cows. Feed intake levels, fibre digestibility, and milk production levels were not affected. The reduced CH_4 emissions persisted over the 12-week trial period. Therefore, 3NOP has been reported as an effective CH_4 mitigation strategy which reduces CH_4 without reducing feed intake or productivity (Hristov et al., 2015b). However, further investigation into this product has suggested that the timing of the dosage of 3NOP relative to feeding causes differential mitigating effects (Reynolds et al., 2014). Therefore, this has caused difficulty in how to incorporate 3NOP into a pasture-based system, such as in New Zealand.

2.3.9 Genetics

Genetic variation between breeds and between animals causes natural variation in CH_4 production rates (de Haas et al., 2011). This opens up potential for reducing CH_4 emissions through genetic selection (Wall et al., 2010; Sejian et al., 2011). Selectively breeding livestock based on CH_4 emissions should reduce emissions without compromising production. Selective breeding is capable of producing annual gains of 1-3% of the mean of the trait under selection (Wall et al., 2010). Genetic improvement is a cost-effective method capable of providing long-term, cumulative results to genetically lower CH_4 emissions (de Haas et al., 2011).

2.3.9.1 Genetic correlations

Studies in beef cattle have reported that there are relatively strong genetic correlations between the desired production trait (BW) and the different CH_4 phenotypes (Donoghue et al., 2013):

- CH_4 production: 0.79
- CH_4 yield: 0.18
- CH_4 intensity: −0.23

These data indicate that progress can be made in changing CH_4 output, but that the trend would be for a reduction in animal production with reduced CH_4 production and yield (Donoghue et al., 2013; de Haas et al., 2017). Donoghue et al. (2013) also reported genetic correlations between CH_4 production, CH_4 yield, and CH_4 intensity ranging from 0.87 to 0.96, which means that selection for one trait will have a similar effect on the other. However, as these were preliminary results, there is more work needed in this area (Donoghue et al., 2013; de Haas et al., 2017).

The results in beef cattle are consistent with those reported in dairy cattle: genetic correlations between the desired production trait (milk yield) and the CH_4 phenotypes of interest are positive (Lassen and Løvendahl, 2016):

- CH_4 production: 0.43
- CH_4 yield: 0.15

This suggests that any selection for CH_4 will be to the detriment of genetic progress for milk production (Lassen and Løvendahl, 2016; de Haas et al., 2017). Lassen and Løvendahl (2016) also reported a very low correlation between CH_4 production and CH_4 yield of 0.07.

2.3.9.2 Methane heritability

The heritability of CH_4 production and yield is moderate in size and consistent across ruminant species. For example:

- In sheep, the heritability (h^2) of CH_4 production has been reported as $h^2 = 0.29$ and for CH_4 yield as $h^2 = 0.13$ (Pinares-Patiño et al., 2013).
- In beef cattle, the heritability of CH_4 production has been reported as $h^2 = 0.21$-0.27, CH_4 yield as $h^2 = 0.19$-0.22 and CH_4 intensity as $h^2 = 0.23$ (Donoghue et al., 2013, 2016).
- In dairy cattle, the heritability of CH_4 production and CH_4 intensity was $h^2 = 0.21$ (Lassen and Løvendahl, 2016).

These low to moderate heritability estimates for the CH_4 phenotypes indicate that there is some potential to use genetic improvement to reduce CH_4 emissions in ruminants (Donoghue et al., 2013, 2016; Pinares-Patiño et al., 2013; Lassen and Løvendahl, 2016).

However, one of the issues faced is that genetic selection based on CH_4 production would require direct measurement of individual cow CH_4 emissions. Currently, there is no accurate, fast, and inexpensive CH_4 measurement method available that is suitable for large populations of animals (Wall et al., 2010). Another limitation is that available measurement methods are not always applicable to grazing animals. This means that CH_4 mitigation by direct genetic selection for that trait is currently limited.

Genetic selection through correlated traits, such as feed efficiency and RFI, is one potential strategy for reducing CH_4 production (Waghorn and Hegarty, 2011). As CH_4 production is an energy cost for the animal, the animals with lower CH_4 emissions may also be more energy efficient. The more energy efficient animals would be expected to direct more energy into weight gain or milk production, and would, therefore, be more productive. Genetic selection based on RFI may, therefore, help to reduce CH_4 emissions per animal (Waghorn and Hegarty, 2011) and improve productivity (Wall et al., 2010).

2.3.9.3 Breed differences

One of the major contributing factors to the difference in CH_4 emissions between breeds is the size difference. Jersey (Jer) cattle have been reported to have lower DMI than Holstein-Friesian (HF) cattle due to physical size differences (Goddard and Grainger, 2003; Prendiville et al., 2010; Beecher et al., 2014; Spaans et al., 2018). Therefore, they would be expected to also produce less CH_4/d. Jerseys also have a larger gastrointestinal tract per unit BW (Beecher et al., 2014) and greater FCE than HF (L'Huillier et al., 1988; Prendiville et al., 2009; Spaans et al., 2018), as will be explained in Section 2.4.3.

There is limited previous research comparing the CH_4 emissions of different breeds of cattle. L'Huillier et al. (1988) compared the New Zealand HF and Jer dairy breeds, reporting no significant differences in losses of energy as CH_4, faeces or urine, but did report a significantly lower heat production in Jer cows. Münger and Kreuzer (2006) investigated the CH_4 emissions in Holstein, Jer, and Simmental breeds in Switzerland. In line with DMI levels, CH_4 production per day was similar in Holstein and Simmental, while that of Jer cows was lower. Therefore, when converted to CH_4/DMI, all three breeds were similar over the entire lactation. However, in the dry period, Holstein cows had a significantly lower CH_4 yield compared with Jer. When estimated on a CH_4 intensity (g/kg ECM) basis, Holstein and Jer were similar and emissions of the Simmental cows were higher (P<0.05; Münger and Kreuzer, 2006). Therefore, the previous research indicates that there is similar CH_4

emissions between HF and Jer dairy cattle, once size or DMI is accounted for; however, the research is limited. Although not strictly breed differences, Robertson and Waghorn (2002) also reported lower CH_4 emissions from Holsteins of Dutch/US origin compared with those of New Zealand origin.

2.4 Residual feed intake

Residual feed intake is a measure of feed efficiency, which is defined as the difference between the actual DMI and the predicted DMI for an animal of a specified size and production level (body weight gain or milk production). This is graphically indicated by the coloured arrows in Figure 2.10. Residual feed intake was first described by Koch et al., (1963) and the concept has been applied to several industries including dairy and beef cattle (Arthur et al., 2001; Nkrumah et al., 2006; Macdonald et al., 2014), pigs (Hoque and Suzuki, 2009; Young et al., 2011), mice (Hughes and Pitchford, 2004), fish (Silverstein et al., 2005; Grima et al., 2010), and poultry (Luiting and Urff, 1991; Bordas and Minvielle, 1999).

- Animals with a negative RFI eat less than predicted and have 'low RFI' or 'high feed efficiency'.
- Animals with a positive RFI eat more than predicted and have 'high RFI' or 'low feed efficiency'.

Efficient animals (low RFI) are beneficial in farm systems because they require less feed per unit of body weight gain (BWg) or milk production (Waghorn et al., 2012; Macdonald et al., 2014).

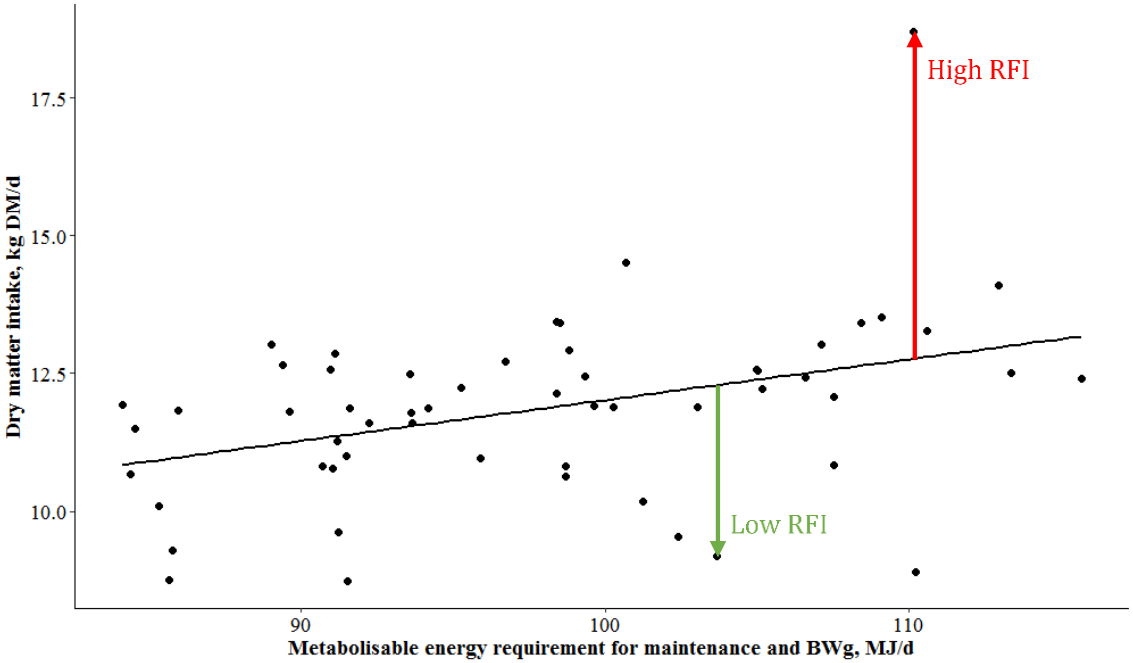


Figure 2.10. Simplified regression model indicating representing the calculation of RFI.

Residual feed intake in growing dairy heifers can be calculated using the residuals of a multiple regression model of the measured DMI against metabolic BW ($BW^{0.75}$) and daily BWg. RFI is defined as the residuals of the model (Green, 2012; Waghorn et al., 2012; Macdonald et al., 2014). The model for DMI is described in Equation 7 where β_0, β_1, and β_2, are regression coefficients and RFI is the residuals:

$$DMI = \beta_0 + \beta_1 BW^{0.75} + \beta_2 BWg + RFI \quad (7)$$

Where the estimation for RFI in growing heifers is based on a reasonably simple regression model, when considering lactating cows, the regression model becomes more complex (Equation 8; Berry and Pryce, 2014; Macdonald et al., 2014):

$$DMI = \text{fat yield} + \text{protein yield} + \text{lactose yield} + BW^{0.75} + \Delta BW + BCS + RFI \quad (8)$$

This is because the net energy used for producing the different components of the milk need to be considered in the calculation, as well as the change in body weight (ΔBW) and body condition score (BCS) of the animal.

Predicted DMI may otherwise be calculated using standard energy requirement formulae for maintenance, body weight gain/loss, physiological state, and production (e.g.; AFRC, 1993; National Research Council, 2001; Freer et al., 2007; Tedeschi et al., 2017). However, estimating protein and energy requirements requires many assumptions and can vary greatly depending on animals' age, size, genetics, physiological state, etc. Therefore, it is a difficult calculation, whereas the regression model is simpler. To account for the type of BWg (i.e. fat vs. muscle, the regression model may be adjusted to include fat depth; Berry and Pryce, 2014).

Residual feed intake is a measure of the between-animal variation in their utilisation of energy for maintenance and growth/production (Korver et al., 1991; Green, 2012) and assumes other nutrients are not limiting. It differs slightly from other terms used to measure feed efficiency: output (i.e. BWg or milk production), which does not consider DMI or BW; or FCE which measures feed consumed for growth/production, but does not take into consideration the animal's size and maintenance requirements. For example, selecting animals for improved FCE (kg DMI/kg BWg) can lead to selecting animals for higher BWg and, hence, larger mature BW, which may lead to complications within the farm system (Arthur et al., 2001; Alende et al., 2016). In comparison, RFI has been reported to have zero correlation with $BW^{0.75}$ and BWg (Berry and Crowley, 2012; Berry and Pryce, 2014). This is sensible because it is not just those animals with higher $BW^{0.75}$ or BWg that will have a low RFI/higher efficiency, as they may eat comparatively more to achieve this weight than an animal of lower $BW^{0.75}$ and BWg. However, RFI is positively correlated with intake (Williams et al., 2011; Waghorn et al., 2012; Green et al., 2013). This is because for animals with similar $BW^{0.75}$ and BWg, those with lower intakes will have lower RFI and vice versa. So, selecting for low RFI could result in a selection for low DMI and, therefore, low production animals, even though they are more efficient (Berry and Crowley, 2012; Berry and Pryce, 2014).

Overall, selecting for decreased RFI should result in animals that utilise energy more efficiently and, as such, should be able to:

- produce the same as their counterparts from less feed, or
- produce more than their counterparts from the same feed.

2.4.1 Heritability of RFI

Residual feed intake would need to be heritable for it to be included as a selection trait in the national breeding objectives. Several authors have reported that RFI is moderately heritable, with studies in beef cattle identifying the heritability of RFI within the range of h^2 = 0.14 to 0.52 (McNaughton and Pryce, 2007; Arthur et al., 2010; Rolfe et al., 2011). For growing dairy heifers, heritability of RFI was reported to be within the range h^2 = 0.22 to 0.38 (Korver et al., 1991; Pryce et al., 2012). In lactating dairy cows the heritability of RFI has been reported across a wider range. Van Arendonk et al. (1991) measured 360 first lactation dairy cattle for the first 105 days in milk and reported a heritability of h^2 = 0.19, and Veerkamp et al. (1995) measured 204 mixed age dairy cows up to 26 of lactation and reported a heritability of h^2 = 0.32. However, Ngwerume and Mao (1992), Svendsen et al. (1993), and Vallimont et al. (2011) all measured mixed age lactating dairy cattle, and reported a heritability of h^2 = 0.00 to 0.02. This is because a major disadvantage with RFI in dairy cows is that the heritability changes across lactation (Macdonald et al., 2014; Hurley et al., 2017). However, if it can be determined the stages of lactation at which the heritability is at its highest, the overall moderate heritability of RFI in growing dairy heifers, and that reported in lactating dairy cows by Van Arendonk et al. (1991) and Veerkamp et al. (1995) indicates that genetic selection for RFI is a possibility in future breeding schemes.

Currently, limited information exists on the association between RFI and fertility or health (Pryce et al., 2007). It would be unprofitable to improve feed efficiency, if it negatively affected another trait such as fertility, health, or production. Therefore, before RFI is incorporated as a selection trait, research is required to ensure that there are no correlated adverse effects. Macdonald et al. (2016) reported that selection for divergent RFI as calves did not affect milk production, reproduction, BW, or BCS in lactating cattle when managed

under an intensive pastoral grazing system. However, there is the potential for more research in this area.

2.4.2 Variation in residual feed intake

The between-animal variation in RFI could be a result of a variety of mechanisms. Theoretically, every physiological step throughout the conversion of a feed's gross energy to animal production (i.e., net energy use) might, potentially, play a part in affecting an animal's RFI. Animals convert the gross energy stored in feed to useable energy for maintenance and production through several digestive and physiological steps, broadly depicted in Figure 2.11. The utilisation of energy during any of these steps may partially contribute to the variation between animals in RFI (Herd and Arthur, 2009). For example, these may include variation in; heat production, body composition, physical activity, or feeding behaviour.

One potential source of variation in RFI could be due to between-animal differences in CH_4 emissions. Due to the nature of this thesis, this warrants its own section and is discussed in Section 2.5.

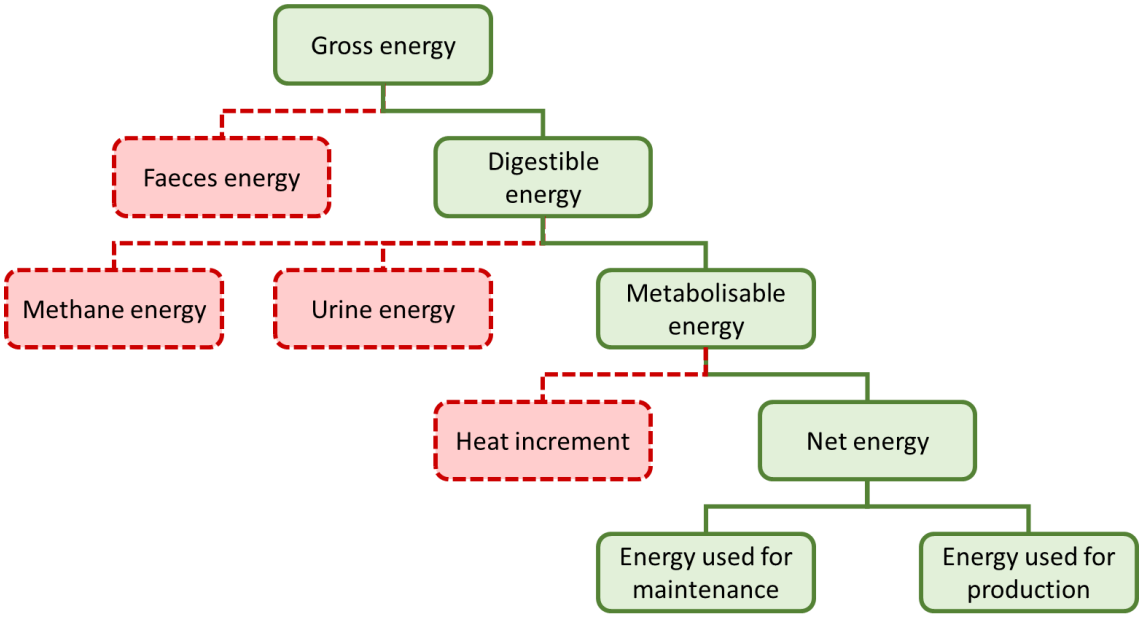


Figure 2.11. The partitioning of feed energy in animals. Energy losses are indicated in the red (adapted from McDonald et al. 2010).

2.4.2.1 Heat production

Heat production is a source of energy loss from the system, as indicated in Figure 2.11, arising from the heat increment and energy used for maintenance (McDonald et al., 2010). Nkrumah et al. (2006) reported that heat production (kcal/kg $BW^{0.75}$), measured indirectly through oxygen consumption, was 21% lower in low RFI steers compared with high RFI steers ($P<0.001$). Basarab et al. (2003) reported similar results; estimating heat production from metabolisable energy intake and composition of gain and concluding that low RFI animals produced 9.3% less heat than high RFI animals ($P<0.01$).

Montanholi et al. (2010) assessed using infrared thermography, which measures the surface body temperature of cattle, to estimate the heat production. Their study reported that low RFI steers had lower cheek (28.1°C vs. 29.2°C) and muzzle temperatures (30.0°C v. 31.2°C) than high RFI steers, and overall evaluation indicated that infrared thermography accounted for 59% of the total variation associated with RFI (Montanholi et al., 2010). However, these results may be implicated by differences in DMI between the low and high RFI steers, as heat production is affected by DMI, and estimations would be more accurate if DMI was controlled (Alende et al., 2016). Overall, infrared thermography may show some promise at estimating heat production and correlating with cattle RFI and feed efficiency (Montanholi et al., 2010).

2.4.2.2 Body composition

Body composition affects RFI because, for the same unit of weight, the deposition of lean tissue (i.e. protein/muscle) and fat have different energy costs (Herd and Arthur, 2009; Alende et al., 2016). Gross energy of fat is 9.4 Mcal/kg and protein is 5.5 Mcal/kg (Garrett and Hinman, 1969). There is greater variation in the efficiency of depositing lean gain than fat gain, due to greater variation in protein turnover (Herd and Arthur, 2009). Therefore, variations in the composition of gain or body composition may affect feed efficiency and RFI (Herd and Arthur, 2009; Alende et al., 2016).

Richardson et al. (2001) investigated steer progeny of parents selected for divergence in RFI and reported that low RFI (high feed efficient) was correlated with steer progeny of lower fat and greater lean body composition than those with high RFI (low feed efficient) parents. Arthur et al. (2001) reported genetic correlations of 0.17 and 0.06, respectively, for the 12/13th rib and rump fat depth with RFI, in post-weaning beef bulls and heifers. Robinson and Oddy (2004) reported stronger genetic correlations in older feedlot steers and heifers: genetic correlations with RFI were reported as 0.48 with 12/13th rib fat depth, 0.72 with rump fat depth, and 0.22 with intramuscular fat percentage.

2.4.2.3 Physical activity

Physical activity levels are positively correlated with RFI in cattle, with a review by Herd and Arthur (2009) reporting that 9% of the variation in RFI in cattle is due to their physical activity. Gregorini et al. (2015) reported that low RFI cows took 23% fewer steps during grazing alone (1,797 vs. 2,323; *P*=0.04) and overall tended to take 14% fewer steps than high RFI cows (3,311 vs. 3,880; P=0.06). Consistent with this, Richardson et al. (1999) reported a positive phenotypic correlation (r=0.32) between RFI and total steps measured by a pedometer. Cows that have lower physical activity expend less energy on walking and thus have a higher net energy for production, corresponding to higher efficiency.

