Animal factors affecting enteric methane production in late lactation pasture based dairy cows in Ireland

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Animal Science

At Massey University, Palmerston North, New Zealand

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2022

Abstract

Ireland currently has a national commitment to reduce 51% of total greenhouse gas emissions by 2030. In order to achieve these reductions, factors that affect enteric methane production in a pasture-based system need to be further investigated. The objectives of this study were to 1) investigate the repeatability on enteric methane emissions in grazing dairy cows, 2) assess the relationship between enteric methane and other animal traits at grass and 3) investigate the potential of a new trait called residual enteric methane emission (RME) to select for lower enteric methane emitting cows without impacting productivity.

Enteric methane emissions were measured on forty-five late lactation grazing dairy cows using the GreenFeed monitoring system at Teagasc, Moorepark, County Cork, Ireland. The average enteric methane produced was 351.8 g per day with a daily coefficient of variation of 13%. The cows were averaging 16.6 kg dry matter intake (DMI) while producing 1.62 kg milk solids (MS; fat plus protein) per day. The repeatability of the enteric methane measurements was 0.67 indicating that the enteric methane measurement is reliable. Through the partial correlations conducted, it showed that milk, MS, fat and protein yields, milk urea, live weight and DMI all have positive correlations with daily CH_4 production. Therefore, it is expected that an increase in any of these traits would lead to an increase in enteric methane production. While, body condition score (BCS) had a significant negative correlation with enteric methane production. This could be due to higher energy demands at a lower BCS for an animal to increase their body condition score leading to an increase in DMI.

Residual enteric methane emissions were estimated through two methods: multiple regression and Irish national inventory calculations. For each method, animals were split into three groups with high, medium and low ranking of RME with 15 animals per group. The rank correlation between the two methods was 0.79 ($P<0.001$) showing that the two methods are able to rank animals to a similar level as each other. The low ranked animals produced between 16.2% and 6.9% less enteric methane per day than both the high and medium ranked animals. Despite this reduction in enteric methane production, there was no effect on the milk production, composition, live weight, BCS or DMI. Therefore, these low ranked animals produced less enteric methane per kg of milk solids, live weight and DMI indicating that the RME has potential be used in future strategies to reduce methane through for example breeding for lower enteric methane producing animals while not affecting the production and income of farmers.

Acknowledgements

Firstly, my greatest appreciation and thank you to my supervisors Dr Laurence Shalloo, Dr Ben Lahart and Dr Nicolas Lopez-Villalobos. I am extremely grateful for your support, guidance and help throughout my masters. Thank you all for being so understanding and supportive throughout this process. Laurence without you planting the idea of me continuing with my studies we wouldn't be here today. You've helped sparked my interest in the enteric methane and research space through your knowledge and amazing opportunities you have given me. Ben, you've been a great friend and supervisor throughout this. It has really helped having you to ask quick stats questions to when I get stuck with my SAS coding. And also, for all the time you have spent explaining different aspects of the analysis to me, thank you! Nicolas, thank you for your quick replies and your constant support and feedback throughout the analysis and writing stages. You have been very understanding of my situation and given me the support and knowledge I needed to complete this masters thesis.

I would also like to thank VistaMilk, Science Foundation Ireland and Ireland's Department of Agriculture, Food and Marine for the financial support throughout this masters.

Also, a big thank you to the Moorepark farm manager, John Paul Murphy, for putting up with all of my requests and making trial work operate seamlessly. I would also like to thank the farm staff at Moorepark. You all lent a helping hand out in the field which was greatly appreciated – whether that be training cows, moving the GreenFeed machines, drafting/sorting cows, etc. You guys helped make trial work very enjoyable through the great banter and the constant help!

To my friends that I made while in Ireland, you all helped me to see that it is enjoyable to continue studying and gave me trust in myself that I could do it as well. It was great being able to talk about stats issues and also being able to switch off with you all at the pub.

Lastly, to my partner Jacob, Mum and Dad. Thank you all for pushing me, supporting, keeping me focused on the end goal when I lost motivation. In particular, to my partner Jacob for being there every day, lending an ear to bounce ideas off, challenge my ideas and of course for helping with intake runs at 5.30am. Your unconditional support throughout this process has been amazing. Mum and dad, despite being on the other side of the world, our video calls always gave me fresh ears to talk to. It helped hugely in the writing stages when translating our conversations into words on paper. The reassurance and encouragement you have all given me throughout this past year has been incredible.

Table of contents

List of abbreviations

CH_4	Enteric methane
CO_2	Carbon dioxide
CO_2-eq	Carbon dioxide equivalent
DMI	Dry matter intake
ECMY	Energy corrected milk yield
FPCM	Fat protein corrected milk
GHG	Greenhouse gas
NDF	Neutral detergent fibre
N_2O	Nitrous oxide
RFI	Residual feed intake
RME	Residual enteric methane emissions
SF_6	Sulphur hexafluoride
VFA	Volatile fatty acid

Chapter 1: General Introduction

Over the past decade there has been an increasing level of awareness and interest into global warming and climate change. These issues are recognized to be as a result of increasing greenhouse gas (**GHG**) emissions. The greenhouse effect is a result of gases being absorbed into the atmosphere and subsequently trap energy and warm the environment (Le Treut *et al.* 2007). The three main GHG are enteric methane (**CH_4**), carbon dioxide (**CO_2**) and nitrous oxide (**N_2O**) (IPCC 2014b). Enteric methane has a short atmospheric half-life and a global warming potential of 28 times greater than CO_2 based on the IPCC's fifth assessment (IPCC 2014b). For example, 1kg CH_4 equates to 28kg CO_2 according to Annex 5.3 of Ireland's National Inventory Report (Duffy *et al.* 2020).

Enteric methane is a GHG that is predominantly produced in the agricultural sector by ruminants and is largest GHG emitted from agriculture in Ireland (Duffy *et al.* 2021). This enteric methane is produced as a by-product of digestive fermentation in the rumen. Rumen microorganisms break down feed to produce volatile fatty acids (**VFA**). These VFAs are absorbed by the rumen and used as an energy source. However, during this procedure, hydrogen gas (**H_2**) is produced as a by-product. A low partial pressure of hydrogen needs to be maintained in the rumen to ensure optimal microbial growth and forage digestion (Clark *et al.* 2005). If a high concentrate of hydrogen occurs, there is a risk of toxicity to the animal. Methanogens have evolved within the rumen to utilize the excess H_2 as they combine CO_2 and H_2 to produce CH_4 in a process called methanogenesis (McAllister *et al.* 1996). At least 80% of CH_4 is produced through methanogenesis in the rumen and the rest is produced in the lower digestive tract (Clark *et al.* 2005). The majority of CH_4 is released from the animal through eructation and less than 5% released through flatulence (Murray *et al.* 1976). Methanogenesis consumes energy and therefore removes some of the available energy for the animal, leaving less energy for maintenance, milk production, pregnancy etc. Although methanogenesis is an important process that helps ruminants remove hydrogen, the process produces a very undesirable gas in the form of enteric methane.

Ruminants such as dairy cows play an important role in the human food supply chain. Ruminants have the unique ability to convert human in-edible protein into a nutritious food source for humans, which is crucial to be able to feed the increasing global population (Hennessy *et al.* 2021). Dairy cattle produce 80% of the global milk consumed (Gerber *et al.* 2013). It is predicted that the demand for animal derived protein will double by 2050 (Henchion *et al.* 2017) as developing countries are including a greater proportion of high quality animal derived protein in their diet (Figure 1.1) and this is forecasted to continue to increase

(Alexandratos and Bruinsma 2012). This means there is a need for an increase in animal derived protein produced along with an increase in production efficiency for sustainability and profitability purposes. This will be important to be able to feed the ever-increasing global population as well as meet climate targets. This will also be important to be able to continue supplying to consumers that are becoming more aware of their environmental footprint. With some consumers perceiving that dairy products have a negative impact on the environment, advancements need to be made on the industry at all levels of production to ensure that there is a continual increase in sustainability.

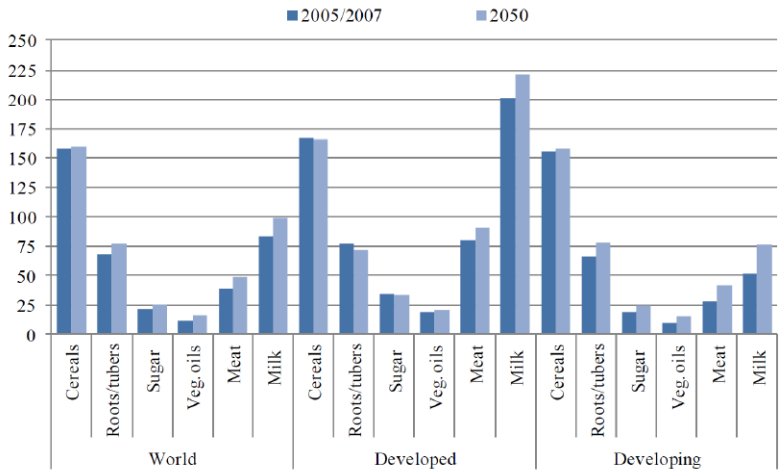

Figure 1.1. Food consumption per capita of major commodities (kg/person/year) in the world, developed and developing countries (Alexandratos and Bruinsma 2012).

Ireland's dairy industry is predominately a pasture-based system. This means that majority of a lactating dairy cows diet comes from grazed grass (O'Brien *et al.* 2018). In 2021, Ireland's dairy industry contributed over €5 billion to the national economy (Bord Bia 2022), which has increased from €2.2 million between 2009 and 2013. This is a result of the EU milk quota being abolished in 2015 and cow numbers/production increasing. Since then, the dairy industry has continued to grow and is currently producing 4 billion litres more milk than in the 2007 to 2009 period (CSO 2022).

There are currently 196 parties involved in the Paris Agreement, which was decided upon in 2015 and adopted at COP 21 in Paris to limit the warming of the globe to 1.5-2 degrees Celsius (United Nations 2016). This international aim to reduce total emissions to mitigate the effect of global warming on the environment is instrumental in combating or mitigating climate change. Not only are there international goals there are also climate goals at a national level

for many countries. Ireland has committed to reduce 51% of total GHG emissions by 2030 relative to 2018 and are also aiming to become a net zero emissions nation by no later than 2050 (Government of Ireland 2021). Within the agricultural sector Ireland has set a sectoral budget for agriculture that shows a decline in emissions of between 21.7% and 30% in overall GHG emissions by 2030 (Climate Action and Low Carbon Developent (Amendment) Act, 2021).

Where there are potential systems level emissions reductions, significant enteric methane missions will be largely reliant on new innovative technologies. As Ireland is based predominantly on pasture-based systems, it is imperative that enteric methane produced during the grazing season is measured accurately. There are three main techniques that measure enteric methane emissions at an individual animal level (Hammond *et al.*, 2015). The gold standard is the respiration chamber method (O'Hara *et al.* 2003; Garnsworthy *et al.* 2019). However, in a grazing situation the animals are removed from their natural environment and placed into an enclosed chamber. This may upset their feed intake behaviour and therefore not produce a true representation of their enteric methane production while grazing (Hammond *et al.* 2016b). Sulphur hexafluoride (SF_6) is another method that involves a gas canister being strapped to the back of the animal and intake tubes located near the muzzle of the animal with a tracer gas emitting bolus being put in the animal's rumen. However, this method is costly, labour intensive, and not capable of measuring large amounts of animals. Sensor technology is also used to quantify enteric methane emissions. One of these techniques is the GreenFeed system (C-Lock) which is able to carry out enteric methane gas measurements in the farm environment and also able to measure multiple animals simultaneously (Coppa *et al.* 2021). This technology provides the most applicable measurements to a pasture-based dairy system over a long period of time with a high throughput of animals.

