animal

Review: Selecting for improved feed efficiency and reduced methane emissions in dairy cattle

P. Løvendahl[1†], G. F. Difford[1,2], B. Li[1,3], M. G. G. Chagunda[4], P. Huhtanen[5], M. H. Lidauer[6], J. Lassen[1,7] and P. Lund[8]

[1]Department of Molecular Biology and Genetics, Aarhus University, DK 8830 Tjele, Denmark; [2]Wageningen University and Research, NL 6708 Wageningen, The Netherlands; [3]Swedish University of Agricultural Sciences, SE 75007 Uppsala, Sweden; [4]University of Hohenheim, DE 70599 Stuttgart, Germany; [5]Swedish University of Agricultural Sciences, SE 90183 Umeå, Sweden; [6]Natural Resources Institute Finland (Luke), SF 31600 Jokioinen, Finland; [7]Viking Genetics, DK 8900 Randers, Denmark; [8]Department of Animal Science, Aarhus University, DK 8830 Tjele, Denmark

It may be possible for dairy farms to improve profitability and reduce environmental impacts by selecting for higher feed efficiency and lower methane (CH_4) emission traits. It remains to be clarified how CH_4 emission and feed efficiency traits are related to each other, which will require direct and accurate measurements of both of these traits in large numbers of animals under the conditions in which they are expected to perform. The ranking of animals for feed efficiency and CH_4 emission traits can differ depending upon the type and duration of measurement used, the trait definitions and calculations used, the period in lactation examined and the production system, as well as interactions among these factors. Because the correlation values obtained between feed efficiency and CH_4 emission data are likely to be biased when either or both are expressed as ratios, therefore researchers would be well advised to maintain weighted components of the ratios in the selection index. Nutrition studies indicate that selecting low emitting animals may result in reduced efficiency of cell wall digestion, that is NDF, a key ruminant characteristic in human food production. Moreover, many interacting biological factors that are not measured directly, including digestion rate, passage rate, the rumen microbiome and rumen fermentation, may influence feed efficiency and CH_4 emission. Elucidating these mechanisms may improve dairy farmers ability to select for feed efficiency and reduced CH_4 emission.

Implications

An increasing number of studies show that feed conversion efficiency(FCE) is a heritable trait of dairy cows. In addition, daily methane (CH_4) emission has been shown in recent studies to be a heritable trait. Both traits are, to some degree, thought to be influenced by the composition of the rumen microbiome. There are indications of a favourable correlation between CH_4 emission per kg of milk produced and FCE, whereby more efficient cows may be emitting less CH_4 per kg milk, which would facilitate the uptake of results by the dairy industry. However, the cost of systematic recording of feed intake and CH_4 emission has hampered data analysis for large cohorts.

Introduction

Target for research
Researchers across multiple disciplines, including nutrition, genetics, breeding and microbiology, have turned their

† E-mail: Peter.Lovendahl@mbg.au.dk

efforts towards understanding and improving dairy cow feed utilisation efficiency. Examples of cross-disciplinary projects tackling these goals include the Nordic 'Feed Utilization in Nordic Cattle' (http://projects.au.dk/func/), Ruminomics (http://www.ruminomics.eu/) and GplusE (http://www.gpluse.eu/) projects. Moreover, international collaborations have been formed to improve the statistical power of genetic studies through large cohorts, such as in the global Dry Matter Initiative (Berry et al., 2014).

Several collaborative CH_4 mitigation and microbiome projects that are aligned with feed utilisation projects are based on the sharing of resources, such as rumen fluid samples (e.g. Ruminomics and EU-COST METHAGENE; http://www.methagene.eu/). It is hoped that these efforts will form a scientific basis for improving feed efficiency and facilitating the implementation of improvements. Prior increases in milk production per dairy cow have diluted maintenance energy requirements per unit of produced milk, thereby reducing CH_4 emissions per unit of milk (e.g. Capper et al., 2009; Gerber et al., 2013). However, further progress may be

possible through the implementation of various new developments. The purpose of this review is to provide an overview, status update and insights into recent developments in dairy cow feed efficiency research, its connection with CH_4 emissions and underlying mechanisms, such as digestibility and rumen microbiome functions. Each of these issues and their interactions are addressed in the review.

Calculation and expression of efficiency

The methodology for calculating and expressing feed efficiency determines the data required and, eventually, how selection based on a given efficiency variable will influence correlated traits. Commonly, efficiency is expressed as a ratio between product (outcome) and feed intake (expenses) in the form of mass or energy value of milk per kilogram of dry matter intake (DMI). A variable known as residual feed intake (RFI) has been applied successfully in chicken, pigs and beef cattle. Recently, there has been an interest in applying RFI measurements in lactating dairy cattle. Residual feed intake is calculated as the difference between measured and expected feed intake. Consequently, RFI values are centred around zero, with negative values indicating better efficiency. A somewhat similar variable called residual milk yield (RMY) has been proposed. For RMY, milk yield is the y-variate in the model and feed intake is a co-variate. In this paper, we provide examples and comparisons between alternative estimates of efficiency.

Greenhouse gases and energy partitioning

The greenhouse gas, CH_4, is often assumed to represent a loss of energy, and thereby a drag on efficiency. If so, CH_4 measurements could be useful as an indicator of energy loss and, by extension, efficiency. However, animal data on this at large scale have not been available or reported, but could have potential as indicator traits. A thorough review of CH_4 measurement methodologies has been provided by Hammond et al. (2016a), supplemented by the works of Bell et al. (2014) and Negussie et al. (2017). New methods with the capacity to test groups of cows have provided estimates of genetic variation in CH_4 emission from Holstein cows (Lassen and Løvendahl, 2016). As new methods are validated and improved (e.g. Difford et al., 2016a), their statuses and associated advances need to be updated continually, but a comprehensive methodology update is beyond the scope of this review.