2.4.2.4 Feeding behaviour

Several researchers have reported differences in feeding and rumination behaviour associated with feed efficiency and RFI (Nkrumah et al., 2006; Green et al., 2013). Herd and Arthur (2009) reported that 2% of the variation in RFI in cattle is due to feeding patterns. Multiple feeding behaviours have been associated with RFI, with more efficient animals spending less time feeding and having less meals. Green et al. (2013) reported a 23% reduction in the number of feeding bouts in low RFI cattle compared with high RFI, and a longer meal duration. Low RFI animals consumed 14% less/min during meals and, ultimately, spent 5% less total time feeding during a 24 h period, resulting in a 20%

reduction in DMI in low RFI heifers compared with high RFI heifers (Green et al., 2013). These behavioural trends of less time spent eating per day (min/d), slower eating rate (g/min) and a reduced number of meals per days in low RFI cattle has been reported by several studies (Robinson and Oddy, 2004; Nkrumah et al., 2006; Golden et al., 2008; Montanholi et al., 2010).

In addition to the reduced overall activity and the reduced time spent eating, Gregorini et al. (2015) also reported that cows selected for low RFI performed fewer and more intense rumination bouts, which would increase the physical breakdown of forage. Such a hypothesis was supported by the faecal particle size distribution in the study: relative to the high RFI cows, low RFI cows excreted faeces with 30% smaller particles, indicating a greater level of ruminal digestion of the forage feed (Clauss et al., 2010).

2.4.3 Breed differences

Previous research has indicated that Jer cattle have a greater FCE than HF (L'Huillier et al., 1988; Oldenbroek, 1988; Prendiville et al., 2009; Spaans et al., 2018), thus, indicating that Jer may have a lower RFI than HF. For example, Spaans et al. (2018) reported that at a system level: 11% less total metabolisable energy was required by the Jer cow for every kg of milk fat and protein produced, compared with the HF cows.

The reported differences in FCE are consistent with reported differences in the comparative mass of the gastrointestinal tract of the two breeds; Jer have a 10.6% larger gastrointestinal tract per unit BW than HF cows (Beecher et al., 2014), thus promoting a 2-3% increase in DM and NDF digestibility in Jer cows (Beecher et al., 2014).

2.5 Methane and residual feed intake

The assumption that lower CH_4 production should result in more energy available for production purposes and a greater production efficiency (Section 2.3.1), has led me to hypothesise that low RFI (high efficiency) might correlate with lower CH_4 production.

The studies published to date on the effects of RFI on CH_4 production in the bovine are limited to beef cattle, except for one experiment of 'non-published' results presented in a review by Waghorn and Hegarty (2011). Previous studies researched and compared the RFI and CH_4 production of:

- beef steers offered high concentrate diets (Nkrumah et al., 2006; Hegarty et al., 2007);
- beef cows grazing pasture of low and high quality (Jones et al., 2011);
- beef heifers fed grass silage (Fitzsimons et al., 2013);
- beef heifers fed three different diets of pasture silage, fresh pasture, and a 30:70 corn silage:concentrate total mixed ration (McDonnell et al., 2016); and
- dairy cows fed lucerne cubes and pasture (Waghorn and Hegarty, 2011).

Previously reported results are summarised in Table 2.5.

2.5.1 Residual feed intake and dry matter intake

In general, DMI was less in low RFI animals compared with high RFI animals: the 10% of growing dairy heifers ranked lowest for RFI (i.e. most efficient) consumed, on average, 22-25% less than the 10% heifers ranked highest for RFI (i.e. least efficient; Williams et al., 2011; Waghorn et al., 2012; Green et al., 2013). Both Hegarty et al. (2007) and Fitzsimons et al. (2013) also reported that low RFI cattle had lower DMI than their high RFI counterparts and, although DMI in kg/d did not differ between RFI categories in McDonnell et al. (2016), DMI/kg $BW^{0.75}$ was significantly less for low RFI than high RFI cattle. Nkrumah et al. (2006) reported that DMI was not affected by RFI category; however, this was because animals were offered a restricted allowance. In the same study, a preceding feedlot experiment identified a lower DMI in low RFI animals (Nkrumah et al., 2006).

However, there were some inconsistencies in this effect of RFI on DMI, as Jones et al. (2011) and Waghorn and Hegarty (2011) both reported that DMI did not differ between RFI categories. The lack of trend in this result may be explained by differences in the size and

production between the two groups of animals in each study. If the two groups of animals had the same average metabolic BW and BWg, the low RFI cows may have eaten less than the high RFI cows. However, if the low RFI cows weighed more or were producing more than the high RFI cows, it would be acceptable for the two groups to be eating the same amount of feed. This hypothesis cannot be confirmed, as the levels of production from studies in which the DMI was not significantly different between RFI categories were not reported (Jones et al., 2011; Waghorn and Hegarty, 2011). Overall, though, most studies support that DMI is less in low RFI animals compared with high RFI animals (Nkrumah et al., 2006; Hegarty et al., 2007; Fitzsimons et al., 2013; McDonnell et al., 2016).

Table 2.5. DMI, CH_4 production (g/d) and CH_4 yield (g/kg DMI) from cattle selected for variance in RFI. (Waghorn and Hegarty 2011).

RFI	n	Dry Matter Intake			Daily CH_4 (g/d)			g CH_4/kg DMI			Source
		Low	High	*P*-val	Low	High	*P*-val	Low	High	*P*-val	
Beef steers	8 + 11	8.6	8.7	0.39	96	127	0.04	11.2	14.7	0.04	1
Beef steers	10 + 10	8.4	14.1	<0.01	142	190	0.01	16.3	14.7	0.37	2
Beef cows[a]	23 + 25	13.1	14	ns	182	227	<0.05	14.1	16.2	<0.05	3a
Beef cows[b]	23 + 25	10.2	10.7	ns	133	125	ns	12.3	12.4	ns	3b
Beef heifers	7 + 7	7.0	8.0	0.02	260	297	0.04	38.0	36.0	0.52	4
Beef heifers	14 + 14	7.2	7.5	0.14	156	146	0.11	22.4	20.2	0.03	5
Dairy heifers[c]	8 + 8	10.8	11.2	0.57	192	194	0.78	18.3	17.5	0.40	6c
Dairy cows[d]	8 + 8	12.2	12.5	0.61	272	259	0.39	22.4	20.9	0.21	6d

References and methodology:

1: Nkrumah et al. (2006); 500 kg beef steers, fed diets containing 80% grain (DM basis), with CH_4 measured in a 4-chamber, open-circuit, indirect calorimetry system for 16 h/d without access to feed.

2: Hegarty et al. (2007); 590 kg beef steers, fed diets containing 75% grain (DM basis), with CH_4 measured by SF_6 tracer.

3: Jones et al. (2011); 500 kg beef cows fed pasture, with CH_4 measured in field using the open path Fourier Transform infrared spectrophotometer technique. Results are presented based on an estimated result for a 500 kg BW animal.

a: beef cows nursing calves, grazing on high quality pasture

b: pregnant beef cows grazing low quality pasture

4: Fitzsimons et al. (2013); 490 kg beef heifers, fed grass silage, with CH_4 measured by SF_6 tracer.

5: McDonnell et al. (2016); 350 kg beef heifers, fed 3 different diets of grass silage, pasture and 30:70 corn silage:concentrate total mixed ration, with CH4 measured by SF6 tracer. The different diets are not separated into treatments as there were no RFI x diet interactions observed.

6: Waghorn and Hegarty (2011); DairyNZ, Hamilton, New Zealand; unpublished data.

c: Nonlactating Holstein-Friesian dairy heifers aged 21 months fed alfalfa cubes.

d: Holstein-Friesian dairy cows aged 26 months at day 60 of lactation fed pasture

2.5.2 Residual feed intake and methane emissions

Most previous research identified a positive relationship between RFI and daily CH_4 production (Nkrumah et al., 2006; Hegarty et al., 2007; Jones et al., 2011 (high-quality pasture); Fitzsimons et al., 2013); however, this effect was not consistent across all studies (Jones et al., 2011 (low-quality pasture); Waghorn and Hegarty, 2011; McDonnell et al., 2016). As presented in Table 2.5, daily CH_4 production (g/d) was reportedly less in low RFI cattle in the research by Nkrumah et al. (2006), Hegarty et al. (2007), Fitzsimons et al. (2013), and similar results were reported in the high-quality pasture treatment of Jones et al. (2011). However, in research by Waghorn and Hegarty (2011) and McDonnell et al. (2016) daily CH_4 production was not affected by RFI category, and similar results were reported by the low-quality pasture treatment of Jones et al. (2011).

The results for CH_4 yield (g CH_4/kg DMI) are also inconsistent across previous research, without a dominant trend. Methane yield was not different between RFI categories in Hegarty et al. (2007), Waghorn and Hegarty (2011), Fitzsimons et al. (2013), nor in the low-quality pasture treatment of Jones et al. (2011). However, CH_4 yield was positively correlated with RFI in the work of Nkrumah et al. (2006) and in the high-pasture quality treatment of Jones et al. (2011). Methane yield was significantly greater in low RFI cattle than high RFI cattle in the study by McDonnell et al. (2016). Despite a lack of statistical significance in the research reported by Hegarty et al. (2007), Waghorn and Hegarty (2011) and Fitzsimons et al. (2013), low RFI animals had numerically higher CH_4 yield. Although this may be due to chance, it is interesting to note this commonality among studies.

It is not possible to determine the reason for the inconsistent relationship between RFI and CH_4, but it might relate to differences in diet. Some studies have been undertaken with cattle fed high concentrate diets (Nkrumah et al., 2006; Hegarty et al., 2007; McDonnell et al., 2016), whereas others have forage based diets (Jones et al., 2011; Waghorn and Hegarty, 2011; Fitzsimons et al., 2013; McDonnell et al., 2016), as summarised earlier. As described

in Section 2.3.7.1, diet plays an important role in the variation of CH_4 emissions. Therefore, the variety of diets across these experiments would result in very different rumen fermentation patterns upon digestion; hence, one could reasonably expect different CH_4 yields/kg DMI for the different diets (Ramin and Huhtanen, 2013; Moate et al., 2017).

2.6 Objectives

After identifying the potential for a relationship between feed efficiency and CH_4 production and yield, and the knowledge gaps in the literature, with the lack of research in this area in the dairy industry, the objectives of my Masters are to:

1. Determine the effect of heifer RFI category on CH_4 production (g/d), yield (g/kg DMI), and intensity (g/kg BWg) (Chapter 4).
2. Investigate the relationship between breed and CH_4 production (g/d), yield (g/kg DMI), and intensity (g/kg BWg) (Chapter 4).

Chapter 3 Methane Measurement Methods

3.1 Introduction

Accurate, quick, and cost-effective CH_4 measurement is essential for CH_4 mitigation. Methane reduction strategies include nutrition adaptation, dietary additives, or genetic selection, as discussed in Section 2.3.8. An efficient measurement method that can accurately determine individual emissions across large populations of animals must be available for these strategies to be evaluated.

There are several techniques for measuring CH_4, each with advantages and disadvantages (Huhtanen et al., 2015). The methods discussed in this chapter include respiration chambers, sulphur hexafluoride (SF_6) tracer technique, GreenFeed system and in vitro gas production technique.

3.2 Respiration chambers

Respiration chambers are the 'gold standard' method for accurate CH_4 emission measurement from individual animals (Hristov et al., 2018). This is because they give reliable and stable measurements under a controlled environment (Brouček, 2014). In respiration chambers, an animal is confined in a chamber and all exhaled air (from nostrils, mouth, and rectum) is collected and the concentration of CH_4 present measured. Currently, whole animal open-circuit respiration chambers are most commonly used (Hammond et al., 2016a), which involves measuring all gas flow in and out of the respiration chamber, and calculating the change in composition to determine CH_4 emissions.

In an open-circuit respiration chamber, individual animals are confined into a chamber and the gas flow in and out of the chamber is measured. Fresh air is pumped into the front of the chamber. This fresh air is either sourced directly from outside the chamber, or it can be sent through an air conditioning system to maintain humidity, temperature and pressure. The

control of these parameters allows data and calculations to be adjusted for atmospheric conditions. Air is pumped out of the back of the chamber through a flow meter and gas sensors, which continuously sample and analyse the outlet gas (Storm et al., 2012; Brouček, 2014; Hammond et al., 2016a).

Methane emissions are calculated from gas flow and concentration measurements from the inlet and outlet ports (Grainger et al., 2007; Garnsworthy et al., 2012; Huhtanen et al., 2015). The gas concentrations in the air are measured using gas analysers, infrared photoacoustic monitors or gas chromatography (Goopy et al., 2015). The difference between the CH_4 concentration of the incoming and outgoing air indicates the CH_4 emitted by the animal (Storm et al., 2012; Brouček, 2014). Figure 3.1 is an example of an open-circuit respiration chamber and the direction of air flow is indicated by arrows.

Figure 3.1. Schematic of an example of open-circuit respiration chamber. The arrows indicate the direction of air flow. Locations 1 and 2 are the intake and exhaust ducts sample points for non-calibration periods; location 3 is the injection point enabling the analytical system calibration; location 4 is the sample point for the system calibration; and location 5 denotes the chamber volume (Grainger et al., 2007).

Respiration chambers are regarded as the most accurate CH_4 measurement method. Previous studies have indicated that respiration chambers give more precise measurements than other methods, such as the SF_6 tracer technique (Storm et al., 2012). All changes in gas concentration in the chamber are measured, including those from the rectum, unlike other methods (Grainger et al., 2007; Hristov et al., 2018).

The main factor reducing the accuracy of respiration chambers is that the confinement of animals can affect their feeding behaviour (Garnsworthy et al., 2012; Goopy et al., 2015; Huhtanen et al., 2015; Hristov et al., 2018). Dry matter intake, feeding frequency, or rumination frequency may change, which can distort both the total CH_4 emissions and the profile of emissions. Feed intake levels has the largest impact on CH_4 production rates. Reduced feed intake decreases total CH_4 emissions, and increases CH_4/kg DMI. This means that results cannot accurately be applied to pasture-based farm systems (Storm et al., 2012; Brouček, 2014). To minimise the change in feeding behaviour, it is suggested that animals be given a period of acclimatisation to the chambers. Chambers can also be constructed from transparent materials to allow visual contact with other animals (Goopy et al., 2015).

Despite their accuracy, respiration chambers are expensive, time-consuming and labour intensive. The construction and maintenance of the chambers is a high cost. For example, the sensitivity of the gas sensors and flow meters used greatly affects the results, and using high-performance instruments required for accurate results is expensive. Each chamber usually holds only one animal and one animal has a measurement period of usually 3-5 days (Goopy et al., 2015). This means that measurements take several days per chamber and per cow and, therefore, are unsuitable for large populations of animals. In addition, the chamber restricts the movement of animals and may cause stress (Brouček, 2014). However, despite all these issues, respiration chambers are still the most accurate and therefore preferred measure for ruminant CH_4 emissions.

3.3 Sulphur hexafluoride tracer technique

The SF_6 tracer technique was developed as a measurement method for grazing animals and larger populations, hence addressing some of the shortfalls of the respiration chambers. This method is, therefore, widely used in New Zealand (Storm et al., 2012; Berndt et al., 2014). The principle behind this technique is that CH_4 emission rates from the rumen can be measured in comparison to the known emissions rates of a tracer gas, SF_6. The tracer gas must be non-toxic to the animal, physiologically inert, stable, and mix with rumen air in the same way as CH_4. The SF_6 gas meets these requirements and is also cheap, has a low detection limit, and can be analysed easily (Storm et al., 2012; Berndt et al., 2014; Brouček, 2014).

The SF_6 tracer technique involves the insertion of a permeation tube containing SF_6 into the rumen (Hammond et al., 2016a). The rate of SF_6 diffusion from the tube is measured in a 39°C water bath prior to insertion by measuring the daily weight loss of the tube for a minimum of six weeks (Berndt et al., 2014). This diffusion rate should be stable (Storm et al., 2012), and once it has been determined, the tube is inserted into the rumen of the animal and CH_4 measurement can begin.

A sample of exhaled air from both respiration and eructation is drawn continuously through a capillary tube positioned near the animal's nostrils into an evacuated PVC canister hanging around the animal's neck or placed on her back (Figure 3.2; Grainger et al., 2007; Goopy et al., 2015). The exhaled air is typically accumulated over a period of 24 hours, and repeated for five to eight consecutive days (Berndt et al., 2014; Hammond et al., 2016a).

Methane emissions are calculated through the ratio of SF_6 and CH_4 present in the canister. The concentrations of the two gases in the canister are measured via gas chromatography; using an electron capture detector for SF_6 and a flame-ionisation detector for CH_4 (Grainger et al., 2007; Pinares-Patiño et al., 2011). The CH_4 emission rate is calculated by multiplying the CH_4/SF_6 ratio by the release rate of SF_6 from the permeation tube (Berndt et al., 2014;

Goopy et al., 2015). This calculation is also adjusted for the background gas concentrations and molecular masses of the two gases.

Figure 3.2. Illustration of the SF_6 tracer technique methodology (Johnson et al., 1994).

The SF_6 tracer technique has a range of advantages and disadvantages. It is a labour-intensive, invasive, and time-consuming method, but it does allow normal movement and feeding behaviour for grazing animals. The method requires regular animal handling, insertion of a rumen bolus, and gas collection equipment attached to the animal's head (Grainger et al., 2007; Garnsworthy et al., 2012). The measurement technique can interfere with normal behaviour and is not suitable for measurement of large populations (Garnsworthy et al., 2012; Huhtanen et al., 2015). Some of the major issues are described below.

The release rate of SF_6 from the permeation tube may vary over long trials (i.e. months), and this will cause inaccurate emission results (Berndt et al., 2014; Hammond et al., 2016a). Studies have indicated that the release rate of SF_6 permeation tubes pre- and post-experimentation can change, and also that permeation tubes with higher SF_6 release rates correlate to higher calculated CH_4 emissions than tubes with lower SF_6 release rates (Storm et al., 2012; Berndt et al., 2014). To reduce these effects, the release rate of SF_6 from the permeation tube should be calibrated for a minimum of six weeks and up to half as long as

the planned experiment duration (with a practical maximum of about 10 weeks), and only tubes with extremely linear release rates (R^2 > 0.997) are used for experiments (Berndt et al., 2014).

Low concentrations of SF_6 and CH_4 in the background air may interfere with the measurements. Therefore, it is not recommended to use the SF_6 tracer technique in enclosed barns, unless there is satisfactory ventilation (Hammond et al., 2016a; Hristov et al., 2018). Regardless of the experimental environment, background air samples should always be obtained and sampled for SF_6 and CH_4 concentrations. These have been done by various methods such as a canister placed upwind of the animals, or by including animals in the trial that are sampled along with other animals but do not have an SF_6 permeation tube inserted (Hristov et al., 2018). Several background measurements are required because CH_4 will disperse and accumulate differently as it is lighter than SF_6 (CH_4 16 g mol^{-1} vs. SF_6 146 g mol^{-1}; Goopy et al., 2015).

Compared with respiration chamber measurements, the SF_6 tracer technique tends to give larger within- and between-animal coefficients of variation (Pacheco et al., 2014; Huhtanen et al., 2015). The study by Grainger et al. (2007) identified a within-animal coefficient of variation of 6.1% for the SF_6 tracer technique, and 4.3% for the chamber method. The between-animal coefficient of variation was larger at 19.6% and 17.8% for SF_6 tracer and chamber methods, respectively. The relatively high within- and between-animal variation limits this method and increases the length of the measuring period required to ensure precise results (Storm et al., 2012; Goopy et al., 2015).

The correlation between SF_6 tracer and respiration chamber measurements is poor: for example, measured CH_4 emissions were 7% less using the SF_6 tracer technique than respiration chamber (Johnson et al., 1994). The reduced measurement is likely because the SF_6 method does not measure CH_4 emissions through the rectum, while the respiration chamber does (Murray et al., 1976; Storm et al., 2012). However, other studies have

reported greater CH_4 emissions using SF_6 tracer measurement compared with the chamber method (Boadi et al., 2002; Grainger et al., 2007; Pinares-Patiño et al., 2011). Grainger et al. (2007) reported CH_4 emissions for dairy cows at 331 and 322 g CH_4/d when measured using SF_6 tracer technique and respiration chambers, respectively. In the same article, reviewed Canadian data indicated that emissions were 8% lower in the SF_6 method than the chamber method (Grainger et al., 2007). Previous studies also differ in the correlation between methods, and this inconsistency is likely due to the larger variation in the SF_6 tracer measurements.

The main advantage of the SF_6 tracer technique is that it allows animals to move and graze normally (Berndt et al., 2014; Goopy et al., 2015). This is beneficial over respiration chamber measurements as it means that the SF_6 tracer method is suitable for exploring the effects of grazing management on CH_4 emissions (Pinares-Patiño et al., 2011; Goopy et al., 2015). However, the SF_6 method is less precise, less accurate and more labour intensive (Goopy et al., 2015).

