

# Review: Genetic and genomic selection as a methane mitigation strategy in dairy cattle

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*Over the last decade, extensive research effort has been placed on developing methane mitigation strategies in ruminants. Many disciplines on animal science disciplines have been involved, including nutrition and physiology, microbiology and genetic selection. To date, few of the suggested strategies have been implemented because: (1) methane emissions currently have no direct or indirect economic value for farmers, with no financial incentive to change practices and (2) most strategies have limited, or no, long-term effects. Consequently, there is a fundamental need for research on methane mitigation strategies across disciplines. Coordinated international initiatives similar to METHAGENE could represent highly relevant coordination tool of collaboration between countries, facilitating knowledge exchange, sharing concerns and building future collaborations.*

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## Implications

This paper presents ideas and perspectives on how genetic selection can become one of many mitigation strategies for methane emission in dairy cattle together with nutrition, management and others. With analysis of routine data recording of methane emission in commercial farms, it will be possible to use these results as a mitigation strategy. The effect of selection is easily widespread with artificial insemination, and the effect is there from day to day. On top of this, genetic effects inherited from generation to generation.

## Introduction

Soon after Steinfeld *et al.* (2006) published the infamous report implicating the production of greenhouse gases (predominantly methane (CH<sub>4</sub>) emissions) by ruminants as an anthropogenic threat to our climate, genetic selection was proposed as a mitigation solution (Wall *et al.*, 2010). Genetic selection is an attractive solution because changes are cumulative and permanent; however, this approach requires additive genetic variation and time to have effect, as selection is carried out over generations. Furthermore, genetic selection requires recording the CH<sub>4</sub> of large numbers of cows, which is costly. Thus, complementary short-term multi-disciplinary approaches and international co-operation

are required to document this phenomenon objectively (Pickering *et al.*, 2015).

Many disciplines within animal science have focused on establishing methods for mitigating methane production in dairy cattle, including nutrition, physiology, microbiology and genetic selection. Examples of multi-disciplinary and international projects are Ruminomics (<http://ruminomics.eu>), the European Union cost action large-scale methane measurements on individual ruminants for genetic evaluations (METHAGENE) ([www.methagene.eu](http://www.methagene.eu)), Animal Selection Genetics and Genomics Network (<http://www.asggn.org/>) and Efficient Dairy Genome Project (<https://genomedairy.ualberta.ca/>). Consequently, many cross-discipline reviews have been compiled on the key challenges faced in mitigating this phenomenon over the last decade. Due to the relatively slow uptake and long-term nature of research on genetic selection, results from pilot genetic selection studies are beginning to emerge following a decade of research, on which this review is focused.

The first, and most obvious, requirement for selective breeding is a method to measure the traits or phenotypes of interest. Such information could be used to establish the biological sources of variation affecting the phenotype, such as non-genetic factors, which must be experimentally eliminated or statistically controlled. Thus, it is necessary to establish whether the phenotype is significantly repeatable and heritable under the environmental conditions in which animals are expected to perform. Repeatability is established

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by recording the same individuals multiple times during their productive lifetime, while heritability is established by recording information about related individuals. Provided the phenotype is heritable, it is then necessary to establish the genetic parameters that are highly correlated (high certainty and low SEs) to existing traits in the selection index. Further avenues of genetic research include methods for improving the accuracy of estimated breeding values (EBVs), through incorporating genetically correlated indicator traits, evaluating prediction methods and models and assessing how potential genotypes interact with the environment. Once these genetic parameters are established, it is possible to determine the selection weighting and conduct cost-benefit analyses to determine the economic value of recording and selecting for certain traits.

This review, first published in an abstract form (Lassen and Difford, 2019), provides an update on the status of genetic selection research for lowering methane emissions in dairy cows. Specifically, we report on the productive and unproductive paths, challenges and future research perspectives.

#### *Biological aspects of methane emission in dairy cattle*

An understanding of the underlying biology of CH<sub>4</sub> emissions in dairy cattle precedes that of recording systems and genetic evaluations, with Hammond *et al.* (2016) presenting a detailed review. In brief, CH<sub>4</sub> release from animals primarily occurs through three routes: (1) direct eructation from the rumen, (2) absorption of CH<sub>4</sub> from the rumen and hindgut to the blood and exhalation from the lungs and (3) emission of CH<sub>4</sub> from the hindgut as flatulence. Using radio-labelled CH<sub>4</sub>, it has been estimated that approximately 98% of CH<sub>4</sub> is expired through the breath and eructation of cattle and sheep, while just 2% is expired from flatulence (Murray *et al.*, 1976). This phenomenon has implications for the methods of measuring CH<sub>4</sub> emitted by cows, because, even though 98% is expired in the breath and eructation, recording only this part of the emission (expiration *v.* flatulence) is not necessary the same as measuring the entire emission (expiration and flatulence) (Muñoz *et al.*, 2012). This issue is particularly important if variation exists in the proportions emitted through these three routes in different animals.