Beyond their importance with respect to climate change, CH_4 emissions are also a conduit of energy loss. Eructed CH_4 has been estimated to account for 2% ~ 12% of ingested feed energy (Johnson and Johnson, 1995). Thus, it may be possible to use direct or indirect selective breeding strategies to reduce such energy losses and thus potentially improve feed efficiency. Such an endeavour will require ample volumes of data tracking feed efficiency subtraits along with good emission data from large cohorts of cows. The case for dairy cows is especially complex owing to tissue mobilisation factors interacting with daily intake and production traits. Consequently, we need to address efficiency beyond short

periods of time, perhaps for whole life-cycles, with intensive recordkeeping throughout. Meanwhile, other traits affected by energy status, such as fertility and disease resistance (treated only briefly here) should be tracked concomitantly.

Feed efficiency and digestion

Feed efficiency depends upon an animal's ability to transform ingested feed into metabolically available nutrients. It was long assumed that digestibility was similar across individuals. However, it has become evident that individual cows differ in their ability to digest various feedstuffs (Huhtanen et al., 2015; Cabezas-Garcia et al., 2017). These differences in digestibility among dairy cows are worthy of further study given that even small improvements can, on a large scale, provide substantial value.

Dairy cows host huge microbiomes in their digestive tracts that enable them to digest feed with a high-fibre content while releasing CH_4 as a by-product. Recent advancements in DNA sequencing technologies have made it possible to examine microbiome organisms as well as host–microbiome interactions. The application of such technology platforms to rumen fluid microbiomes from many cows may provide further insights into both digestion and CH_4 production. This review is intended to provide insights into aspects of feed efficiency, CH_4 emission and rumen–microbiome interactions with a focus on the variation and co-variation of traits across individual cows.

Defining and estimating feed efficiency variation

Definition of feed efficiency

A simple, oft-used definition of feed efficiency is based on FCE, a trait-based ratio calculated as energy-corrected milk (ECM) per kilogram DMI (Hurley et al., 2016). The RFI definition — in its simplest form, the difference between actual intake and expected intake based on diet composition, BW change and milk energy production — is gaining favour. Unless live weight changes (ΔLW) are accounted for, this simple definition of RFI resembles that of energy balance, which may have confounding responses to fertility and health. Residual milk yield (the converse of RFI) has also been described as an efficiency trait (Hurley et al., 2016). Each of these definitions has advantages and disadvantages. Because of time-related fluctuations in variables, such as BW, the error associated with any of them can be reduced by extending recording periods. In the following section, we will address these issues in more detail based on findings in the literature and a phenotypic example from Jersey cow data.

Residual feed intake

Efficiency trait estimation depends upon the recording of feed intake and some traits accounting for energy sinks. Commonly, RFI calculations are done by a two-step procedure (e.g. Berry and Crowley, 2013), where the first step is a linear regression of DMI encompassing the factors of metabolic BW (MBW), ECM and ΔLW to account for body tissue

mobilisation (equation (1)). Other definitions have also included body condition score (BCS) changes, as well as combinations of ΔLW and BCS.

$$DMI = \mu + \beta_1\,MBW + \beta_2\,ECM + \beta_3\,\Delta LW + RFI \qquad (1)$$

The partial regressions in equation (1) are β_1, β_2 and β_3, making RFI a residual from the regression model. In the second step (equation (2)), RFI is modelled against relevant fixed effects, such as feeding regime, herd, season and parity. The random part of the model includes animal effects at two levels if cows are recorded repeatedly, where genetic relationships (COW$_A$) form one level and 'permanent animal effects' (COW$_{PE}$) form another.

$$RFI = \sum Fixed\ effects + COW_{PE} + COW_A + \varepsilon \qquad (2)$$

As a residual of equation (1), RFI has a mean of zero. The random genetic effect of cows (COW$_A$) and the permanent animal effect (COW$_{PE}$), which includes learnt and non-genetic effects that persist in the life of the animal, come out as random solutions from equation (2). If this model is used at the phenotypic level only, the COW$_A$ plus COW$_{PE}$ effects are joined in a single COW effect. The two steps can be joined into a unified model, as shown in equation (3).

$$DMI = \mu + \beta_1\,MBW + \beta_2\,ECM + \beta_3\,\Delta LW$$
$$+ \sum Fixed\ effects + COW_{PE} + COW_A + \varepsilon \qquad (3)$$

In equation (3), all effects are estimated simultaneously, reducing effect biases and residual variances (Tempelman *et al.*, 2015). If researchers employ serial (e.g. weekly) data recorded over a period of lactation (Berry *et al.*, 2014; Li *et al.*, 2017), the linear regression coefficients in equation (1) change systematically with lactation stage, which impacts the estimated variance components, resulting, generally, in larger errors (Li *et al.*, 2017). Factors that change with lactation stage include, in addition to co-varying fixed regressions, a set of variance components, namely the genetic (COW$_A$), permanent animal (COW$_{PE}$) and residual components (Liinamo *et al.*, 2015; Tempelman *et al.*, 2015; Hurley *et al.*, 2016; Li *et al.*, 2017). Some studies have included records from multiple parities, assuming a repeatability model to be adequate (e.g. Hurley *et al.*, 2016), whereas others have focussed on primiparous cows (e.g. Li *et al.*, 2017). Although Holstein cows are common in Europe and used for most RFI studies, other breeds are also in commercial use, including Red Dairy cattle and Jersey cows. However, there are few reports on feed efficiency in these breeds (e.g. Liinamo *et al.*, 2015).

Residual milk yield

The RFI approach focusses on production cost, dividing it over a range of sinks, including milk production. Shifting focus from cost to income, DMI and ECM can exchange positions in the RFI model to obtain an estimated RMY (equation (4)) (Hurley *et al.*, 2016):

$$ECM = \mu + \gamma_1\,MBW + \gamma_2\,DMI + \gamma_3\,\Delta LW + RMY \qquad (4)$$

This RMY model can be extended in a manner similar to that used with the RFI model to include cow effects and fixed effects in one unified model.

$$ECM = \mu + \gamma_1\,MBW + \gamma_2\,DMI + \gamma_3\,\Delta LW$$
$$+ \sum Fixed\ effects + COW_{PE} + COW_A + \varepsilon \qquad (5)$$

Note that, in (equation (5)), the partial regressions changed from β in the RFI model to γ in the RMY model, whereas the other terms remain similar. Consequently, efficient cows with negative RFI values would have positive RMY values. The interpretation of RMY becomes the deviation in milk yield at a constant feed intake, adjusted for MBW and ΔLW. Owing to the favourability of positive values, RMY is easier to comprehend than RFI.