3.4 GreenFeed system

GreenFeed is a patented system (C-Lock Inc., USA) that combines a feeding system and CH_4 analyser. The system continuously analyses air exhaled into a hood over feed troughs or automatic milking systems (Goopy et al., 2015; Hristov et al., 2015a). The animal enters the feeding system and is recognised by an electronic ID reader. The hood captures the exhaled air and measures gas concentrations. The readings are short-term (usually 3-6 minutes each feeding session) and repeated over a longer period of days or weeks. Air is continuously pumped through the system to measure both flow and gas concentrations as background and sample measurements (Goopy et al., 2015). This system uses the flux method for measuring gas emissions, a method similar to that used in respiration chambers. Measurements of air flow, gas concentrations and muzzle position are all used to measure

the CH_4 flux and calculate the CH_4 emission per animal visit to the system (Huhtanen et al., 2015). An illustration of the GreenFeed system is presented in Figure 3.3.

Figure 3.3. Layout of the GreenFeed system (Hristov et al., 2015a).

The GreenFeed system measures short, but repeated samples over several weeks or months. This helps to reduce the potential sampling bias due to CH_4 production patterns. The system has been reported to estimate CH_4 emissions comparable to results measured by respiration chambers or SF_6 tracer methods (Hammond et al., 2013; Goopy et al., 2015).

The main limitation of the GreenFeed system is that it only measures CH_4 concentration while the animals are eating and have their head under the hood. This means that the individual measurements must be estimated to a 'whole day' emission rate before the results are comparable. The position of the animal's head also has a large effect on the readings, as it affects whether exhaled air is all captured in the hood or not (Storm et al., 2012; Huhtanen et al., 2015). Another limitation is that feed is supplied to lure the animal

to the system. Therefore, the animal's complete diet and feed intake levels can vary, which will affect CH_4 production rates (Goopy et al., 2015).

In recent years, DairyNZ commissioned C-Lock Inc., USA to develop a system that combined the GreenFeed technology for measuring CH_4 emissions (Huhtanen et al., 2015) with the technology currently used for measuring RFI as described by Waghorn et al. (2012). Zimmerman et al. (2015) describes these DMI-CH_4 stations, and it is depicted in Figure 3.4. Briefly, they are a feeding station, accessible by one animal at a time via a narrow chute, with the feed bin mounted on load cells. Bin weight and animal ID (by an electronic ID reader) are recorded continuously when an animal accesses feed, so that intake, time, and duration of eating are recorded. The feeding station has a sealed lid and air is extracted from the bin using a fan and the system integrates measurements of air flow and gas concentrations to allow direct measurement of CH_4 fluxes during each animal visit to the feed station (Huhtanen et al., 2015).

This system compensates for the identified shortfalls of the original GreenFeed system in that the animals sole diet can be fed from these DMI-CH_4 stations, instead of as a lure to entice a visit. This means that the exact intake and feeding behavior of each individual animal is recorded, instead of having to estimate it by other means. It also means that frequency of visitation to the units is likely to be greater.

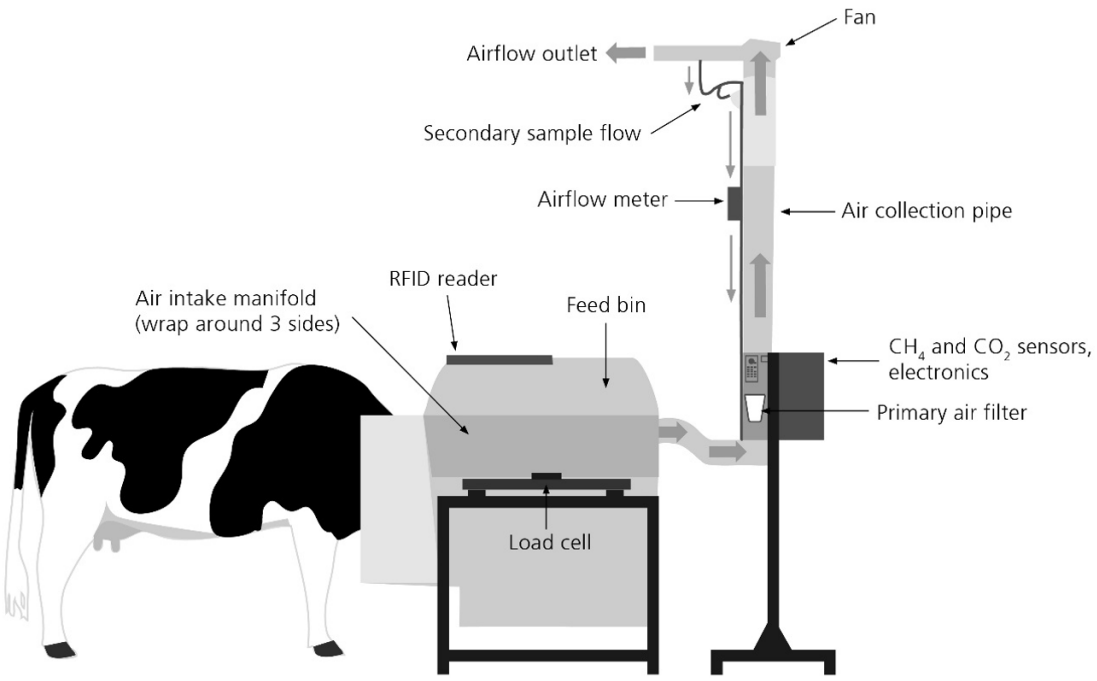


Figure 3.4. Diagrammatic representation of the GreenFeed system for measuring CH_4 production incorporated into the custom-built feed stations (C-Lock Inc., Rapid City, SD, USA).

3.5 In vitro gas production technique

The in vitro gas production technique (IVGPT) is a laboratory procedure that simulates rumen fermentation processes, and has traditionally been used to evaluate the nutritive value of feeds. In the last decade, however, this method has evolved to encompass CH_4 measurement (Yáñez-Ruiz et al., 2016).

This method involves the incubation of a feed in a solution containing rumen fluid, buffer and minerals in an anaerobic environment, simulating the rumen. The mixture is incubated at 39°C for a 16-72 hours (Yáñez-Ruiz et al., 2016). Gas products accumulate throughout the incubation period from the fermentation, and the total amount of gas produced, and its composition, are analysed to estimate the in vitro CH_4 production rate (Storm et al., 2012; Brouček, 2014).

This method requires access to fresh rumen fluid, which is usually provided from fistulated ruminants, but may be collected by oesophageal tubing on live animals or from slaughtered animals. During IVGPT, it is possible to analyse the degradation of feedstuffs alongside CH_4 production. This helps to examine whether a reduction in CH_4 production is related to lower digestion rates or differences within the feed. The results of these measurements are often given as g CH_4/g DM, g CH_4/g DM degraded, or g CH_4/g degraded NDF. Studies have reported close agreement between IVGPT measurements and chamber or SF_6 tracer methods (Storm et al., 2012).

The measurements of IVGPT are useful, but not completely applicable to in vivo CH_4 production. Only small proportions of the animal population can be experimented with, as fresh rumen fluid is continually required and the measurements take weeks to complete. This means that IVGPT cannot be used to compare between animals, but it does give good guidance on the dietary factors affecting CH_4 emissions.

Chapter 4 Selecting for low residual feed intake did not affect daily methane production, but increased methane yield in dairy heifers

This chapter has been submitted to Journal of Dairy Science as a Hot Topic. Additional information has been added to the Materials and Methods section and Results section. Also, it has been presented at the annual meeting of the American Dairy Science Association in Knoxville, Tennessee (June 2018; Appendix 1), will be presented at the International Symposium for the Nutrition of Herbivores in Clermont-Ferrand, France in September 2018 (Appendix 2), and has been accepted for presentation at the biennial Australasian Dairy Science Symposium in Palmerston North in November 2018.

4.1 Abstract

Reducing enteric CH_4 emissions and improving FCE of dairy cows is important. Residual feed intake is one measure of feed efficiency, with low RFI animals being more efficient converters of feed to body weight or milk. Enteric CH_4 is an important source of digestible energy loss in ruminants, and because research in beef cattle has reported a positive relationship between RFI and daily CH_4 production, I hypothesized that low RFI dairy heifers, which are more feed efficient, would also produce less CH_4. I measured the daily CH_4 production (g/d), CH_4 yield (g/kg DMI), and CH_4 per kg BWg for 56 heifers (20-22 mo old) in a 2 x 2 factorial arrangement: factors included two breeds (HF and Jer; n=28/breed), with equal numbers of animals previously determined as either high (+2.0 kg DM) or low RFI (-2.1 kg DM; n = 28/RFI category). All heifers were co-mingled and offered the same diet of dried lucerne cubes. Between RFI categories, heifers did not differ in BW or BWg; but low RFI heifers had 9.3% and 10.6% lower DMI and DMI/kg BW, respectively, than high RFI heifers. Similarly, RFI category did not affect CH_4/d or CH_4/kg BWg; but, CH_4/kg DMI was greater in low RFI heifers because of their lower DMI. These results might reflect more

complete digestion of ingested feed in more efficient, low RFI heifers, consistent with previous reports of greater apparent digestibility of organic matter. Holstein-Friesian heifers were heavier and consumed more total DM than Jersey heifers, but breed did not affect DMI/kg BW or BWg. Jersey heifers produced less CH_4/d, but not CH_4/kg DMI or CH_4/kg BWg. In conclusion, selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d), but may increase CH_4 yield (g/kg DMI).

4.2 Introduction

Rapid growth in the global human population and a concomitant rising demand for animal products are generating concerns that enteric CH_4 emissions are contributing to climate change (Garnsworthy et al., 2012; Ramin and Huhtanen, 2013; Huhtanen et al., 2015). Reducing these emissions, while maintaining current production, requires improved FCE (Waghorn et al., 2012; Potts et al., 2015). Agriculture and land use change (i.e. converting forest or permanent pasture to annual crops) is estimated to contribute 14 to 22% of global GHG emissions and agricultural CH_4 is reported to be almost 6% of global GHGs (Knapp et al., 2014). Therefore, there is considerable interest in genetic or management-level strategies to reduce this source of GHG.

Ruminants eruct 5-10% of their gross energy intake as CH_4 (van Soest, 1994; Pacheco et al., 2014). Therefore, selective breeding for more feed efficient animals could both reduce CH_4 emissions and increase productivity. Residual feed intake (RFI) is a measure of FCE that describes the difference between an animal's actual DMI and its predicted DMI for its productivity. Residual feed intake has been positively associated with daily CH_4 production in beef steers (i.e. low RFI = low CH_4/d; Nkrumah et al., 2006; Hegarty et al., 2007; Fitzsimons et al., 2013). Between-animal differences in RFI have been reported within dairy cow breeds (Pryce et al., 2012; Davis et al., 2014; Macdonald et al., 2014). Differences in FCE have been also reported between breeds, with Jer cows requiring less feed/kg of milk components produced than HF cows (L'Huillier et al., 1988; Prendiville et al., 2009; Spaans

et al., 2018). This is probably due to the larger gastrointestinal tract per unit BW of Jer cows compared with HF cows, thus promoting an increase in NDF and DM digestibility (Beecher et al., 2014). It is, therefore, plausible that dairy breeds differ in daily CH_4 production and yield per unit DMI. I hypothesized that high feed efficient (low RFI) animals would emit less CH_4/kg DMI than the lower efficiency (high RFI) animals, and that Jer heifers would have lower CH_4 yield than HF heifers.

4.3 Materials and methods

To test this hypothesis, I measured daily CH_4 production (g CH_4/d) in a 2 x 2 factorial arrangement of breed and pre-defined RFI category: 28 HF and 28 Jer heifers (20-22 mo old) previously identified as being either high or low for RFI (i.e., n = 14 HF-High, 14 HF-Low, 14 Jer-High, and 14 Jer-Low). The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (1999). The experiment was undertaken at Lye Farm, Hamilton, New Zealand. (37°46'S 175°18'E) between March and June 2017.

I selected fifty-six 20-22 mo old dairy heifers from an experiment designed to measure the RFI of HF and Jer heifers. Briefly, I procured 280 heifers (140 HF and 140 Jer), representing 30 elite sires (n = 5 to 10 heifers per sire), on loan from commercial dairy farms across the North Island of New Zealand and transported them to the Dairy Trust Taranaki (Hawera, Taranaki, New Zealand 39.59 ° S, 174.28° E) research facility to measure RFI. The Hawera research facility was described in detail by Waghorn et al. (2012). Briefly, the facility contained 28 x 42 m^2 pens bedded with coarse wood shavings on top of stones and drainage pipes. Each pen contained a single feeding station, accessible to one animal at a time, with the feed bin mounted on load cells. Bin weight and animal ID were recorded continuously when an animal accessed feed, so that intake, time, and duration of eating were recorded. Access to the feed was via a narrow chute and individual ID was recorded by an electronic ID reader (Gallagher G03103 R series; Gallagher Group Ltd., Hamilton, New Zealand)

mounted above the feed bin. Four load cells supported each bin and weights were recorded at 0.02-s intervals to an accuracy of 0.1 kg. Water was freely available.

I divided the 280 heifers into two cohorts (n=140/cohort), with equal representation of HF and Jer in each cohort, and offered unrestricted access to dried lucerne cubes (*Medicago sativa*; supplied by MultiCube Stockfeeds Ltd.; Yarrawonga, Victoria 3730, Australia). I measured heifer intake over a 52-d period and assessed BW 3 times/wk. At the end of each cohort's measurement period, individual heifer RFI was calculated by regressing DMI against $BW^{0.75}$ and BWg and the top 10% (+2.0 kg DM/d) and bottom 10% (-2.1 kg DM/d) for RFI within each breed and cohort were selected for assessment of CH_4 production (n = 7 heifers per breed and RFI category within each cohort group; see Figure 4.1).

Immediately following assessment for RFI, selected heifers were transported to DairyNZ Lye Farm, Hamilton, New Zealand (37°46'S 175°18'E) (n=14 heifers/breed per cohort) and housed in the DairyNZ CH_4 measurement facility. Photos of experimental work in the CH_4 measurement facility are included in Figure 4.2. The CH_4 measurement facility consisted of a free-stall barn with 30 raised cubicle sand beds. The barn was split into two halves with 15 stalls per pen. Seven feed stations with similar dimensions to the feed bins described previously were installed; with three feed stations in one pen and four in the other pen. The setup of these stations within the barn is demonstrated in Figure 4.3. These feed stations identified animals automatically via an electronic ID reader placed on top of the feed bin and recorded feed disappearance and CH_4 production as the animal ate.

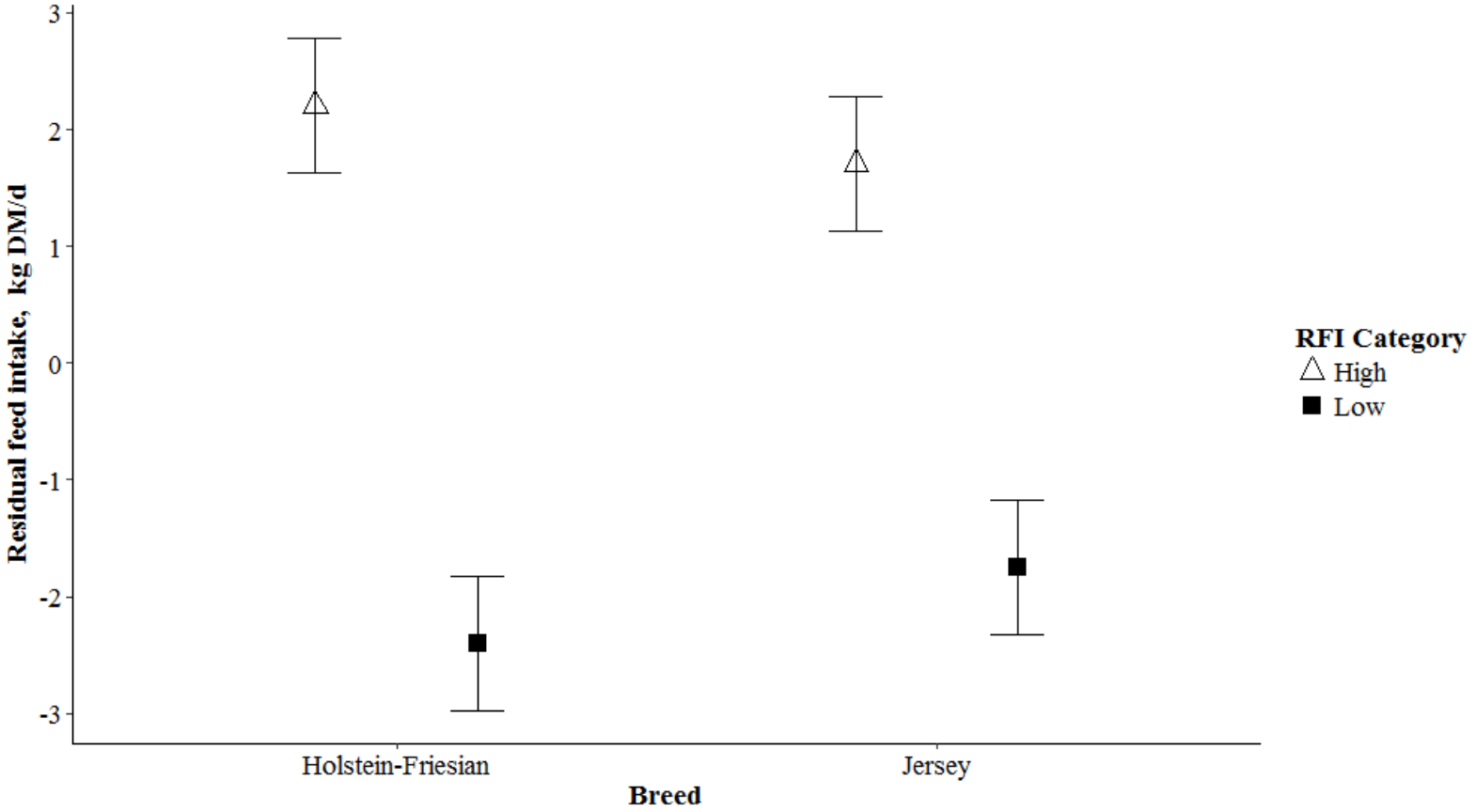
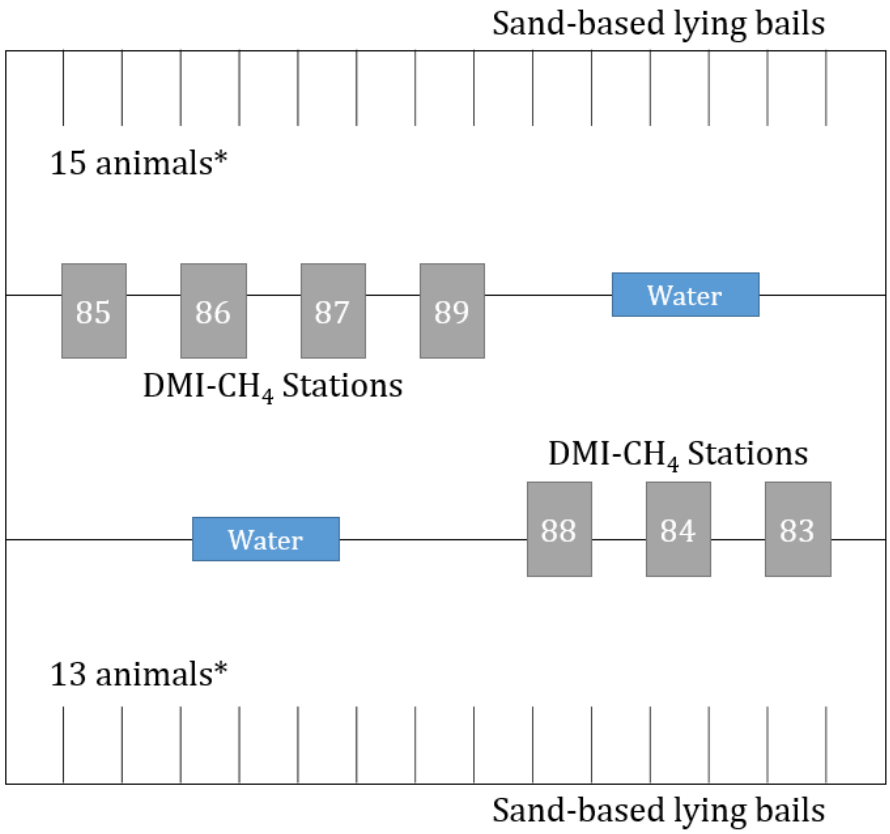


Figure 4.1. Residual feed intake (RFI) of four groups of dairy heifers representing two breeds (Holstein-Friesian and Jersey) and two previously-determined RFI categories (High: +2.0 kg DM; Low: -2.1 kg DM). Heifers were 20-22 mo old; BW = 480 and 408 for HF and Jer, respectively, and 439 and 448 for High and Low RFI, respectively. The RFI is the difference between amount of feed DM required for biological processes and estimated feed requirements based on a regression of feed DM against BW and daily BW gain. Midpoint in each vertical bar is the least square mean for the group; the error bars are the 95% confidence interval.

Figure 4.2. Photos of experimental work in the CH_4 measurement facility at Lye Farm, DairyNZ, Hamilton, New Zealand, using the DMI-CH_4 units (C-Lock Inc., Rapid City, SD, USA).



*Animals in the two halves of the barn were not split into two even halves due to the uneven number of DMI-CH_4 stations. The animals were split on a ratio of 15:13 rather than 16:12 due to only 15 lying bails being available on each side of the barn.

Figure 4.3. Schematic of the setup of the CH_4 measurement facility.

