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Solutions to enteric methane abatement in Ireland

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Abstract

The efficiency of Ireland's grass-based livestock systems can be attributed to high outputs, low production costs and a low carbon footprint relative to housed systems. Methane (CH_4) is a potent greenhouse gas (GHG) of which enteric fermentation from livestock production is a key source, being directly responsible for 57% of Irish agricultural GHG emissions. There are a number of strategies including dietary manipulation and breeding initiatives that have shown promising results as potential mitigation solutions for ruminant livestock production. However, the majority of international research has predominantly been conducted on confined systems. Given the economic viability of Irish livestock systems, it is vital that any mitigation methods are assessed at pasture. Such research cannot be completed without access to suitable equipment for measuring CH_4 emissions at grazing. This review documents the current knowledge capacity in Ireland (publications and projects) and includes an inventory of equipment currently available to conduct research. A number of strategic research avenues are identified herein that warrant further investigation including breeding initiatives and dietary manipulation. It was notable that enteric CH_4 research seems to be lacking in Ireland as it constituted 14% of Irish agricultural GHG research publications from 2016 to 2021. A number of key infrastructural deficits were identified including respiration chambers (there are none currently operational in the Republic of Ireland) and an urgent need for more pasture-based GreenFeed™ systems. These deficits will need to be addressed to enable inventory refinement, research progression and the development of effective solutions to enteric CH_4 abatement in Ireland.

Keywords

Abatement • livestock • methane • research • sustainability

Introduction

Although methane (CH_4) has a relatively short atmospheric residence time (half-life of 9.1 yrs), it is a potent greenhouse gas (GHG) with 84 times the global warming potential (GWP) of carbon dioxide (CO_2) over its atmospheric residence time and 28 times that of CO_2 over a 100-yr time horizon (GWP_{100}) (IPCC, 2014). Methane is the second-largest contributor to anthropogenic climate change with global emissions estimated at 559 [540–568] Tg CH_4/yr for the decade 2003–2012 (Saunois *et al*., 2016). The principal anthropogenic sources are fossil fuel extraction and burning, with biogenic contributions associated with eructation from ruminants during fermentation of feed, management of organic wastes and manures and rice paddy cultivation (IPCC, 2014). In 2019, agriculture was responsible for 35.5% of Ireland's GHG emissions, of which enteric CH_4 comprised 57.45% (EPA, 2021).

Agricultural GHG emissions belong to the non-emissions trading sector (non-ETS), meaning that emissions from the sector are not subject to the European Union cap and trade system. This also means that national governments, rather than EU, are directly responsible for emission reductions in these sectors with the extent of each country's reductions established under the 2030 Effort Sharing Regulation (ESR) (PE/3/2018/REV/2). Under the ESR, Ireland is obliged to reduce non-ETS GHG emissions by 30% relative to 2005 over the 2021–2030 commitment period. This will require agriculture to limit emissions to between 17.5 and 19 million tonnes CO_2 equivalent, while also establishing a downward trajectory to reach net zero emissions by 2050. In order to identify pathways for emissions reductions, a marginal abatement cost curve (MACC) analysis was conducted to

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establish the potential mitigation capacity of the Agriculture, Forestry and Other Land-use (AFOLU) sectors and the associated costs (Lanigan *et al*., 2018). While other GHG such as nitrous oxide have been well researched regarding point sources and mitigation methods (Harty *et al*., 2016; Krol *et al*., 2016), there are many unanswered questions regarding the development of a suitable Irish CH_4 abatement strategy.

There are a number of metrics for the expression of CH_4 emissions. These include: absolute emissions (total quantity of CH_4 emissions produced), daily emissions (g CH_4/day^{-1}) (Cottle *et al*., 2015), emissions yield (g CH_4/kg dry matter intake [DMI]) (Y_m) (Hegarty *et al*., 2007; Løvendahl *et al*., 2018) and emissions intensity (g CH_4/unit output i.e. milk or meat) (Gerber *et al*., 2011; Hayes *et al*., 2013). Current abatement strategies focus on improving efficiency measures, such as the dairy economic breeding index (EBI), increasing beef live weight gain, extended grazing and the use of sexed semen all of which can significantly reduce CH_4 emissions intensity and associated production costs by increasing overall farm efficiency (Holden and Butler, 2018). However, these measures may not lead to reductions in absolute CH_4 emissions, as increasing GHG efficiency can result in increased activity (and hence emissions) via so-called "rebound effects" (Paul *et al*., 2019). Reductions in absolute CH_4 emissions require reductions in methane output per head and for the overall herd size not to increase. Although there has been progress in achieving this in confinement systems, reducing daily emissions from pastoral systems remains challenging, due to reduced control over animal feed consumption. Researchers in New Zealand are trying to address these challenges and have been doing so for many years (Buddle *et al*., 2011; Pacheco *et al*., 2014). In order to achieve carbon neutrality, major mitigation efforts will have to be established and implemented, particularly for CH_4 emissions resulting from enteric fermentation and offsetting of residual emissions through enhanced carbon sequestration. Thus, there is a requirement in Ireland to conduct research on mitigation strategies to reduce absolute CH_4 emissions while cattle are at pasture. This will require the development of infrastructure and acquisition of necessary equipment to conduct high-level research.

Therefore, the specific aims of this review are: (1) to identify and critically review CH_4 mitigation strategies that may be suitable for Irish livestock production systems, (2) to review enteric CH_4 publications and projects conducted in Ireland to date, (3) to document the current Irish enteric CH_4 inventories, and (4) to determine the infrastructures required in Ireland to allow for high-level research on decoupling CH_4 emissions from livestock production to progress.