Challenges associated with using residual feed intake and residual milk yield

The method of recording feed intake and feeding level may affect RFI and RMY results. Hurley *et al.* (2016) used data from pasture-maintained Holstein cows and recorded intake by way of the indirect *n*-alkane method. Although this method is well documented, the grazing situation differs from that of housed total mixed ration-fed cows. International consortium findings indicate that environmental differences may be so substantial that genetic correlations with DMI deviate strongly from unity (Berry *et al.*, 2014). The timeframe for estimation is also important. Recording windows may be shortened (to days or weeks) to enable more animals to be tested within a given facility each year. Thus, there is a need to select a timeframe likely to reflect the whole lactation-period or life-span efficiency, such as the more stable mid-lactation period (Hooven *et al.*, 1972; VandeHaar *et al.*, 2016; Hardie *et al.*, 2017).

Comparison of efficiency measures based on a study with Jersey cows

We have used recent data from Jersey cows kept at the Danish Cattle Research Centre (Foulum, Denmark) to compare efficiency measures. The calculations began with raw data, using weekly averages of traits (Li *et al.*, 2017), DMI, ECM, LW and MBW = LW$^{0.75}$. Daily ΔLW was considered more useful than LW and therefore calculated with smoothed data. Data recording began just after calving (5 days in milk (DIM)) and continued throughout lactation until drying off, or culling or 305 DIM. Cows in parity 1 and 2 with at least 32 weeks of data in the first 40 weeks post-parturition were included. All cows were fed *ad libitum* with partial mixed rations plus 3 kg of concentrates per day during milking with an automated milking system (see Li *et al.*, 2017 for recording procedures). Additional analyses included variables as lactation means: average DMI (AVG_DMI), average ECM (AVG_ECM) and average FCE (AVG_FCE), based on ECM/DMI ratios.

The specific models used were of the unified type, including an RFI model (6) and an RMY model (7), with each parity modelled separately.

$$DMI_{jkl} = \mu + Y_m + S_j + \beta_1 \, MBW + \beta_2 \, ECM(S_j) + \beta_3 \, \Delta LW(S_j)$$

$$+ W_l + COW_{kj} + \sum_{n=1}^{3} \begin{pmatrix} \tau_{1n} \, \cos(n\varphi) \\ + \tau_{2n} \, \sin(n\varphi) \end{pmatrix} + \varepsilon \qquad (6)$$

$$ECM_{jkl} = \mu + Y_m + S_j + \gamma_1 \, MBW + \gamma_2 \, DMI(S_j) + \gamma_3 \, \Delta LW(S_j)$$

$$+ W_l + COW_{kj} + \sum_{n=1}^{3} \begin{pmatrix} \omega_{1n} \, \cos(n\varphi) + \\ \omega_{2n} \sin(n\varphi) \end{pmatrix} + \varepsilon \qquad (7)$$

The effects are as in models (4) and (5), with added factors for recording year (Y_m), seasons modelled as order-3 Fourier terms, where φ is the day of the year measured in radians, τ and ω are regression coefficients. Lactation curves were modelled by week of lactation (W_l). Random cow effects (COW) were also nested within the segments to allow variance components to change during lactation. Random solutions for each cow within the segment were extracted and used to estimate correlations between and within traits. Estimates of fixed and random effects were obtained by the MIXED procedure in SAS software (SAS Institute Inc., Cary, North Carolina, USA).

An overview of the raw data is given in Table 1, with 269 first and 189second parity cow-lactations contributing to the data. Cows in second parity had a higher feed intake and larger yield, but gained less weight during the lactation period than first lactation cows.

The partial regressions of DMI on ECM and ΔLW reflect the amount of DMI per kilogram ECM or per kilogram ΔLW, respectively. The coefficients changed over the trajectory of lactation in similar ways in first and second parity cows (Figure 1a and b). Similar partial regression changes were described by Li *et al.* (2017) in first parity Holstein cows. The changes in regression coefficients were gradual over the lactation period for regression of DMI on ECM (Figure 1b). The regressions of DMI on ΔLW showed a peak in early lactation and another peak 6 to 8 months into lactation (Figure 1b). There is no obvious, simple reason for these unexpected peaks, which need to be studied further, especially given that they were seen in both in first and second

parity cows. Regressions on MBW (and thereby also on LW) were constant over the lactation period ($0.0974 \pm 0.0055 \, kg$ DMI/MBW × day) and therefore not shown in figures. Changes in regression coefficients over the lactation period may reflect interactions with mobilisation of body energy (body composition) that were ignored in the models, or may reflect digestibility changes that were also not accounted for in the models.

Partial regressions of ECM on DMI and ΔLW during lactation (Figure 2a and b) differed clearly from those of the DMI regressions presented in Figure 1. The regression coefficients cannot be compared directly because they have different units, and the pattern of development over lactation differs for the covariates DMI and ΔLW. Although they are supposed to be mirror images, they are not exactly so, possibly due to correlations between these covariates. The regression of ECM on MBW was small and not significant.

Trait consistency over weeks in lactation was examined *vis-à-vis* correlations between random cow solutions from each of 10 4-week segments in first parity cows (Figure 3), and for each segment with an overall estimate per lactation (AVG). For RFI, adjacent segments correlated strongly, whereas distant segment correlations approached zero, especially between early and late lactation stages (Figure 3). However, from the fourth 4-week lactation segment onwards, correlation coefficients surpassed 0.40, and correlations with average lactation were high by the third segment.