Zimmerman et al. (2015) described the feeding stations. Briefly, the commercial GreenFeed system (Figure 3.4; C-Lock Inc., Rapid City, SD, USA) for measuring CH_4 production (Huhtanen et al., 2015; Hammond et al., 2016b) was incorporated into a custom-built feed station (92 x 92 x 81 cm^3) with a sealed lid. Air was extracted at 45 L/s using a fan and the system integrated measurements of air flow and gas concentrations to allow direct measurement of CH_4 fluxes during each animal visit to the feed station (Huhtanen et al., 2015). Each feed bin was supported on two load cells (C-Lock Inc., Rapid City, SD, USA) and weights were recorded at 0.02-s intervals to an accuracy of 0.1 kg. Validation of bin accuracy during the experiment was based on weekly calibration with known weights and by checking whole-pen intake data against cumulative daily disappearance from each bin.

The 28 heifers in each cohort were randomly assigned to one of two pens, ensuring approximately equal representation of each breed and RFI category in each pen. However, the heifers were not split evenly into the two halves of the barn due to the uneven number of DMI-CH_4 stations. The heifers were split on a ratio of 15:13 rather than 16:12 due to only 15 lying bails being available on each side of the barn (Figure 4.3).

Heifers had free access to at least three feed stations in their pen. All feed was consumed from these feed stations enabling daily intake for each animal to be calculated. Water was freely available. Heifers were offered unrestricted access to the same batch of dried lucerne cubes as used in the original RFI measurement. Samples of lucerne cubes were dried thrice weekly in triplicate, with one batch dried at 95˚C for 48 h to determine DM content (%) and another batch dried at 65˚C for 72 h for nutritional analyses by NIR. Nutritional analyses indicated reasonable feed value: DM, 88 ± 0.2% fresh (mean ± standard deviation); CP, 20.6 ± 0.30% DM; NDF, 36.2 ± 0.80% DM; ADF, 30.6 ± 0.91% DM; Fat, 1.6 ± 0.0% DM. Feed disappearance/animal (kg) was multiplied by the most recently-measured feed DM% to determine individual animal daily DMI. Heifers were weighed thrice weekly. Animals were in the pens for 32 d for Cohort 1 and 25 d for Cohort 2.

The measurement period of the two cohorts differed in length due to the requirement to return the animals in Cohort 2 to their farm of origin, but were of sufficient length for intake and CH_4 determination. Power calculations based on previous data (B. Kuhn-Sherlock, Personal Communication) recommended a trial period of 28 days with a 1-week adaptation period, giving a 21-day measurement period (± 3 days).

4.3.1 Data exclusion

The first seven days for each cohort were an adaptation period and excluded from the statistical analysis. The length of the subsequent measurement period was 25 days for Cohort 1 (Day 8 to 32) and 18 days for Cohort 2 (Day 8 to 25). Intake and CH_4 data were

downloaded from the feed stations and manually checked for accuracy. Care was taken to ensure valid bases for data exclusion, and incorrect values were removed.

4.3.1.1 Intake data

Poor reconciliation of intake data related to occasional failure of electronics, an animal bumping or moving the feed bins, or an animal being present in the unit while the feed bin was being refilled. Data exclusion criteria applied were:

- Feed disappearance records with a start or end mass for visit of <-1 kg or >150 kg
- Intakes <0 kg/visit (due to data recorded while bins were being filled)

Intake data were summed by day and multiplied by the most recent DM% to provide daily DMI for each heifer. The mean of the daily DMI for each animal was used for further analyses. Following implementation of the data exclusion criteria, 11% of intake data were excluded from analysis, leaving 1,111 individual heifer daily DMI measures (an average of 20 d/heifer) for the 56 animals.

Intake data for any animals with access to units with temporary failure of electronics were removed for all affected days. As intake data are additive throughout the day, and the heifers had free access to multiple feeding stations, if one unit went offline for even part of the day, I could not be certain of the total daily intake of any animal that had access to that unit while it was offline.

Figure 4.4 depicts the start mass of the lucerne cubes that were in the feed bin of one DMI-CH_4 station over the dates of 13-21 March 2017: (1) before and (2) after data exclusion criteria were applied. The start mass in the feeding station slowly declined as the animals ate from the feed bins. The step-change increase in the start mass coincided with the refilling of the feed bins. The heifers were given unrestricted access to the lucerne cubes in the feed bins; therefore, there were only short periods where one of the multiple units were empty.

Highlighted in yellow and labelled with (a) is a section of the plot in which the start mass of the unit dropped to below -50 kg, which is an unrealistic change. However, when looking at the rest of the day's data, it was apparent that the bin was empty as the points immediately preceding this section were approaching 0 kg. Therefore, it was likely that an animal had moved the feed bin (as they had been observed doing on-farm), and this was the reason for the large negative values.

As the feed bin was empty at the time of these unusual readings, no feed was available for the animals from this single unit, so their total daily intakes will not have been affected by the misreporting of the intake data over this short period. Once the feed bin was refilled the intake measurements resumed as normal. Therefore, only the data points with the unusual start mass (<-1 kg or >150 kg) were removed from analyses, not the entire days' worth of data. The lower limit of -1kg was imposed to allow for the slight off-zero error of the scales, and the upper limit of 150 kg was above the holding capacity of the feeding bins.

Another problem noted in Figure 4.4 was highlighted in blue and labelled with (b). This problem was due to an animal being present in the unit while the feed bin was being refilled. This means that a large, negative intake was recorded. Subsequently any intakes <0 kg/visit were removed from analyses.

Figure 4.4 is a subset of the data, and similar problems were faced with the rest of the intake data across the other units and measurement days. Plot (1) represents the original data, and plot (2) represents the 'clean' data after the data exclusion criteria were applied. As indicated by the differences in the two plots, the data exclusion criteria only remove the incorrect data points that were highlighted in plot (1) and do not remove the rest of the correct data.

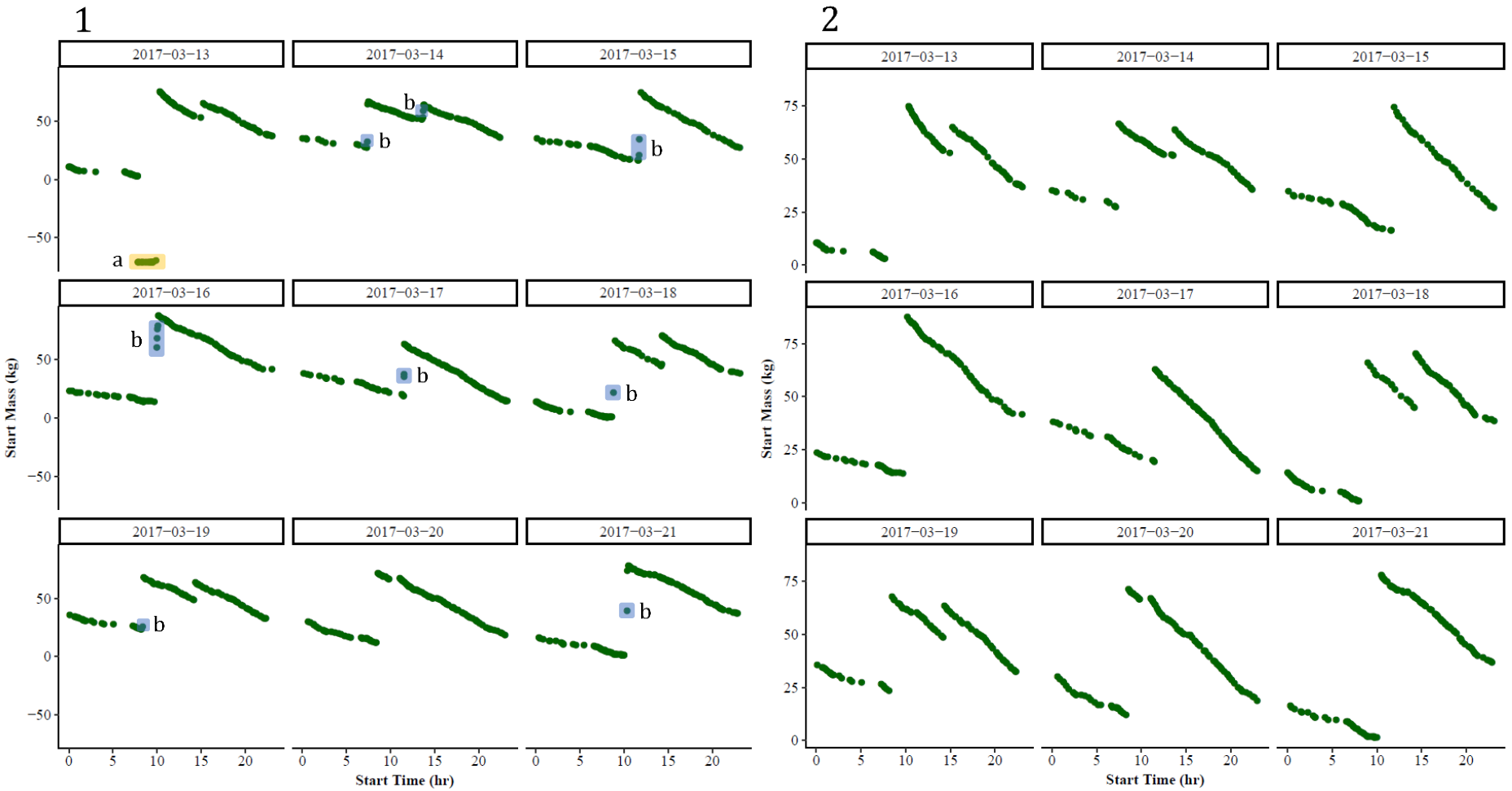


Figure 4.4. Start mass of the lucerne cubes that were in the feed bin of one DMI-CH_4 station over the dates of 13-21 March 2017: (1) before and (2) after data exclusion criteria were applied, with (a) and (b) highlighting the data that were excluded from analyses.

4.3.1.2 Methane data

Poor reconciliation of CH_4 data related to failure of electronics and short visit times. The extraction fan failed for all days in one of the seven feed stations; therefore, no CH_4 data were available for this unit. Feed disappearance data were still collected and CH_4 data were available from the other feed stations. The CH_4 data were removed when individual animal visits were <1 min. Following implementation of the data exclusion criteria, 33% of CH_4 data were excluded from analysis and there were 6,110 spot measures of CH_4 (an average of 109 per heifer for the duration of the measurement period) for the 56 animals.

The CH_4 data were removed when individual animal visits were <1 min. Table 4.1 indicates that, despite a similar mean to the rest of the data, CH_4 measurements <1 min are more variable (coefficient of variation = 44.9%), indicating poor quality data. Removing data <1 min did not affect the overall mean but reduced the standard deviation (from 88.4 to 71.9) and coefficient of variation (from 34.6% to 28.1%).

Table 4.1. Number of observations of CH_4 spot measurements from the GreenFeed units and the mean, median and standard deviation when removing data of different minimum visit duration.

CH_4, g/d	Number of observations	Mean	Median	Standard deviation	Coefficient of variation
All visit data	9182	256	254	88.4	34.6%
Visit duration >1 min	6110	256	253	71.9	28.1%
Visit duration <1 min	*3072*	*255*	*257*	*114.3*	*44.9%*

Figure 4.5 illustrated the CH_4 production data for all spot measurements, plotted over the hour of the day with all animals and all measurement days overlaid on top of each other. Within this Figure, (A) plots all the CH_4 visit data, and (B) plots only the CH_4 visit data with a duration >1 min. The red reference lines indicate the mean ±2 standard deviations. The absence of visits around 5.00 am is due to daily calibration of the units from 4.30 am to 5.40

am. The bulk of the CH_4 data sits around 200-300 g/d, with smaller tails either side and there is a slight diurnal pattern with a peak at approximately 7.30-8.00 pm.

In Figure 4.5 plot (A), the red reference lines (mean ±2 standard deviations) should cover 95% of the data (Freund, 1992). However, significantly more than 5% of the data are outside the ±2 standard deviation reference lines (P<0.001). After removing data from visits <1 min (Plot (B)), the extremities of the data were removed, because these were the data with the largest variation, as previously explained (see Table 4.1). This reduced the proportion of data outside the ±2 standard deviation reference lines to within 5% at a 95% confidence interval. This is because the spot measurement of CH_4 requires a period of calibration before a precise measurement of CH_4 can be recorded.

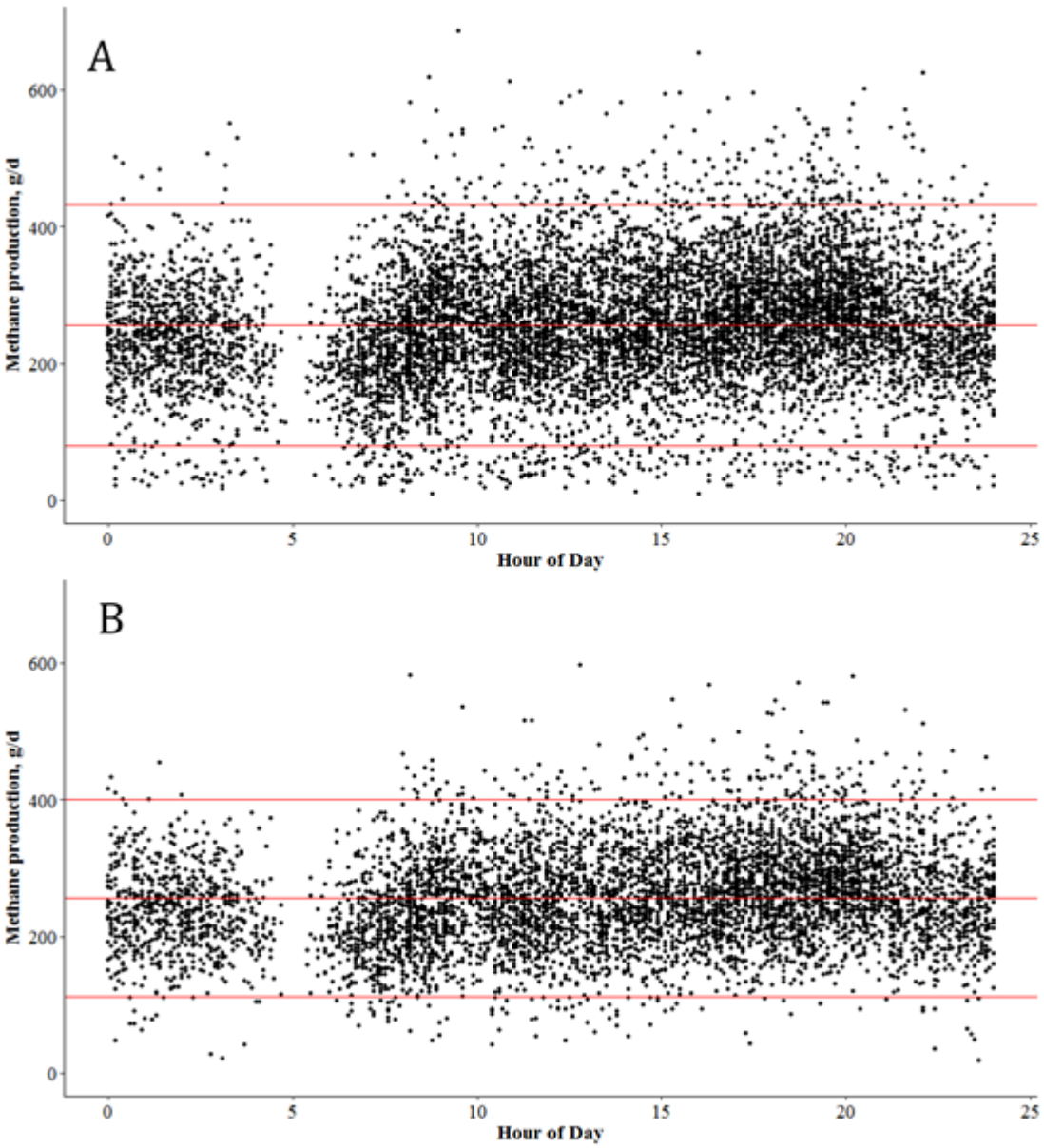


Figure 4.5. Methane production for all spot measurements plotted over hour of the day for all animals over all measurement days with: (A) all visit data; (B) data with individual animal visit duration >1 min. The red reference lines refer to mean ±2 standard deviations.

4.3.1.2.1 Methods for estimating mean daily methane production

The CH_4 spot measurements typically give a range of CH_4 measurements per cow throughout the day. To calculate the representative CH_4 for a particular heifer in the experiment, I evaluated three different methods of estimating CH_4. These were:

- **Arithmetic mean:** the average of all CH_4 spot measurements for a particular animal across all measurement days.
- **Daily mean:** the arithmetic mean of each day's CH_4 spot measurements for a particular animal, averaged across the whole measurement period.
- **Trapezoidal mean:** a weighted average of each day's CH_4 spot measurements for a particular animal using the trapezoidal approach to calculate area under the curve. Daily estimates are averaged across the whole measurement period. Refer to Appendix 3 for details on methodology.

Between the three methods, the estimate of CH_4 production results (g/d) were very similar, as indicated by the similar mean and between-animal standard deviation, and high correlation coefficient and R-squared values in Table 4.2, and the similar distributions in the box plots (A) in Figure 4.6.

Although all three methods appear to be similar, the arithmetic and daily means are the most similar and the trapezoidal mean gives a slightly lower estimate of CH_4 production (P <0.01), as highlighted in Figure 4.6. Figure 4.6 plot (B) is the plot of daily mean against arithmetic mean for CH_4, with a y = x line. As the y = x line sits through the middle of the data like a 'line of best fit', this suggests that that there is minimal difference between the two methodologies, as supported by the same mean between the two methodologies (255 g/d; Table 4.2). Figure 4.6 plot (C) is the plot of trapezoidal mean against arithmetic mean for CH_4, with a y = x line that indicates that the trapezoidal mean is a slightly lower estimate of CH_4 compared with the arithmetic mean. This is because the bulk of the data sits below the y = x line. This is supported by the trapezoidal mean (249 g/d) being lower than the

arithmetic mean (255 g/d; Table 4.2). Figure 4.6 plot (D) is the plot of trapezoidal mean against daily mean for CH_4, with a y = x line again indicating that the trapezoidal mean is a slightly lower estimate of CH_4 compared with the daily mean. This is because the bulk of the data sits below the y = x line. This is also supported by the trapezoidal mean (249 g/d) being lower than the daily mean (255 g/d; Table 4.2).

One possible explanation for the lower estimate in the trapezoidal mean is because of the low frequency of CH_4 spot measurements recorded for some animals, due to the extraction fan failing for all days in one of the seven feed stations and no CH_4 data being available for this unit. Therefore, some of the animals had very low numbers of spot measurements and, hence, this hindered the accuracy of the trapezoidal method for averaging CH_4 (Table 4.3 and Table 4.4). Therefore, I chose to use the arithmetic mean because it was the simplest method for averaging CH_4 without losing accuracy and it was the best method for calculating the average for those cows with low frequency of data across the trial.

Table 4.2. Averages and correlations for the three different approaches to estimate the CH_4 emissions from each animal (n = 56). Along the diagonal is the mean ± between-animal standard deviation. Above the diagonal is the correlation coefficient. Below the diagonal is the R-squared.