There have been multiple studies conducted indoors where animals are fed a total mixed ration diet. Many of these studies report that dry matter intake is one of the major drivers of daily enteric methane emissions (Hegarty *et al.* 2007; Manafiazar *et al.* 2016). It is also identified that when residual feed intake is low, daily enteric methane emissions are also reduced (Hegarty *et al.* 2007), however, residual feed intake is only explaining a small proportion of the variation in enteric methane emissions. Not only does dry matter intake have an effect on daily enteric methane emissions but so does breeding and genetic parameters. A beef study conducted indoors highlights a positive genetic correlation between live weight and daily enteric methane emissions (Donoghue *et al.* 2013). A dairy study also conducted indoors

reported a strong relationship between milk yield with daily enteric methane emissions (Lassen and Lovendahl 2016). Contrastingly, a study that was carried out on lactating pasture based cows reported a phenotypic correlation between dry matter intake and daily enteric methane emissions to be -0.02 (Herd *et al.* 2014). This difference between indoor and outdoor studies shows that there are animal and environmental factors that are influencing the enteric methane produced in each system. There is limited research carried out to identify these animal factors in a grazing system, which results in a lack of information for countries like Ireland that are predominantly based on pasture. Therefore, there is a need to investigate the animal factors that are affecting enteric methane emissions to be able to work towards reducing total enteric methane emissions.

Residual enteric methane (**RME**) is the difference in estimated enteric methane production and true enteric methane produced. The measurement of enteric methane provides the true enteric methane production and the estimated enteric methane emissions is predicted through modelling. RME has been identified as a trait that is independent of animal production traits and therefore could have the potential to be used in selection indices in the future.

The aim of this study was to identify animal factors that influence enteric methane emissions at an individual animal level. Residual enteric methane was also used in this study to determine the enteric methane efficiency of the animals (grams of enteric methane per kilogram of output; milk, live weight, dry matter intake). Residual enteric methane was selected to be included in this study due to the fact that it is independent of production traits and therefore would be a more equitable comparison between animals. From these findings, animals with low enteric methane emissions can be identified.

Chapter 2: Literature Review

2.1 Greenhouse gas emissions

Greenhouse gases (GHG) are atmospheric gases that affect the climate by altering the incoming solar radiation and out-going infrared radiation. The incoming and outgoing radiation has created the earth's energy balance (Forster *et al.* 2007). Increasing the concentrations of these GHG in the atmosphere can lead to global warming (Raval and Ramanathan 1989). Since the start of the industrial era (1750), the overall effect of human activities has resulted in global warming (Forster *et al.* 2007).

2.1.1 Main greenhouse gases

The principle GHG's are carbon dioxide (CO_2), enteric methane (CH_4), and nitrous oxide (N_2O) (IPCC 2007; IPCC 2014b). Each of these gases have a different global warming potential (GWP), which is based on CO_2-equivalent (CO2-eq) emissions. The GWP of CO_2 is 1, compared to enteric methane and nitrous oxide of 28 and 265 respectively as shown in Table 2.1 (IPCC 2014b). This means that for every 1kg of CH_4, it is equivalent to 28kg of CO_2 and 1kg of N_2O is equivalent to 265kg of CO_2 (Duffy *et al.* 2020). There are other minor greenhouse gases such as hydrofluorocarbons (HFCs) and carbon tetrafluoride (IPCC 2014b).

Table 2.1. Global warming potential of different greenhouse gases (IPCC 2014b).

Greenhouse gas[1]	Lifetime (years)	Global warming potential
CO_2	[a]	1
CH_4	12.4	28
N_2O	121	265
HFCs	1.5	138
CF_4	50,000	6630

[1] CO_2 = carbon dioxide, CH_4 = enteric methane, N_2O = nitrous oxide, HFCs = hydroflourocarbons, CF_4 = carbon tetraflouride

[a] no single lifetime can be given for CO_2 (IPCC 2014b)

2.1.2 Global emissions

Global emissions have been increasing since the industrial revolution in the 1750's as shown in Figure 2.1 with historical data from 1970. In 2010, CO_2 accounted for 76% of the world's greenhouse gas emissions. This CO_2 is mostly from fossil fuel and industrial processes (65%) and the remaining 11% is CO_2 emitted through forestry and other land use (FOLU) and land use change (IPCC 2014a). It is estimated that between 2007 – 2016 agriculture, forestry and other land use produced approximately 44% of enteric methane emitted internationally (IPCC 2021). A total of 3.5% increase in enteric methane produced from all sectors internationally has been seen from 2011 to 2019 (IPCC 2021). Emissions from enteric methane are estimated to be 109Tg/year for the period 2008 to 2017 whereas it was 87Tg/year from 1990-1999 (IPCC 2021). This increase is reported due to an increase in total animal numbers and productivity globally.

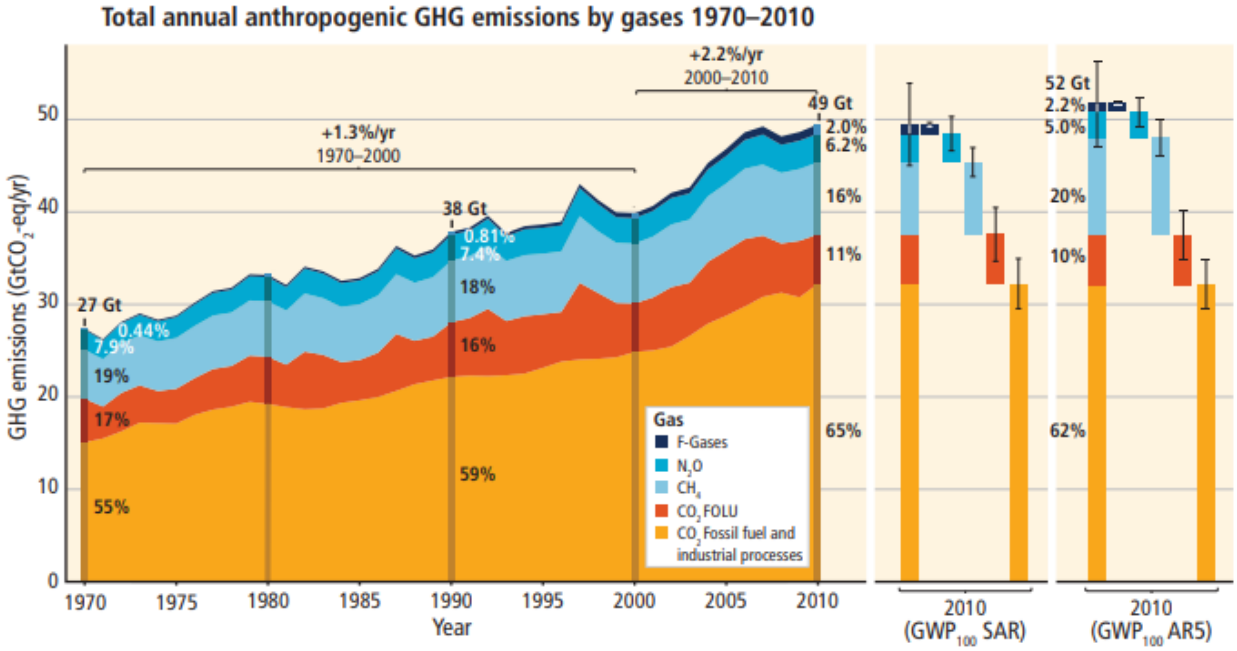

Figure 2.1. Global emission trend by gas type 1970-2010 (IPCC 2014b).

2.1.3 Ireland's agricultural greenhouse gas emissions

In 2019, Ireland's agricultural industry contributes 34.3% (20.5 Mt CO_2-eq) to total national emissions according to the Irish national greenhouse gas inventory report 2021 (Duffy *et al.* 2021) as shown in Table 2.2. The sector with the largest contribution is energy which encompasses energy production, household energy consumption, and transport. The energy sector produces 35.2Mt CO_2-eq which equates to 58.9% of national emissions. Within the agricultural sector, 59.3% of emissions are from enteric methane (Duffy *et al.* 2021).

In 2021, Ireland's dairy industry contributed over €5 billion in exports (Bord Bia 2022), which has increased from €2.2 million in 2009-2013. This is a result of the EU milk quota being abolished in 2015. Since milk quota abolishment, the dairy industry has continued to grow and is currently producing 4.0 billion litres more milk than over the 2007 - 2009 period (CSO 2022). Over the past 10 years from 2007/2009 to 2020 cow numbers in Ireland have increased by 43% while milk production increased by 77% (Shalloo 2021). Ireland's agriculture emissions peaked in 2018 at 21.3Mt CO_2-eq, however, 2019 showed a 3.8% decrease down to 20.5Mt CO_2-eq. As the industry continues to expand, enteric methane mitigation strategies need to be investigated to decouple methane emissions from production.

Table 2.2. Irish national greenhouse gas emissions comparing 1990 to 2019 (Duffy *et al.* 2021).

	Emissions (Mt CO_2-eq)		Change from 1990 to 2019	
Category[1]	1990	2019	(Mt CO_2-eq)	(%)
Energy	31.0	35.2	4.2	13.5
IPPU	3.3	3.2	-0.1	-3.8
Agriculture	18.5	20.5	2.0	10.6
Waste	1.6	0.9	-0.7	-41.7
Gross (excluding LULUCF)	55.4	59.8	4.4	-7.4
LULUCF	5.1	4.4	-0.7	-13.4
Net (including LULUCF)	60.4	64.2	3.8	7.9

[1] IPPU = Industrial processes and product use, LULUCF = Land use, land use change and forestry.

2.2 Emissions reduction targets

The global targets are to reduce the effect that GHG emissions are having on climate change and global warming and ultimately their effect on the environment. There are currently 189 parties involved in the Paris Agreement. This agreement was decided upon in 2015 to limit the warming of the globe to 1.5-2 degrees Celsius (United Nations 2016). This international aim to reduce total emissions to mitigate the effect that the warming of the globe is having on the environment is instrumental to combating climate change. Not only are there international goals there are also climate goals at a national level for many countries. Ireland has committed to reduce total emissions by 51% relative to a baseline year of 2018 during the period of 2021 and 2030. The overall aim for Ireland is to become climate neutral by 2050 which was set by the Climate Action and Low Carbon Development (Amendment) Bill 2021. This Bill has also set in law that the agricultural industry needs to achieve an emissions reduction between 21.7% and 30% by 2030. 2030. A separate enteric methane reduction target of 10% has been set for enteric methane as part of the international efforts to reduce enteric methane.