The rate at which CH<sub>4</sub> is emitted changes throughout the day, and from day to day. Consequently, cows are in a continually changing biological state in terms of CH<sub>4</sub> emissions. Within day diurnal variation is affected by feeding behaviour, diet, and feeding allowance and patterns (Crompton *et al.*, 2010; Bell *et al.*, 2018). The simplest way to avoid diurnal variations, in principle, is to record CH<sub>4</sub> emissions every second of the 24 h period to obtain true daily CH<sub>4</sub> emissions. In practice, few approaches sample or record emissions continually throughout a 24 h period, instead relying on timestep average estimates, which are subject to experimental error. The number of measurements and timing of sampling required to obtain a representative sample of daily CH<sub>4</sub> emissions vary in relation to many factors, such as feeding time, feeding behaviour, feed intake and activity (e.g. eating and ruminating) (Hegarty, 2013). One approach is to sample

throughout the day over multiple consecutive days and obtain an average estimate or moving average (Arthur *et al.*, 2017). Another approach is to model the time of day, for instance, using sine-cosine curves, regression or class effects within day (Lassen *et al.*, 2012; van Engelen *et al.*, 2018). Using these approaches, the effects of diurnal variation are reduced or corrected.

The rate of CH<sub>4</sub> emission also changes across days (Grainger *et al.*, 2007), with physiological state (growing, lactating and non-lactating) (Ricci *et al.*, 2013), during lactation (early, mid, peak and late) (Rischewski *et al.*, 2017), and from one lactation period to the next. Thus, it is important to understand the phenotypic and genetic relationships between methane emissions recorded at different points in time during an animal's life to understand the implications for selection based on CH<sub>4</sub> emissions recorded at a particular point in time, allowing the optimization of recording strategies.

Further sources of systematic variation in CH<sub>4</sub> production by an individual cow include total feed intake, DM content, feed composition, and the proportion and rate of fermentation of feed in the rumen and rate of passage (for reviews, see Hristov *et al.* 2013; Cabezas-Garcia *et al.* 2017). In some cases, the size of the cow and other metabolic-related traits (such as feed efficiency and energy-corrected milk (ECM) production) explain large amounts of variation in CH<sub>4</sub> production (de Haas *et al.*, 2011). This information has led many authors to try to 'correct' estimates of CH<sub>4</sub> production for these traits known as residual phenotypes (main residual feed intake), by dividing them in relation to CH<sub>4</sub> or regressing them in a multiple linear regression model. However, the consequent ratios and residual phenotypes require careful consideration.

## Methods of recording

### *Respiration chambers as the gold standard*

Many methods are available for recording the CH<sub>4</sub> emissions of individual dairy cattle *in vivo*. However, each method has its own set of advantages, disadvantages and scope of application, as reviewed by Hammond *et al.* (2016). It is well established that the gold standard is indirect calorimetry in respiration chambers (RCs), which have been in use in livestock research for more than a century (Krogh, 1916) and are regarded as the most accurate and precise method from which to benchmark other methods. Respiration chambers are ideal for small-scale experiments, in which the number of animals is low, and the need for accuracy and precision is high. However, RCs are costly, time consuming, not necessarily representative of all environmental conditions (like grazing systems) and are not comparable across facilities. Not surprisingly, many potential technologies are under development, which might be cheaper, less invasive, easier to implement or have a wider scope of applications than the gold standard method. These alternative methods are evidenced by frequent reviews of methods (e.g. Patra, 2012; Storm *et al.*, 2012; Hammond *et al.*, 2016; Hill *et al.*, 2016).

Two main sources of measurement error associated with RC exist, namely (1) airflow rate or ducting efficiency and (2) the mixing of gases within the chamber. Both issues are reflected in the response time (Hammond *et al.*, 2016). In a joint calibration 'ring testing' procedure in the UK, high variation within and between chambers and across facilities was observed for the airflow rate and chamber mixing at 15.3% and 3.4%, respectively (Gardiner *et al.*, 2015). If the absolute accuracy of the CH<sub>4</sub> release rate of the test gas is known with certainty and is constant over time, the recovery rate could be used as a correction factor to calibrate measurements. After correction for differential recovery rates was made in a UK study, the combined uncertainty between chambers and facilities was reduced to 2.1% (Gardiner *et al.*, 2015). However, the use of correction factors is discouraged, and with good practice being to identify the source of error and correct it (McLean and Tobin, 1987). Thus, routine and expensive ring testing is required when using multiple RC testing facilities and would hold for genetic evaluations.

Confinement within a chamber can stress animals and alter their feeding behaviour, resulting in a drop in DM intake (DMI), which is the largest driver of CH<sub>4</sub> emissions. This issue has led many to question how these results are extrapolated to commercial conditions, particularly grazing systems (Pinares-Patiño *et al.*, 2013). Some developments in RC methods have led to animal friendly chambers constructed from cheaper transparent materials. As a result, the cost and invasiveness of the method is lowered, while minimally disrupting the accuracy and precision of the measurement, with no drop in the DMI of cows under confinement (Hellwing *et al.*, 2012).