CH_4, g/d	Arithmetic mean	Daily mean	Trapezoidal mean
Arithmetic mean	255 ± 25.6	0.97	0.93
Daily mean	0.94	255 ± 25.4	0.96
Trapezoidal mean	0.87	0.92	249 ± 25.6

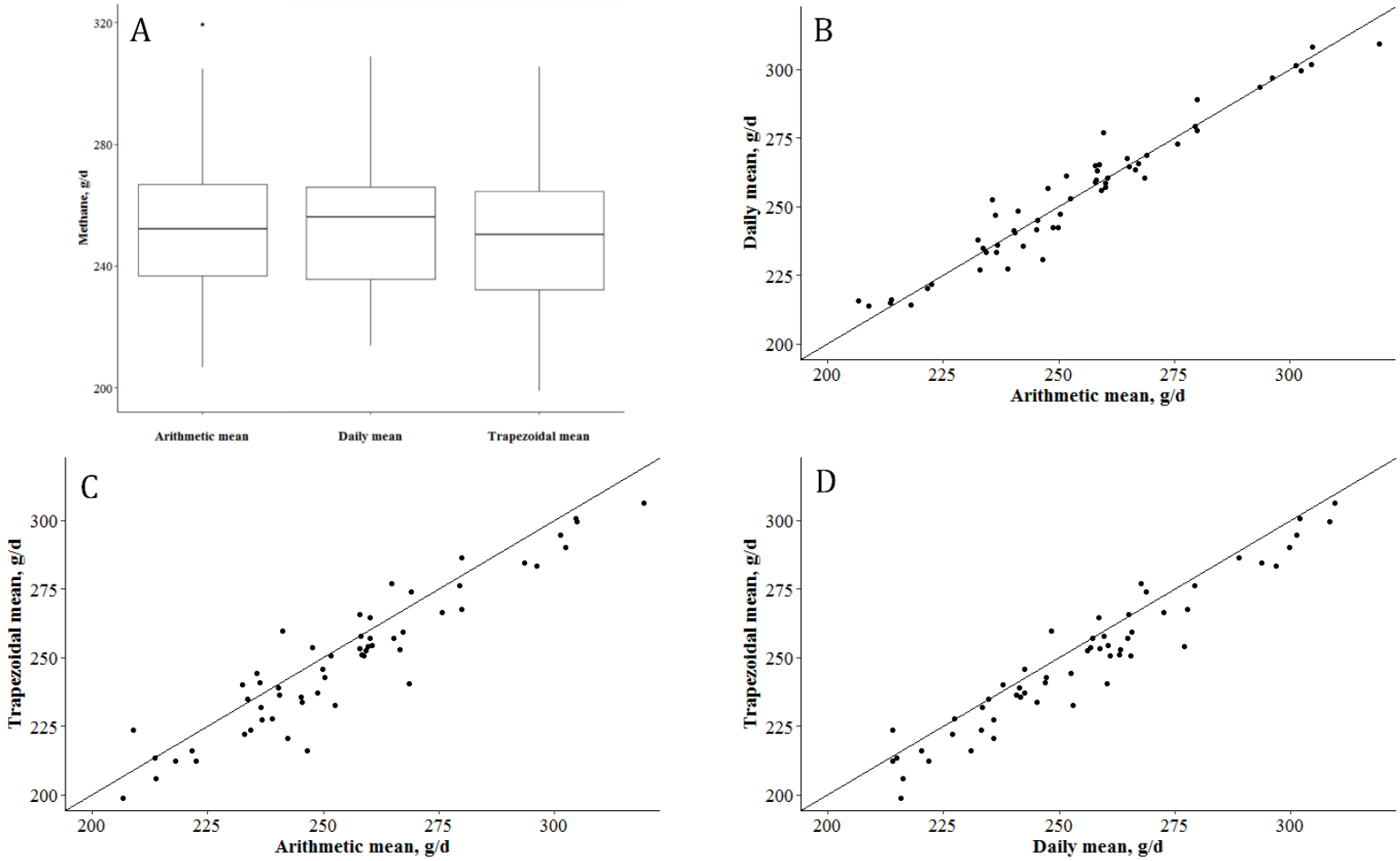


Figure 4.6. Plots comparing the three different approaches to estimate the CH_4 emissions from each animal (n = 56). (A) Box plot comparing the distributions of the three methods. (B) Plot of daily mean against arithmetic mean. (C) Plot of trapezoidal mean against arithmetic mean. (D) Plot of trapezoidal mean against daily mean. Each plot has a y = x line through the 45°.

Table 4.3. Heat map for the frequency of the CH_4 spot measurements per animal per day for Cohort 1.

Animal	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10	Day11	Day12	Day13	Day14	Day15	Day16	Day17	Day18	Day19	Day20	Day21	Day22	Day23	Day24	Day25	Average	Sum
4	8	8	9	5	12	10	7	10	8	8	9	12	10	8	8	5	8	5	7	8	7	7	7	7	8	8	201
17	1	1	0	0	0	2	0	2	1	2	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	12
19	7	6	7	3	4	11	8	5	6	8	8	4	4	6	6	9	5	8	5	6	3	4	5	10	9	6	157
21	10	11	11	6	13	15	14	11	16	11	10	12	10	10	10	13	13	12	9	8	11	12	8	10	12	11	278
22	10	11	10	12	12	9	11	13	17	12	10	11	9	7	8	11	11	15	10	13	12	12	12	6	9	11	273
27	4	2	5	5	2	3	1	4	3	0	4	5	1	2	0	2	1	0	0	0	1	0	0	0	0	2	45
32	1	3	7	4	0	9	8	2	4	1	1	2	2	1	0	0	1	2	3	7	6	5	7	4	5	3	85
46	9	9	9	6	13	8	9	7	9	8	10	10	9	7	10	8	12	13	6	9	10	10	13	13	10	9	237
50	6	8	3	1	0	3	2	4	2	0	0	0	4	0	1	1	2	0	1	5	1	0	1	0	0	2	45
53	3	5	3	2	3	4	1	2	2	0	0	2	1	1	1	1	1	0	1	1	0	1	0	0	0	1	35
58	2	2	0	1	1	2	1	1	2	0	0	5	0	2	1	2	3	3	1	2	1	1	1	2	0	1	36
62	4	3	7	3	3	1	4	2	1	3	1	0	3	1	0	0	1	2	0	1	1	2	3	3	0	2	49
63	13	15	9	9	13	12	10	15	12	15	9	14	14	13	10	12	14	11	12	10	14	10	14	14	13	12	307
69	2	3	1	3	4	1	2	0	4	2	1	1	3	3	2	1	4	2	0	3	5	3	6	4	1	2	61
82	7	6	5	7	2	6	6	2	4	7	9	5	4	4	3	2	1	1	5	5	4	2	1	4	5	4	107
83	6	6	5	2	3	8	4	3	5	1	5	1	3	0	3	4	1	3	2	4	2	2	1	1	6	3	81
85	7	4	4	7	6	6	7	9	8	6	4	6	8	7	8	2	2	6	2	3	2	1	2	4	3	5	124
93	6	4	6	4	4	3	4	6	2	5	5	5	7	6	3	5	1	3	0	1	1	2	3	0	2	4	88
95	7	12	12	12	7	11	12	15	7	9	10	7	4	4	4	1	1	1	3	4	0	4	6	8	7	7	168
96	8	10	11	8	5	11	10	7	7	8	6	6	5	6	7	8	9	7	8	10	6	4	6	5	5	7	183
101	1	3	4	6	2	1	5	4	6	2	0	2	3	1	5	3	1	2	2	1	2	3	4	4	5	3	72
114	2	8	5	3	0	2	3	3	4	3	2	2	3	1	0	2	1	1	4	0	2	1	3	1	2	2	58
118	3	10	3	5	6	8	8	7	6	7	6	8	10	8	6	8	6	4	9	3	3	6	10	6	8	7	164
121	9	7	14	9	7	9	6	6	10	8	10	8	6	6	6	8	4	2	5	8	5	3	9	6	5	7	176
124	4	6	5	8	8	2	4	4	2	5	6	4	5	3	4	6	2	2	4	1	0	1	2	1	1	4	90
130	6	11	12	12	15	10	14	4	4	8	1	4	3	2	4	1	5	2	2	3	5	6	3	2	5	6	144
137	3	8	10	4	7	4	1	3	8	8	4	8	3	7	6	2	5	6	0	4	7	4	2	1	1	5	116
141	13	15	15	14	12	13	11	10	11	13	13	12	12	12	11	7	13	8	9	12	13	7	10	13	11	12	290

Table 4.4. Heat map for the frequency of the CH_4 spot measurements per animal per day for Cohort 2.

Animal	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Average	Sum
151	1	1	0	9	4	3	6	6	3	0	3	0	3	1	4	3	2	4	3	53
158	0	0	2	0	0	0	0	3	5	5	1	3	1	2	5	2	2	1	2	32
160	7	5	9	7	10	13	10	6	5	8	9	11	6	6	6	5	8	5	8	136
163	3	2	4	7	3	8	5	0	0	1	3	3	0	5	2	2	3	0	3	51
165	2	0	1	6	3	1	0	0	1	0	0	0	0	0	1	1	0	1	1	17
169	8	7	6	4	7	6	4	10	10	10	10	5	9	8	7	6	10	7	7	134
170	4	5	6	3	4	3	5	4	2	3	4	1	4	5	4	3	8	4	4	72
172	6	6	7	14	6	9	12	14	9	8	7	8	9	8	10	11	10	9	9	163
173	4	4	4	7	1	3	4	0	2	0	1	1	0	3	0	2	1	1	2	38
176	3	2	5	4	7	4	6	7	6	8	6	7	9	5	9	10	9	9	6	116
181	0	4	1	1	0	2	4	2	0	2	2	1	0	0	0	0	0	1	1	20
185	1	3	4	1	1	4	1	1	1	3	1	1	1	1	1	0	1	2	2	28
188	1	1	1	0	1	0	0	0	0	0	1	1	3	1	0	0	0	0	1	10
190	1	0	1	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	5
211	4	3	3	6	6	6	2	1	6	8	9	7	4	7	7	6	6	6	5	97
222	10	12	12	8	9	8	7	9	7	10	5	8	6	12	12	15	10	10	9	170
239	12	7	7	10	7	6	5	2	7	4	1	2	3	2	3	1	2	3	5	84
242	4	6	8	10	2	6	3	5	6	7	9	7	11	9	11	8	6	7	7	125
247	9	6	12	9	6	11	7	12	14	10	14	15	14	8	15	11	13	12	11	198
250	14	9	9	8	9	11	12	9	16	11	13	10	7	7	11	8	9	8	10	181
255	1	0	0	2	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	6
261	12	9	11	9	7	8	12	7	5	8	9	9	6	11	4	6	7	6	8	146
267	11	10	8	10	0	7	9	10	10	9	12	9	8	12	9	13	8	9	9	164
268	4	6	5	2	6	4	3	6	6	6	6	6	7	8	10	11	13	9	7	118
274	3	1	8	5	5	3	3	10	11	10	9	9	12	7	8	11	11	7	7	133
286	6	4	3	3	3	3	1	0	0	0	1	1	0	5	3	1	0	2	2	36
288	7	5	7	6	1	3	6	3	3	2	3	5	3	1	6	6	10	4	5	81
298	2	2	2	1	2	0	2	0	0	0	1	0	0	0	0	0	0	2	1	14

4.3.2 Calculations and statistical analysis

The average CH_4 for each animal was calculated from the arithmetic mean of all measurements for all days. Linear regression analyses were used to describe the relationship between BW and day of trial, with the slope equal to average daily BWg. The average BW for each animal for the trial was estimated as the predicted BW at the midpoint of the experimental period (day 20 for Cohort 1, and day 16.5 for Cohort 2). Methane yield (expressed as g/kg DMI) was calculated by dividing average CH_4 production (g/d) by average DMI, and CH_4/kg BWg was calculated by dividing average CH_4 production (g/d) by the average BWg.

Dependent variables were analyzed with a linear model that included breed, RFI category, cohort, and the interaction of breed and RFI category as fixed effects. Statistical analyses were performed using R (version 3.3.3, R Core Team, 2017). Results are presented as least-squares means and SE of the difference or 95% confidence intervals. Significance was declared at $P < 0.05$.

4.4 Results and Discussion

Average RFI results for the HF and Jer breeds and the high and low RFI categories are presented in Figure 4.1 and CH_4, DMI, and BWg summary data are presented in Table 4.5. Breed x RFI category interactions were not significant; therefore, only breed or RFI differences are presented. I found no significant differences between RFI categories in BW or daily BWg, but DMI and DMI/kg BW were 9.3% and 10.6% lower in low RFI heifers compared with their high RFI counterparts (see Figure 4.7), confirming the accuracy of their designation to RFI category. On average, low RFI heifers ate 1.2 kg DM/d less than heifers in the high RFI category. This effect of RFI was anticipated and has been reported previously (Green et al., 2013). Consistent with my results, Waghorn et al. (2012) reported that the DMI difference between the top and bottom 10% RFI heifers selected in a similar manner from a cohort of 1,052 heifers was 1.5 kg DM/d.

Table 4.5. Least square means for groups representing two breeds (Holstein-Friesian: HF; and Jersey: Jer, n = 28/breed) and two pre-determined residual feed intake (RFI[1]) categories (Low: -2.0 kg DMI/d and High: +2.0 kg DMI/d, n = 28/RFI category). Animals were 20-22 mo old dairy heifers and were offered unlimited access to dried lucerne cubes.

	Breed				RFI category			
	Jer	HF	SED	*P*-val	Low	High	SED	*P*-val
BW[2], kg	408	480	8.2	<0.001	448	439	8.2	0.27
DMI, kg DM/d	11.3	12.4	0.37	<0.01	11.3	12.4	0.37	<0.01
BWg[3], kg/d	1.2	1.3	0.08	0.077	1.2	1.3	0.08	0.25
CH_4, g/d	242	267	6.2	<0.001	253	256	6.2	0.6
CH_4, g/DMI	21.6	21.9	0.64	0.62	22.7	20.7	0.64	<0.01
CH_4, g/kg BWg	220	212	13.8	0.6	224	207	13.8	0.23

[1] The RFI is the difference between amount of feed in kg DM required for biological processes and estimated feed

[2] BW = average BW estimated as the predicted BW at the midpoint of the experiment.

[3] BWg = average daily BW gain

Contrary to my hypothesis, there were no significant differences between RFI categories on CH_4 production/d (Figure 4.8) or CH_4/kg BWg (Figure 4.10). However, because of the similar CH_4 production, but a lower DMI, low RFI heifers produced 2.0 g more CH_4/kg DMI, or had a 9.7% greater CH_4 yield than heifers in the high RFI category (Table 4.5; Figure 4.9). Therefore, my results do not support genetic selection for low RFI to reduce enteric CH_4 emissions. The lack of effect of RFI category on daily CH_4 production was surprising, but may indicate that differences in ruminal digestive efficiency are the key reason for the greater FCE in low RFI heifers. If the lower RFI was a result of greater ruminal feed digestibility, particularly NDF digestibility in a high forage diet, this would be expected to increase CH_4 yield (g/kg DMI; Ramin and Huhtanen, 2013; Moate et al., 2016). My new hypothesis is that greater CH_4 yield/kg DMI in low RFI heifers is a result of greater ruminal

digestion of DM and NDF, and this is supported by previous work investigating phenotypic differences between animals selected for low and high RFI. For example, cows selected for low RFI performed fewer and more intense rumination bouts, which would be expected to increase the physical breakdown of forage (Gregorini et al., 2015). This was supported by the fecal particle size distribution measured in their study: relative to the high RFI cows, low RFI cows excreted feces with 30% less large particles, indicating a greater level of ruminal digestion of the forage (Clauss et al., 2010) and the potential for greater CH_4 yield. Furthermore, Rius et al. (2012) reported that low RFI dairy heifers had a greater apparent total tract nitrogen digestibility and a tendency toward greater DM and organic matter digestibility. Nkrumah et al. (2006) also reported a tendency towards greater DM digestibility with greater efficiency in beef steers and numerically higher NDF and ADF digestibility. These data are consistent with a greater ruminal digestion of NDF and, as a result, increased CH_4/kg DMI in low RFI animals on a high forage diet. Therefore, although selecting dairy heifers for low RFI increases FCE and reduces the inputs/kg BWg (Waghorn et al., 2012) and milk (Macdonald et al., 2014), it is unlikely to reduce total daily CH_4 production because the animals produce more CH_4/kg DMI.

Previously reported effects of RFI on enteric CH_4 production in cattle are limited to beef cattle. Although most studies report a positive relationship between RFI and both DMI and CH_4 production (Nkrumah et al., 2006; Hegarty et al., 2007; Fitzsimons et al., 2013), the effect of RFI on CH_4 is not consistent across all studies (McDonnell et al., 2016). The reason for the inconsistent effect of RFI on CH_4 production is not known, but it might relate to differences in diet. Both Nkrumah et al. (2006) and Hegarty et al. (2007) offered growing beef steers a diet of predominantly cereal grains compared with the dried lucerne cubes offered in my experiment. These dietary differences would result in very different rumen fermentation patterns and expected CH_4 yields/kg DMI (Ramin and Huhtanen, 2013; Moate et al., 2017). Fitzsimons et al. (2013) offered unrestricted access to pasture silage, a forage with no starch, and not dissimilar to the diet used in my experiment in terms of rumen

fermentation products. Yet, they also reported a reduction in CH_4 production in low RFI steers. In comparison, McDonnell et al. (2016) offered pasture silage, fresh pasture, or a TMR and reported no effect of RFI on CH_4 production, and an increase in CH_4 yield in low RFI animals, similar to my results. Gender may be another source of difference, with many of the animals evaluated in the beef studies being male castrates. These may have a different physiology underpinning RFI compared with dairy heifers. Age and production stage may also be a factor in the inconsistency of results between studies. Age and production stage have been reported to affect RFI in dairy cattle. For example, Macdonald et al. (2014) reported that the animals identified as being 21% different in RFI as calves were only 3% different in their RFI as lactating cows. Although, I cannot determine with any certainty why my results differ from those reported for beef cattle, my results do not support a positive effect of RFI on daily CH_4 production by dairy heifers approaching 2 years of age when consuming dry lucerne cubes.

Dairy breed affected BW, DMI, and CH_4 production. The HF heifers were approximately 70 kg heavier than their Jer counterparts and there was a trend ($P < 0.10$) for HF to have a higher average daily BWg. The trend for a BWg difference is consistent with HF heifers consuming 1.1 kg DM/d more than Jer heifers (Figure 4.7). Due to their lower DMI, Jer heifers produced about 25 g less CH_4/d (approximately 9.3%) than the HF heifers (Figure 4.8). However, breed did not affect CH_4/kg DMI (Figure 4.9) or CH_4/kg BWg (Figure 4.10). A breed effect on DMI has been previously reported (Goddard and Grainger, 2003; Prendiville et al., 2010; Beecher et al., 2014; Spaans et al., 2018); so, I hypothesized that the relatively larger gastrointestinal tract of the Jer (Beecher et al., 2014) and the greater FCE of the Jer breed (L'Huillier et al., 1988; Prendiville et al., 2009; Spaans et al., 2018) would result in a lower CH_4/kg DMI. The experimental results do not support this.

The lack of breed effect on CH_4 yield in my study, despite reported breed differences in FCE, is consistent with previous reports. In their review, Goddard and Grainger (2004) concluded that, despite reported differences in FCE and metabolic heat production, no

difference existed in CH_4 yield (g/kg DMI) between HF and Jer breeds. Further research is required to understand the mechanisms supporting the greater FCE in the Jer breed; however, my data indicate that dairy breed itself does not affect CH_4/kg DMI and any difference in daily CH_4 production is associated with differences in DMI.

4.5 Conclusion

In conclusion, consistent with previous beef cattle experiments, a considerable variation occurs in RFI in growing dairy heifers. Even with a large difference in DMI in divergent animals, there was no effect on daily CH_4 production (g/d). As a result, CH_4 yield (g/kg DMI) was greater in low RFI animals. I hypothesize that this is because of increased ruminal NDF digestibility. Jersey heifers have a lower DMI than their heavier HF comparison and, therefore, produce less CH_4/d; however, there was no effect of breed on CH_4 yield (i.e., g CH_4/kg DMI). Selecting dairy genetics for lower RFI will not reduce daily CH_4 production.

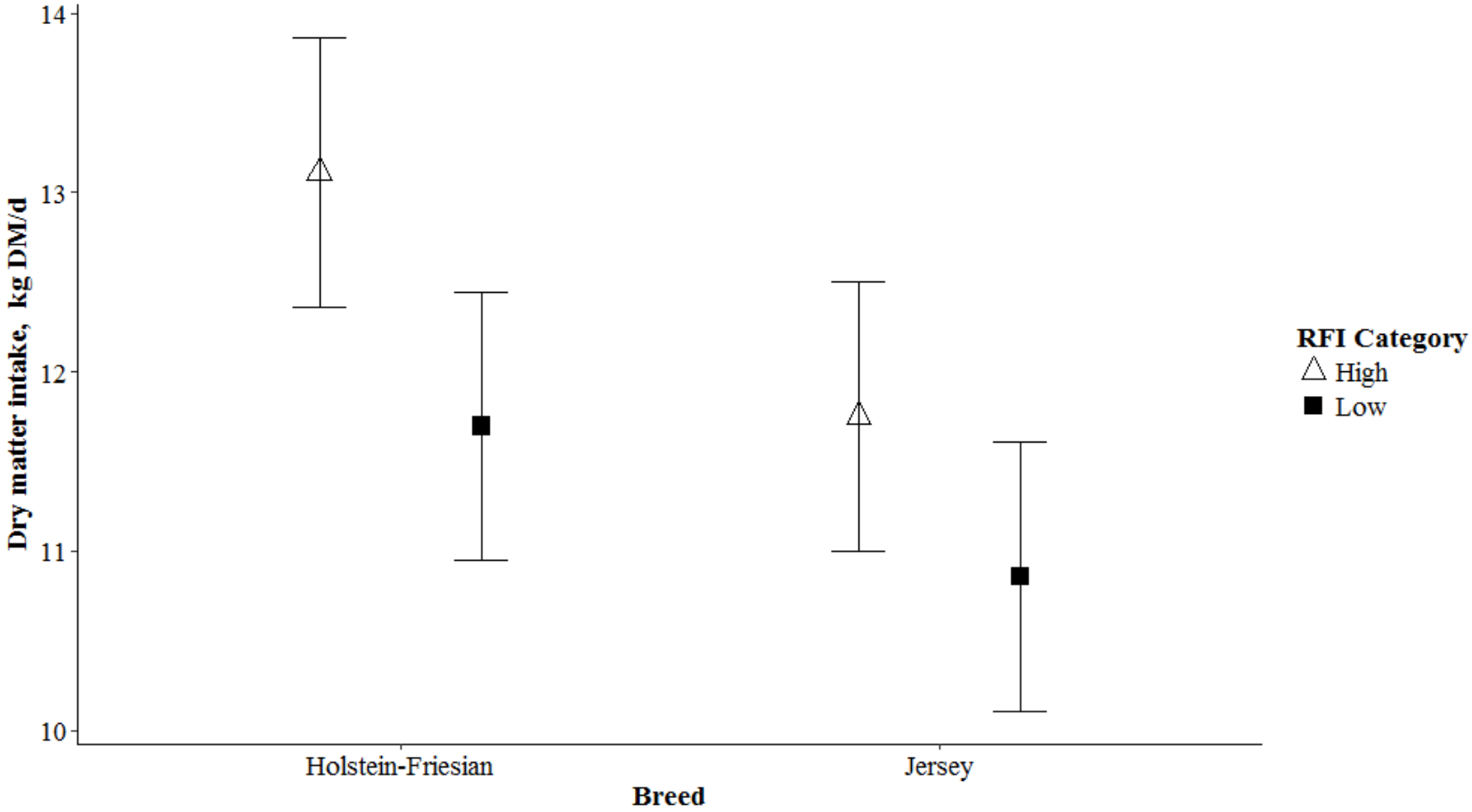


Figure 4.7. Dry matter intake (kg DM/d) of four groups of dairy heifers representing two breeds (Holstein-Friesian and Jersey) and two previously determined RFI categories (High: +2.0 kg DM; Low: -2.1 kg DM). Heifers were 20-22 mo old; BW = 480 and 408 for HF and Jer, respectively, and 439 and 448 for High and Low RFI, respectively. Midpoint in each vertical bar is the least square mean for the group; the error bars are the 95% confidence interval.