The process of enteric methane production

Enteric CH_4 originates as a by-product of rumen microbial fermentation, the process through which ruminant livestock digest feed (Tapio *et al*., 2017). The majority (87%) of enteric CH_4 is eructated from the forestomach (rumen). A smaller proportion (13%) originates in the hindgut where it is absorbed into the blood and exhaled from the lungs (Hammond *et al*., 2016). Fermentation is carried out by a complex anaerobic microbial ecosystem, consisting of bacteria, archaea, protozoa and fungi, in the forestomach (rumen) of ruminant livestock (Huws *et al*., 2018). Individual members of the rumen microbial community play a key role in the fermentation of complex plant structures, and subsequent supply of nutrition, for the ruminant host. Indeed, during enteric (rumen) fermentation, ingested feed is converted into volatile fatty acids (VFA), which provide an estimated 63% of the ruminant animal's energy requirements (Bergman, 1990). In addition to the supply of VFA to the ruminant host, rumen microbial fermentation yields carbon dioxide (CO_2) and hydrogen (H_2) as metabolic end-products (Newbold & Ramos-Morales, 2020). The production of CH_4, methanogenesis, acts as a H_2 sink in the rumen, which facilitates the progression of further fermentation by preventing an accumulation of excess H_2 which may otherwise hinder electron transport (Morgavi *et al*., 2010). Methanogens, belonging to the kingdom archaea, are the sole producers of CH_4 in the rumen with the majority of enteric CH_4 believed to originate from the hydrogenotrophic methanogenesis pathway (Equation 1), whereby CO_2 is reduced by H_2 (Janssen & Kris, 2008). Finally, the majority of CH_4 originating from ruminant livestock is expelled, to the atmosphere, via the breath of the animal (Hammond *et al*., 2016).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{1}$$

The relationship between the rumen microbial community composition and CH_4 output has been investigated in a variety of studies (Kittelmann *et al*., 2014; Wallace *et al*., 2015; Kamke *et al*., 2016; Wallace *et al*., 2019). As highlighted in many reviews (Morgavi *et al*., 2010; Tapio *et al*., 2017; Waters *et al*., 2020), individual members of the rumen microbiome, rather than the overall size of any one microbial kingdom, have been linked to the methanogenic potential of an animal. As a result, practices which are capable of reducing the abundance of microbes that produce substrates for methanogenesis, or alter the methanogen community in favour of a reduced CH_4 output, offer promise as successful CH_4 mitigation strategies. Dietary regimes and animal selection are promising CH_4 mitigation strategies as both dietary management (Carberry *et al*., 2014; Henderson *et al*., 2015; Lyons *et al*., 2017; Liu *et al*., 2019; Smith *et al*., 2020a) and host genetics (Weimer *et al*., 2010; Henderson *et al*., 2015; Roehe *et al*., 2016; Li *et al*., 2019) have been demonstrated to alter rumen microbial composition.

Methods for measuring methane

The main methods to measure CH_4 emissions from ruminants include: (1) respiration chambers (RCs); (2) portable

Table 1: Summary of methane measurement equipment and associated cost, advantages and disadvantages

Method	Advantages	Disadvantages	Approximate cost
Respiration chambers	Measures all CH_4 emissions from the animal	Does not represent field or grazing patterns Artificial environment for the animal	€50,000–€60,000 per chamber including individual air-conditioning, gas meters and associated devices
SF_6	Measures CH_4 at pasture	Large variability and difficult to maintain at pasture	~ €10,000 for 20 sets. Equipment include individual measurement sets including canisters, absorption pipes (excluding gas chromatography techniques and pump required for analyses)
GreenFeeds	Measures CH_4 at both indoor and at pasture High accuracy of measurements Easy to maintain relative to SF_6	Potential bias due to animal behaviour Short-term measurements require long-term trials	€65,000 per unit for indoor systems (up to €125,000 in total for additional costs of adding trailers for pasture-based systems)
Portable accumulation chambers (sheep)	High levels of correlation with RC Quick measurements cause less stress on the animal Easy to operate	Artificial environment for the animal which can cause stress. Unsuited for longer and/or repeated daily measurements	~ €80,000 per chamber including individual air-conditioning, gas meters and associated devices

accumulation chambers (PACs) (for smaller livestock); (3) GreenFeed™ (GF) systems; and (4) the sulphur hexafluoride tracer technique (SF_6). Respiration chambers have been used to estimate energy losses and CH_4 emissions from livestock for over a century (Hammond *et al*., 2016). The underlying principles of the RC involves keeping an animal in a pressurised chamber, with the enteric emissions of the animal estimated as the difference in the concentration of gases entering and leaving the chamber, with the fluctuation in gas concentrations assumed to be those emitted from the animal (eructated, exhaled and flatulence) (Storm *et al*., 2012). The experimental time period may vary but is typically ~96 h (Muñoz *et al*., 2012). Emissions are determined as the difference in CH_4 gas concentrations entering and leaving the chamber with and corrected for temperature, humidity and pressure. RCs are considered the “gold standard” for recording CH_4 measurements due to high levels of repeatability, robustness and precision (Gardiner *et al*., 2015; Patra, 2016). However, animals are required to be contained within a standardised chamber for the experimental period. Therefore, the method is unsuited to estimating emissions from animals under grazing conditions. One of the main disadvantages to RC is the changes in the behaviour of contained animals and DMI can drop, meaning in-chamber CH_4 measurements may not reflect actual animal CH_4 output (Table 1). Portable accumulation chambers act as airtight chambers that measure CH_4 and CO_2 emissions from small animals such as sheep (Image 1). This method is considered a rapid (1 h), straightforward and highly

Image 1. A sheep in a portable accumulation chamber, Teagasc, Athenry, Co. Galway.