The throughput and cost of RCs are the biggest challenge for their use in genetic evaluations. Assuming a single day of acclimation and two consecutive days of recording, a single chamber can record the CH<sub>4</sub> production of 120 cows over a year (Garnsworthy *et al.*, 2019). In practice, this quantity is likely to be far less (30 to 50 cows) a year, as reported in the only large-scale genetic evaluation of CH<sub>4</sub> emissions of 1042 growing angus steers and heifers (Donoghue *et al.*, 2016a). This cohort of cattle showed that CH<sub>4</sub> production is repeatable ( $t=0.97$ ) over consecutive days (Donoghue *et al.*, 2016b), heritable ( $h^2=0.27\pm0.07$ ) (Donoghue *et al.*, 2016a) and had moderate genomic prediction accuracy  $0.32\pm0.04$  (Hayes *et al.*, 2016).

#### Critical overview of the sniffer method

Most results reported in the published literature over the last decade were based on the sniffer method (Garnsworthy *et al.*, 2012 and Madsen *et al.*, 2010). The power of the sniffer method is that: (1) equipment can be installed in commercial farms without disturbing the behaviour and everyday life of cows or the farmer and (2) 1000s of animals can be registered with relatively small investment. The expensive component of registrations is the salary for technicians to install and remove equipment, rather than the equipment itself. These two assets are extremely important for inclusion in next generation measurement equipment on commercial farms. If

farmers will be taxed on methane emissions, accurate recording equipment must be available, so that actions taken by the farmer to reduce methane emissions can be assessed and inventoried. This cannot be accomplished with expensive equipment requiring the handling or training of dairy cows, removing them from everyday life, not even for a few hours. However, while affordable at large scales, the sniffer method is not equivalent to the gold standard RC, in terms of accuracy and precision. Consequently, it has been excluded from inventory studies and has limited application in small-scale dose response studies, such as nutritional trials (Hristov *et al.*, 2018; Garnsworthy *et al.*, 2019).

Sniffers take spot samples of methane emissions when the cows are milked; consequently, the data are not necessarily representative of methane emissions over a full day. Also, the lack of flux (active airflow with measured volume) information means that methane concentration, not production, is recorded. Subsequently, researchers use calibration equations (using weight and milk production data) or recovery factors to estimate CH<sub>4</sub> production (Madsen *et al.*, 2010; Garnsworthy *et al.*, 2012). Because the air that is sampled is very low, certain factors (such as dilution due to the movement of cow heads, barn air dynamics and wind speed) affect the accuracy and precision of readings (Huhtanen *et al.*, 2015, Wu *et al.*, 2018). Furthermore, many different types of sensors and installations are used. However, as there are no accepted standard practices for sniffers, each set-up must be validated against RC (Difford *et al.*, 2016 and 2019; Negussie *et al.*, 2016). Despite this, Difford *et al.* (2019) reported individual level correlations (proxies for genetic correlations) of  $0.77\pm0.18$  between sniffer CH<sub>4</sub> production and RC CH<sub>4</sub> production, as well as  $0.75\pm0.20$  of sniffer CH<sub>4</sub> concentration with RC CH<sub>4</sub> production. These results support the scope for using sniffers for the large-scale measurement of CH<sub>4</sub> emissions under commercial conditions.

Sniffers are often criticized and dismissed for their high experimental variation and random errors (Huhtanen *et al.*, 2015; Huhtanen and Hristov, 2018; Wu *et al.*, 2018). This issue tends to arise when researchers use sniffers outside the scope of genetic evaluation, failing to take repeated measures into account. For instance, Hammond *et al.* (2016) stated 'the need for high throughput methodology, e.g. for screening large numbers of animals for genomic studies, does not in itself justify the use of methods that are inaccurate, imprecise, or biased'. Huhtanen and Hristov (2018) stated 'We conclude that true between-cow variation in CH<sub>4</sub> emissions is too small to be reliably measured by the sniffer method with its low precision'. These statements are only partially true, as imprecision can be overcome at the individual cow level by repeated measurements, as demonstrated in classic equation (1) (Falconer and Mackay, 1996; Bovenhuis *et al.*, 2018):

$$V_{p(n)} = \left( t + \frac{1-t}{n} \right) V_p \quad (1)$$

where  $n$  is the number of records,  $t$  is the repeatability, while  $V_p$  and  $V_{p(n)}$  are phenotypic variance before and after repeated

measures, respectively. As  $n$  increases,  $V_{p(n)}$  decreases, due to a decrease in residual error  $V_e$  (imprecision). The increase in accuracy, due to high throughput, is further compounded when viewed at the bull breeding value level in equation (2) (Mrode, 2003):

$$r_{ay} = \frac{0.5h^2 V_{p(n)}}{\sqrt{h^2 V_{p(n)} \left( 0.25h^2 + \frac{1-0.25h^2}{N} \right) V_{p(n)}}} \quad (2)$$

where  $r_{ay}$  is the accuracy of the bull breeding value,  $h^2$  is the heritability,  $V_{p(n)}$  is the phenotypic variation in the presence of repeated measures and  $N$  is the number of daughters. By evaluating (1) and (2) together, increasing  $n$  and/or increasing  $N$  causes  $V_{p(n)}$  to decline, while  $r_{ay}$  will fast approach a maximum of 1. Clearly, high-throughput screening of phenotypes can overcome imprecision in genetic evaluations, to a certain extent. A certain threshold for when this is due is very difficult to set. It will depend highly on the economic value of the trait and the interest in changing the trait in one or another direction.