2.3 Enteric methane production

2.3.1 Ingestion and rumen fermentation

The Irish dairy system is predominantly pasture based, therefore the majority of a dairy cow's diet is from fresh forage (mainly perennial ryegrass) (Roche *et al.* 2017; O'Brien *et al.* 2018). When the ryegrass is initially ingested, some of the cells are ruptured which allows micro-organisms and enzymes to access the cells for digestion (Beha *et al.* 2002). While the animal is chewing the fresh forage, saliva mixes with the forage and forms a bolus. This helps with swallowing and to buffer the rumen pH which is usually balanced at 6.8 (Beha *et al.* 2002; Beauchemin *et al.* 2008). The digestion of forages occurs as a result of symbiotic associations between the host ruminant and also the gut microflora such as bacteria, archaea, fungi and protozoa (Beauchemin *et al.* 2004).

The majority of the digestion occurs in the rumen and the remainder of the digestion takes place in the caecum and colon. Approximately 55% to 65% of the organic matter digestion occurs in the rumen whereas 20-30% in the small intestine and 5-15% in the large intestine (Waghorn *et al.* 2007; Janssen 2010). The rumen is the largest stomach compartment and is the site of microbial fermentation. The pH range between 6 and 6.8 provides the ideal environment for the microflora and enzymes to effectively ferment and digest feed (Leng and Nolan 1984).

The rumen walls contract together which mixes its contents, ensuring all substrate becomes in contact with the multitude of micro-organisms in the rumen which maximises fermentation and digestion (Leng and Nolan 1984).

During the fermentation and digestion process substrates are broken down through glycolysis which generates adenosine-tri-phosphate (ATP) (Russell and Wallace 1997). This process produces volatile fatty acids (VFA) as well as by products of CO_2 and H_2. As the hydrogen can change the pH of the rumen, it is important for the ruminant to remove the H_2 to avoid acidosis and environmental changes to the rumen (Janssen 2010). The main VFAs produced are propionate, acetate and butyrate and their ratio can affect the composition of milk and energy efficiency (Sutton *et al.* 2003). The by-products (CO_2 and H_2) are then converted into enteric methane through the methanogenesis process.

2.3.2 Methanogenesis

Rumen microorganisms break down feed to produce volatile fatty acids (VFA), which are then absorbed and used as an energy source (Van Soest 1994). A low partial pressure of H_2 needs to be maintained in the rumen to ensure microbial growth and forage digestion (Clark *et al.* 2005). Methanogens are archaea that are responsible for the production of enteric methane (methanogenesis) as they convert H_2 and CO_2 into CH_4. Methanogenesis is a form of anaerobic respiration that converts adenosine di-phosphate (ADP) to ATP. This process occurs simultaneously with the reduction of nicotinamide adenosine dinucleotide (NAD^+) to NADH. NADH donated electrons to hydrogen ions (H^+) to form H_2 (Russell and Wallace 1997). Methanogenesis has seven different steps that need to be completed in order for enteric methane to be produced as shown in Figure 2.2. The carbon molecule from the carbon dioxide is sequentially modified, reduced and transferred through the cycle to form the enteric methane molecule at the end of the process. These seven steps reduce CO_2 to CH_4 with four reductive intermediates; formyl, methenyl, methylenyl, and methyl (Rouviere and Wolfe 1988). The stoichiometric equation for this conversion of CO_2 and H_2 to CH_4 is $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (Janssen 2010) which only occurs under anaerobic conditions in the rumen (McAllister et al., 1996). Typically, at least 80% of CH_4 is produced through this process (methanogenesis) in the rumen and the rest is produced in the lower digestive tract (Murray *et al.* 1976; Clark *et al.* 2005).

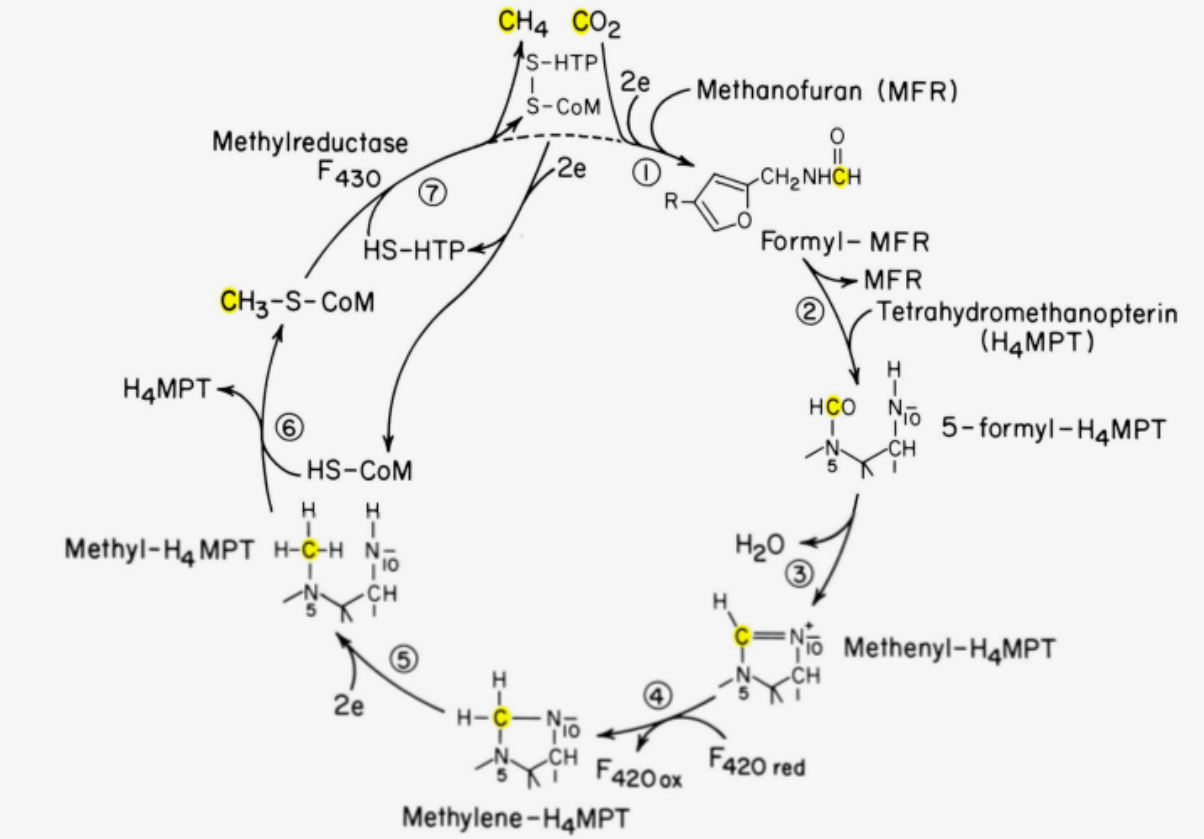

Figure 2.2. Proposed mechanism for the reduction of carbon dioxide (CO_2) to enteric methane (CH_4) (Rouviere and Wolfe 1988).

2.3.3 Enteric methane release

A number of studies show ranging partitioning routes for the release of enteric methane emissions. Murray *et al.* (1976) reported that 83% of CH_4 is released through eructation, 16% through exhalation and 1% through flatulence. This author also showed that 89% of the CH_4 produced in the lower digestive tract is absorbed into the blood and then released into the lungs and sequentially exhaled. Another study reported 98% of CH_4 is released through eructation or exhalation and 2% through flatulence in ewes on a chopped lucerne hay diet (Kempton *et al.* 1976). A range of studies conclude that the majority of enteric methane is released from the animal through a combination of eructation and exhalation through the mouth (Kempton *et al.* 1976; Murray *et al.* 1976; Thorpe 2008; Smith *et al.* 2010). Due to this, enteric methane measurement techniques focus on measuring and sampling from the animals breathe.

2.4 Enteric methane measurement techniques

There are three main ways to measure enteric methane emissions from cattle; respiration chambers, sulphur hexafluoride (SF_6) tracer technique and sensor technology placed on the farm in areas that the animal may visit or where the animal is bated to visit (e.g., GreenFeed emissions measurement).

2.4.1 Respiration chambers

Respiration chambers are known as the 'gold standard' of enteric methane measurement for individual animals (O'Hara *et al.* 2003; Garnsworthy *et al.* 2019). This is because they operate by measuring the absolute emissions from the animal in a sealed chamber shown in Figure 2.3. However, the respiration chambers have limitations when measuring enteric methane from naturally grazing animals as feeding behaviour may change (Hammond *et al.* 2016b). This could affect the diurnal pattern of enteric methane production in these grazing animals as enteric methane production is reflective of feeding behaviour and intake (Clapperton and Czerkawski 1969). Intake may also be restricted as there is an allowance of feed whereas in a grazing system there is generally *ad libitum* feed available (Hammond 2011). Also, these chambers are very expensive and only one animal can be measured at a time.

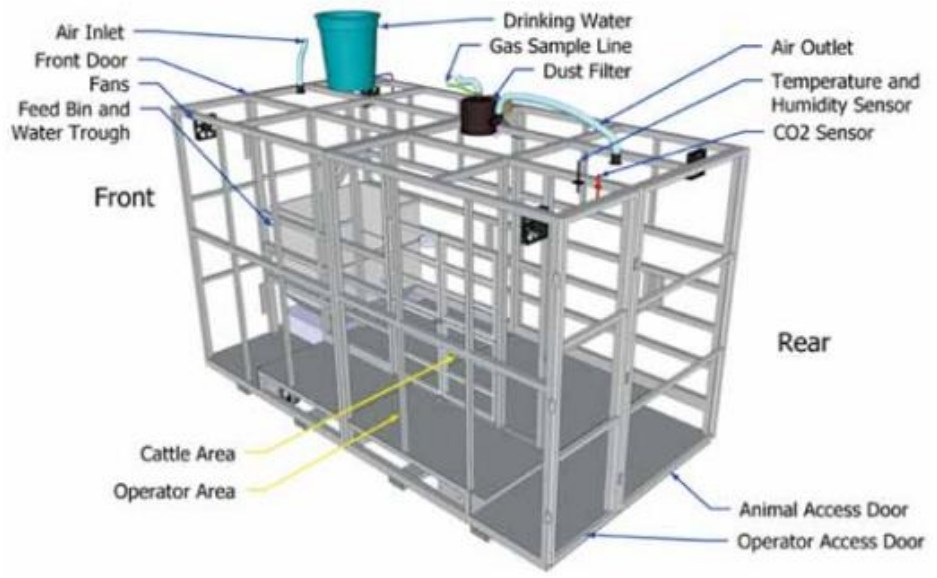

Figure 2.3. Design of a cattle respiration chamber that is covered with clear walls and roof (Pinares-Patiño *et al.* 2011).

2.4.2 Sulphur hexafluoride tracer technique

Sulphur hexafluoride (SF_6) tracer technique is another method to measure enteric methane from individual animals. This method works by detecting SF_6 released from a rumen bolus in the animal's breath (Johnson and Johnson 1995, Lassey *et al.* 1997). The cow is fitted with a halter with a sample tube located close to the nose. This sample tube is connected to a collection canister which is strapped on the animals back as shown in Figure 2.4. Air is drawn up into the canister and manually analysed every 24 hours which includes a release of the tracer gas. This method allows enteric methane emissions to be measured in a grazing system and feeding behaviour to remain the same. Therefore, this method is more suitable for quantifying enteric methane emissions in a grazing system rather than the respiration chambers (Grainger *et al.* 2007). However, this technique is very labour intensive which results in the measurement period being restricted (Hammond *et al.* 2016b).