effective technique as results show high comparability with RC results (Goopy *et al*., 2016; Jonker *et al*., 2018). However, PAC cannot be deployed for longer periods as increased CO_2 concentration can negatively influence measurements, thus PAC only allows for measurements over a single time point (Hammond *et al*., 2016). Breath sampling during feeding can be analysed through GreenFeed™ (GF) systems. They operate as open-circuit head-chambers baited with feed pellets to attract the animal. Airflow inside the feed troughs is collected via an extractor fan, with the analyses of CH_4 and CO_2 fluxes determined with the use of infra-red sensors, allowing for the calculation of CH_4 concentration (Hristov *et al*., 2015; Huhtanen *et al*., 2015; Jonker *et al*., 2016). GreenFeed™ units can also be fitted with sensors to measure H_2, O_2 and H_2S. The accuracy of CH_4 measurements, obtained with the use of the GF system, can potentially be compromised, if visitation to the unit is not reflective of the diurnal pattern of enteric emissions (Hristov *et al*., 2018). As a result, it is imperative animals are permitted access to the unit at even intervals throughout a 24-h period over the duration of the measurement period. Feeding of bait to attract the animal to the GF may also cause issues, for example, if investigating forage diets, feeding concentrate feed may impact on the results. Similarly, if the cattle are on an *ad libitum* concentrate diet, they may not visit the GF unit and training the animals may be difficult (Table 1). GreenFeed™ systems are not restricted to housed environments and have been successfully used to measure CH_4 emissions on intensive pasture-based dairy systems (Waghorn *et al*., 2016). Although GFs have been used primarily on cattle to date, there has been development of systems for use with sheep and calves (Nguyen *et al*., 2018; Meale *et al*., 2021). Another breath sampling method termed "the sniffer technique", where a gas sampling inlet is placed within the feed manger of a robotic milking unit, measures CH_4 emissions from the animal at milking events. From these measurements, the daily emission rates can be determined. However, as the sniffer technology has primarily been developed for assessing the methanogenic output of dairy cows at milking (Garnsworthy *et al*., 2012), its use within a pasture setting, to estimate emissions from non-lactating ruminants, is limited.

The SF_6 technique involves placing a small permeation tube containing SF_6 inside the rumen, and collection tubes with sample lines are used to collect breath samples from the animal. This allows the CH_4 emission rate to be calculated from the known SF_6 emission rate and the measured SF_6 and CH_4 concentrations (Johnson *et al*., 1994). The use of SF_6 is a labour-intensive method for measuring CH_4 as it involves daily gas canister collections, some loss of canisters from animals and animal handling issues (Table 1). In a comparative study by Deighton *et al*. (2013), it was reported that prolonged deployment of SF_6 tubes can result in an overestimation of CH_4 emissions from animals if the declining release rate of SF_6 from permeation tubes over time is not accounted for. Additionally, Hristov *et al*. (2016) found higher variability of results generated by the SF_6 technique compared to the GF method.

The comparative study by Garnsworthy *et al.* (2019) found that all methods are highly correlated with RC, but levels of correlation between non-RC methods are lower. Although Jonker *et al.* (2016) found no difference between average emission yields from the SF_6 technique, the GF system or from those of RC, there are benefits and drawbacks associated with all methods. Additionally, correcting CH_4 emissions for either intake or output can provide a more contextualised comparison between individual animals than daily emissions alone (outlined in section 1). In order for such calculations to be made, individual feeding units for use in housed systems and during experiments are vital to determine the total feed intake and DMI associated with CH_4 output.

Mitigation strategies – farm efficiency

O'Brien & Shalloo (2016) recommended a number of on-farm efficiency measures that can aid in reducing CH_4 emissions from Irish livestock production systems such as extending the length of the grazing season, increasing the daily live weight gain of beef cattle and lambs, optimising the age and rate of calving and lambing, and reducing the age at slaughter. Such strategies are complementary and when used in conjunction can provide effective CH_4 emissions savings. Albeit, these measures only confer a benefit on a short-term basis and at fixed livestock numbers as increasing the stocking rate will increase overall emissions.

Livestock breeding initiatives are an effective farm management practice that can aid in CH_4 mitigation in a number of ways including the following: (1) breeding more productive animals can reduce emission intensity (less CH_4 emissions per unit milk/meat produced), for example, increasing dairy cow genetic merit via the EBI and therefore milk production; (2) breeding for increased health, fertility and productivity, that is, faster-growing animals reduces the age at slaughter and thus cumulative CH_4 emissions over the animal's lifespan; and (3) selecting for more CH_4-efficient traits in animals, which will in turn reduce overall CH_4 emissions. Regarding the latter, there are a number of studies, particularly in New Zealand, which highlight the association between the rumen microbiome, genotype and phenotype of sheep bred for low CH_4 output (Xiang *et al*., 2016; Jonker *et al*., 2019a; Rowe *et al*., 2020). Goopy *et al*. (2014) found that certain physical traits are associated with sheep selectively bred for low CH_4 output, with such animals having smaller rumens and a shorter rumen retention time. In the study by Smith *et al.* (2021), cattle ranked low for residual CH_4 output (difference between animals predicted, and actual level of CH_4 output, based on DMI and body weight) had an ~30% reduction in daily CH_4 emissions and emissions intensity (g/kg of carcass weight) in comparison to their high residual CH_4 counterparts. In addition, the previous authors noted feed intake and animal performance were not compromised by residual CH_4 output ranking. Although it can be slow to select for such low-emitting animals, nevertheless, such breeding strategies offer a long-term, highly effective solution to CH_4 abatement (González-Recio *et al*., 2020).

Mitigation strategies – dietary manipulation

Dietary manipulation involving additives and feed/sward type are of fundamental importance in CH_4 mitigation strategies as emissions are highly correlated to animal feed intake and digestibility. Thus, dietary manipulation (i.e. changing the amount or proportion of carbohydrate, protein and roughage in animal diets) has been previously explored by numerous studies and remains an important avenue of further investigation.