The results presented using sniffers are promising, because the measurements are repeatable (Lassen *et al.*, 2012) and even heritable (Lassen and Løvendahl, 2016; Pszczola *et al.*, 2019). The correlations to other traits are as expected (Lassen and Løvendahl, 2016; Zetouni *et al.*, 2017), with the distribution over the lactation period following biological lactation curves (Negussie *et al.*, 2016; Pszczola *et al.*, 2017). Furthermore, correlation between different sniffers, as well as other methods (including flux methods), shows that sniffers explain in excess of 60% of phenotypic variation in methane emission, with a potentially higher portion of genetic variance (Difford *et al.*, 2016; Negussie *et al.*, 2016). Still, there are many applications in which sniffers add value, improving accuracy and precision, which could facilitate expansion to other applications (Løvendahl *et al.*, 2018). This value includes the level and change of mean and variation during lactation. Some initial results have been obtained, but more research based on data from more cows is required, along with genetic correlations between different methods (Pickering *et al.*, 2015).

#### Alternative methods of recording methane emissions

For genetic analyses, it is very important to utilize precise and consistent phenotypes, where possible. The better the phenotype, the better the genetic evaluation. In parallel, less precise phenotypes can sometimes be used for selection purposes, if the less precise phenotype is very cheap to measure and describes a proportion of variation that is present in the phenotype one wants to improve (de Haas *et al.*, 2017). So, genetic selection is only possible if 100% of genetic variance in the target trait is described. This phenomenon is determined by the square of the genetic correlation between two traits. For instance, if a correlation between two traits is 0.8, then 64% of variance between traits is described by the other trait. In comparison, if the correlation is 0.2, only

4% of variance in one trait is described by the other trait. The threshold of genetic correlation between two traits that determines whether the alternative trait is an indicator or a direct measures is termed 'the break even correlation' and is traditionally estimated at around 0.80 in progeny testing schemes (Robertson, 1959; Mulder *et al.*, 2006).

However, under genomic selection schemes, this threshold of 0.80 between two traits for them to be appropriate to use one trait as an indicator for the other tends to be far higher under certain conditions (Slagboom *et al.*, 2019). Yet, measurements using both methods on  $10^3$  to  $10^4$  related individuals are required to estimate genetic correlations with meaningful SEs (Visscher, 1998). Larger numbers are required if measurements are made on different animals or animals at different points in time, or environments (Bijma and Bastiaansen, 2014). Estimating genetic correlations between RC and alternative methods is largely prohibited by the cost of recording suitably large numbers of individuals with both methods. To date, only Jonker *et al.* (2018) achieved this, by recording CH<sub>4</sub> production in 3601 lambs with portable accumulation chambers (alternative method) and RC, and obtained a genetic correlation of  $0.67 \pm 0.11$ . Despite calls for genomic reference populations and genetic correlations between RC and other methods, this requirement has not been achieved in dairy cattle (Pickering *et al.*, 2015).

One way to overcome these cost limitations is to either test whether similar results are obtained across methods directly (method agreement) at the phenotype level or estimate individual level correlations as a proxy for genetic correlations (Difford *et al.*, 2019). When assessing method agreement between the RC and alternative methods, it is important to assess the relative accuracy, precision and linear association between alternative methods and the gold standard (Barnhart *et al.*, 2007a). This is achieved by combining these metrics into Lin's concordance correlation coefficient (CCC) or coefficient of individual agreement (Barnhart *et al.*, 2007b; Difford *et al.*, 2016). When these values are suitably high ( $>0.90$ ), it is likely that the alternative method is equivalent to RC. A review by Garnsworthy *et al.* (2019) compared methods used for dairy cattle, obtaining a low CCC (0.38 to 0.88) between alternative methods and the RC. However, when evaluating phenotypic correlations and individual level correlations, the sulphur hexafluoride technique (SF6), GreenFeed™ and sniffers were highly correlated with RC (0.72 to 0.89), indicating potentially high genetic correlations between methods. However, true genetic correlations between methods to validate which methods are appropriate alternatives to RC are still needed.

Substantial work is needed to determine the genetic equivalent, or lack of, between different methods, before recommendations can be made on selection strategies. Thus, it is necessary to genotype animals and estimate genetic correlations between methods and countries. In particular, a genomic reference population based on the RC is needed to benchmark methods genetically. For instance, Niu *et al.* (2018) collated RC, GreenFeed, and SF6 data on 5233



lactating dairy cattle. Even though their primary objective was an intercontinental database, the scope of their database did not include the genetic benchmarking of methods.