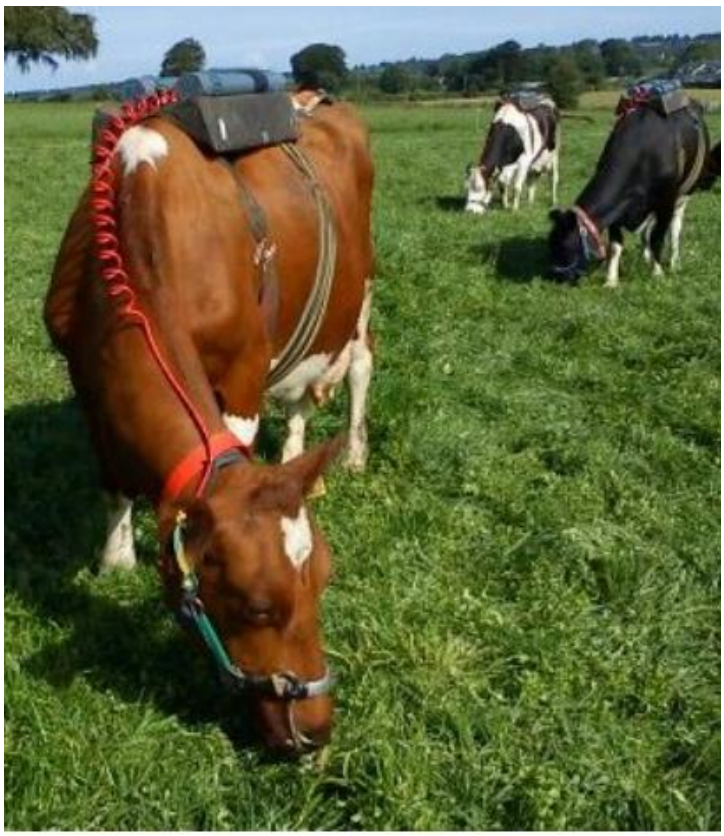

Figure 2.4. SF_6 tracer technique on a grazing dairy cow at Teagasc Moorepark, Ireland (Berndt *et al.* 2014)

2.4.3 Sensor measurement technology

New methodologies have enabled enteric methane measurement to be carried out using sensor technology. These sensor technologies are non-invasive and allow the animals to remain in their natural environment and routines through the measurement period. In a grazing situation, these aspects are important to be able to measure enteric methane that is representative in a pasture based system (Waghorn *et al.* 2016). When animals are moved from their environment, their feed intake and behaviour may change which will also affect the level of enteric methane produced (Waghorn *et al.* 2016). The sensor technologies are less intense on the animals as it relies on spot measurement throughout the day to estimate enteric methane emissions rather than an 'all-day' measurement (Goopy *et al.* 2016a). Two of the main sensor technologies used are the GreenFeed emissions system and sniffer method. The accuracy of these sensor technologies are comparable to the SF_6 and respiration chamber results which indicates that it can be used to reliably quantify enteric emissions (Goopy *et al.* 2016a, Hammond *et al.* 2016b).

2.4.3.1 GreenFeed emissions measurement

GreenFeed emissions measurement technology (C-Lock Inc, South Dakota, United States of America) is a type of sensor measurement technology that has been used in estimating enteric methane emissions in both inside and outdoor environments. The GreenFeed operates by continuously measuring the emissions expelled by the animal while their head is inside the feed bin (Hristov *et al.* 2015). An air sample is drawn through an air filter and into the electronic sensors in the system as shown in Figure 2.5. Air is continuously sampled with and without the animal to account for background emissions (Goopy *et al.* 2016b). Respiration chambers typically measure the enteric methane fluxes 70 to 100 times a day, whereas the GreenFeed relies on 2 to 4 flux measurements per day (Hammond *et al.* 2016b). However, results from the GreenFeed system are comparative to the respiration chambers and SF_6 technique (Hammond *et al.* 2015, Goopy *et al.* 2016b).

The data being collected from the GreenFeed has been shown to be as accurate as either SF6 or respiration chamber methods (Hammond *et al.* 2015; Goopy *et al.* 2016b; Manafiazar *et al.* 2016). Manafiazar *et al.* (2016) reported a repeatability of 0.79 and 0.67 when the data is averaged over 14 and 7 days respectively. Another study reported a 14-day average repeatability of 0.78 (Coppa *et al.* 2021) indicating that the measurements from the GreenFeed are reliable and accurate. Both of these studies have been carried out in an indoors system where there would be less environmental factors, such as wind, affecting the spot measurements compared to being outdoors. As a result of this, it would be expected that the

variability of estimated enteric methane may increase in outdoor measurement, however there is limited research in a grazing system. The GreenFeed appears to be the most practical and accurate technique to measure enteric methane emissions from grazing animals as it can sample up to 40 animals per machine and can continuously sample for a long measurement period (Waghorn *et al.* 2016).

Figure 2.5. Components of the GreenFeed emissions measurement method (Hristov *et al.* 2015).

2.4.3.2 Sniffer measurement method

The Sniffer method involves an enteric methane and/or carbon dioxide sensor mounted to the feed bin of automated milking systems or in the feed bin of the milking shed. The majority of studies that have used this technique have been using the automated milking system for ease of farm and research management as the animals are milked 1-4 times a day (Jonker *et al.* 2020). The principle behind this method is to be able to measure enteric methane emissions from cows without disrupting their behaviour or complicating the farm management. Due to the fact that this method does not use an active air flow to create a vacuum to draw in the animal's expelled air the capture rate is low. This means that there will only be a small proportion of the animal's breath per sample, and this can be reduced further with the effect of wind, head position and breathing rate (Jonker *et al.* 2020). This is one of the main limitations with this method as the air pump is only drawing in 1-4L/minute which is at least 22L/minute less than the GreenFeed system (Goopy *et al.* 2016a; Jonker *et al.* 2016). This results in a large variability between samples and therefore the repeatability is relatively low. Recovery rates and gas calibration have been carried out in studies which have slightly improved the results (Garnsworthy *et al.* 2019), however, to improve the reliability of this method an active airflow system would need to be installed, similar to the GreenFeed system.

2.4.4 Enteric methane relationship with carbon dioxide

There is an indirect approach to estimate enteric methane emissions through the carbon dioxide emitted (Madsen *et al.* 2010; Jonker *et al.* 2016). The estimated CO_2 emissions combined with the CO_2:CH_4 ratio expelled in the breath can be used to calculate total enteric methane emissions (Madsen *et al.* 2010). However, the ratio can be affected by differences in digestion, fermentation and metabolic activities as well as a change in feed efficiency, which can skew the estimated enteric methane production if the amount of carbon dioxide produced changes and enteric methane remains constant (Huhtanen *et al.* 2015; Hristov *et al.* 2018). Therefore, this technique may result in inaccuracies of enteric methane estimation as it depends on the state of the animal. The results from this method are comparable to the SF_6 technique, however, with respiration chambers there are slight differences that could lead to a change in treatment significance (Madsen *et al.* 2010; Haque *et al.* 2017; Hristov *et al.* 2018). This method is not as robust as estimating enteric methane emissions due to the indirect nature of the method as well as a variation in results when compared to respiration chamber studies.

2.5 Animal factors affecting enteric methane production

There are multiple different factors that affect enteric methane production, ranging from environment, diet composition as well as within animal variation (Molano and Clark 2008; Herd *et al.* 2014; Flay 2018). The variation between animals has limited research compared to the effect that dietary components have on enteric methane emissions, with many studies showing the latter.

2.5.1 Feed intake

The level of feed intake for an individual animal varies significantly due to the physiological state as well as the maintenance and milk production energy demands (Nicol and Brooks 2007). Therefore, it is expected that all cows will have a slightly different level of intake. Multiple studies in beef, sheep and dairy systems have reported a positive relationship between enteric methane emissions and level of intake, therefore an increase in intake may lead to an increase in enteric methane production (Hammond *et al.* 2016a; Molano and Clark 2008, Herd *et al.* 2014). One study investigated this relationship in sheep with a diet of ryegrass and white clover, which indicated that an increase in dry matter intake may lead to an increase in daily enteric methane production but less methane per kilogram of dry matter intake (Hammond *et al*. 2013).

Animals that have a lower intake than predicted (residual feed intake, RFI) have been reported to be more efficient animals and produce less enteric methane per kilogram of dry matter intake (Hegarty *et al.* 2007). The metabolic differences between the high and low RFI animals may also be affecting the level of enteric methane produced. Therefore, the low RFI animals are also low enteric methane emitting animals as a result of their metabolic and physiological processes (Xi *et al.* 2016). This study suggested that there was potential to be able to abate animals based on their residual feed intake and therefore select for animals that are more efficient leading to a reduction in enteric methane produced. However, other studies have found contrasting evidence that an improved RFI did not have an effect on overall enteric methane produced per day (Flay *et al.* 2018). However, this same study reported a significantly higher enteric methane per kilogram of dry matter eaten for lower RFI animals. Flay *et al.* (2018) suggests that this may be a result of greater digestion of dry matter and neutral detergent fibre (NDF) in the low RFI animals which could lead the substrate to become more exposed to methanogens and increase enteric methane production.

2.5.2 Diet and digestion

The diet of the cow will have an effect on the amount of enteric methane produced per kilogram of dry matter and also totally daily production of enteric methane. Some animals genetically have the ability to digest particles faster which could be attributing to a lower enteric methane yield per day and hence have a faster rate of passage (Moss *et al.* 2000). Animals or diets that are easily digested have less retention time in the rumen and therefore the substrate will spend less time in the rumen. This leads to an environment that methanogens are not able to compete in (Moss *et al.* 2000) and are not able to fully utilise the substrate in the rumen resulting in a reduction in enteric methane production. Diets that are high in fibre will generally have a slower rate of passage than starch rich diets (Molano and Clark 2008). This is reported to lead to an increase in acetate production which favours methanogenesis (Benchaar *et al.* 1998; Molano and Clark 2008). Therefore, the diet composition also has an effect on enteric methane production. The animal itself will have an influence on how the feed is digested and therefore the variations in rumen size, chewing rate, and feeding patterns will affect that level of enteric methane produced (Waghorn *et al.* 2006).

Multiple studies have reported that legume forages produce less enteric methane than grass forages (McAllister *et al.* 1996; Molano and Clark 2008). It is reported that this is a result of an increase in digestibility with legume forages which leads to a faster rate of passage (Molano and Clark 2008). This may reduce the amount of enteric methane produced per kilogram of dry matter eaten but the total enteric methane produced per day may not reduce as the daily intake may increase. There have also been numerous studies that have investigate the effect of feeding maize and/or grass silage (Hammond *et al*. 2016a; Reynolds *et al*. 2010; Livingstone *et al.* 2015). These results show that cows fed maize silage will likely produce less methane per kilogram of dry matter intake compared with high grass silage diets. This links back with the effect of fibre as maize silage often has a higher NDF content and therefore theoretically results in an increase in methane production (Hammond *et al*. 2016a).