Rank correlations between RMY solutions at different lactation stages were, in general, somewhat higher than those for RFI, albeit with similar reductions for correlations between distant segments (Figure 3). A similar pattern of results with very similar values was obtained for second parity cows (data not shown). These results indicated that treating RFI and RMY as trait characteristics based only on a single 4-week segment for each cow does not yield results that are highly consistent across short-term lactation periods, though the results correlate well with complete lactation values. These findings support previous findings in Holstein cows (Li *et al.*, 2017), indicating that if RFI or RMY is evaluated in only part of the lactation period, it is best done in the more stable part of lactation (months 3 ~ 6) when segments correspond most closely with the entire lactation period. However, limiting subjects to the stable lactation

Table 1 *Feed efficiency data from first- and second-parity Jersey cows*

Parameters	Parity 1 (269 cows, 10 440 records)		Parity 2 (189 cows, 7322 records)	
	Mean (SD)	Range	Mean (SD)	Range
DMI (kg/day)	16.1 (2.8)	5.6 to 34.5	19.0 (3.2)	5.95 to 39.7
ECM (kg/day)	25.8 (5.0)	2.8 to 48.6	30.8 (6.3)	5.21 to 51.2
LW (kg)	441.0 (45.0)	254 to 631	485.0 (46.0)	260 to 694
ΔLW (kg/day)	0.191 (0.512)	−12.2 to 4.2	0.150 (0.483)	− 6.3 to 5.4
MBW (kg$^{0.75}$)	96.1 (7.4)	63.5 to 125.9	103.2 (7.4)	64.7 to 135.3
FCE	1.62 (0.35)	0.23 to 5.98	1.63 (0.34)	0.45 to 6.04

DMI = dry matter intake; ECM = energy corrected milk yield; LW = live weight; ΔLW = daily change in live weight; MBW = metabolic BW; FCE = feed conversion efficiency. Data in the table reflect weekly records of DMI, ECM, LW, ΔLW, MBW and FCE (FCE = ECM/DMI).
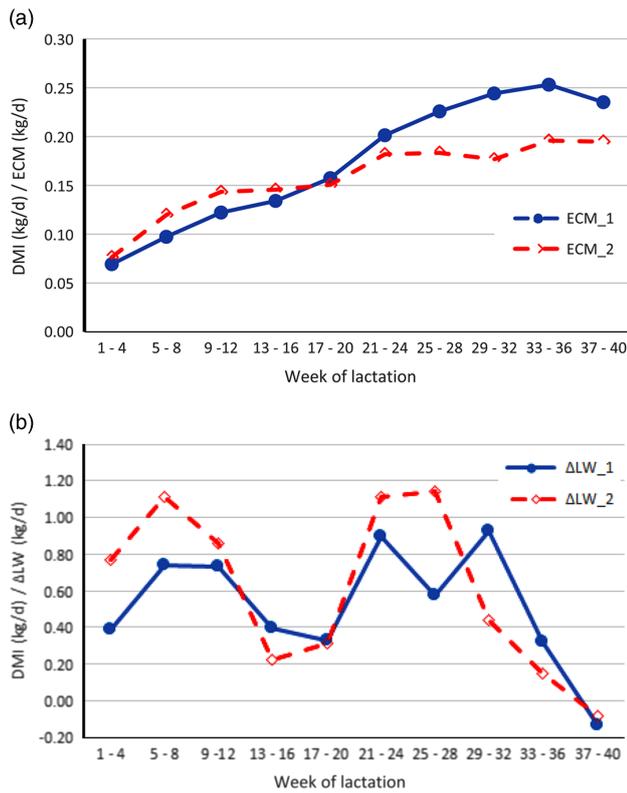
Figure 1 Estimated regression coefficients for dry matter intake (DMI) *v.* energy-corrected milk (ECM) (a) and DMI *v.* live weight changes (ΔLW) (b) across ten 4-week lactation periods in first-parity (_1) and second-parity (_2) Jersey cows.
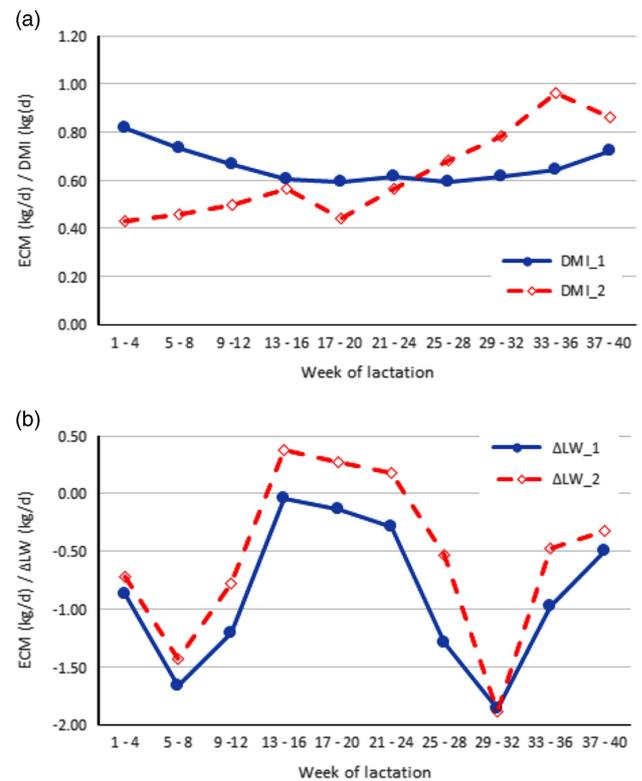
Figure 2 Estimated regression coefficients for energy-corrected milk (ECM) *v.* dry matter intake (DMI) (a) and ECM *v.* live weight changes (ΔLW) (b) ratios across ten 4-week lactation periods in first-parity (_1) and second-parity (_2) Jersey cows.

phase may reduce contemporary group sizes due to cows being in different stages of lactation at the time of recording.

Although RFI and RMY are both used as indices of efficiency, there are divergences in the animal rankings they yield (Figure 3). All rank correlations were in the range of −0.25 to 0.25, with positive and negative correlation values being observed mainly in early and late lactation phases, respectively. Theoretically, we would expect negative correlations between RFI and RMY values. However, in second parity cows, we tended to see RFI–RMY correlation values close to zero (data not shown). These findings contrast with prior results seen in grazing Holstein cows (Hurley *et al.*, 2016). Notwithstanding, the lack of a strong correlation indicates that the two measures are distinct indicators of efficiency.