### Phenotypes for measurement

The phenotypes of various methane emissions have been reviewed to reduce CH<sub>4</sub> emissions (de Haas *et al.*, 2017). These phenotypes are included here, as they have practical implementation in breeding programmes. The four main phenotypes are (1) methane production as a mass flux rate per day (litres or grams per day), (2) methane yield (MY), which is CH<sub>4</sub> production divided by feed intake (e.g. CH<sub>4</sub> production/kilogram DMI), (3) methane intensity (MI) per unit product (e.g. CH<sub>4</sub> production per kilogram ECM yield and (4) residual methane production (RMP) (e.g. methane regressed on DMI, BW and ECM). But other measures are also known for such methane production per unit of digestible DM.

Defining methane emission traits as ratios is a useful metric for describing groups of animals, such as different treatment groups, herds, breeds and species. However, ratio traits typically violate two statistical assumptions, which can have consequences on defining the linear relationship (correlation or regression) between the two sets of traits, making them unsuitable for incorporation in selection indices (Gunsett *et al.*, 1981; Zetouni *et al.*, 2017). First, it is assumed that a ratio is independent, or uncorrelated, to its numerator or denominator. Second, it is assumed that the relationship between a ratio and its component traits is linear. Sutherland (1965) demonstrated the genetic interdependence between a ratio and its component traits. The severity of nonlinearity between a ratio and its denominator trait is a function of the genetic correlation between the two component traits and the relative difference between their genetic and phenotypic variances. Consequently, there is a very narrow range where a ratio is independent of its denominator traits and when the relationship between the traits is linear. Furthermore, adding a biased correlation to a selection index results in suboptimal index weightings, preventing the response to a prediction being predictable (Gunsett, 1987). The implications of this issue are that the correlation estimates, and thus the relationship between feed efficiency and methane emissions when either or both are expressed as ratios, are likely to be a biased reflection of the relationship between traits. Unfortunately, the use of ratio traits is perpetuated in genetic research, as these traits are used by other disciplines, with it often being necessary to compare results across disciplines.

Residual methane production can be estimated using multiple linear regression models in conceptually similar ways to estimating RFI. When using this approach, CH<sub>4</sub> becomes phenotypically independent of production and other related traits that are corrected for. Another method comparable to gRFI is to make genetic corrections using selection indices (Kennedy *et al.*, 1993). Genetic residual traits result in genetic independence between gRMP and

regressor traits, such as DMI, ECM and BW. Still there might be a phenotypic correlation structure between the traits. This metric is useful for breeders because it indicates how much progress can be made in reducing methane emissions, while having no correlated changes to other economically important traits, such as DMI, ECM and BW. A limitation of this method is that substantial amounts of data are needed to make proper corrections and to estimate appropriate parameters to generate accurate models.

Studies estimating the response to selection for feed efficiency-based ratios and phenotypic and genetic residual traits in pigs (Shirali *et al.*, 2018) observed that genetic residual traits had consistent direct and correlated responses to selection for all traits. However, the phenotypic residual traits had some suboptimal correlated responses to other traits, while the ration traits have unpredicted responses to selection. In a study where ways to obtain the highest response for a ratio trait were simulated, the breeding goal was only represented by two traits, methane and milk production (Zetouni *et al.*, 2017). This simulation did not aim to mimic a complete breeding goal but showed the consequences of selection for simply selecting to improve a ratio trait (e.g. MI) without it being influenced by other traits (Zetouni *et al.*, 2017). Zetouni *et al.* (2017) showed that CH<sub>4</sub> production that was genetically independent of milk production yielded a high reduction in CH<sub>4</sub> production without compromising milk production, and the ratio trait performed the worst. Future research verifying the extent to which methane-based phenotypic residual and ratio traits deviate from genetic restricted selection indexes is needed. In particular, an over reliance on ratio traits should be avoided, which is also the general case in practical breeding.

With pedigree-based selection, there is a huge dependency on direct information from animals in, preferably, the whole population. This approach generates the highest genetic improvement and generates the best structure to the population. With the introduction of genomic selection, in which selection is based on DNA information, not all animals must be phenotyped. It might be more beneficial to find herds with good registrations and then use these to generate prediction models. With these genomic prediction models, it is then possible to take a DNA sample of a new born calf and predict the genetic/genomic merit of that animal, even before decisions on whether this animal will be used for mating are made. The genomic prediction models today is in many ways black box biology and markers as such are not needed to be identified for genomic selection to work. In the future, genomic selection will be less black box biology since pathways, networks and interaction will be incorporated in the models. Genomic selection has slightly lower accuracy since breeding values to a much larger extent are predicted to selection candidates without known performance but strongly impact the generation interval, leading to much higher genetic progress. These methods could also be applied to methane emissions, for which data seem more complex to obtain than, for instance, milk yield, carcass traits and BW.