2.5.3 Breed and genetic variation

The breed of cow has an effect on the level of enteric methane production (Flay 2018). Multiple studies have reported that Jersey cows produce less enteric methane per day but do not appear to be more efficient on a 'per kilogram of body weight' or 'per kilogram of dry matter intake' (Flay 2018; Olijhoek *et al.* 2018). This shows that there is a potential to breed or cross breed for cows that produce less enteric methane in total per day. However, Jersey cows have a lower body weight and therefore can be stocked at a higher rate (Coffey *et al.* 2017). Through having Jersey and Jersey/Friesian cross-bred cows, farmers may be inclined to increase their herd size to maximise milk production from their farm. Although enteric methane per cow will have reduced, there will be more cows per hectare and therefore the overall enteric methane will not change or increase in this situation. The energy lost through methanogenesis can be redirected into milk production and this could lead to a potential milk yield increase. The varying enteric methane production between animals should be seen as an opportunity to select animals best suited to the pastoral sustainable and profitable agriculture.

Enteric methane production can be largely variable, and studies have indicated that there may be a genetic influence. Some animals are able to produce the same level of enteric methane production per kilogram of dry matter intake throughout a lactation and others do not have this same stability (Waghorn *et al.* 2006). Despite being exposed to the same diet and environmental conditions (temporary effects) there is still variation within the herd, which indicates that the there is potential for a genetic influence to affect enteric methane production. Multiple studies have investigated the heritability of daily enteric methane produced in dairy cows and their findings have ranged from 0.12 to 0.30 and repeatability ranging from 0.25 to 0.67 (Lassen and Lovendahl 2016; Pszczola *et al.* 2017; Breider *et al.* 2019). Specific heritability and repeatability numbers are reported and detailed in Table 2.3. This shows that enteric methane production is influenced by genetic parameters which could be used in selective breeding towards a lower emitting animal.

Table 2.3. Comparison of the estimated heritability and repeatability for daily enteric methane emissions in dairy cows reported.

Study	Heritability	Repeatability
Lassen and Lovendahl, 2016	0.21	0.35
Breider ***et al***. 2019	0.12 – 0.43	0.50 – 0.67
Pszczola ***et al***. 2017	0.27	0.25

2.5.4 Live weight

Enteric methane emissions are affected indirectly by the live weight of the animal. The main indirect effect is through the level of intake. A larger or heavier animal will have greater energy requirements to be partitioned towards maintenance than smaller animals (Penno 1999). This is a result of the larger body mass needing more energy to maintain its body's functions. Therefore, as energy requirements increase, dry matter intake increases proportionally (Nicol and Brooks 2007). Due to the positive relationship between dry matter intake and enteric methane production, the enteric methane will also increase (Molano and Clark 2008; Herd *et al.* 2014). Therefore, it would be expected that a heavier cow would produce more enteric methane due to the daily intake being higher than a smaller cow.

2.6 Residual enteric methane emissions

Residual enteric methane emissions (RME) have been identified as a potential breeding selection index when aiming to lower enteric methane emissions from cows (Ross *et al.* 2020; Smith *et al.* 2021; Herd *et al.* 2014). Residual enteric methane emissions was first proposed as a trait by Herd *et al.* (2014) as a trait that is worth considering due to its independence from dry matter intake. RME is independent of dry matter intake, animal production and body weight which have been identified to affect methane production. Therefore there is potential for this trait to be used to rank and select animals for a low emitting breeding scheme.

RME is calculated by actual (measured) methane production minus the estimated enteric methane production. Estimated enteric methane production is predicted through modelling (Bird-Gardiner *et al.* 2017; Smith *et al.* 2021) and actual enteric methane production is measured through such methane measurement techniques discussed in Section 2.4. This is similar to how residual feed intake (RFI) is calculated in many studies (Arthur *et al.* 2001; Hegarty *et al.* 2007); the difference between estimated and actual dry matter intake. A low

RME means that an animal is producing less enteric methane than estimated and a high RME is an animal that is producing more enteric methane than estimated (Smith *et al.* 2021).

Selecting animals to reduce enteric methane production based solely on enteric methane production per day, could lead to detrimental effects on animal production (Bird-Donoghue *et al.* 2017; Donoghue *et al.* 2016). RME has the potential to limit these detrimental effects while still selecting for lower emitting animals due to the independence of production traits. The RME trait has the ability to be able to rank animals based on their RME and select animals accordingly without affecting their production (Smith *et al.* 2021). Therefore, animals are able to be identified with varying efficiencies through the RME ranking. The benefit of RME is animals are able to be identified as low emitting and therefore selected for, while there is no difference in production. These animals are therefore more efficient (less enteric methane per day or kilogram of output) than their contemporaries. These efficient animals may be able to be used in a breeding scheme to reduce methane emissions in the next generation while having the same level of productivity (Renand *et al.* 2019).

2.7 Research opportunity

The effect that animal traits such as milk production, live weight and intake have on enteric methane production is relatively unknown in a pasture based system. This research will identify and highlight different animal factors that influence enteric methane emissions. From this identification, farming management decisions, further research and policies can become more informed on what is happening in the Irish pasture based system. As consumers are becoming more aware of their environmental footprint, they are seeking food that is environmentally sustainable. The dairy industry has to continue working towards a more sustainable industry to ensure that the demand for the dairy products produced is still there. Farmers also have an obligation to reduce their emissions to reach the national emission reduction targets and therefore by understanding these animal factors, farmers can be more informed about their decision making. Researchers will be able to use this information in breeding decisions, while working towards low emitting animals. All of these factors are imperative to the continued success of the Irish dairy industry. Therefore, this research is important not only for the understanding of enteric methane production, but also for future of the Irish dairy industry.

2.8 Objectives

The aim of this study was to identify animal factors that influence enteric methane emissions in pasture based dairy cows. The first objective of the study was the investigate the repeatability of enteric methane emissions and other animal traits in pasture based dairy cows. This repeatability measure allows animal variation and equipment reliability to be investigated. This is particularly important for enteric methane emissions due to the potential for environmental conditions to impact on accuracy of measurement while using GreenFeed system outdoors. The second objective was to assess the relationship between enteric methane and other animal traits in pasture-based cows. These relationships will be able to identify key traits that impact on the amount of enteric methane produced for an individual animal basis. The third objective was to investigate the potential of a new trait called residual enteric methane (RME) to select for lower enteric methane emitting cows without impacting on productivity. Reducing enteric methane is a crucial part of achieving the national and international emission reduction targets. Identifying a trait like RME that has the ability to reduce enteric methane emissions while maintaining the same level of production, could have the potential to be influential in achieving these targets in the future.

Chapter 3: Materials and Methods

3.1 Experimental design

A grazing experiment involving forty five mid lactation dairy cows to measure enteric methane production using two outdoor GreenFeed units (C-Lock Inc, Rapid City, SD, USA) was conducted at Teagasc, Moorepark, County Cork, Ireland from 3rd August to 18th October 2020. The cows ranged in age from their first to eighth lactation with a mean of 3.02 ± 1.67 including twenty seven Friesian and eighteen Jersey Friesian crossbred cows. The mean bodyweight was 540kg ± 55.96kg and body condition score of 3.14 ± 0.26. BCS is measured on a 1 to 5 scale. All cows were managed in a rotational grazing system, similar to that described by Roche et al. (2017). The lactating dairy cows were stocked at 2.6 livestock units per hectare on the grazing platform. The paddock sizes ranged from 0.4 to 0.9 hectares with temporary water troughs located in every pasture break. The pasture consisted of perennial ryegrass (*Lolium perenne*). The forty five cows were managed amongst a total of eighty cows (45 were involved in the enteric methane study), which were split in two different herds of forty and randomised and blocked evenly between the two herds. These animals were split into two herds because of a previous grazing study and also to maintain a stocking rate of 23 animals on one GreenFeed machine and 22 on the other. All procedures and animal management were approved by the Teagasc Animal Ethics Committee and the Health Products Regulatory Authority (HPRA).

3.2 Animal measurements

Emissions of enteric methane (CH_4), carbon dioxide (CO_2) and hydrogen (H_2) were estimated for each cow several times during the day through the two outdoor GreenFeed systems (C-Lock Inc, Rapid City, SD, USA). All cows had access to the GreenFeed systems 24 hours a day and could get an allocation of concentrate at most once every four hours. Concentrate offered was dispensed at a rate of 34 grams every 20 seconds. Only visits that were longer than 2 minutes were used in the data analysis (Manafiazar *et al.* 2016; Velazco *et al.* 2016). The data collected from the GreenFeed was sent electronically to C-Lock Inc (Rapid City, SD, USA) where it was processed and the results were cleaned and verified. These results were then available for data analysis.

Throughout this study, the cows were milked twice daily between 07.00 and 09.30 in the morning and 14.30 and 17.00. Individual milk yield was recorded daily at each milking (Dairymaster, Causeway, CO. Kerry, Ireland). Milk composition was sampled weekly through Dairymaster milk sampling equipment (Dairymaster, Causeway, CO. Kerry, Ireland) every

Tuesday afternoon and Wednesday morning milking. These individual animal milk samples were analysed for protein, fat, lactose percentages and yields as well as somatic cell count, and milk urea using a Pro-Foss FT 6000 instrument (MilkoScan ^{TM} FT). Milk solids yield (MSY) was calculated as the sum of fat protein yields.

Live weight (LWT) and body condition score (BCS) was recorded weekly throughout the experiment. The cows were weighed after morning milking before returning to the paddock using an electronic portable weighing scale with the Winweigh software package (Tru-test, Auckland, New Zealand). BCS was scored weekly by a trained personal on a scale of 1 to 5 (where 1 = emaciated and 5 = extremely fat) with 0.25 increments (Lowman and Scott 1976).

Individual dry matter intakes were estimated using the n-alkane technique (Mayes *et al.* 1986) as modified by Dillon and Stakelum (1989) for cows twice over the study. All cows were dosed twice daily, before milking, for twelve consecutive days with a paper bullet (Carl Roth GmbH, Karlesruhe, Germany) containing 500 mg of dotriacontane (C32 – alkane). From day seven of dosing, faecal samples were collected from each cow twice daily (before both milkings) for the remaining five days. The faecal samples were bulked (12 g of each collected sample) and dried for 48 hours at 60^{o}C and milled through a 2 mm screen and stored for chemical analysis.

In conjunction with the faecal collection, the diet of the cows was also sampled. Two herbage samples of approximately fifteen individual grass snips were manually collected with Gardena hand shears mimicking the grazing defoliation pattern observed on previously grazed swards, on days 6 to 11. The daily samples were stored at -18^{o}C. The frozen herbage samples were bowl-chopped (Muller, Type MKT 204 Special, Saabrücken, Germany), freeze-dried at -500^{o}C for 120 hrs, and milled through a 2 mm screen and analysed for alkane content. The content of C31 and C32 in the faeces and herbage samples and the amount of the C32 dosed was used to estimate dry matter intakes using the equation stated by Mayes *et al.* (1986).

3.3 GreenFeed management

Before the beginning of the experimental period there was a training period to ensure each animal had an adequate visitation frequency. After the 4 weeks of training, cows were selected to continue into the experimental period providing they were visiting the GreenFeed over 1 visit/day over a seven day period. During the training period all 80 cows had access to a GreenFeed which was positioned on the roadway next to the grazing paddock. Although many

of the cows used the GreenFeed machines, others did not. All of the non-user cows were blocked from the GreenFeed at the beginning of the experimental period. Enteric methane estimates continued for the duration of the experimental period for the selected cows.