Summary data covering entire lactation periods may be more relevant than data limited to particular phases within the lactation period. Therefore, additional analyses were carried out with models (6) and (7), where the cow effect was assumed to be constant across the entire lactation period, and the results were aligned with the simple means of DMI, ECM and FCE (Table 2).

As expected, milk yield and feed intake correlated strongly in both first and second parity cows, such that higher yield and higher feed intake followed each other. The FCE ratio (ECM/DMI) correlated robustly with milk yield but not DMI, reflecting the fact that more efficient cows had higher milk

yields, but not higher feed intake. Residual feed intake correlated positively with feed intake, but not with milk yield, MBW or ΔLW, suggesting that efficient (i.e. low RFI) cows eat less. In contrast, efficient RMY cows have higher milk yields and, by definition, at similar feed intake levels, higher MBW and ΔLW values, suggesting that efficient RMY cows (i.e. cows with a high RMY) produce more milk. Residual feed intake and RMY tended to correlate negatively with each other, as found by Hurley *et al.* (2016), demonstrating that the choice of efficiency index can have a pronounced impact on data interpretation and the ranking of animals. It is noteworthy that ECM/DMI ratio data, a traditional measure of FCE, correlated substantially better with RMY data than with RFI data. Furthermore, FCE-associated traits, with the exception of ΔLW, were found to have moderate to high repeatability, across first and second parity cows.

The strengths of our estimated RFI–DMI correlations were similar to or higher than those reported previously for lactating Holstein cows (Connor, 2015; Hurley *et al.*, 2016). Our RMY–ECM correlations were strong in both first and second parity cows (Table 2), largely in agreement with the findings of Hurley *et al.* (2016). Furthermore, we found that RMY correlated positively with FCE, extending the findings of Hurley *et al.* (2016) to Jersey cows housed indoors and sustained on total mixed ration feed.
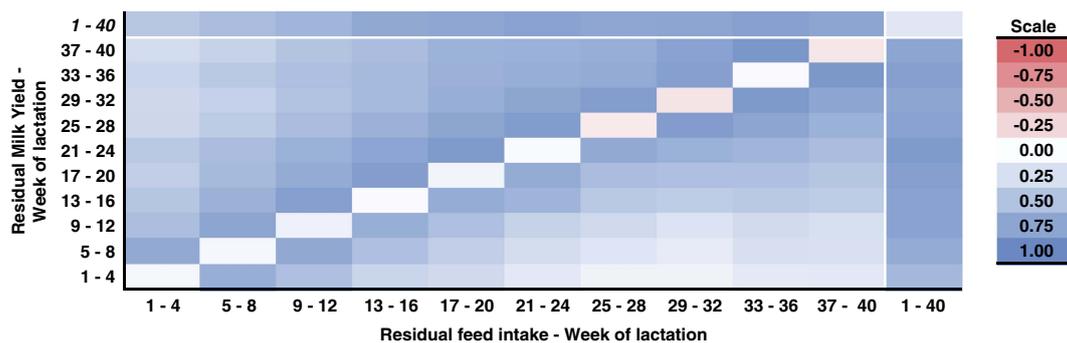
Figure 3 Rank correlations between random animal solutions for residual feed intake (below diagonal) and residual milk yield (above diagonal) for ten 4 weeks of lactation segments in first-parity Jersey cows. Correlations between RFI and RMY in the same segment are in the diagonal. Correlations to complete lactation solutions are indicated as '1–40'. Colour intensity reflects correlation strength. Correlations above 0.12 or below −0.12 were significant ($P < 0.05$).

Table 2 *Correlations among cow random solution parameters in first- and second-parity (italics) Jersey cows*

Parameters	DMI	ECM	FCE	ΔLW	MBW	RFI	RMY
DMI	**0.67**	0.58	− 0.03	− 0.14	0.18	0.80	0.0
ECM	*0.64*	**0.67**	0.79	− 0.23	0.16	0.0	0.79
FCE	*− 0.11*	*0.68*	**0.47**	− 0.18	0.03	− 0.59	0.97
ΔLW	*− 0.15*	*− 0.18*	*− 0.10*	**0.03**	0.10	0.0	0.0
MBW	*0.25*	*0.24*	*0.03*	*0.10*	**0.84**	0.0	0.0
RFI	*0.76*	*0.0*	*− 0.70*	*0.0*	*0.0*	**0.47**	− 0.56
RMY	*0.0*	*0.76*	*0.97*	*0.0*	*0.0*	*− 0.61*	**0.57**

DMI = dry matter intake; ECM = energy-corrected milk; FCE = feed conversion efficiency; ΔLW = live weight change; MBW = metabolic BW; RFI = residual feed intake; RMY = residual milk yield.
Analysed parameters include RFI, RMY and lactation means for DMI, ECM, FCE (FCE = ECM/DMI), ΔLW and MBW.
In the diagonal are correlations between first and second parity for the same trait (bold).
Correlations with absolute value above 0.13 were significant.

It is tempting to suggest that efficiency can be seen in two dimensions, namely an input and an output. If RMY is used for selection, cows with a higher yield at a fixed feed intake will be found to be the more efficient ones. If RFI is used, cows who eat less while producing a given fixed yield will be found to be the more efficient ones. In any case, the key to identifying the most efficient cows is to have comprehensive records of both milk yield and feed intake for large groups of cows, preferably over most or all lactation stages.

The presently observed close correlation between RFI and energy balance, representative of efficient cows having a more negative energy balance, might impact fertility trait selection negatively. This aspect needs to be investigated further using empirical data before solid recommendations can be made as to the implementation of an efficiency trait-based breeding programme.

Measuring emissions from animals en masse

The preferable method of CH_4 emission assessment depends upon the purpose of the assessment. Target emission levels differ across countries, farm inventories and individual animals' current life stages. The relative importance of performance specifications, such as precision, accuracy and capacity, may depend on the application of measurements, which determines the statistical power of key parameter estimates.