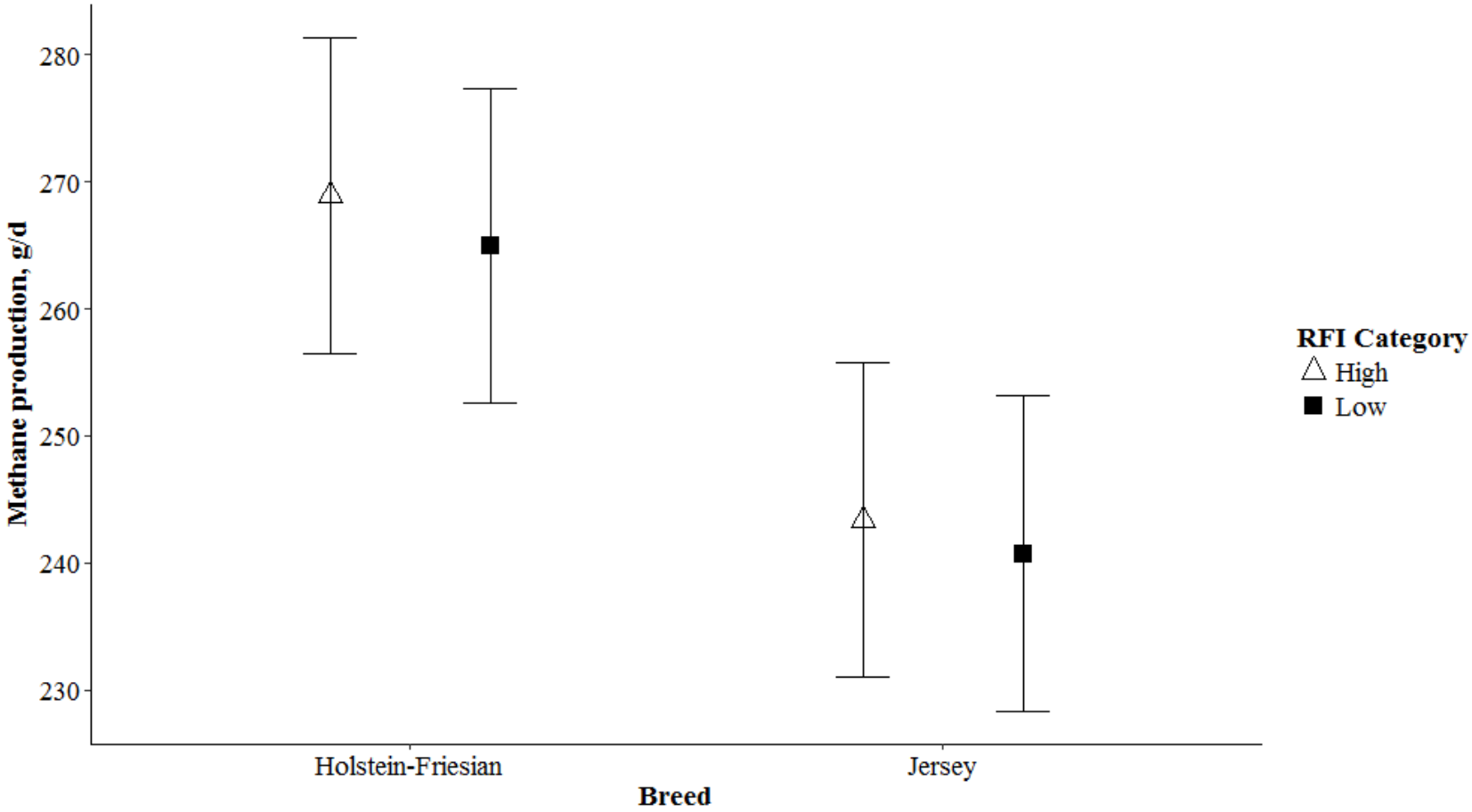


Figure 4.8. Methane production (g/d) of four groups of dairy heifers representing two breeds (Holstein-Friesian and Jersey) and two previously determined RFI categories (High: +2.0 kg DM; Low: -2.1 kg DM). Heifers were 20-22 mo old; BW = 480 and 408 for HF and Jer, respectively, and 439 and 448 for High and Low RFI, respectively. Midpoint in each vertical bar is the least square mean for the group; the error bars are the 95% confidence interval.

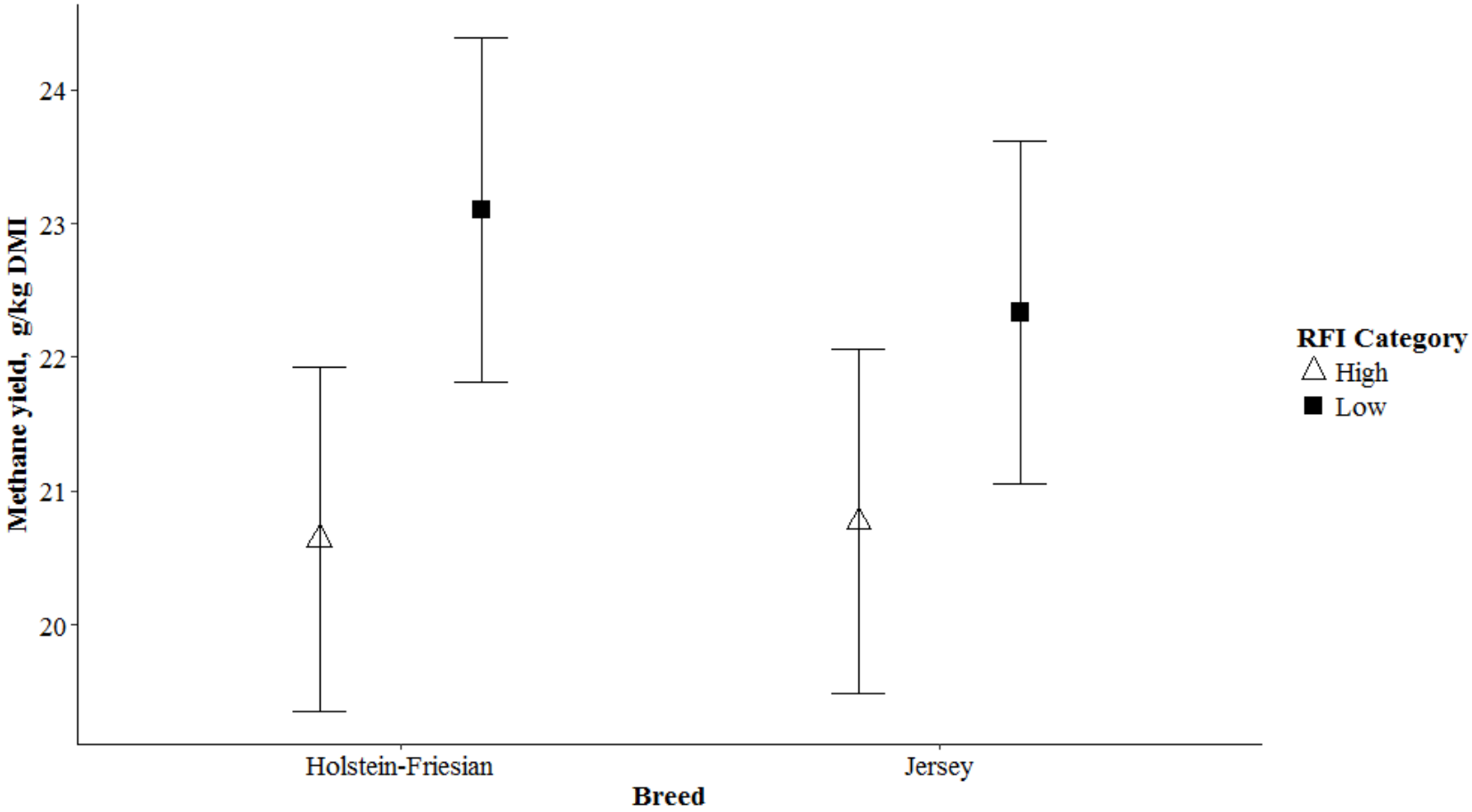


Figure 4.9. Methane yield (g/kg DMI) of four groups of dairy heifers representing two breeds (Holstein-Friesian and Jersey) and two previously determined RFI categories (High: +2.0 kg DM; Low: -2.1 kg DM). Heifers were 20-22 mo old; BW = 480 and 408 for HF and Jer, respectively, and 439 and 448 for High and Low RFI, respectively. Midpoint in each vertical bar is the least square mean for the group; the error bars are the 95% confidence interval.

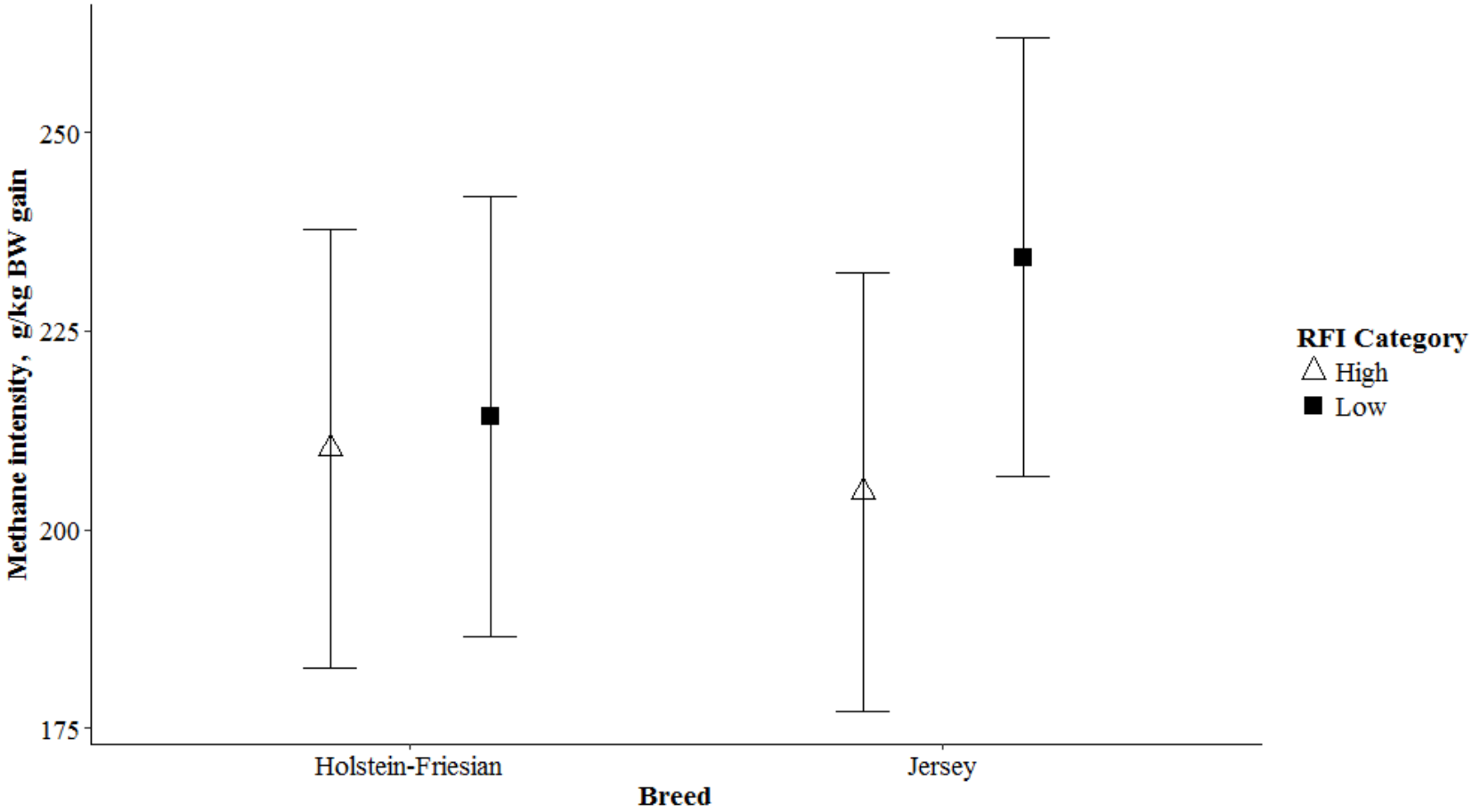


Figure 4.10. Methane intensity (g/kg BWg) of four groups of dairy heifers representing two breeds (Holstein-Friesian and Jersey) and two previously determined RFI categories (High: +2.0 kg DM; Low: -2.1 kg DM). Heifers were 20-22 mo old; BW = 480 and 408 for HF and Jer, respectively, and 439 and 448 for High and Low RFI, respectively. Midpoint in each vertical bar is the least square mean for the group; the error bars are the 95% confidence interval.

Chapter 5 General Discussion

5.1 Residual feed intake

One of the main objectives of this thesis was to determine the relationship between enteric CH_4 emissions from dairy heifers and their RFI (Chapter 4). As discussed, enteric CH_4 is an important source of digestible energy loss in ruminants. Because research has identified a positive relationship between RFI and daily CH_4 production in beef cattle, I hypothesized that low RFI dairy heifers, which are more feed efficient, would also produce less CH_4.

I measured the CH_4 emissions from dairy heifers previously designated as either high or low RFI, and, contrary to my hypothesis, there were no effects of RFI category on CH_4 production/d or CH_4/kg BWg. However, RFI did affect daily DMI, with low RFI heifers eating less DM daily than their high RFI category herdmates. Because of the similar CH_4 production, but a lower DMI, low RFI heifers had a 9.7% greater CH_4 yield (g/kg DMI) than heifers in the high RFI category (Table 4.5). Therefore, my results do not support genetic selection for low RFI to reduce enteric CH_4 emissions.

The lack of effect of RFI category on daily CH_4 production was initially surprising. However, there is a plausible reason for the measured effect: that is, the RFI trait in dairy cows, at least in New Zealand, is, largely, a representation of between-animal differences in ruminal digestive efficiency. On reviewing additional research undertaken with different RFI populations in New Zealand, it appears that low RFI animals have a more efficient fermentation process, extracting more value from the feed eaten. For example, Rius et al. (2012) reported that low RFI heifers in New Zealand had greater ruminal DM, organic matter and nitrogen digestibility. Similarly, Nkrumah et al. (2006) reported increased fibre and DM digestibility in low RFI animals in Canada. This is also consistent with Gregorini et al. (2015), who reported differences in chewing behaviour and faecal particle size that were consistent with increased physical breakdown of feed in the mouth and rumen.

Therefore, the results from this study indicate that low RFI animals require less feed because they are able to extract a greater digestible energy from the feeds consumed, which increases both metabolisable and net energy availability. However, the results from this study indicate that there is no reduction in the percent of digestible energy lost as CH_4.

Despite the negated hypothesis, RFI is still a beneficial trait to the New Zealand dairy industry. This is indicated through the lower DMI for the same BWg in the low RFI heifers. Macdonald et al. (2014) investigated that cattle selected for divergence in RFI:

- as calves displayed a 21% difference in RFI during growth,
- as lactating cows displayed a 3% difference in RFI during lactation.

This demonstrated that, although the difference is reduced, divergence in RFI based on growth traits as young animals persist through to when the animal is lactating (Macdonald et al., 2014). Therefore, over the lifetime of the cow, selection for low RFI cattle will save feed and/or improve animal productivity. However, although selecting dairy heifers for low RFI increases FCE and reduces the inputs/kg BWg (Waghorn et al., 2012) and milk (Macdonald et al., 2014), it is unlikely to reduce total daily CH_4 production because the animals produce more CH_4/kg DMI.

Although some research has identified an association between RFI and CH_4 yield, the effect is inconsistent, with many manuscripts reporting no effects (Hegarty et al., 2007; Jones et al., 2011 (low quality pasture treatment); Waghorn and Hegarty, 2011; Fitzsimons et al., 2013), negative effects (McDonnell et al., 2016), and positive effects of RFI on CH_4 yield (Nkrumah et al., 2006; Jones et al., 2011 (high quality pasture treatment)). I can't deduce the reason for the inconsistency from my work, but, if allowed to speculate, I assume it points to different mechanisms underpinning RFI in different breeds, genders, and, possibly even in different physiological states. Nevertheless, my results, in conjunction with previous research undertaken on independent populations on New Zealand, highlight that a large proportion of the variation in RFI in New Zealand dairy cattle can be explained by

differences in rumen fermentation. Considering the inconsistencies in CH_4 yield in different RFI categories, it is imperative that research needs to be undertaken to ascertain the reasons for differences in RFI whenever populations are being characterised.

5.2 Breed

The second objective of this thesis was to determine the effect of breed on enteric CH_4 emissions from dairy heifers (Chapter 4). As discussed, Jer cows have a larger gastrointestinal tract per unit BW compared with HF cows, thus promoting an increase in NDF and DM digestibility (Beecher et al., 2014). This led to my hypothesis that HF heifers would have lower CH_4 yield than Jer heifers, and to test this I measured the CH_4 emissions of Jer and HF dairy heifers.

As expected, HF heifers were heavier and ate more than the Jer heifers, for the same BWg. Due to their lower DMI, Jer heifers produced approximately 9.3% CH_4/d less than the HF heifers. However, breed did not affect CH_4/kg DMI or CH_4/kg BWg (Table 4.5). Therefore, my results do not support a difference in enteric CH_4 emissions between Jer and HF dairy breeds, beyond their size and DMI differences.

The lack of breed effect on CH_4 yield in my study, is consistent with previous reports: despite reported differences in FCE and metabolic heat production, no difference existed in CH_4 yield (g/kg DMI) between HF and Jer breeds (Goddard and Grainger, 2003). Therefore, Jer and HF do not differ in CH_4 yield and further research is required to understand the mechanisms supporting the greater FCE in the Jer breed. Overall, the difference in daily CH_4 production is associated with differences in DMI and BW of the two breeds.

5.3 Limitations

5.3.1 Technology

One of the major issues confronted in my Masters was the technological failure of the extraction fan in one of the seven feed stations and, thus, no CH_4 data were available for this unit. Feed disappearance was still collected and CH_4 data were available from the other feed stations. However, because of the failure of this unit, some of the animals had very low numbers of spot measures for the duration of the experiment (see Table 4.3 and Table 4.4). Unfortunately, due to a miscommunication, DairyNZ understood that the trouble with the fan was not going to affect the measurement of the CH_4.

In future experiments, a better troubleshooting system and communication process must be established to ensure that this does not happen. The diet adaptation period could be utilised to ensure that not only the animals are adapted to their situation but, also, that the units are all functioning correctly.

There were also some further technological issues around the strength of the internet connection on the farm; therefore, there is a need to install a more effective and weather-proof internet router.

However, these limitations did not greatly affect the results, as:

- both breeds and both RFI categories were co-mingled and, therefore, the unit did not differentially affect any of the treatment groups;
- removing animals with low frequency of CH_4 spot measurements from the analysis did not change the overall conclusions of the trial.

5.3.2 Proximity to calving

By the end of the Cohort 2 experimental period, some of the heifers were approaching their calving dates (approximately 1-month away from calving). This added the implication of

their proximity to calving meaning that the Cohort 2 experimental period had to be cut short to allow for the heifers to be safely transported back to their home farms as per DairyNZ recommendations of not transporting pregnant cows within 4 weeks of their calving date, for more than 2 hours. This is in accordance with the Code of Welfare: Transport within New Zealand (Ministry for Primary Industries, 2016; DairyNZ, 2018b). This meant that the two cohorts' experiment durations differed in length.

The proximity to calving and shorter duration of Cohort 2 should not have impacted the validity of the trial as:

- both breed and RFI categories were represented equally in both cohorts and therefore any differences between the two cohorts should not differentially affect any of the treatment groups; and
- as animals are the experimental units, it is the number of animals more so than the number of days or individual spot measurements per animal that determine the power of the trial.