Feed additives

Historically, halogenic compounds such as bromoform and chloroform have been studied for their efficacy as anti-methanogenic compounds (Bauchop, 1967; Russel & Martin, 1984). At present, such compounds are mainly used as experimental controls (Martinez-Fernandez *et al*., 2018) due to their strong anti-methanogenic albeit toxic and carcinogenic qualities. This has also been the case for ionophores such as monesin (an antibiotic) which deplete the rumen microbiome and therefore methanogenesis. Antibiotics were previously fed to animals as growth promotors, particularly in the USA. However, since 2006, the use of ionophores is prohibited within the EU due to issues with resistance and human health concerns (EC, 2005).

Hristov *et al*. (2013) list the efficacy of various livestock dietary additives for reducing CH_4 emissions. Their study highlights the efficacy of including seaweed in ruminant diets to substantially reduce CH_4 emissions. It has been found that certain seaweeds, particularly red and brown species such as *Asparagopsis taxiformis* (red) and *Sargassum flavicans* (brown), contain compounds with anti-methanogenic properties as outlined in Abbott *et al*. (2020). Bromoform (a haloform mostly found in red seaweeds) is known to inhibit the enzymes involved in methanogenesis. Various other compounds found in seaweeds such as lipids, peptides and phlorotannins also play a role in reducing CH_4 emissions, although the modes of action are less understood (Machado *et al*., 2016; Abbott *et al*., 2020). Many studies have seen significant reductions in CH_4 emissions from livestock receiving seaweed-based additives at various administration rates and concentrations (Machado *et al*., 2016; Li *et al*., 2018; Roque *et al*., 2019; Kinley *et al*., 2020; Roque *et al*., 2021). However, there are concerns surrounding the potential for compounds such as iodine (toxic at high levels) and bromoform (carcinogen) to carry through the food chain and adversely affect human health (Antaya *et al*., 2019; Abbott *et al*., 2020). Additionally, importing tropical seaweed species such as *A. taxiformis* risks negatively impacting bioactive compounds during transit. There are also monetary costs and risk of "pollution swapping" through increased GHG emissions associated with transport. The supply of native seaweeds (such as temperate brown species) could present issues, for example, harvesting wild crops or commercially growing seaweeds could have negative environmental impacts. To date, seaweed has not been tested in Irish pasture-based production systems as an anti-methanogenic additive. Overall, further work will be required to understand the potential long-term effects of seaweed additives on animal productivity and human health.

The addition of fats and oils as CH_4 abatement compounds to ruminant diets has shown promising results. Lipids can reduce CH_4 emissions, as they are toxic to and therefore reduce methanogens and protozoan numbers within the rumen (Beauchemin *et al*., 2009; Broucek, 2018). Overall, results from lipid addition have been variable, but up to 20% reduction in emissions have been reported (Beauchemin *et al*., 2020). However, fat addition can negatively affect animal feed intake, carbohydrate digestion in the rumen and overall milk quality. As regards plant-based oil seeds, Kliem *et al*. (2019) found only linseed-based supplements reduced CH_4 emissions (across production, yield and emissions intensity) when comparing the administration of linseed, palm and rapeseed oil products to dairy cows. Similarly, Boland *et al*. (2020) reported an 18% decrease in emissions intensity (g CH_4/kg milk) from pasture-fed dairy cows receiving linseed oil-based concentrates compared with cows receiving stearic acid or soy oil-based concentrates.

There are a range of industrially formulated products with the potential to reduce methanogenesis such as Mootral (a feed additive containing allicin from garlic and citrus extracts) and Agolin Ruminant (an essential oil blend). Studies have shown positive, albeit variable, effects of both products on reducing the amount and rate of enteric CH_4 production in both dairy and beef cattle (Castro-Montoya *et al*., 2015; Hargreaves *et al*., 2019; Belanche *et al*., 2020). Agolin Ruminant is an affordable solution that has also been shown to improve livestock productivity (particularly dairy), which can reduce CH_4 emission intensity. However, both of these additives have primarily been tested as part of total mixed ration (TMR) diets and are in need of testing at pasture level.

The synthetic, non-toxic, organic compound 3-nitrooxypropanol (3-NOP) has proven to be an effective feed additive for the reduction of enteric CH_4 emissions (up to 30% reductions), without compromising animal performance. 3-NOP inhibits methane formation by binding with the enzyme methyl-coenzyme M reductase (MCR) (the catalyst for methane formation) during the final stages of methanogenesis (Meale *et al*., 2021). The efficacy of 3-NOP has been assessed in multiple experimental trials (Romero-Perez *et al*., 2015; Jayanegara *et al*., 2018; Martinez-Fernandez *et al*., 2018; Vyas *et al*., 2018; McGinn *et al*., 2019; van Gastelen *et al*., 2020). The recent study of Meale *et al*. (2021) found that a daily oral administration of 3-NOP to dairy calves from birth to 3 wks post-weaning (14 wks of age) significantly reduced daily CH_4 emissions up until at least 1 yr of age. It is likely that this early-life administration imprinted on the rumen microbiome, which resulted in lasting alterations of the methanogenesis process. Similar reductions in CH_4 emissions from adult cattle receiving 3-NOP have not shown long-term persistence once administration ceased as reported in the study on housed beef cattle by Romero-Perez *et al.* (2015). Most 3-NOP studies have been carried out on indoor/confined systems; however, beneficial results are harder to achieve at pasture level as additives such as 3-NOP need to be combined within animal feed or administered directly after feeding. Therefore, the development of suitable technologies such as slow-release boluses will be required for pasture-based administration as direct feeding cannot take place outside housed periods (Leahy *et al*., 2020). Additionally, consumer behaviour will need to be considered before 3-NOP or other synthetic additives are adopted as a mitigation option, that is there may be issues surrounding the consumption of products that arise from animals fed on synthetic compounds (Beauchemin *et al*., 2020).