There is an ongoing debate about methods and priorities. As new methods continue to be developed, there has been a growing emphasis on accuracy among nutrition-focussed scientists, whereas geneticists, who are relatively new to this field, have been more focussed on high capacity. Comparisons of the available methods have pointed to the importance of comparing and validating any new technique against an accepted gold standard, such as respiration chambers, to gauge relative differences in performance specifications (Hammond *et al.*, 2016a; Cabezas-Garcia *et al.*, 2017; Negussie *et al.*, 2017; Hristov *et al.*, 2018). Although it is a less attended-to issue in the literature, it is similarly important for genetic studies to compare animals, and estimate the differences between them, accurately under standardised conditions.

Although the performance specifications (i.e. precision, accuracy and capacity) of alternative techniques are being estimated through comparison with a gold standard, it is important to remain mindful of parameter inter-relatedness and joint influences on technique applicability across different target levels. All CH_4 emission measures have some level of error. However, the type of error can differ. For instance, a systematic error or mean bias in accuracy refers to the circumstance where a method records emission data that differ from data obtained by the gold standard reference method by a consistent value. If the systematic error relative to the gold standard is known, then it can be corrected with a calibration equation or statistical modelling of a fixed effect (Barnhart *et al.*, 2007). Errors of precision, including random errors or noise, refer to the presence of a higher residual/ background error rate than is seen with the gold standard. Correcting imprecision is far more challenging than correcting inaccuracy because doing so requires mechanical or technical changes (e.g. improving hardware), augmenting the method's capacity for processing repeated

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measurements, or improving statistical modelling of random effects on a per subject basis (Roy, 2009). Hence, a method that is less accurate than the gold standard, but precise, can be used in place of the gold standard method if the performance specifications relative to the gold standard are known and able to be adjusted appropriately.

Measures of CH_4 emission traits include daily emission rate or daily flux (l or g/day animal), CH_4-yield related to feed intake (e.g. CH_4 g/kg DMI) and CH_4-intensity related to production (g or l/kg ECM) (Negussie *et al.*, 2017). Daily CH_4 emission rate measurement has the advantage of sharing features with milk yield per day, whereas the two latter parameters are simple ratios between emission rate and feed intake or milk yield. Although basic measurements obtained from most techniques are converted to an emission rate, an emission rate is sometimes estimated by multiplying a basic measure by an estimate of energy intake derived from milk yield (e.g. Madsen *et al.*, 2010). In such cases, estimated fluxes are then converted to CH_4-intensity in rather circular way, by dividing the emission rate by milk energy output. In such instances, it would preferable to continue with the basic measures (e.g. the CH_4:CO_2 ratio used by Madsen *et al.*, 2010; Lassen *et al.*, 2012 and Bell *et al.*, 2014). Not only would it be easier, but it avoids the possibility of assumptions creating bias, and perhaps inaccuracy, in the conversions (Arndt *et al.*, 1991; Raubenheimer, 1995). The critical requirements for geneticists are capacity and similarity in the ranking of animals across methods (Difford *et al.*, 2016a).

There has been a great interest among geneticists in using milk fatty acid composition or milk IR spectra as an indicator of CH_4 emissions (e.g. Dehareng *et al.*, 2012). These approaches are attractive because they can be obtained for all cows within a milk recording programme and doing so is inexpensive because the data are already generated for milk component analysis. Although the predictive value of such data are very good when calibrated relative to experimental data (Vanlierde *et al.*, 2015), the validity of the approach was only partly confirmed in larger studies of cows kept under conditions more similar to commercial herd conditions (Shetty *et al.*, 2017). The potential for using mid-IR spectrometry data as an indicator of DMI or feed efficiency has initially shown some promise, though the findings of Shetty *et al.* (2016) raise concerns regarding whether that approach can be experimentally reliable.

Rumen microbiome and rumen fermentation

Between-animal variation in CH_4 emissions can be attributed to a variety of factors, including individual differences in rumen microbiome, digestive functions and metabolic regulation, and the interactions between these factors. Rumen microbiome effects on CH_4 emissions have been studied intensively, but the results remain inconclusive. Firkins and Yu (2006) concluded that the abundance of methanogens might not be a reliable indicator of ruminant CH_4 emissions. Methanogen densities did not differ between rumen samples from high and low emitters, but archaeal community structure did differ across rumen samples associated with

different CH_4 yields (Kittelmann *et al.*, 2014). Part of the inconsistency between CH_4 emissions and archaea abundance could be related to differences in the expression of genes involved in the methanogenic pathway. Shi *et al.* (2014) found that although the abundance of methanogens and methanogenesis pathway genes were similar between low and high CH_4 emitters, the transcription of these genes was substantially higher in high emitters.

If rumen microbiomes play a major role in explaining interanimal variations in CH_4 emissions, then such differences should be reflected in rumen fermentation patterns because molar proportions of rumen volatile fatty acids (VFAs) reflect the balance between H_2 production (acetate and butyrate) and H_2 utilisation (propionate). In a recent meta-analysis of rumen fermentation data from dairy cow-digestion studies, Cabezas-Garcia *et al.* (2017) found a between-cow CV in CH_4 yield per mole VFA (calculated according to Wolin, 1960) of only 0.01, with low repeatability ($t = 0.11$). Such results suggest that rumen microbiome differences are not strongly related to the end-products of fermentation and thus CH_4 emission. Pinares-Patiño *et al.* (2003) found a CV for CH_4 yield of 0.098 in a study of 10 sheep with low variability in molar proportions of acetate, propionate and butyrate (0.011, 0.047 and 0.036, respectively). Small betweenanimal variation in rumen fermentation patterns relative to CH_4 emission variation is inconsistent with the rumen microbiome being a major factor in determining betweenanimal differences in CH_4 emission.