I chose to keep the two cohorts different lengths instead of reducing Cohort 1 down to the same length as Cohort 2, as it would have meant removing useable data from Cohort 1.

5.3.3 Refilling the feed bins

One further implication that will be improved upon in further experiments using the DMI-CH_4 stations is to use a barrier across the unit to ensure that there is not an animal in the unit while the feed bins are being refilled. This relatively simple on-farm change will be beneficial to reduce the amount of data that need to be removed before analyses.

5.4 Conclusion

The results from this Masters study provide new information on the effects of RFI on enteric CH_4 emissions from dairy heifers, which has previously only been investigated in beef herds. Consistent with previous research, there is considerable variation in RFI in growing dairy

heifers, within both Jer and HF breeds. Even with a large difference in DMI in divergent animals, there was no effect of RFI on daily CH_4 production. As a result, CH_4 yield (g/kg DMI) was greater in low RFI animals.

Jersey heifers had a lower DMI than the heavier HF heifers and, therefore, produced less CH_4/d; however, there was no effect of breed on CH_4 yield.

These results indicate that genetic selection for lower RFI will not reduce daily CH_4 production in dairy heifers.

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Appendices

Appendix 1: American Dairy Science Association Abstract and Presentation

This abstract and presentation was presented at the annual meeting of the American Dairy Science Association in Knoxville, Tennessee, in June 2018

215 Relationship between residual feed intake and CH_4 production in dairy heifers. H. Flay*[1,2], B. Kuhn-Sherlock[1], K. Macdonald[1], M. Camara[1], D. Donaghy[2], N. Lopez-Villalobos[2], and J. R. Roche[1,3], *[1]DairyNZ, Hamilton, New Zealand, [2]Massey University, Palmerston North, New Zealand, [3]University of Auckland, Symonds St, Auckland, New Zealand.*

There is growing interest in improving feed conversion efficiency, through reducing residual feed intake (RFI), and in reducing agricultural methane (CH_4) emissions. As CH_4 is a major source of digestible energy loss in ruminants, it is plausible that selection for low RFI (i.e., high feed efficient) would also reduce CH_4 emissions. CH_4 production (g/d) and yield (g/kg DMI) for 56 heifers (20–22 mo old) were measured in a 2 × 2 factorial arrangement, including 2 breeds of dairy cattle (Jersey: Jer and Holstein-Friesian: HF; n = 28/breed) previously designated as either high (+2.0 kg DM) or low RFI (−2.1 kg DM; n = 28/RFI category). Breed × RFI category interactions were not significant; main effects are presented (see Table 1). HF heifers were significantly heavier and consumed more than Jer, but breed did not significantly affect DMI/kg BW or daily BW gain (BWg). Because of the lower DMI, Jer heifers produced less CH_4/d, but not per kg DMI or per kg BWg. RFI category had no significant effect on BW or BWg, but DMI and DMI/kg BW were 9.2% and 10.6% less in low RFI heifers. RFI category had no significant effect on CH4/d or CH_4/kg BWg; but CH_4/kg DMI was greater in low RFI heifers because of their lower DMI. Results probably reflect more complete digestion of ingested feed in low RFI heifers, consistent with previously reported greater physical breakdown of feed and a higher apparent digestibility of organic matter in low RFI animals. In conclusion, selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d), but could increase CH_4 yield (g/kg DMI). This research was funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

Key Words: feed conversion, environmental sustainability, greenhouse gas

Table 1 (Abstr. 215). LSM (± SEM) for BW, DMI, BWg and CH_4 in Jer and HF heifers from low or high RFI categories.

	BW, *kg*	DMI, *kg DM/d*	BWg, *kg/d*	CH_4, *g/d*	CH_4, *g/kg DMI*	CH_4, *g/kg BWg*
Jer	408 ± 7.0	11.3 ± 0.29	1.2 ± 0.06	242 ± 4.3	21.6 ± 0.51	219 ± 9.6
HF	479 ± 7.0	12.4 ± 0.29	1.3 ± 0.06	267 ± 4.3	21.9 ± 0.51	211 ± 9.6
P	<0.001	0.01	0.09	<0.001	0.65	0.57
Low RFI	448 ± 9.8	11.3 ± 0.29	1.2 ± 0.06	253 ± 4.9	22.7 ± 0.47	222 ± 9.5
High RFI	439 ± 9.8	12.4 ± 0.29	1.3 ± 0.06	256 ± 4.9	20.7 ± 0.47	208 ± 9.5
P	0.50	<0.01	0.31	0.63	<0.01	0.30

Relationship between residual feed intake and CH_4 production in dairy heifers

H. E. Flay, B. Kuhn-Sherlock, K. A. Macdonald, M. Camara, N. Lopez-Villalobos, D. J. Donaghy, and, J. R. Roche

MASSEY UNIVERSITY
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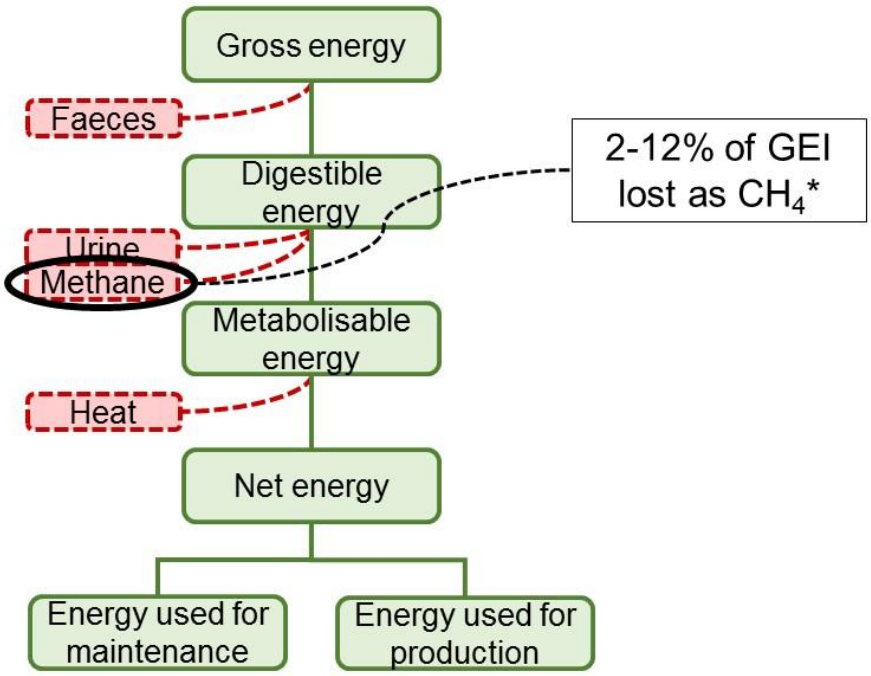
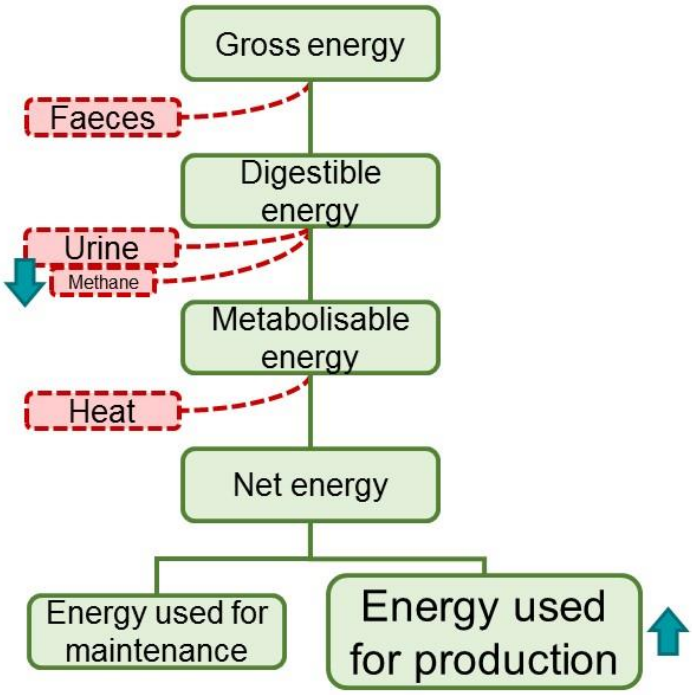
DairyNZ



*Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483–2492.



Methane – CH_4

- By-product of ruminant enteric fermentation;
- Greenhouse gas – warming effect 25 x CO_2;
- Agriculture and land use change contributes ~22% of global greenhouse gas emissions. (Knapp et al., 2014)

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

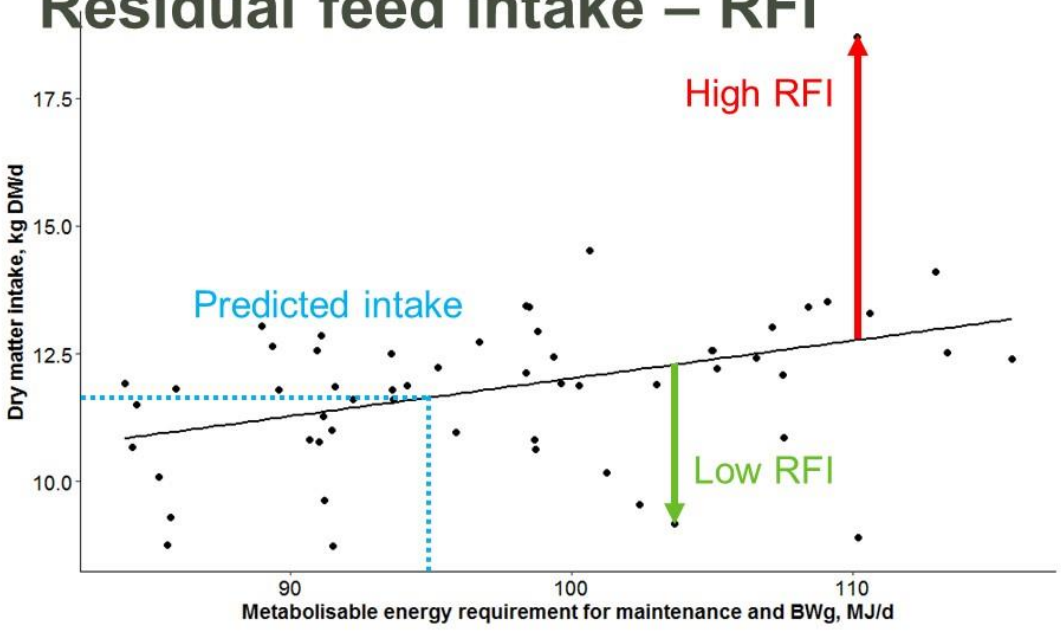
DairyNZ

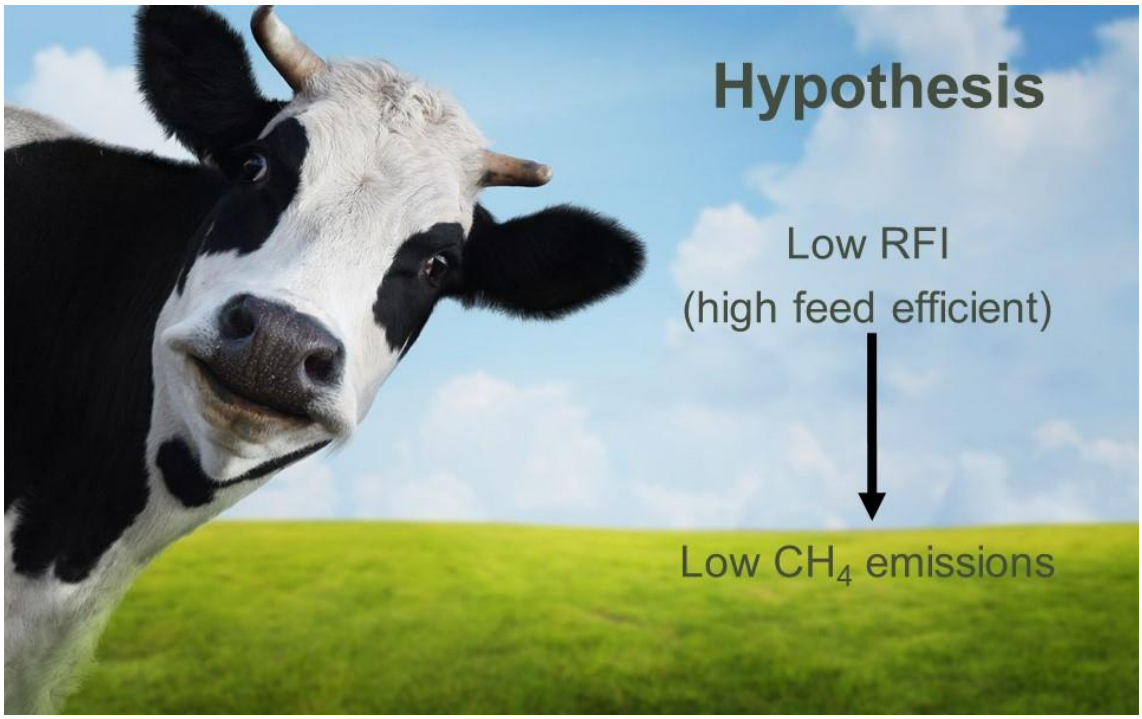
Residual feed intake – RFI

- Measure of feed efficiency;

DairyNZ

Residual feed intake – RFI





Trial Design (1)

- 2 x 2 factorial → RFI and Breed
  - High and Low RFI
  - Holstein-Friesian and Jersey
- Top and bottom 10% RFI for each breed
  - Measured in preceding RFI trial
- 2 Cohorts - 7 animals per group/cohort
- Housed in free-stall barn for ~30 days
  - 7 days adaptation and remainder experimental

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Trial Design (2)

- Fed Lucerne hay cubes
- Methane and DMI measured continuously
  - Modified GreenFeed® Methane Units
- BW measured 3X-weekly
- Linear model
  - breed, RFI category, cohort, and the interaction of breed and RFI category as fixed effects,
  - heifer as the random effect
  - Statistical analyses were performed using R (version 3.3.3, R Core Team, 2017)

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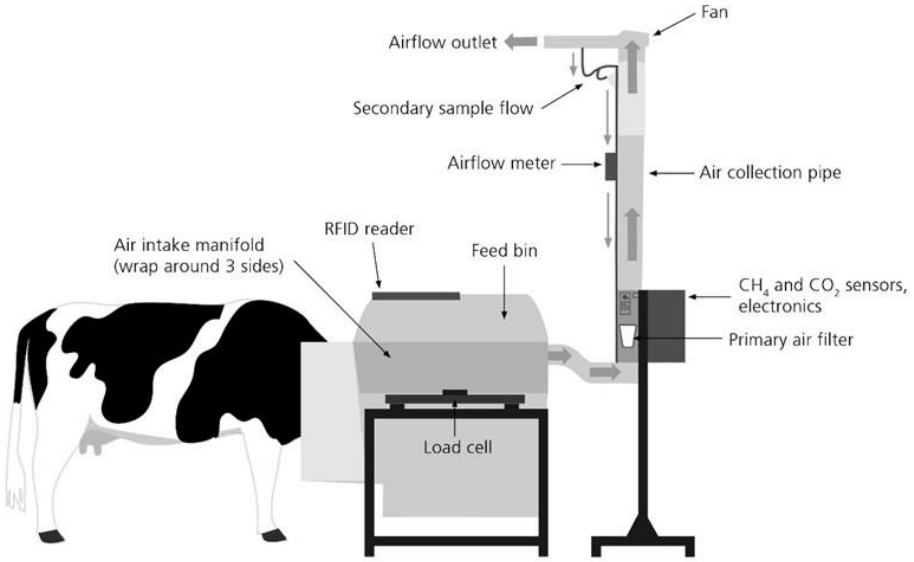
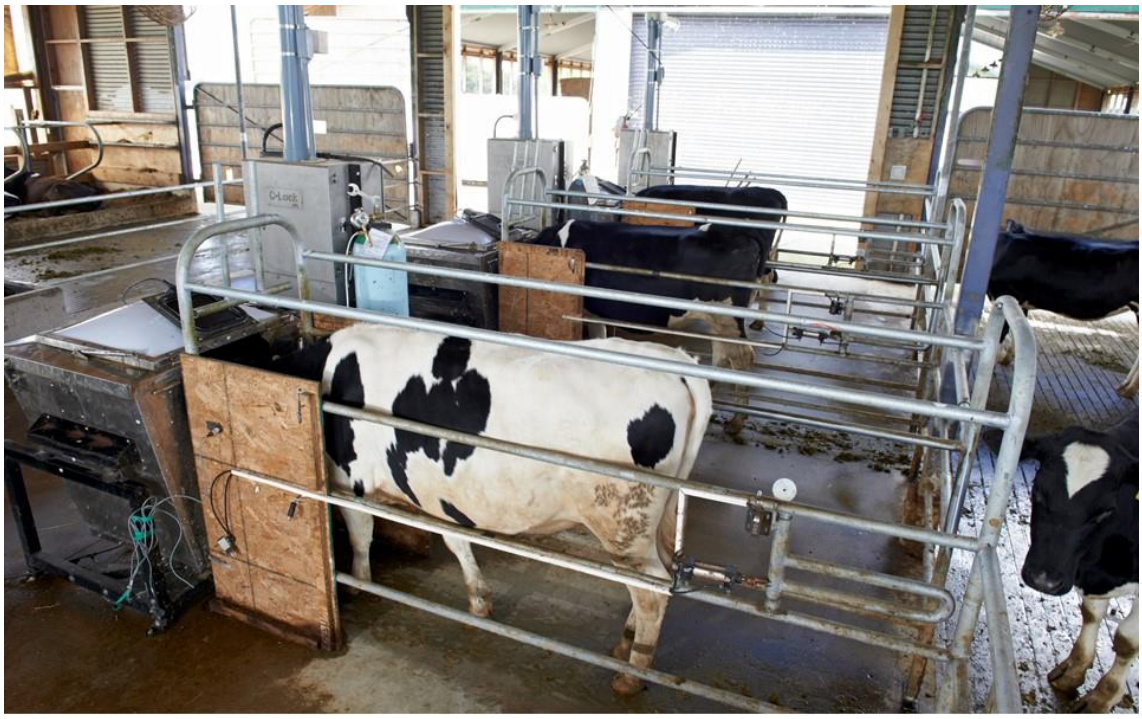
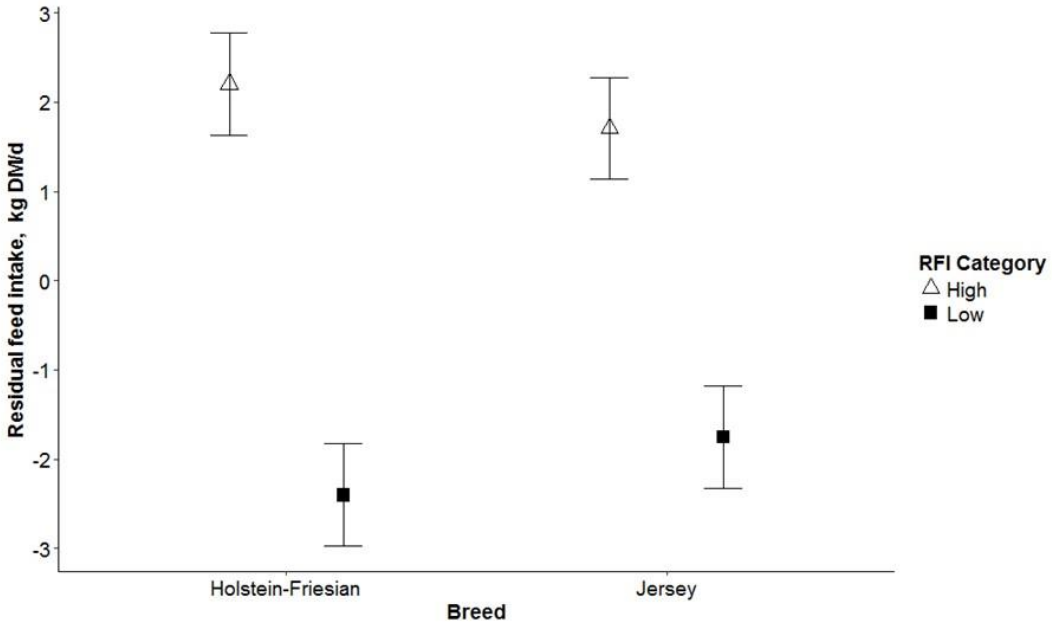
GreenFeed® Methane Units





Results



Results

	Breed		RFI category		SED	*P*-value	
	J	HF	Low	High		Breed	RFI
BW, kg	408	480	448	439	8.2	<0.001	0.27
DMI, kg DM/d	11.3	12.4	11.3	12.4	0.37	<0.01	<0.01
BWg, kg/d	1.2	1.3	1.2	1.3	0.08	**0.08**	0.25
g CH_4/d	242	267	253	256	6.2	<0.001	0.60
g CH_4/DMI	21.6	21.9	22.7	20.7	0.64	0.62	<0.01
g CH_4/kg BWg	220	212	224	207	13.8	0.60	0.23