Two outdoor GreenFeed units were positioned on the lane directly outside the grazing paddock. The GreenFeed constantly followed the grazing rotation allowing continual access for the cows. However, the GreenFeed was removed from the cows for 2 days as a result of extreme weather (Force 9 storm, Met Eireann). The GreenFeed was moved approximately every 2 days depending on rotation length.

The two GreenFeed units were identical and followed the same herd throughout the experiment. The cows stayed in the same herd with the same GreenFeed for the duration of the experiment with 23 and 22 cows allocated per GreenFeed machine. The GreenFeed alleyway ensured that only one animal was able to access the machine at a time. When moving the machines, the alleyway was folded up to allow ease of movement.

Standard gas calibrations are carried out using span (20% oxygen and 80% nitrogen) and zero (10ppm hydrogen, 500ppm enteric methane, 5000ppm carbon dioxide and 21% oxygen and the balance of nitrogen) gases. These calibrations are carried out automatically every 3 days at 04.00. A carbon dioxide recovery was carried out manually every month. These calibrations ensure the sensors do not drift away from the baseline concentrations over time.

The visit frequency was closely monitored throughout the experiment to ensure that concentrate intake was similar across all animals. This is also important that there are enough visits across a period to ensure maximum repeatability and accuracy. The visitation frequency of each cow was closely monitored daily. If the visit frequency was less than 2 for each day these cows were encouraged to visit the GreenFeed between milking during the day through human interaction. However, this did not occur regularly. The average visit frequency was 2.20 per day for the period of the study.

Figure 3.1. GreenFeed located next to grazing plot with a cow using the machine.

Figure 3.2. GreenFeed set up on farm race

3.5 Statistical analysis

All statistical analysis were performed using the SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Individual estimated enteric methane production values were excluded from the study if the average visits frequency was ≤1 visit per day for each 7 day period. The diurnal pattern for the 10 week period was normally distributed throughout the day and therefore no adjustments had to be made. After cleaning the dataset all data was averaged per week of the experiment. Descriptive statistics (mean, standard deviation and coefficient of variation) were obtained using the MEANS procedure.

Partial correlations of daily enteric methane production with daily production of carbon dioxide, hydrogen, milk, fat, protein, lactose, milk solids, percentages of fat, protein and lactose, milk urea, live weight, body condition score, live weight change and dry matter intake were obtained using the GLM procedure with a linear model that included the fixed effect of parity, week, breed group and herd and deviation from median calving date of the herd as covariate.

As live weight is measured weekly, daily weight estimations were made through a polynomial of order 3 for each cow using the REG procedure. Daily live weight change was estimated from the predicted live weights at each day of the lactation.

Within and between individual cow variances was calculated using MIXED procedure in SAS. The model included the fixed effect of parity, breed, herd, week and the deviation from median calving date of the herd as covariates and the random effect of cow. In the model, the random effect of the cow was assumed with mean zero and varianceσ_c^2, and residual error with mean zero and varianceσ_e^2. The repeatability (t) was calculated using the following formula: $t = \sigma_c^2 / (\sigma_c^2 + \sigma_e^2)$.

3.4 Residual enteric methane emissions

Measures of residual enteric methane emissions (RME) production were obtained by two methods, residuals from a multiple liner regression model, and differences between actual measures of enteric methane production and the enteric methane production estimated using animal energy requirements formulae used by the Irish inventory.

In the first method daily enteric methane emissions were modelled with a linear multiple regression model that included herd, breed, lactation number and week of trial as class effects and visitation frequency, energy corrected milk, metabolic live weight, body condition score, predicted live weight change and deviation from median calving date as covariates, and the residual. Daily measures of all variables were averages per week.

Other effects were considered in the multiple regression such as milk composition but were not included because they introduced multicollinearity in the model. Factors that had variance inflation factors greater than 4.0 were not included in the model (Hair *et al.* 2010).

Energy corrected milk yield (ECMY) was calculated through the following formula (Sjaunja *et al*. 1990):

ECMY (kg) = Milk yield × [(383 × fat% + 242 × protein% + 165.4 × lactose% + 20.7)/3140]

and metabolic live weight (mLWT) was calculated as:

$mLWT = LWT^{0.75}$

The residual errors were extracted from the model and became the measures of residual enteric methane. The output of the model had 11 residual enteric methane values for each animal representing each week of the trial. These residual enteric methane values were then averaged per animal to obtain the average residual enteric methane value per animal. This overall averaged residual enteric methane was then used for subsequent ranking. Animals were ranked on RME and were split into 3 groups. The highest RME animals were classified as high, the lowest RME animals were ranked low and the 15 animals that were in the middle were ranked medium. Therefore, the herd was split in 3 groups with 15 cows in each group. This resulted in the range for each rank group being:

Group	Range of residual enteric methane emission (g CH_4/day)
High	11 to 61
Medium	-11 to 11
Low	-49 to -11

In the second method, the RME were the difference between the actual measure of enteric methane production (aCH_4) obtained by the GreenFeed machine and the calculated measure of enteric methane emission (cCH_4) obtained formulae used for the Irish national inventory calculations (Duffy *et al.* 2021). That is,

$RME = aCH_4 – cCH_4$

where cCH4 = energy requirement × 6.5% × dry matter intake

The inventory calculations estimated the energy requirements based on the cow production and live weight and assumed 6.5% of this energy is consumed through methanogenesis. Similarly, as in the previous method, the highest RME animals were classified as high, the lowest RME animals were ranked low and the 15 animals that were in the middle were ranked medium. The herd was split in 3 groups with 15 cows in each group. This resulted in the range for each rank group being:

Group	Range of residual enteric methane emission (g CH_4/day)
High	22 to 68
Medium	18 to -5
Low	-11 to -49

Least squares means and standard errors for enteric methane, milk production and composition traits, and live weight, dry matter and feed efficiency traits for each for the RME groups were obtained with a linear model that included the fixed effect of RME group and residual error. The least squares means were used for multiple mean comparison suing the Fisher's least significant difference test. Significant differences were declared at $P<0.05$. The rank correlation between the two measures of RME were obtained using the Spearman rank correlation coefficient.

Chapter 4: Results

4.1 Descriptive statistics

Mean, standard deviation and coefficient of variation values for enteric methane, live weight, milk production and composition traits are shown in Table 4.1. Forty-five late lactation (early August to mid-October) grazing dairy cattle produced 0.65g CH_4/kg live weight throughout this study. The coefficient of variation for enteric methane (13%) is relatively low in comparison with milk yield (20%) and milk solids (18%). Somatic cell count exhibits a large coefficient of variation (137%), however when converted to somatic cell score the coefficient of variation decreases (29%). The mean grams of enteric methane produced per kilogram of dry matter intake was 21.28 g/kg DMI.

4.2 Repeatability

Estimates of repeatability for all of the studied animal production traits are given in Table 4.2. These repeatabilities tended to be relatively high as the majority of the traits ranged from 0.56 to 0.95. Live weight change displayed the lowest repeatability of 0.20. However, liveweight has the highest repeatability of 0.95. The weekly repeatability of estimated enteric methane production in this study (0.66) is comparative to other traits such as milk yield (0.76) and milk solids production (0.59). This shows that the measurement technique and quality of the estimated enteric methane produced is reliable and accurate in comparison to milk production traits.

4.3 Partial phenotypic correlations

The phenotypic correlations between enteric methane and animal production traits are shown in Table 4.3. Estimated enteric methane production exhibits a highly significant correlation between daily milk yield, fat yield, protein yield, lactose yield, lactose percentage, and milk urea all have a correlation of at least 0.20. Somatic cell count and body condition score both have a significant negative relationship with enteric methane production (p-value <0.05). Milk fat percentage, lactose percentage and yield, somatic cell count and live weight change all show no correlation with enteric methane production. The correlation between dry matter intake and enteric methane production is 0.24 which is also slightly greater than milk yield correlation.

Table 4.1. Number of observations (N) Mean, standard deviation (SD) and coefficient of variation (CV) for enteric methane, milk production and composition traits, and live weight traits measured in late lactation dairy cows in Ireland.

Trait	N	Mean	SD	CV (%)
Enteric methane production (g/day)	495	351.8	45.67	13
Milk yield (kg/day)	389	17.42	3.41	20
Fat yield (kg/day)	495	0.92	0.18	20
Protein yield (kg/day)	495	0.70	0.13	19
Lactose yield (kg/day)	495	0.81	0.17	21
Milk solids (kg/day)	495	1.62	0.29	18
Energy corrected milk (kg)	389	20.95	3.56	17
Fat percent	495	5.31	0.82	15
Protein percent	495	4.03	0.40	10
Lactose percent	495	4.61	0.15	3
Milk urea (mg/dl)	491	23.57	7.85	33
Somatic cell count (x1,000 cells/ml)	469	182.1	302.9	137
Somatic cell score[1]	469	6.30	1.82	29
Live weight (kg)	446	540.3	55.96	10
Body condition score	450	3.14	0.26	8
Live weight change (kg)	405	0.71	0.48	25
Dry matter intake (kg/day)	43	16.60	2.03	12
Methane/dry matter intake (g/kg)	43	21.28	2.71	13
Estimated methane production Method1[2] (g/day)	45	350.0	29.76	9
Estimated methane production Method2[2] (g/day)	45	345.7	33.95	10
Estimated residual methane Method1[2] (g/day)	45	-0.30	22.81	- [a]
Estimated residual methane Method2[2] (g/day)	45	6.03	27.42	- [a]

[1]Somatic cell score = log-transformed somatic cell count (Wiggans and Shook, 1987)

[2] Estimated enteric methane production calculated by Method1 was through a multiple regression model and enteric methane production measured by the GreenFeed machine. The model fitted enteric methane emission (g/day) as the dependent variable, and herd, breed, lactation number, visit frequency, energy corrected milk, predicted live weight, body condition score, live weight change and deviation from median calving date as independent variables. Estimated enteric methane production calculated by Method2 was through the enteric methane

production calculated by using the energy requirement formulae described in the Irish national inventory method (Duffy *et al.* 2021).

[a] No coefficient of variation due to the average residual methane expected to be 0 and therefore the standard deviation is showing the deviation from the mean. When the mean is 0, or expected to be 0, then no coefficient of variation exists.

Table 4.2. Estimated weekly repeatability for estimated enteric methane production, live weight traits, milk production and composition traits for late lactation dairy cows.

Trait	Repeatability
Enteric methane (g/day)	0.66
Milk yield (kg/day)	0.76
Fat yield (kg/day)	0.56
Protein yield (kg/day)	0.84
Lactose yield (kg/day)	0.68
Milk solids (kg/day)	0.59
Fat percent	0.75
Protein percent	0.84
Lactose percent	0.68
Milk urea (mg/dl)	0.62
Somatic cell count (x1,000 cells/ml)	0.62
Live weight (kg)	0.95
Body condition score	0.80
Live weight change (kg)	0.20

Table 4.3. Partial phenotypic correlations (r) and p-values between animal production traits and estimated enteric methane production from late lactation grazing dairy cows. All correlations are reported after adjusting for parity, breed, calving date, herd and week of study.