Sward type

In Ireland, cattle consume >80% of their DMI requirement from grassland forage (O'Brien *et al*., 2018) with the majority of improved pastures consisting of perennial ryegrass (*Lolium perenne* L.) monocultures. In recent years there has been increased interest in the use of multi-species swards (a forage mixture consisting of two or more species from different functional groups) for livestock production. This is due to the associated multi-functional benefits which include increased agronomic productivity, livestock health benefits, drought resilience and environmental benefits (Haughey *et al*., 2018; Grace *et al*., 2019; Suter *et al*., 2021). Legumes including white clover (*Trifolium repens* L.) are often used in multi-species swards and have been shown to contain high tannin levels (Woodfield *et al*., 2019). Recently, Ku-Vera *et al.* (2020) presented the potential of plant secondary metabolites such as tannins to modify the rumen microbiome and its function. Stewart *et al*. (2019) also found that hays with high tannin content have the potential to reduce enteric CH_4 emissions from beef cattle. It has been found that tannins can lower CH_4 emissions through a reduction in fibre digestion (which decreases H_2 production), and by inhibiting the growth of methanogens (Naumann *et al*., 2017; Jafari *et al*., 2019). However, Patra & Saxena (2010) recommend that the proportion of tannin in the diet does not exceed 50 g/kg DMI to avoid negative implications on animal performance. It is also worth noting the role of pasture management in CH_4 mitigation with animals grazing on low pre-grazing herbage mass swards having reduced CH_4 emissions intensity due to increased forage digestibility (Wims *et al*., 2010; Boland *et al*., 2013).

The benefits of white clover on animal performance could potentially impact CH_4 emissions intensity, that is, increased milk yield/solids (Lee *et al*., 2004; Egan *et al*., 2017; Dineen *et al*., 2018) but with the same/lower level of CH_4 output. White clover also increases the passage rate through the rumen which can impact methanogens (Dewhurst *et al*., 2003; Smith *et al*., 2020a). Research has also been conducted on legume species other than white clover; Huyen *et al*. (2016a,b found that Sanfoin (*Onobrychis viciifolia*, a tanniniferous legume forage) inclusion within the diet of dairy cows at 50% silage proportion significantly reduced CH_4 emissions intensity. Aside from emissions intensity, Montoya-Flores *et al.* (2020) found a significant decrease in daily CH_4 emissions from cattle fed high legume (*Leucaena leucocephala)* proportion diets.

Herbaceous species *Cichorium intybus* and *Plantago lanceolata* contain high levels of bioactive compounds, in particular condensed tannins (Totty *et al*., 2013; Peña-Espinoza *et al*., 2018; Ineichen *et al*., 2019). A multi-species mixture of sorrel (*Rumex acetosa*), ox-eye daisy (*Leucanthemum vulgare*), yarrow (*Achillea millefolium*), knapweed (*Centaurea nigra*) and ribwort plantain (*Plantago lanceolata*) fed as haylage resulted in a 10% reduction in Y_m (SF_6 technique) compared with a perennial ryegrass monoculture (Hammond *et al*., 2014). Wilson *et al*. (2020) reported no CH_4 mitigation effects when lactating dairy cows were fed legume or herb-rich swards, compared to grass-based swards. Jonker *et al.* (2019b) reported elevated Y_m for cows fed a diverse sward (containing ryegrasses, [both *Lolium perenne* and *Lolium multiflorum*], white clover, lucerne, chicory and plantain) compared to a ryegrass (*L. perenne* and *L. multiflorum*) and white clover mixture. Similarly, Loza *et al.* (2021) found that CH_4 emissions per kg energy corrected milk produced (emissions intensity) to be 11% higher from dairy cows grazing diverse mixtures compared to grass-clover swards. The aforementioned studies present some contrasting results. However, verifying the effect of multi-species swards and different types of species at grazing will be important to determining solutions to CH_4 abatement in Ireland.

Methodology

We undertook a comprehensive literature survey of recent (2016–2021) research publications that focus on enteric CH_4 emissions and rumen microbiome function. This was performed through online searches of relevant databases (SCOPUS and Scholar) using specific key words ("rumen"

AND "livestock" OR "methane" OR "microbiome"). The search was limited to research articles from Irish and Northern Irish research-performing organisations (RPOs). In addition, further relevant published scientific articles were identified from cited references in relevant review or meta-analysis papers including Waters *et al*. (2020). The relevant papers were added to an excel sheet and the methodology sections were analysed to determine the equipment and methodology used. Contact was then made with the corresponding authors of relevant publications to identify current projects and facilities. The proposals of the main Irish CH_4 research projects were also evaluated to determine the facilities and equipment available for use. Site visits to the relevant research centres took place where CH_4 projects, research infrastructure and facilities were documented. The current Irish bovine and ovine GHG inventory methodologies were reviewed according to the United Nations Framework Convention on Climate Change (UNFCCC, 2021) Irish National Inventory Reports.

Results

Global warming potential and inventory

The current metric used to assess the impact of individual gases towards climate change is termed the GWP of CH_4 which is "GWP100". This method equates one GHG unit to its CO_2 equivalent, averaged over a 100-yr time horizon. This results in a GWP of 28 in the case of CH_4 (IPCC, 2014). However, this method fails to account for the short-lived atmospheric persistence of CH_4 (9.1 yrs) relative to other GHGs such as N_2O, which persists in the atmosphere for more than 100 yrs and CO_2, which has a residence time of several centuries. This means that whilst CH_4 has a strong radiative forcing impact when first emitted, this warming impact diminishes as CH_4 oxidises to CO_2 and H_2O. Therefore, there are concerns that the GWP100 overestimates the contribution of CH_4 to long-term radiative forcing (Allen *et al*., 2016; Allen *et al*., 2018).