It is reasonable to expect that VFA concentrations may be related to animal factors such as rumen volume, passage rate and absorption rate, whereas VFA proportions are more likely to be influenced by the rumen microbiome than animal factors. Cabezas-Garcia *et al.* (2017) found that betweencow variability and repeatability of ruminal VFA concentrations was much greater than the corresponding parameters for molar proportions of VFA, suggesting that diet and animal factors have a greater impact on CH_4 emissions than the rumen microbiome. Notwithstanding, it remains a concern that currently available microbiome characterisation methods are not adequate for determining what traits are predictive of CH_4 emission.

Animal factors

Cabezas-Garcia *et al.* (2017) found that the between-cow variability and repeatability of the NDF pool (g/kg BW) of rumen and the indigestible-NDF passage rate were much greater than the variability and repeatability of variables describing rumen fermentation patterns. Smuts *et al.* (1995) reported that rumen digesta retention time data were repeatable ($t = 0.45$). Levels of CH_4 produced appear to be strongly inversely related to the rate of digesta passage from the rumen (Pinares-Patiño *et al.*, 2003 and 2011; Goopy *et al.*, 2014). Pinares-Patiño *et al.* (2003) obtained a particulate passage rate-to-CH_4 yield correlation coefficient of −0.75, a level of CH_4-relatedness similar to that seen with feeding level. It is well known that increased feeding reduces digesta retention time and CH_4 yield (Johnson and Johnson,

1995). Analyses of Yan *et al.* (2000) and of Ramin and Huhtanen (2013) showed that CH_4 yield decreases about 10% per multiple of maintenance increase in feeding level. Increasing feeding raises the digesta passage rate, which reduces diet digestibility and increases the efficiency of microbial cell synthesis partitioning of fermented carbon from gasses and VFA to microbial cells (Russell *et al.*, 1992); microbial cells are more reduced than fermented carbohydrates (Czerkawski, 1986; Van Soest, 1994).

Animals as hosts for the microbiome

Modern ruminants and the diverse ecosystems of rumen microorganisms within them have been coevolving for some 50 million years. The rumen provides an anaerobic environment hospitable to microorganisms, which ferment and digest plant material into nutrients that are absorbed by the host. This commensal relationship has been highly successful in allowing ruminants and rumen microorganisms to thrive in harsh and widely distributed environments on high cellulose plant diets, which are indigestible to most other domains of life. This co-dependence is such that the ruminant host cannot survive without the microorganisms and some rumen microorganisms are not found outside of ruminants (e.g. rumen protozoa) or are highly differentiated from related microbes (e.g. methanogenic archaea) (Knapp *et al.*, 2014).

The rumen microbial ecosystem, henceforth referred to as the rumen microbiome, contains many domains of life, most of which have not been cultured or annotated, but can be described by culture-independent molecular techniques, such as ribotyping, metagenomics or meta-transcriptomics (Denman and McSweeney, 2014). The relative microbial domain biomass percentages have been estimated as follows: protozoa, 40% to 50%; bacteria, 26% to 42%; fungi, 1% to 20%; and archaea, 0.3% to 4.0% (Wallace *et al.*, 2014; Tapio *et al.*, 2017). From the limited annotations and cultured representatives described, it has been determined that plant structural carbohydrates are degraded to 5- and 6-carbon sugars and then further converted into VFAs for host energy metabolism, with CO_2 and H_2 by-products being absorbed primarily by bacteria, protozoa and fungi (Moss *et al.*, 2000). Fermentation efficiency is limited to a narrow range of H_2 partial pressure and pH, such that a drop in pH inhibits fermentation (McAllister and Newbold, 2008). Methanogenic archaea are thought to play an important role in modulating the partial pressure of H_2 and regulating pH in the rumen by converting H_2 and CO_2 into CH_4. This interspecific hydrogen transfer is seen as a link between CH_4 production and the efficiency of fibre digestion.

Digestibility

Herd *et al.* (2004) suggested that diet digestibility may underlie, at least in part, RFI variation. Herd and Arthur's (2014) review paper also provided evidence showing that digestibility is negatively associated with RFI (low residual feed intake = high efficiency), indicating that more efficient animals have greater diet digestibility. To have a major contribution to variation in feed efficiency, digestibility

should vary between animals and be repeatable. Based on carefully conducted digestibility experiments, Van Soest (1994) concluded that between-animal variation is about 20 g/kg with respect to starch digestibility. Part of this variation appears to be random when measurements are based on one replicate per animal. In a meta-analysis by Cabezas-Garcia *et al.* (2017), between-cow variation in starch digestibility was small (starch digestibility = 10 g/kg; CV = 0.013). These data originated from changeover studies in which digestibility was determined mostly by the total faecal collection method. Mehtiö *et al.* (2016) reported a between-cow organic matter digestibility (OMD) variation of 12.3 g/kg, determined using acid-insoluble ash as an internal marker. Huhtanen *et al.* (2016) obtained lower variability (SD = 8.8 g/kg; CV = 0.012). Berry *et al.* (2007) reported a greater variability for grazing cows when digestibility was determined using alkanes as a marker, probably because they used only one replicate per animal, and the alkane technique is less precise than intake recorded using weigh bins. Repeatability of digestibility was moderate (0.28 and 0.37) when digestibility was determined with acid-insoluble ash as an internal marker (Huhtanen *et al.*, 2015) or a total faecal collection method (Cabezas-Garcia *et al.*, 2017), respectively. These studies indicate that between-cow variation in digestibility alone is too small to explain the observed variation in feed efficiency.

Digestibility was positively associated with ECM production in Huhtanen *et al.*'s (2015) data set when the effects of DMI, LW and DIM were used as covariates in a mixed model analysis with random effects of experiment, diet within experiment and period within experiment, as follows:

$$\text{ECM} \, (\text{kg} \, / \, \text{day}) = 8.82 + 1.69 \times \text{DMI} - 0.0148 \times \text{LW} - 0.0289 \times \text{DIM} + 0.0249 \times \text{OMD}$$

When these factors are held constant, a 10 g/kg increase in OMD increases ECM production by 0.25 kg/day. The difference in digestibility corresponds to an increase in metabolisable energy (ME) intake of ~ 3 MJ (DMI 21 kg/day organic matter, 0.95 dry matter (DM); 1 kg digestible organic matter = 16 MJ of ME). The marginal response to additional ME owing to improved digestion was 0.083 kg ECM/MJ of ME. This value is slightly lower than the typical marginal efficiency of ME utilisation for milk production (0.10 kg ECM/MJ of ME). This slightly lower value could might be associated with a greater marginal CH_4 production and increased digesta retention time in the rumen. The relationship between CH_4 yield and digestibility suggests that some 25% to 30% of incremental digestible energy can be lost as CH_4 in response to a slower passage rate.