Trait	Enteric methane (g/day)	
	r	P-value
Carbon dioxide (kg/day)	0.76	<0.001
Hydrogen (g/day)	0.42	<0.001
Milk yield (kg/day)	0.21	<0.001
Fat yield (kg/day)	0.27	<0.001
Protein yield (kg/day)	0.21	<0.001
Lactose yield (kg/day)	0.23	<0.001
Fat percent	0.06	0.340
Protein percent	-0.07	0.257
Lactose percent	0.28	<0.001
Milk urea (mg/dl)	0.24	<0.001
Somatic cell count	-0.18	0.002
Live weight (kg)	0.12	0.056
Body condition score	-0.19	0.002
Live weight change (kg)	-0.11	0.078
Dry matter intake (kg/day)	0.24	<0.001

4.4 Residual enteric methane production

4.4.1 Multiple regression method

The estimates of regression coefficients of animal traits on enteric methane production are presented in Table 4.4. The regression coefficients show that for every unit increase in energy corrected milk, metabolic live weight and live weight change enteric methane production will increase by 4.68 g, 1.64 g, and 1.75 g, respectively. Contrastingly, a unit increase in BCS will result in a decrease of 39.77 g in enteric methane production.

Table 4.4. Estimates of regression coefficients of animal traits on enteric methane production in late lactation grazing dairy cows.

Trait	Regression coefficient	Standard error	P-value
Energy corrected milk	4.68	0.84	<0.001
Body condition score	-39.77	9.57	<0.001
Metabolic live weight	1.64	0.28	<0.001
Live weight change	1.75	4.47	0.696

The least squares means and standard errors for enteric methane, milk production and composition traits, live weight and feed efficiency traits measured in late lactation dairy cows classified into three groups of RME derived from linear regression model, are shown in Table 4.5. The daily enteric methane produced for the high and medium were both significantly higher than the low ranked group by 59.25 g and 38.04 g, respectively (p-value <0.001). There was a reduction of daily enteric methane emissions by 16% and 11% in the low ranked cows in comparison to the high and medium groups. Despite this large reduction of enteric methane, there was no differences between the high and low ranked groups in milk production, milk composition, dry matter intake or live weight traits.

As a result, the efficiency of enteric methane production between groups were significantly different. Efficiency can be measured by the amount of enteric methane produced for kilogram of milk solids produced, kilogram of live weight or kilogram of dry matter intake. In all cases, the low ranked group are more efficient than the high ranked group. The low group produced 33.02 g of CH_4/kg MS less than the high rank group ($P < 0.001$). The low ranking is also significantly more efficient and produces less enteric methane per kilogram of live weight in comparison to both the high and medium group. There is an increase of 0.09 g CH_4/kg LWT and 0.08 g CH_4/kg LWT for the high and medium group respectively to the low group. The amount of enteric methane produced per kilogram of dry matter intake shows the same trend as the enteric methane per milk solids, where the low group is significantly lower (2.01 g CH_4/kg DMI and 2.95 g CH_4/kg DMI) than both medium and high groups.

Table 4.5. Least squares mean and standard errors (SE) for enteric methane, milk production and composition traits, live weight, dry matter intake (DMI) and feed efficiency traits measured in late lactation dairy cows classified into three groups of residual enteric methane production. Residual methane production was calculated through a multiple regression model[1].

	Group of residual enteric methane production						
	High		Medium		Low		
	Mean	SE	Mean	SE	Mean	SE	P-value
Enteric methane (g/d)	367.1[a]	5.58	345.9 [b]	5.63	307.9[c]	6.29	<0.001
ECMY[2] (kg/d)	8.71	0.29	9.08	0.30	8.39	0.33	0.343
Milk yield (kg/d)	16.09[ab]	0.63	17.55 [a]	0.64	15.35[b]	0.71	0.097
Fat yield (kg/d)	0.92	0.03	0.91	0.03	0.86	0.04	0.467
Protein yield (kg/d)	0.69	0.03	0.71	0.03	0.65	0.03	0.492
Lactose yield (kg/d)	0.78	0.03	0.82	0.03	0.73	0.04	0.204
Milk solids (MS) (kg/d)	1.51	0.05	1.62	0.06	1.47	0.06	0.232
Fat percent	5.61	0.18	5.22	0.18	5.61	0.20	0.267
Protein percent	4.04	0.07	4.00	0.07	4.16	0.08	0.345
Lactose percent	4.65	0.04	4.61	0.04	4.66	0.04	0.668
Milk urea (mg/dl)	25.65	1.09	23.26	1.10	22.72	1.23	0.111
SCS[3]	6.41	0.51	6.19	0.51	6.11	0.58	0.903
Live weight (LWT) (kg)	531.8	11.23	512.0	11.10	511.6	12.33	0.301
Body condition score	3.13	0.07	3.07	0.07	3.17	0.07	0.648
Live weight change (kg)	0.67	0.06	0.57	0.06	0.68	0.07	0.504
Dry matter intake (kg/d)	16.10	0.47	15.96	0.51	15.68	0.54	0.804
Methane-MS (g/kg)	248.2[a]	6.61	218.1[b]	6.67	215.2[b]	7.45	<0.001
Methane-LWT (g/kg)	0.69[a]	0.01	0.68[a]	0.01	0.60[b]	0.01	<0.001
Methane-DMI (g/kg)	22.91[a]	0.60	21.97[ab]	0.65	19.96[b]	0.68	0.004
Feed efficiency (kg/kg)	0.09	0.003	0.09	0.003	0.10	0.003	0.259

[1] Residual enteric methane emissions were the residuals of a multiple regression model fitting enteric methane emission (g/day) as the dependent variable, and herd, breed, lactation number, visit frequency, energy corrected milk, predicted live weight, body condition score, live weight change and days in milk as independent variables.

[2]Energy corrected milk yield

[3]Somatic cell score = log-transformed somatic cell count (Wiggans and Shook, 1987).

[a, b, c] Means with different superscripts within the same row are significantly different (P<0.05).

4.4.2 Irish national inventory method

Residual enteric methane was also calculated through the Irish national inventory calculations and the same comparison was carried out as the multiple regression prediction. Table 4.6 shows similar relationships between the different rankings as Table 4.5. The high group is producing 16 % more enteric methane than the low group and 7 % more than the medium group. There are significant differences between all rank groups with the high group producing the greatest enteric methane per day and the lowest group producing the least enteric methane. There are no significant differences between either groups for any milk production, composition and live weight traits which is consistent with Table 4.5.

However, similar to Table 4.5, there is a significant difference in enteric methane-MS efficiency and enteric methane-DMI efficiency between the low group to both the medium and high ranked group. This relationship trend is also the same for enteric methane-LWT efficiency, with the low group being 0.07 less than the high group and 0.03 less than the medium group. Therefore, this is showing that the low group is more efficient at producing less enteric methane per milk solid and per kilogram of live weight. The low group are producing 18% less enteric methane per kilogram of dry matter eaten than the high ranked group. This shows huge reductions in enteric methane emissions without compromising production.

4.4.3 Rank correlation

For each method of estimating RME (multiple regression and national inventory) the animals were ranked in high, medium or low groups according to their residual enteric methane. The rank correlation between the different estimation methods and subsequent ranking was 0.77 (P<0.001), showing that the ranking between the two methods to estimate RME was relatively similar.

Table 4.6. Least squares mean and standard errors (SE) for enteric methane, milk production and composition traits, live weight, dry matter intake (DMI) and feed efficiency traits measured in late lactation dairy cows classified into three groups of residual enteric methane production. Residual enteric methane production was calculated through the Irish national inventory formula[1].

	Group of residual enteric methane production						
	High		Med		Low		
	Mean	SE	Mean	SE	Mean	SE	P-value
Enteric methane (g/d)	368.5 [a]	7.28	341.0 [b]	6.81	317.6 [c]	7.08	<0.001
ECMY[2] (kg/d)	8.59	0.31	8.70	0.29	9.02	0.30	0.564
Milk yield (kg/d)	15.99	0.69	16.40	0.65	17.00	0.68	0.561
Fat yield (kg/d)	0.89	0.04	0.90	0.03	0.91	0.04	0.923
Protein yield (kg/d)	0.65	0.03	0.70	0.03	0.71	0.03	0.377
Lactose yield (kg/d)	0.75	0.03	0.80	0.03	0.80	0.03	0.492
Milk solids (MS) (kg/d)	1.53	0.06	1.51	0.06	1.59	0.06	0.609
Fat percent	5.53	0.19	5.44	0.18	5.40	0.19	0.880
Protein percent	4.04	0.08	4.04	0.07	4.10	0.08	0.790
Lactose percent	4.59	0.04	4.66	0.03	4.66	0.04	0.284
Milk urea (mg/dl)	24.70	1.16	24.71	1.08	22.13	1.13	0.169
SCS[3]	6.91	0.52	5.92	0.49	5.94	0.50	0.292
Live weight (LWT) (kg)	526.9	12.05	522.9	10.84	504.1	11.14	0.303
Body condition score	3.11	0.07	3.17	0.07	3.08	0.07	0.638
Live weight change (kg)	0.68	0.07	0.65	0.06	0.56	0.06	0.406
Dry matter intake (kg/d)	15.51	0.50	16.26	0.46	15.93	0.49	0.532
Methane-MS (g/kg)	243.9 [a]	6.81	232.3 [a]	6.37	204.4 [b]	6.62	<0.001
Methane-LWT (g/kg)	0.70 [a]	0.02	0.66 [b]	0.01	0.63 [b]	0.02	0.007
Methane-DMI (g/kg)	23.86 [a]	0.56	21.10 [b]	0.52	20.20 [b]	0.55	<0.001
Feed efficiency (kg/kg)	0.10	0.003	0.09	0.003	0.10	0.003	0.166

[1]Residual enteric methane emissions were the differences between the inventory estimate enteric methane emissions and the true measured enteric methane.

[2]Energy corrected milk yield

[3]Somatic cell score = log-transformed somatic cell count (Wiggans and Shook, 1987).

[a, b] Means with different superscripts within the same row are significantly different (P<0.05).

Chapter 5: Discussion

Understanding the factors that affect enteric methane emissions from lactating dairy cows is important to be able to continually increase the sustainability of dairy products and to also be able to achieve the national and international emission reduction targets. As Ireland is a predominantly grazing based dairy industry enteric methane emissions need to be quantified and understood in this system. Different animal traits such as milk production, milk composition and live weight were investigated. Indoor studies have shown that milk production and live weight have the largest effect on enteric methane production (Herd *et al.* 2014; Bird-Gardiner *et al.* 2017). Body condition score, parity and breed have also been identified to affect daily enteric methane production indoors (Bird-Gardiner *et al.* 2017; Flay 2018). Given the associations between enteric methane indoors on productivity related traits. Some authors have proposed RME as a method of selecting animals for lower daily enteric methane without impacting on production or live weight (Herd *et al.* 2014; Bird-Gardiner *et al.* 2017; Smith *et al.* 2021). At pasture, however, there is sparse literature relating enteric methane output to various animal traits as well as the capability of ranking animals on RME. The objectives of this study were to 1) investigate the repeatability on enteric methane emissions in grazing dairy cows, 2) assess the relationship between enteric methane and other animal traits at grass and 3) investigate the potential of RME to select for lower enteric methane emitting cows without impacting productivity.