In terms of CH_4 inventory accounting, sheep are currently reported using Tier 1 methodologies in the Irish National Inventory (UNFCCC, 2021). However, there are data available on Irish sheep populations, finishing ages, concentrate usage, housing periods and manure storage systems which could potentially be utilised in the progression to Tier 2 methodologies (O'Brien & Shalloo, 2016). Ireland currently uses Tier 2 inventory estimation for cattle, which disaggregates enteric and manure CH_4 emissions from the bovine herd between numerous categories as described in O'Mara (2006). The principal subdivisions are made between dairy and non-dairy (beef) animals. Dairy is subsequently divided in terms of calving date and region with a separate category for dairy heifers. The non-dairy herd is partitioned in terms of suckler cows (subdivided as per dairy cows) and heifers, with bovines for finishing classified based on age and gender. Future developments for reporting of CH_4 emissions and incorporating mitigation may require a Tier 3 or modelling approach. Dynamic models based on the mathematical modelling of rumen processes have the potential to incorporate a myriad of feed strategies such as carbohydrate, protein, fat and fibre metabolism. Such models have been simulated in detail and incorporated into US, Dutch and French inventories (Kebreab *et al*., 2008; Bannink *et al*., 2011; Eugène *et al*., 2019). These models are complex and must describe both the dynamics and interactions between various substrate pools and microbial pools and the consequences for the end-products of fermentation. Turnover rates between different pools are generally based on enzyme kinetics and degradation characteristics of different feed types.

As outlined by O'Brien & Shalloo (2016), there is potential to develop Tier 3 inventories for cattle. Data gathered and held by the CSO, Teagasc NFS, Bord Bia and the animal feed industry could potentially be integrated into the development of more accurate, Tier 3 CH_4 emission factors for cattle. There is already an extensive body of work on the variation in forage quality on production (Hart *et al*., 2009; Muñoz *et al*., 2016). Therefore, the national data required for inputting into a Tier 3 system are already available.

Irish enteric methane research to date

Over the last 5 yrs (2016–2021), 14% of Irish agricultural GHG research publications have been based on enteric CH_4 emissions (Figure 1). In total, 43 research papers have been published on studies using methodologies such as RCs, SF_6 (Images 2 and 3), RUSITEC *in vitro* rumen simulation and 16S rRNA amplicon sequencing (Table 2). Work carried out in Ireland, which furthers the understanding of the rumen microbiome, include the study by Kumar *et al*. (2018) and reviews by Huws *et al*. (2018) and Leahy *et al*. (2019). A number of studies have investigated strategies for decoupling enteric CH_4 emissions from livestock production. The studies by Popova *et al*. (2017), Smith *et al*. (2020b) and Boland *et al*. (2020) examine the effects of dietary supplementations, that is, linseed oil and industrial by-products. Other dietary manipulations strategies including sward composition, sward N application, concentrate type and protein content have been assessed (Hynes *et al*., 2016; McDonnell *et al*., 2016; Zhao *et al*., 2016; Günal *et al*., 2019; O'Connor *et al*., 2019; Ferris *et al*., 2020; Smith *et al*., 2020a). There have been a number of studies that focused on the genetic selection for production efficiency and low CH_4 output animals. The traits investigated include phenotype, age and breeding index of cattle (Morrison *et al*., 2017; Rubino *et al*., 2017; Quinton *et al*., 2018; Ferris *et al*., 2020). Additionally, there has also

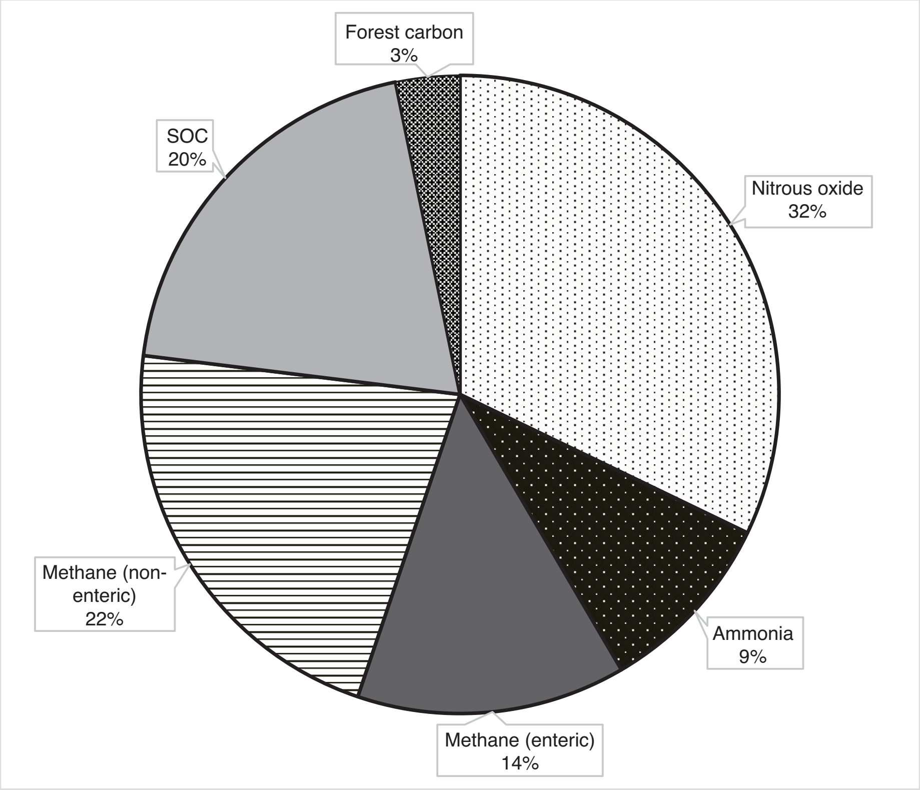

Figure 1. Research publications from Irish and Northern Irish research-performing organisations (2016–2021) that focus on agricultural greenhouse gases (GHGs): nitrous oxide, ammonia (pollutant and indirect GHG), carbon (soil organic carbon (SOC) and forest C) and methane (enteric and non-enteric).

Image 2. SF_6 equipment on grazing sheep, Teagasc, Athenry, Co. Galway.