**Table 1** Heritability estimates for methane emissions in dairy cows, including SEs, number of cows in the analysis, measurement unit, breed and measurement type

Authors	Number of cows	Measurement unit	Breed	Measurement type	Heritability ± SE
Lassen <i>et al.</i> (2012)	1745	g/day	Holstein	Sniffer	0.21 ± 0.06
Pickering <i>et al.</i> (2015)	1308	mg/kg	Holstein	Laser methane detector	0.05 ± 0.07
Lassen <i>et al.</i> (2016)	339	g/day	Holstein	Sniffer	0.25 ± 0.16
Manzanilla-Pech <i>et al.</i> (2016)	205	g/day	Holstein	Sulphur hexafluoride	0.23 ± 0.23
Pszczola <i>et al.</i> (2017)	485	g/day	Holstein	Sniffer	0.27 ± 0.09
van Engelen <i>et al.</i> (2018)	355	ppm/day	Holstein	Sniffer	0.11 (0.02)
Difford <i>et al.</i> (2018)	750	g/day	Holstein	Sniffer	0.21 ± 0.09
Breider <i>et al.</i> (2019)	184	g/day	Holstein	Sniffer	0.12 ± 0.16 to 0.45 ± 0.11
Difford <i>et al.</i> (2019)	434	ppm/day	Holstein	Sniffer	0.26 ± 0.11
Saborio-Montero <i>et al.</i> (2019)	337	ppm/day	Holstein	Sniffer	0.12 ± 0.01

### Repeatable and heritable genetic variation in methane emissions

Different approaches to obtain the data needed for genetic analyses have been performed (for overview, see Table 1). These data are derived using different approaches to quantify methane emission phenotypes, but all were similar. Methane emission is under some genetic control, with the surrounding environment being the strongest controlling factor. Genetic selection for a trait has the potential to make changes, because the effect is cumulative and lasting. Thus, the effect is persistent over days, with the inherited effect cumulating across generations. Not all methods of recording CH<sub>4</sub> production have resulted in a heritability estimate, with most genetic research in dairy cattle being conducted with sniffer and SF6, followed by laser methane detector methods.

### Genetic correlations between the CH<sub>4</sub> emissions of existing traits

There is extensive debate on how methane should be placed in the context of breeding goals. Of note, selection will never be put on methane or any other single trait. Selection will always be part of the existing total merit index and will be given a proper weight to ensure balanced breeding. Therefore, starting to select for decreased methane emissions would not lead to cows that do not digest roughage or cows that select differential concentrate in their diet, because this would have enormous consequences for many other traits in the existing total merit index. The same is the case for selecting for lower resistance to mastitis. In principal, this should lead to cows that have very low milk yield, but because of the balance that is put into a breeding goal it is still possible to select cows that produce substantial amounts of milk and have low incidence of mastitis. Bulls that produce offspring that cannot live up to the general breeding goal would not be selected as superior bulls. Current results on correlations between methane emission and other traits (Table 2) show that selection for reduced methane emissions likely has minimal consequences on other traits, such as reproduction and

health (Zetouni *et al.*, 2018b; Pszczola *et al.*, 2019); however, CH<sub>4</sub> production is related to milk production (Lassen and Løvendahl, 2016; Breider *et al.*, 2019; Difford *et al.*, 2019), as well as DMI (Breider *et al.*, 2018; Difford *et al.*, 2019). Still more analyses on the correlation structure to other traits are needed on larger data sets to confirm or deny such relationships. With more certainties on these correlation structures, it will be more relevant and appropriate to give the right weight to methane emission in a breeding goal without reducing milk production or decreasing fertility or health.

### Improving the selection accuracy of CH<sub>4</sub> emissions

Improving the accuracy of EBVs could be achieved via several routes. Such routes include multi-trait genetic evaluations with genetically correlated traits, indicator traits and the incorporation of genomic information (Gebreyesus *et al.*, 2016). A number of indicator traits for CH<sub>4</sub> production have been suggested (see review by Negussie *et al.* 2017). These traits largely include milk IR spectra, rumination time, feed efficiency and rumen microbiota. The most well researched and promising are milk IR spectral predictions using partial least square regression models trained on RC, SF6 and GreenFeed data (Dehareng *et al.*, 2012; Vanlierde *et al.*, 2018). High prediction accuracies ( $R^2_{cv}$  of 0.70) in 532 CH<sub>4</sub> measurements of 165 Holstein, Jersey and Holstein-Jersey cows measured with the SF6 method were reported and were significantly heritable (Vanlierde *et al.*, 2015). A subsequent data set of 584 measurements of 148 Holstein cattle across multiple countries yielded a phenotypic prediction accuracy of  $R^2_{cv} = 0.64$  (Vanlierde *et al.*, 2018). The potential of using milk IR spectral information to predict CH<sub>4</sub> emissions is facilitated by the fact that many countries already use milk IR spectra to determine total milk fat and protein for pricing milk. Infrared spectral records are obtained weekly on every cow in some country. However, other studies have not been as successful in replicating the results of Vanlierde *et al.* (2018). For instance, van Gastelen *et al.* (2018) obtained an  $R^2_{cv}$  of 218 cows recorded with RC. Similarly, Shetty *et al.* (2017) obtained an  $R^2_{val}$  of 0.13 from