The average enteric methane reported per day for this study is 352 g per day and 222 g CH4/kg milk solids. O'Neill *et al.* (2012) quantified enteric methane during late lactation and reported enteric methane emissions to be between 349 g CH4/day and 384 g CH4/day, across high and low herbage allowances. Although O'Neill *et al.* (2012), recorded enteric methane through a different technique to this study (SF_6) the results are comparative. The level of enteric methane produced per kilogram of DMI O'Neill *et al.* (2012) reported is 25.0-26.1 g CH_4/kg DMI which is 17% to 23% greater than this study at 21.3 g CH_4/kg DMI. A similar study at this research centre reported approximately 21.28 g CH_4/kg DMI for mid lactation grass fed cows which agrees with this study's findings (Wims *et al.* 2010). Waghorn *et al.* (2016) carried out a study in New Zealand with a similar pasture-based system and reported a range of 23.6-24.8 g CH4/kg DMI and a mean daily enteric methane production of 318 g CH4/day in late lactation using DMI calculated off of energetic requirements. The daily intake calculated is estimated to be between 12.8 kg and 13.5 kg (Waghorn *et al*. 2016), which is relatively low compared to these other studies. However, the discrepancy across studies may be due to a higher intake in

this study in comparison, which results in less enteric methane being emitted per unit of intake (Smit *et al.* 2005).

The absolute accuracy of herd average enteric methane values are important when evaluating enteric methane for the purpose of national inventories. For breeding purposes however, reliable ranking of animals is needed. High repeatability is often used to measure precision and assess the reproducibility of a measurement over consecutive periods (Wolak *et al.* 2012). The repeatability of estimated enteric methane emissions within the current study (0.66) over a weekly period is similar to Manafiazar *et al.* (2016), who reported a repeatability of 0.69 using the GreenFeed system in an indoor dry feedlot system with a total mixed ration diet. This indicates that the GreenFeed monitoring system is reliable and accurate with similar within animal variations in both indoor and outdoor environments. Another study (Arbre *et al.* 2016) reported 0.72-0.77 for a 5 to 10 day period, respectively. When comparing the repeatability between milk and enteric methane production, there are also minimal differences. This indicates that there are the same within animal variations existing between milk traits and enteric methane production and the reliability of measurement indoors vs outdoors is not affected.

Few studies have estimated the partial phenotypic correlations between enteric methane production against live weight, milk production and composition traits. An indoor study where beef heifers were fed a total mixed ration with concentrate (Manafiazar *et al.* 2016) reported a correlation between CH_4 and standardised dry matter intake as 0.79 over a seven day period which was higher than the current study (0.30). Nonetheless, Renand *et al.* (2019) reported an average correlation ranging between 0.36 to 0.48 between enteric methane and DMI indoors on a high forage diet. This indicates that associations between enteric methane and DMI may differ across differing environments. It should also be highlighted that DMI was estimated using markers, opposed to being directly measured in the current study, which can lead to discrepancies in the measurement (Mayes and Dove 2000). The negative correlation with body condition score (-0.19) results in lower body condition score cows producing higher enteric methane in comparison to high body condition score cows. These low BCS animals are having to have a higher intake to meet energy requirements as body fat stores are not as readily available as animals with a high BCS (Nicol *et al.* 2007). Therefore due to the higher intake for lower BCS animals, they will have higher enteric methane emissions as there is a positive relationship between DMI and enteric methane production. Another study predicted enteric methane production through an energy requirement calculation and estimated that the partial

phenotypic correlation with FPCM and DMI (De Haas *et al.* 2011). Collectively, this suggests that for sustainable reductions in enteric methane to occur without negatively impacting productivity or live weight, there is potential for animal selection to be carried out after accounting for live weight, body condition score and milk production.

Residual enteric methane emissions (RME) was used to compare enteric methane production on an individual animal level and identify differences between the predicted and actual enteric methane. The multiple linear regression model that was developed in this study estimates individual animal's enteric methane production accounting for herd, animal factors, milk production, live weight and body condition score. The estimated regression coefficients of enteric methane production from the RME model show that energy corrected milk yield, metabolic liveweight, liveweight change and body condition score all have a significant effect on enteric methane production. Within this population of dairy cows every kilogram increase in energy corrected milk yield (ECMY) was associated with a 4.68 gram increase in daily enteric methane production. This positive relationship between milk production and enteric methane production indicates that a high milk producing cows will likely have greater enteric methane emissions than low milk producing cows. Metabolic liveweight also has a positive regression coefficient. For every unit increase in metabolic liveweight, enteric methane production is estimated to increase by 1.64 g/day. This indicates that heavier cows will be producing more enteric methane than lighter cows. The live weight change regression coefficient was not significant, which indicates that liveweight change does not have a significant effect on the level of enteric methane produced. Contrastingly, there is a significant negative relationship with body condition score and enteric methane production. The regression model indicates that for every unit increase in body condition score, daily enteric methane produced is expected to decrease by 39.77 grams per day. The combination of all of these factors suggest that a high enteric methane emitting cow is on average heavier, higher yielding and thinner than her contemporaries.

Residual enteric methane could have the potential to provide a more even ranking of an animal's enteric methane production, while being independent of animal production traits such as milk production, live weight and dry matter intake (Smith *et al.* 2021). Within the current study, DMI was not used in the regression as it is not routinely available on commercial dairy farms. Given that DMI is closely associated with milk production and live weight (Holmes 2002). It was envisaged that the incorporation of these traits within the regression may account for the majority of variation in DMI, negating the requirement to include it. The results agree

with this hypothesis as there was no significant difference DMI across the three RME groups. Previous studies indoors have shown there is variation of residual enteric methane between individual animals which is independent of production traits (Ross *et al.* 2020; Smith *et al.* 2021). The same level of variation was observed in the current study between the three rankings with no significant difference in animal production. This means that the variation in enteric methane production did not affect the milk solid production, dry matter intake, and live weight which are all key drivers of performance and efficiency within grazing dairy systems (Delaby *et al.* 2021). There are cows that are more efficient producers in terms of lower enteric methane produced per kilogram of milk production and/or live weight (Dijkstra *et al.* 2013). One study has identified that some beef heifers are more enteric methane efficient when comparing their live weight, daily intake and daily live weight gain to their contemporaries (Renand *et al.* 2019). This study shows a similar trend that animals can be more enteric methane efficient whilst the animal production traits are not compromised. The variation in enteric methane efficiency amongst the individual animals' shows that if animals were selected to be lower enteric methane producers or more efficient based on their residual enteric methane there will likely be no negative effects on dry matter intake, milk production or live weight. This is an important trait that needs to be highlighted in a pasture based system where milk production and feed efficiency are key drivers of profitability (Hanrahan *et al.* 2018). Therefore, low emitting/highly efficient animals can be selected for while maintaining the overall profitability of the pasture based dairy farm.

The coefficient of determination (R^2) of the multiple regression model used to estimate RME was 0.50, indicating that 50 % of the variation in CH4 production was explained by the independent factors (herd, breed, lactation number, visit frequency, week of measurement, energy corrected milk, metabolic live weight, body condition score, live weight change and deviation from median calving date) and that the remaining 50% of variation would be explained by other factors. This variation may be explained by feeding behaviour, digestibility, or rumen microbial populations. Further in-depth rumen microbial and metabolomic analysis is needed to be able to identify the key microbes and metabolites that are associated with the decrease in enteric methane and increase in enteric methane efficiency. Studies in sheep have shown that the size of the rumen significantly impacts the level of enteric methane production, with smaller rumens producing less daily enteric methane while maintaining the same intake (Goopy *et al.* 2014; Waite *et al.* 2018).

Residual enteric methane emissions were also calculated using a similar methodology to that used in the national inventories. The inventory is currently operating on the IPCC Tier 2 method which estimates enteric methane is produced from 6.5% of gross energy intake while grazing (O'Brien and Shalloo 2019; Duffy *et al.* 2020). Therefore, the calculation estimates the gross energy requirements based on the level of production and average live weight. However, based on this study it is clear that other animal factors could be included in this calculation to improve the accuracy of the prediction despite the current ability to estimate daily enteric methane emissions. This could potentially lead Ireland into using the IPCC Tier 3 approach to improve the estimation of enteric methane emissions. The IPCC Tier 3 method is a more dynamic and complex model that increases the precision of estimating emissions produced by incorporating more data. To enable Ireland to move towards the Tier 3 method there needs to be additional data available to the model. Entities such as Teagasc national farm survey, Irish cattle breeding federation, Bord Bia sustainability survey, central statistics office, department of agriculture, food and marine as well as the livestock and feed processors are all collecting data currently that could feed into the model (O'Brien and Shalloo 2019). All of these organisations would be able to produce further insight into the dairy industry to be able to develop representative models which will enable improved accuracy of estimated enteric methane production. Other countries such as Switzerland and France are using this Tier 3 method to estimate their national enteric methane emissions which is accepted by the IPCC (O'Brien and Shalloo 2019). Ireland needs to adopt this new methodology to incorporate more animal factors that affect enteric methane production which will lead to more precise estimations of enteric methane emissions from the national dairy herd.

The rank correlation between RME estimated by the two methods was strong (0.77) indicating that the two methods to estimate RME result in similar ranking of cows for RME. Therefore, the two residual enteric methane rankings are identifying majority of the animals in the same rank: high, medium or low residual enteric methane. As a result, either method can be used in the future to quantify this residual enteric methane trait.

It should be highlighted that the energy sinks milk production and live weight are routinely available, whereas enteric methane emissions are not at present. The use of a large quantity of less accurate phenotypes may outweigh the benefits of less routinely available but precise phenotypes. It is also important to consider the effect of selecting low emitting animals on other production factors. This study has shown that there is a positive correlation with milk production and dry matter intake. Therefore, milk production and dry matter intake would be

expected to decrease under this selection criteria. This will result in a reduction of profit for the dairy industry which is not desirable. The RME trait is independent of production and dry matter intake and therefore it could have the potential to be used as a selection index. However, further research needs to be conducted to be able to establish if the RME trait is influenced through genetic or phenotypic parameters.

Conclusions

The findings of this study indicate that the level of enteric methane produced in grazing late lactation cows is influenced by animal factors such as the level of milk production, dry matter intake, live weight, body condition score, parity and breed of the cow. Despite this, there are variations between animals with the same production traits (milk solid production, live weight, dry matter intake, milk composition) due to other factors that are not identified in this study. Further microbial and metabolomic research needs to be conducted to establish the relationship between the microbes and metabolites that are associated to the reduction of enteric methane in these highly efficient animals.

The multiple regression model and Irish national inventory calculations that were used to estimate enteric methane emissions are both able to predict daily emissions. The correlation (0.79) between both methods for ranking animals as high, medium or low residual enteric methane indicates that there is somewhat agreement between the two ranking calculations. Through these two methods it is evident that there are animals that have the ability to produce less daily enteric methane. However, these animals are able to produce less enteric methane while maintaining the same dry matter intake, milk solid production and live weight and therefore, becoming more efficient enteric methane producers. This attribute is very important in the dairy industry as milk production, dry matter intake and live weight are key drivers of profitability.

The Irish national inventory calculation relies on assuming that 6.5% of gross energy intake is consumed during methanogenesis. Relying on this assumption may result in unreliable results across the industry. Therefore, to improve this calculation more information needs to be included in the model to develop an improvement in reliability of enteric methane prediction. By moving from a Tier 2 approach to the Tier 3 method, more data will be incorporated into the estimations and therefore that accuracy would be improved and could include within year

variation. Many different organisations throughout Ireland are currently collecting information that could be utilised in the inventory calculation. Through the integration and collaboration of the different Irish entities, an improvement can be made to the current national inventory calculation.

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