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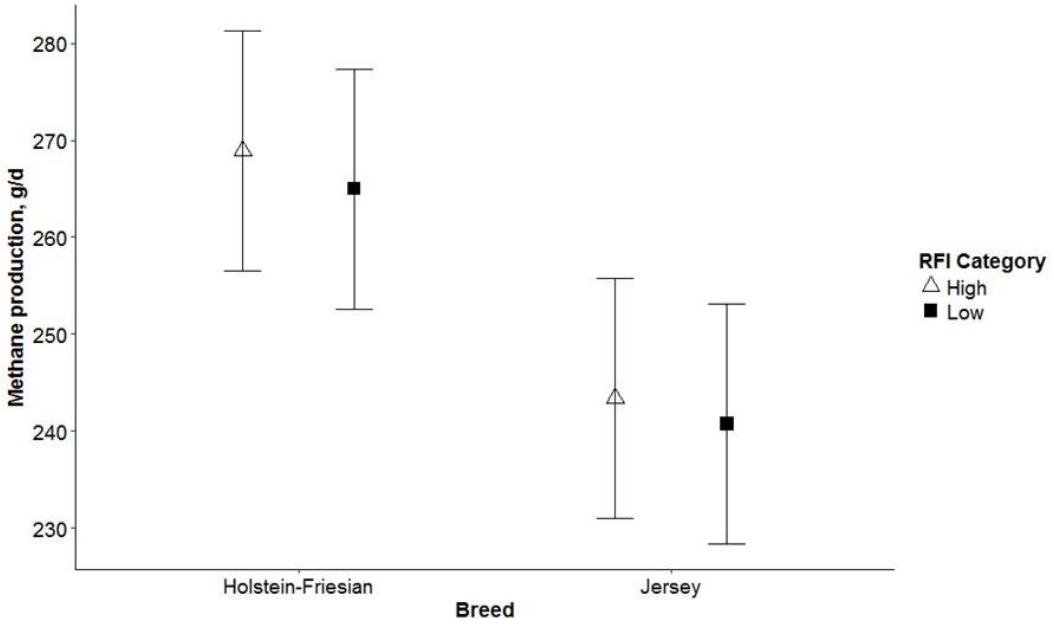
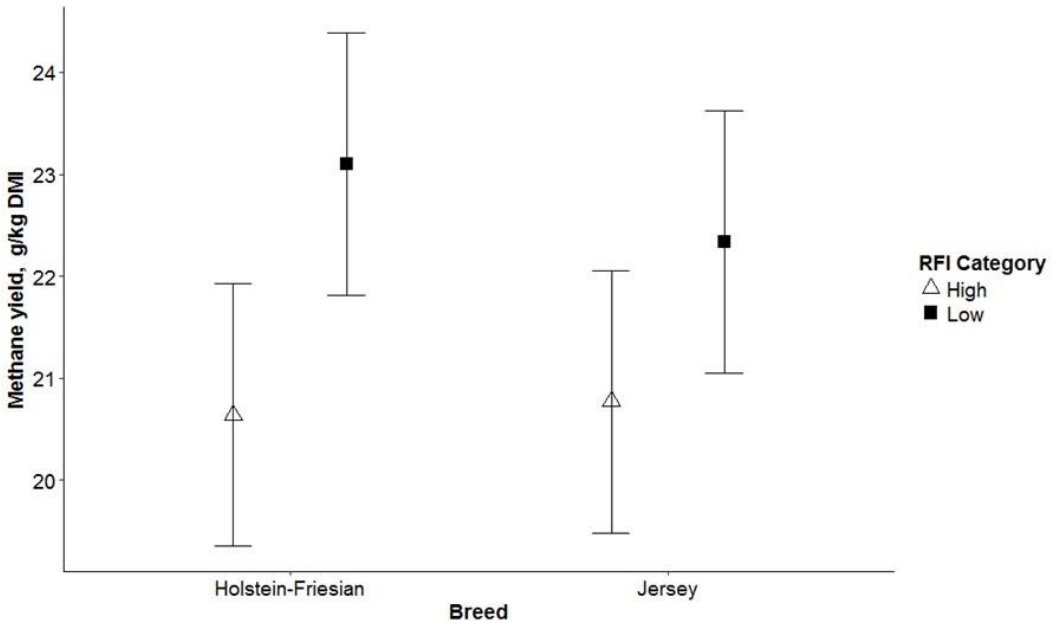

Methane production, g/d
280
270
260
250
240
230
Holstein-Friesian
Jersey
Breed
RFI Category
High
Low


Methane yield, g/kg DMI
24
23
22
21
20
Holstein-Friesian
Jersey
Breed
RFI Category
High
Low

Conclusions – RFI

- No difference in daily CH_4 production
- Lower DMI in low RFI animals
- CH_4 yield (g/kg DM) higher in low RFI animals

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Conclusion - Breed

- Differences in daily CH_4 production due to size/DMI
- No difference in CH_4 yield

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Take home point

- Low RFI:
  - did not reduce daily CH_4 production (g/d);
  - increased CH_4 yield (g/kg DMI).

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Acknowledgements

The authors acknowledge all the help afforded them by Lye Farm staff and the technical support of Olivia Jordan and Mark Bryant.

This research was funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

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Appendix 2: International Symposium for the Nutrition of Herbivores Abstract and Poster

This abstract and poster will be presented at the International Symposium for the Nutrition of Herbivores in Clermont-Ferrand, France in September 2018.

Title Relationship between residual feed intake and methane production in dairy heifers

Take home Message Dairy heifers categorised as low residual feed intake (RFI; i.e., high feed efficiency) have lower dry matter intake (DMI) for the same live weight gain (BWg), the same daily methane (CH_4) production (g/d), and higher CH_4 yield (g/kg DMI) than heifers categorised as high RFI (i.e., low feed efficiency).

Introduction An increasing demand for animal products has increased interest in improving feed conversion efficiency through reducing residual feed intake (RFI; Macdonald et al., 2014). Further, reducing the contribution of agricultural methane (CH_4) production to climate change requires understanding the cow-level factors that contribute to CH_4 emissions. Considering that CH_4 is a major source of digestible energy loss in ruminant animals, it is plausible that these traits are favourably correlated and that selection for reduced RFI would also reduce CH_4 emissions. Our objective was to compare the CH_4 production between dairy cow breeds and RFI categories (i.e., high or low) to elucidate this relationship.

Material and methods We estimated CH_4 production (g/d) and yield (g/kg dry matter intake; DMI) for 20-22 month old heifers in a 2 x 2 factorial arrangement including two breeds of dairy cattle (Jersey: Jer and Holstein-Friesian: HF) that had been previously designated as either high RFI (+2.0 kg DM) or low RFI (-2.1 kg DM; n = 14/treatment). The heifers (split into two periods) were housed in a free stall facility for approximately 30 d with unrestricted access to dried lucerne cubes from feeding stations. The stations electronically monitored feed disappearance in real-time and extracted all exhaled gas for real-time CH_4 analysis (DMI-CH_4 stations; C-Lock Inc., Rapid City, SD, USA). Individual animal CH_4 and DMI were measured for the duration of both periods and heifers were weighed thrice weekly. Mean daily CH_4 emissions were calculated for each animal. We estimated the fixed effects of breed, RFI category, and the interaction between breed and RFI category, and the random effect of animal using a linear model that tested for significant ($P < 0.05$) differences between the fixed effect means.

Results & Discussion The interactions between breed and RFI category were not statistically significant; therefore, only means of main effects are presented in Table 1. HF heifers were significantly heavier and consumed more than Jer, but there were no significant effects of breed on DMI/kg BW or BWg. RFI category had no significant effect on BW or BWg, but DMI and DMI/kg BW were 9.2% and 10.6% less in low RFI heifers. Because of the lower DMI, Jer heifers produced less CH_4/d, but not per kg DMI or per kg BWg. RFI category had no significant effect on daily CH_4 production (g/d) or CH_4/kg BWg; but, CH_4/kg DMI was greater in low RFI heifers because of their lower DMI. The results probably reflect more complete digestion of ingested feed in low RFI heifers, consistent with previously reported greater physical breakdown of feed (Gregorini et al., 2015) and a higher apparent digestibility of organic matter (Rius et al., 2012) in low RFI animals.

Conclusion Selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d), but could increase CH_4 yield (g/kg DMI).

Table 1 Least square means (± SEM) for BW, DMI, BWg and CH_4 in Jer and HF heifers from low or high RFI categories.

	BW, *kg*	DMI, *kg DM/d*	BWg, *kg/d*	CH_4, *g/d*	CH_4, *g/kg DMI*	CH_4, *g/kg BWg*
Jer	408 ± 7.0	11.3 ± 0.29	1.2 ± 0.06	242 ± 4.3	21.6 ± 0.51	219 ± 9.6
HF	479 ± 7.0	12.4 ± 0.29	1.3 ± 0.06	267 ± 4.3	21.9 ± 0.51	211 ± 9.6
P	<0.001	0.01	0.09	<0.001	0.65	0.57
Low RFI	448 ± 9.8	11.3 ± 0.29	1.2 ± 0.06	253 ± 4.9	22.7 ± 0.47	222 ± 9.5
High RFI	439 ± 9.8	12.4 ± 0.29	1.3 ± 0.06	256 ± 4.9	20.7 ± 0.47	208 ± 9.5
P	0.50	<0.01	0.31	0.63	<0.01	0.30

Acknowledgements Funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

Selecting cattle for low residual feed intake did not affect daily methane production, but increased methane yield

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Introduction

- Residual feed intake (**RFI**) is the difference between an animals actual and predicted feed intake.
  - Low RFI = High feed efficiency
- Agriculture and land use change is estimated to contribute to 22% of global greenhouse gas emissions, and agricultural methane (**CH_4**) is reported to be almost 6% of global greenhouse gases (Knapp et al., 2014).
- Research in beef cattle has reported a positive relationship between RFI and CH_4 production (Nkrumah et al., 2006; Hegarty et al., 2007).
- Jerseys have been reported as more feed efficient than Holstein-Friesians (Spaans et al., 2018).

Hypotheses

- Low RFI (i.e., higher feed efficiency) animals emit less CH_4/kg DMI than lower efficiency, high RFI animals.
- Jersey heifers have lower CH_4 yield than Holstein-Friesian heifers.

Methods

- 56 heifers (20-22 mo old) in a 2 x 2 factorial arrangement:
  - Jersey (**J**) and Holstein-Friesian (**HF**); n=28/breed
  - High (+2.0 kg DM) or low RFI (-2.1 kg DM); n = 28/RFI category
- Housed in a free stall facility for 20-30 days
- Measured body weight (**BW**), BW gain (**BWg**), DMI, and CH_4 production
- GreenFeed (C-Lock Inc., Rapid City, SD, USA) installed in feeding stations
  - Electronically monitored feed disappearance in real-time and extracted all exhaled gas for real-time CH_4 analysis.
- RFI estimated based on a regression of DMI against BW and BWg

Acknowledgements

The authors acknowledge all the help afforded them by Lye Farm staff and the technical support of Olivia Jordan and Mark Bryant. This research was funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.

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Nkrumah, J.D., E.K. Okine, G.W. Mathison, K. Schmid, C. Li, J.A. Basarab, M.A. Price, Z. Wang, and S.S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J Anim Sci 84:145–153.

Spaans, O., K.A. Macdonald, J.A.S. Lancaster, A.M. Bryant, and J.R. Roche. 2018. Dairy cow breed interacts with stocking rate in temperate pasture-based dairy production systems. J. Dairy Sci. 101:4690–4702.

Results & Discussion

RFI

- Low and high RFI heifers did not differ in BW or BWg
- Low RFI heifers had ~10% lower DMI
- RFI category did not affect CH_4/d or CH_4/kg BWg
- Low RFI heifers had greater CH_4/kg DMI
- Results might reflect
  - More complete digestion of ingested feed in more efficient, low RFI heifers

BREED

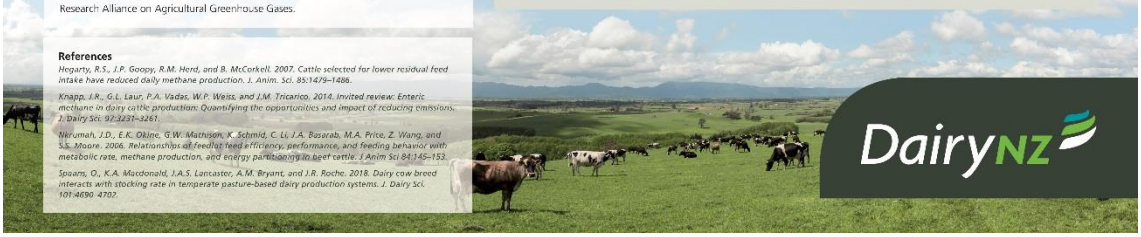
- HF heifers were heavier and consumed more than J heifers
- Breed did not affect DMI/kg BW or BWg
- Jersey heifers produced less CH_4/d, but not per kg DMI or per kg BWg.

Table 1. Least square means for groups representing two breeds (Jersey: J; and Holstein-Friesian: HF) and two pre-determined residual feed intake categories. There were no breed x RFI category interactions.

	BREED		RFI CATEGORY		SED	P-VALUE	
	J	HF	Low	High		Breed	RFI
BW, kg	408	480	448	439	8.2	<0.001	0.27
DMI, kg DM/d	11.3	12.4	11.3	12.4	0.37	<0.01	<0.01
BWg, kg/d	1.2	1.3	1.2	1.3	0.08	0.077	0.25
CH_4, g/d	242	267	253	256	6.2	<0.001	0.60
CH_4/DMI, g/DMI	21.6	21.9	22.7	20.7	0.64	0.62	<0.01
CH_4/BWg, g/kg BWg	220	212	224	207	13.8	0.60	0.23

Take Home Message

Selecting dairy heifers for low RFI is unlikely to affect daily CH_4 production (g/d), but may increase CH_4 yield (g/kg DMI)



Appendix 3: Trapezoidal Mean Methodology

The trapezoidal mean is a weighted average of each days CH_4 spot measurements for a particular animal using the trapezoidal approach to area under the curve, with a linear interpolation for midnight for each animal, as depicted by Figure A3.1.

Firstly, the data were separated into each individual animal and ordered by the start time of the visit. Then, the CH_4 production was linearly interpolated to give an estimate at precisely midnight on each day of the trial, as indicated by the black crosses in Figure A3.1. The trapezoidal approach was used to calculated the area under the curve for each day, as indicated by the area shaded in blue for day 8 or orange for day 9 in Figure A3.1. The trapezoidal area under the curve is an estimate for daily CH_4 production, which gives a weighted average due to the diurnal pattern of the CH_4 spot measurements. Daily CH_4 estimates were averaged across the whole measurement period to get an average for the whole trial. The R-code used for this is as follows:

```
-      setwd("~/My Documents/Masters/Data Analysis")
#PACKAGES####
#install.packages("dplyr")
library(dplyr)

-      #install.packages("pracma")
library(pracma)

-      #install.packages("ggplot2")
library(ggplot2)

-      #COW INFO####
cow.info <- read.csv("Cow_Info.csv")

-      #METHANE DATA SETUP####
methane.data <- read.csv("Methane_data.csv")
methane.data <- subset(methane.data, select = c(Tag, Unit, DayofTrial,
                                                 StartDate, StartJulian,
                                                 StartTime, Duration,
                                                 HourofDay, CO2, CH4))

-      # CH4 Trap####
tags <- unique(cow.info$Tag)
results <- vector("list", length(tags))
for(i in 1:length(tags)){
  print(tags[])

  thistag <- methane.data %>%
    filter(Tag==tags[i]) %>%
```

```
    arrange(StartJulian) %>%
    select(Tag, DayofTrial, StartJulian, CH4)
  xi <- (min(thistag$DayofTrial)+1):max(thistag$DayofTrial)

  thistaginterp <- interp1(thistag$StartJulian,
                           thistag$CH4,
                           xi,
                           method = "linear")
  thistaginterp <- as.data.frame(thistaginterp)
  colnames(thistaginterp) <-  ("CH4")

  thistaginterp$DayofTrial <- (min(thistag$DayofTrial)+1):max(thistag$DayofTr
ial)
  thistaginterp$Tag <- tags[i]
  thistaginterp$StartJulian <- thistaginterp$DayofTrial

  thistag2 <- rbind(thistag, thistaginterp)
  thistag2 <-  thistag2[order(thistag2$StartJulian),]

  thistag2$cumtrapz <- as.vector(cumtrapz(thistag2$StartJulian, thistag2$CH4)
)
  thistag2$DayofTrial <- lag(thistag2$DayofTrial,1)

  tmp <- aggregate(cumtrapz ~ DayofTrial, thistag2, max)
  tmp$CH4 <- tmp$cumtrapz - lag(tmp$cumtrapz,1)
  tmp <- subset(tmp, DayofTrial !=7 & DayofTrial != 31 & DayofTrial != 74 & D
ayofTrial != 91)

  tmp$Tag <- tags[i]
  results[[i]] <- tmp
}

-      #end of loop

ch4.trap <- subset(bind_rows(results), select = c(Tag, DayofTrial, CH4))
ch4.trap <-  ch4.trap[order(ch4.trap$DayofTrial),]
ch4.trap <-  ch4.trap[order(ch4.trap$Tag),]

mean.ch4trap <- aggregate(CH4~Tag, ch4.trap, mean)
colnames(mean.ch4trap) <- c("Tag", "CH4trap")
```

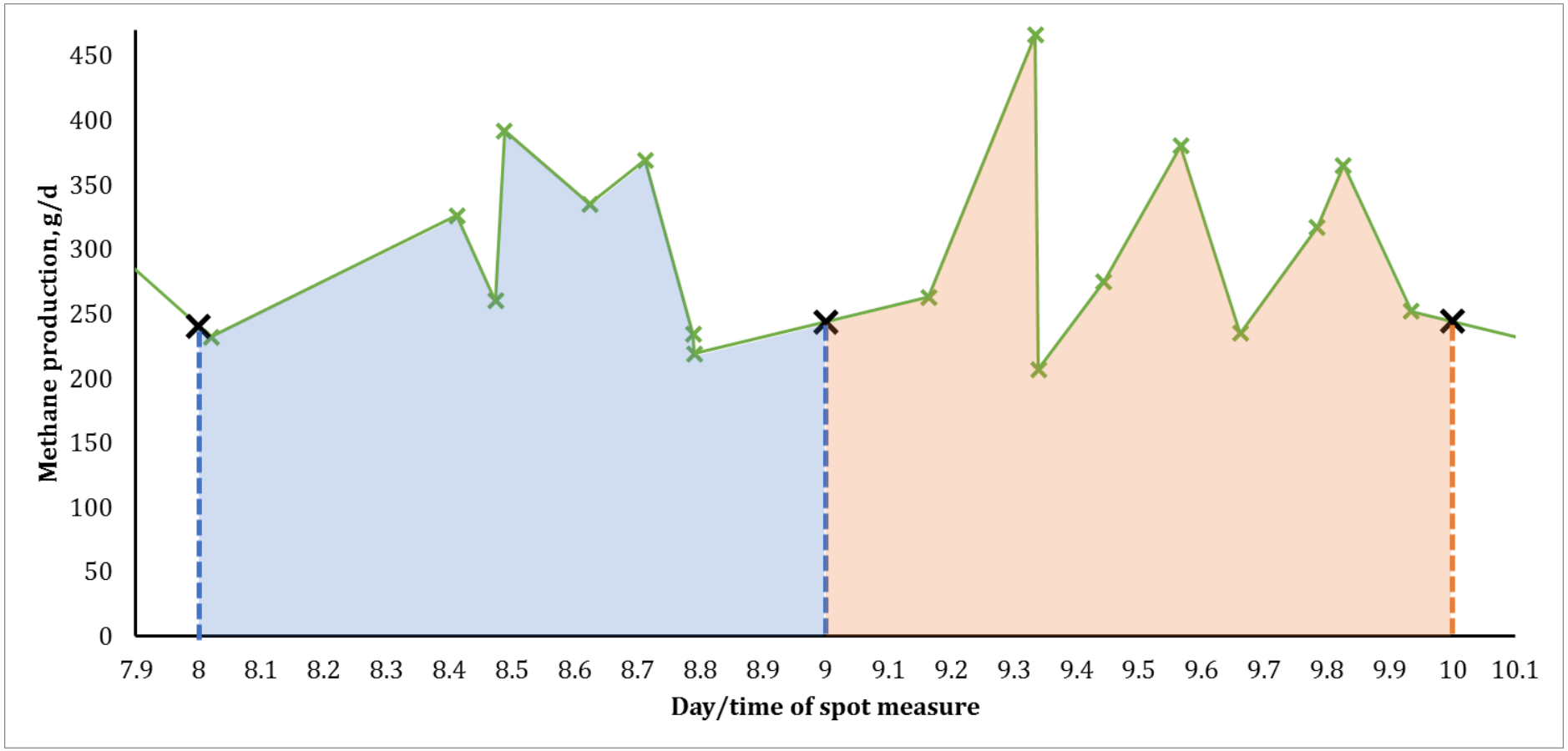


Figure A3.1. Plot of CH_4 spot measurement data for Animal 4 over day of trial 8-10. The plot depicts the trapezoidal mean approach through the linear interpolation for midnight by the black crosses, and the area under the curve by the shading.