**Table 2** Genetic correlations between methane emission traits and existing selection index traits in dairy cattle

Authors	Number of cows	Measurement unit	Measurement type	Trait	Genetic correlation $\pm$ SE
<b>Methane production</b>					
Pszczola <i>et al.</i> (2017)	485	g/day	Sniffer	Methane production DIM 5 – DIM 200	0.30 $\pm$ NA
Pszczola <i>et al.</i> (2017)	485	g/day	Sniffer	Methane production DIM 5 – DIM 305	0 $\pm$ NA
Pszczola <i>et al.</i> (2017)	485	g/day	Sniffer	Methane production DIM 200 – DIM 305	0.60 $\pm$ NA
<b>Milk production</b>					
Lassen and Løvendahl (2016)	1745	g/day	Sniffer	Energy-corrected milk yield	0.37 $\pm$ 0.07
Breider <i>et al.</i> (2019)	184	g/day	Sniffer	Milk yield	0.49 $\pm$ 0.12
Difford <i>et al.</i> (2019)	432	ppm/day	Sniffer	Fat- and protein-corrected milk yield	0.37 $\pm$ 0.15
Difford <i>et al.</i> (2019)	432	ppm/day	Sniffer	Fat- and protein-corrected milk yield	0.61 $\pm$ 0.32
<b>BW</b>					
Lassen and Løvendahl (2016)	1745	g/day	Sniffer	BW	–0.16 $\pm$ 0.07
Breider <i>et al.</i> (2019)	184	g/day	Sniffer	BW	0.01 $\pm$ 0.43
Difford <i>et al.</i> (2019)	432	ppm/day	Sniffer	BW	0.34 $\pm$ 0.16
Difford <i>et al.</i> (2019)	656	ppm/day	Sniffer	BW	0.16 $\pm$ 0.25
<b>DM intake</b>					
Difford <i>et al.</i> (2019)	432	ppm/day	Sniffer	DM intake	0.60 $\pm$ 0.13
Difford <i>et al.</i> (2019)	656	ppm/day	Sniffer	DM intake	0.08 $\pm$ 0.38
<b>Body type traits</b>					
Zetouni <i>et al.</i> (2018b)	1397	g/day	Sniffer	BCS	–0.28 $\pm$ 0.10
Zetouni <i>et al.</i> (2018b)	1397	g/day	Sniffer	Chest width	–0.20 $\pm$ 0.13
Pszczola <i>et al.</i> (2019)	483	g/day	Sniffer	Chest width	0.16 $\pm$ 0.06
Pszczola <i>et al.</i> (2019)	483	g/day	Sniffer	Height	0.15 $\pm$ 0.06
<b>Health</b>					
Zetouni <i>et al.</i> (2018b)	1397	g/day	Sniffer	Udder health	–0.32 $\pm$ 0.16
Pszczola <i>et al.</i> (2019)	483	g/day	Sniffer	Somatic cell score	0.11 $\pm$ 0.07
Pszczola <i>et al.</i> (2019)	483	g/day	Sniffer	Longevity	–0.06 $\pm$ 0.07

DIM = days in milk; NA = not available; BCS = body condition score.

2200 records of 490 Holsteins using the sniffer method. Wang and Bovenhuis (2019) estimated CH<sub>4</sub> emissions from milk IR spectra in 1508 dairy cows using cross-validation strategies, obtaining an  $R^2_{cv}$  of 0.49; however, when random block validation was used, the  $R^2_{cv}$  dropped to 0.01, demonstrating the importance of validation.

Milk IR spectral wavelengths were confirmed to be heritable in over 200 000 cows (Rovere *et al.*, 2019). This information opens up the possibility of using genetic covariance between informative spectral wavelengths and CH<sub>4</sub> emissions, if estimated. Based on the initial success of milk IR spectra, milk volatile fatty acids were suggested as potential indicator traits of CH<sub>4</sub> production. van Engelen *et al.* (2015) predicted methane emissions from saturated fatty acids in the milk of 1900 cows and obtained significant heritability estimates, ranging from 0.12 to 0.44, indicating a strong link for exploitation. Lassen *et al.* (2016) detected a direct genetic correlation between specific saturated fatty acids and methane production with tridecanoic acid (–0.77  $\pm$  0.37) and pentadecanoic acid (0.87  $\pm$  0.30).

Rumination was proposed as a potential indicator of CH<sub>4</sub> emissions (Negussie *et al.*, 2017). Rumination is recorded using acoustic tags mounted on the collar of dairy cows. The tags record the amount of time a cow spends chewing the cud and are thus implicated in CH<sub>4</sub> emissions through

the digestion and fermentation of fibre. Interestingly, Zetouni *et al.* (2018a) compared the rumination time (415.1  $\pm$  116.7; mean  $\pm$  SD) with CH<sub>4</sub> production (405.2  $\pm$  115.8), with these similar means and variances being promising. However, estimates of individual level correlations were very close to zero (–0.10  $\pm$  0.07). Thus, little to no exploitable covariation existed between the two measurements.