Image 3. SF_6 equipment on grazing dairy cattle, Teagasc, Moorepark, Co. Cork.

been Irish involvement with international enteric CH_4 database and model development (Niu *et al*., 2018).

Current research projects in Ireland

There are currently a number of projects focusing on CH_4 abatement underway in Ireland (Table 3). Both EU-funded projects, RumenPredict (ERA-GAS) and *MASTER* (Horizon 2020 – European Commission), have members of Teagasc, University College Dublin (UCD) and the Irish Cattle Breeding Federation (ICBF) working in collaboration to better understand the link between the rumen microbiome composition and CH_4 output. There are a number of other ERA-GAS-funded projects including *SeaSolutions*, a Teagasc co-ordinated project which aims to determine the potential of seaweeds to reduce enteric CH_4 emissions from sheep, beef and dairy cattle, METHlab which focuses on the use of lactic acid bacteria as an approach to reduce CH_4 emissions from ruminant livestock and GrassToGas which aims to identify individual animal, feed and environmental attributes associated with feed and water intake efficiency for pasture-based sheep production systems using portable accumulation chambers (Image 1). The Teagasc-led Department of Agriculture, Food and the Marine (DAFM)-funded *Meth-Abate* project seeks to develop new technologies to reduce enteric CH_4 emissions from ruminants and emissions from stored manure and slurry. This project is focused on investigating the potential of feed and slurry additives as CH_4 mitigation solutions. *Meth-Abate* has research partners from National University of Ireland, Galway (NUIG), Teagasc, Agri-Food and Biosciences Institute (AFBI), Queens University as well as a number of industry stakeholders. Greenbreed (DAFM funded) is a collaborative project between UCD, Teagasc and the ICBF which aims to determine strategies for the breeding of more CH_4-efficient animals. There are a number of GF systems in place at the confinement facilities at the ICBF used to carry out this research (Images 4 and 5). VistaMilk is an Science Foundation Ireland (SFI) research centre, with the aim to “facilitate the development and deployment of new knowledge, new technologies and new decision support tools to maximise the efficiency and effectiveness of the entire dairy production chain”. VistaMilk funds a number of projects investigating feed additives at pasture in dairy cattle at Teagasc, Moorepark (Image 6). APC microbiome is also an SFI-funded centre which has links with CH_4 abatement projects such as METH-lab. SMARTSWARD (DAFM funded) is a collaborative project between UCD, TUD and AFBI and is investigating the impact of multi-species swards on enteric CH_4 emissions of beef cattle and lactating dairy cows using the SF_6 technique. The current (as of 2021) infrastructure available in Ireland to conduct CH_4 research is listed in Table 2.

Discussion

Irish livestock systems are primarily grass-based owing to the temperate climate that promotes grass growth (O’Donovan

Table 2: Irish methane research infrastructure as of 2021: a summary of current research projects in Ireland that focus on enteric methane

Research centre	Facilities
Teagasc Grange	5 × GreenFeed (GF) systems, 4 × Rusitec *in vitro* simulators, SF_6 equipment, digestibility crates for measurements
Irish Cattle Breeding Federation Tully	10 × GF systems (indoor), cattle digestibility units
Teagasc Moorepark	4 × GF (pasture), SF_6 equipment
Teagasc Athenry	Portable accumulation chambers for sheep, SF_6 equipment for sheep
UCD	4 × GF (pasture/indoor), SF_6 equipment, 4 × Rusitec *in vitro* simulators, digestibility crates for measurements (dairy and sheep)
AFBI	2 × large respiration chambers, 6 × medium respiration chambers, 3 × GF systems, SF_6 equipment, dairy cow digestibility units, digestibility crates for measurements
Queens University Belfast	Rumen microbiology laboratory facilities

Table 3: A summary of current research projects in Ireland that focus on enteric methane

Project title	Coordinator	Funder	Duration	Collaborators
Rumenpredict	Prof Sharon Huws	ERA-GAS	3 yrs	QUB, Teagasc, ICBF, UCD, Natural Resources Institute Finland, Agresearch NZ, Swedish University of Agricultural Sciences, Wageningen University, INRA, France
MASTER	Prof Paul Cotter	EU Horizon 2020	4 yrs	Teagasc, ICBF
Seasolutions	Dr Maria Hayes	ERA-GAS	3 yrs	Teagasc, IT Sligo, QUB, AFBI, Norwegian Institute of Bioeconomy Research, Agriculture and Agri-Food Canada, Department of Agricultural Sciences Sweden, Friedrich-Loeffler-Institut Germany, SINTEF Norway
Methlab	Prof Catherine Stanton	ERA-gas	3 yrs	Teagasc, UCC, Wageningen University, INRA, France, Agresearch NZ, SACCO Italy
Meth-Abate	Prof Sinead Waters	DAFM/DAERA	4 yrs	Teagasc, NUIG, AFBI, Queens, Industry
Greenbreed	Prof Donagh Berry	DAFM	4 yrs	Teagasc, ICBF, UCD, WIT, CIT
Grasstogas	Joanne Conington	ERA-GAS	3 yrs	Sheep Ireland, Teagasc, INRA France, National Agriculture Research Institute Uruguay, Norwegian University of Life Sciences, AgResearch NZ, International Center for Livestock Research and Training Turkey
Greengrowth	Dr Fiona McGovern	Teagasc	4 yrs	Teagasc, UCD
SMARTSWARD	Prof Tommy Boland	DAFM/DAERA	4 yrs	UCD, Technological University of Dublin, AFBI
VistaMilk SFI Research Centre	Prof Donagh Berry	Science Foundation Ireland (SFI) and DAFM, national and international industry funding	Ongoing	Teagasc, UCC, UCD, WIT, multiple national and international industry collaborators
Water-based delivery of rumen modifiers to enhance the sustainability of ruminant production systems	Prof Tommy Boland	Enterprise Ireland	2 yrs	UCD

et al., 2021). There are also lower economic and environmental costs associated with grass-based systems relative to confinement/feedlot systems (O’Brien *et al*., 2012; O’Brien *et al*., 2014; Herron *et al*., 2021). Thus, investigating how different sward compositions impact CH_4 emissions/emissions intensity from grazing livestock is certainly an avenue worth further investigation. Some research has shown that multi-species swards can decrease daily CH_4 emissions/head and/or directly lower CH_4 emissions intensity through the improved animal performance achieved with grazing multi-species swards (e.g. Hammond *et al*., 2014). Although, some research does not support these findings, e.g. the study of Loza *et al*., 2021. Nonetheless, legume-based and multi-species swards require less N fertiliser inputs to maintain yield production and have lower associated N_2O emissions and emissions intensity than conventional *L. perenne* monocultures (Cummins *et al*., 2021). Thus, there is the potential that more diverse swards could give dual GHG abatement.

Anti-methanogenic feed additives could potentially have a role in Irish CH_4 abatement strategies. However, issues with social acceptance (Beauchemin *et al*., 2020), cost and on-farm delivery should be taken into account. Due to the constant turnover of the ruminal contents (van Soest, 2018), the efficacy of additives may depend on their residency time in the rumen/frequency of intake. It is easier to deliver additives as part of a TMR diet in housed systems where additives can be supplemented at feeding. This is harder to achieve at pasture level where grazed forage makes up the majority of the animals’ diet. To overcome this, early-life supplementation strategies with additives such as 3-NOP may offer an effective

Image 4. A beef steer visiting a GreenFeed™ system at the ICBF facilities, Tully, Co. Kildare.

Image 5. Teagasc GreenFeed™ systems at the ICBF facilities, Tully, Co. Kildare.

solution as recently established by Meale *et al*. (2021). The technological development of slow-release boluses could also provide an option for pasture-based delivery as is often the case with mineral supplementations (Grace & Knowles, 2012; Aliarabi *et al*., 2019). The effects of additives on animal productivity and welfare will need to be evaluated alongside farm-level cost-effectiveness (i.e. life cycle assessments) to determine the most effective and practical mitigation strategy at farm level.

Selecting for low CH_4 emissions in livestock breeding programmes, that is, including animal CH_4 output in breeding indexes, is a cumulative, permanent and effective CH_4 abatement strategy (González-Recio *et al*., 2020). In addition, some authors have advocated the benefits of selecting more feed-efficient ruminants as a mitigation strategy (Basarab *et al*., 2013). Residual CH_4 output is a useful phenotype for use in selection processes, as it is strongly related to daily CH_4 output but also independent of body weight and feed intake (Bird-Gardiner *et al*., 2017). The work, currently ongoing with Teagasc and the ICBF, will contribute to the development of breeding values for low CH_4 output for both beef and dairy sires as presented in Smith *et al*. (2021). The benefits of selecting for lower CH_4 output animals would be further complemented with CH_4-inhibiting additives and grazing on multi-species swards. However, breeding indexes will require validation at pasture as there is the possibility of a genetic × feed type effect. Therefore, it is vital that further resources are allocated to continuing and expanding on this research.

A major increase in CH_4 research capacity and output is required. For example, between 2016 and 2021, only 14% of agricultural GHG research publications in Ireland were focused on enteric CH_4 (Figure 1). Therefore, research outputs seem to be disjointed from research requirements given that enteric CH_4 emissions from livestock production constitute 57% of Irish agricultural GHG emissions. Thus, considerable investment towards research infrastructure and facilities is urgently required if the Irish CH_4 research capacity is to expand and progress. Regarding specific infrastructures (Table 2), there are currently no RCs in use in the Republic of Ireland. These will be required as RCs are a fundamentally important methodology for measuring CH_4 emissions and for validating other CH_4 measurement methodologies. More individual feeding and feed intake recording systems would be useful to assess further the relationship between genotype, feed use efficiency and CH_4 emissions from a large cohort of animals for breeding purposes. There is also a need for more accurate methodologies for measuring feed intake from grazing animals and further investment in pasture-based GF systems for large-scale field trials. Additionally, there is a need to investigate and further develop upcoming technologies for measuring CH_4 emissions (Neethirajan, 2020) for application to grazing systems. Research would ideally take place at a multi-institutional level and capture CH_4 emissions from dairy, beef and sheep production systems (both intensive and extensive) across Ireland.

Image 6. Pasture-based GreenFeed™ system, Teagasc, Moorepark, Co. Cork.

In order to improve and refine the national enteric CH_4 inventory, the working data (i.e. yearly livestock numbers from the CSO) should be combined with existing research output to develop a dynamic CH_4 model for inclusion within the national inventory. This would ideally be performed for sheep, beef and dairy cattle aiming to progress sheep inventory from Tier 1 to Tier 2 (with the aim of further progression to Tier 3) and cattle from Tier 2 to Tier 3. Further emission factor and inventory development will provide increased accuracy in Ireland's GHG emissions estimations and therefore mitigation efforts. An accurate inventory accounting is essential for determining the efficacy of mitigation efforts and meeting carbon neutrality in Ireland by 2050.

Thus in summary, research is required in the following areas: (1) on-pasture deliverance of anti-methanogenic substances in the form of additives, for example, 3-NOP, oilseeds, seaweeds; (2) the effects of sward type on CH_4 emissions and emissions intensity at pasture level including the effects of different species; and (3) breeding efficiency: selecting for low CH_4-emitting genetics while retaining production and profitability. The capabilities and limitations of all mitigation options should be considered during the development of a national CH_4 abatement strategy and a holistic approach should be taken rather than a "silver bullet" solution. The meeting of GHG commitments will be dependent on policy drivers and technology adaptation. Knowledge transfer and advisory will also have key roles with the on-farm delivery of abatement strategies.

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