Other traits that have received considerable research interest are rumen microbiota. This is because rumen bacteria and protozoa produce the hydrogen and carbon dioxide converted to CH<sub>4</sub> by archaea (see Wallace *et al.*, 2017). Roehe *et al.* (2016) identified 20 microbial genes associated with MY in beef cattle using whole genome sequencing on ~8 extreme samples in relation to MY. In addition, the authors employed 16S rRNA ribotyping on 68 cattle *postmortem* and identified the influence of sire indicating a genetic component in the rumen microbial composition. Consequently, a research field was founded on potential microbial selection for reducing methane emissions. Difford *et al.* (2018) used 16S rRNA ribotyping on 750 Holstein cows and found that certain bacteria and archaea taxa were associated with CH<sub>4</sub> and were significantly heritable. These observations were recently confirmed in a multi-country multi-breed study by Wallace *et al.* (2019). A separate study used nanopore

technology to assess the whole metagenomes of 334 dairy cows. This study found genetic correlations between CH<sub>4</sub> concentrations and genera in protists, archaea, anaerobic fungi and bacteria.

Furthermore, by contrasting the heritability of CH<sub>4</sub> production and microbiability (proportion of total variance due to rumen microbiota), estimated jointly and separately, and assessing the relative change in variance, Difford *et al.* (2018) estimated the overlap between host genetic and rumen microbial influences on host CH<sub>4</sub> production. In other words, the methane emission is affected by the host genotype, the rumen microbial composition and the interaction between the host genotype and the rumen microbial composition. Specifically, Difford *et al.* (2018) proposed a quantitative genetic framework for inferring whether cattle act as a holobiont or one unit under selection for particular phenotypes. Bordenstein and Theis (2015) review the concepts of holobionts and hologenomes, where the cow is seen upon as a unit and not split into nutrition, genetics, microbiology, etc. Selection for optimal microbial community content is a new concept in animal breeding, whereas selection for resistance against specific microbiota, such as pathogens, is not. Further research on the stability of rumen microbiota throughout the lifespan of cows, as well as models of inheritance, and studies where diet are included are needed in future research.

After Johnson and Johnson (1995) first estimated that CH<sub>4</sub> production constitutes a net energy loss of 2% to 12% of the gross energy intake of cows, links have been inferred between CH<sub>4</sub> production and feed efficiency. de Haas *et al.* (2011) predicted methane emissions from feed intake and phenotypic RFI, reporting favourable genetic correlations of 0.72. These findings suggest a win-win situation, where concurrent improvements could be made to feed efficiency (which has a high economic value) and reduced methane production (which, currently, has no economic value). However, nutritionists and physiologists were quick to warn that reduced methane emissions are associated with reduced cell wall degradation and faster passage rates (Huhtanen and Hristov, 2018). In other words, cows that are poor at digesting fibre and quick to pass fibre from the rumen are likely to have reduced CH<sub>4</sub> production. This could also lead to bad feed efficiency since DM digestion is decreased. Recently, Difford *et al.* (2019) estimated genetic correlations between feed efficiency traits and CH<sub>4</sub> concentration (ppm) in two separate Holstein populations. The authors found CH<sub>4</sub> concentration was a strong indicator trait for feed efficiency. However, in Denmark, strong favourable genetic correlations were estimated (range: 0.42 to 0.69) for different definitions of RFI. However, in the Netherlands where different recording periods and diets were used, genetic correlations ranged from -0.69 to 0.46, depending on the RFI definition used. For grazing Holsteins, Breider *et al.* (2018) obtained a genetic correlation between RFI and CH<sub>4</sub> production, which was very close to zero.

These results imply that relationship between feed efficiency and CH<sub>4</sub> emissions is not straightforward, with

considerable research being needed to define these relationships over time and under different production and feeding conditions before selection for reduced methane emissions.

### Future perspectives

We iterate previous calls for an international genomic reference population on CH<sub>4</sub> production in dairy cattle to benchmark genetic correlations between methods of recording methane emissions and potential indicator traits. Such an exercise would be invaluable in unravelling genetic relationships between methane and existing selection traits, as well as potential new traits, like feed efficiency. The development of methods must continue to improve existing methods (such as sniffers), increase the scope of applications and decrease the costs of large scale recording (such as RC and SF6). Initial findings in rumen microbial ecosystems and feed efficiency offer exciting new fields of genetic research but require considerably larger studies in the future. Genetic selection is a powerful tool to change the level of trait of economic importance. This is also the case for methane emission, but we are not there yet. Genetic selection cannot stand alone as a mitigation strategy and solve all problems. Other initiatives will also have effect on the release of greenhouse gases from agriculture. In the future, it will be even more important to collaborate across disciplines within animal science and related areas to improve mitigation strategies.

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### Declaration of interest

This paper has no conflicts of interest.

### Ethics statement

Approval from an ethics committee was not required, as this was a review.

### Software and data repository resources

No data or models were deposited in an official repository, as this is a review of existing literature.

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