Overall, the potential to improve feed efficiency by selecting cows for low CH_4 emission or high digestibility at a constant feed intake level appears to be limited, especially given the positive relationship between CH_4 yield and diet digestibility. Considering that differences in digesta passage rate affect both CH_4 yield and digestibility, and that digestion rate is primarily a feed characteristic, rather than an animal trait, it seems unlikely that one can increase ME supply by

selecting for improved digestibility while reducing CH_4 emissions. The potential to improve digestibility is limited mainly to the cell wall fraction of feed because true digestibility of neutral detergent soluble materials is almost complete (Weisbjerg et al., 2004). Because the digesta passage rate is the main (perhaps only) animal factor contributing to cell wall digestibility, the potential for improving feed efficiency through genetic selection on digestibilty in high yielding dairy cows could be limited. Slowed passage will increase ruminal digesta pool size (rumen fill) and, consequently, limit intake potential.

Diet digestibility has been shown to be lower in low CH_4 emitters than in high CH_4 emitters in sheep (Pinares-Patiño et al., 2011), although one study found only a non-significant trend (Goopy et al., 2014). In a modelling study, Huhtanen et al. (2016) observed a negative relationship of digesta passage rate with CH_4 yield and digestibility, with a positive relationship between CH_4 yield and digestibility. The model simulations were based on observed variability in digesta passage rate determined by the rumen evacuation technique. The aforementioned model simulations and studies with sheep (Pinares-Patiño et al., 2003; Goopy et al., 2014) indicated that CH_4 yield was positively associated with rumen digesta pool size. On average, a decrease of 1.0 g/kg DM in CH_4 yield was associated with a 10 g/kg reduction in diet digestibility (Table 3). Assuming a gross energy concentration of 18.5 MJ/kg DM, a reduction of the CH_4 yield by 1.0 g/kg DM would be expected to be associated with a decrease ME intake by 0.185 MJ (dietary gross energy concentration 18.5 MJ/kg DM), an effect three times greater than that associated with reducing CH_4 (0.055 MJ; 1 g $CH_4 = 0.055$ MJ).

Positive correlations (0.66 and 0.74) have been reported between CH_4 yield and cellulose digestibility (Pinares-Patiño et al., 2003) and between CH_4 yield and NDF digestibility (Pinares-Patiño and Clark, 2010). Strong positive relationships between CH_4 yield expressed as a proportion of gross energy intake and energy digestibility could be derived from individual cow data published by Schiemann et al. (1970a and 1970b; Figure 4), indicating that the relationship between CH_4 yield and digestibility is rather similar when

differences in CH_4 result from differences between cows at the same intake level or differences in feeding level, with differences in passage rate being the most likely causative factor. Hence, selection of low-emitting animals may reduce NDF digestibility, a more important characteristic of ruminants in human food production over monogastrics.

Energy supply is affected by methane emission
Johnson and Johnson's (1995) finding that energy losses in CH_4 ranged from 2% to 12% of gross energy intake is often interpreted as indicating a great potential for improving efficiency of feed utilisation. However, this range represents extreme conditions, rather than variation among animals fed the same diet at the same level of intake. The lowest values were obtained from animals consuming a high concentrate *ad libitum* diet (>90% on a DM basis) and feedlot cattle, whereas the highest values were related to highly digestible diets fed at maintenance levels. For dairy cows eating 20 kg of DM/day (gross energy,18.5 MJ/kg DM), each cow loses about 24 MJ of energy as CH_4 each day (6.5% of gross energy intake) that otherwise could supply ME. Assuming that between-cow CV in CH_4 yield is 10% at the same feeding level, 1 SD unit reduction in CH_4 represents 2.4 MJ of ME. Using a value of 0.62 for the efficiency of ME utilisation for milk production, incremental ME supply from reduced CH_4 would support 0.48 kg/day greater ECM yield, assuming that all incremental ME is partitioned towards the mammary glands and that diet digestibility is unchanged. Although this calculation assumes that there is no relationship between CH_4 yield and digestibility, all experimental and modelling evidence indicates otherwise. It could be argued that it is possible to select animals that have low CH_4 emissions and high digestibility. However, this is not very likely since digestibility is a function of digestion (k_d) and passage (k_p) rates: digestibility $= k_d/(k_d + k_p)$. Both CH_4 yield and digestibility are reduced when k_p is increased, unless k_d is increased enough to compensate for the change in k_p. However, k_d is a feed-specific characteristic and therefore unlikely to be influenced by animal characteristics. The only feasible animal-related change that can positively influence digestibility would be increasing the buffering of rumen contents to

Table 3 *Diet digestibility and methane (CH_4) yield (g/kg dry matter intake (DMI)) in high emitter (HE) and low emitter (LE) groups, and difference (Δ) in digestibility between HE and LE groups*

Study	*In vivo* diet or model species	Diet digestibility (g/kg)		CH_4 (g/kg DMI)		Δdigestibility (g/kg per ΔCH₄)
		HE	LE	HE	LE	
In vivo						
Goopy et al. (2014)		664	648	23.5	20.8	5.9
Pinares-Patiño et al. (2011)	Pasture	629	595	24.6	21.7	11.7
Pinares-Patiño et al. (2011)	Pellet	634	581	10.8	6.4	12.0
Modelling						
Huhtanen et al. (2016)	Cow	758	715	24.4	20.4	10.8
Huhtanen et al. (2016)	Sheep	773	731	29.2	25.0	10.0
Mean		692	654	22.5	18.9	10.1
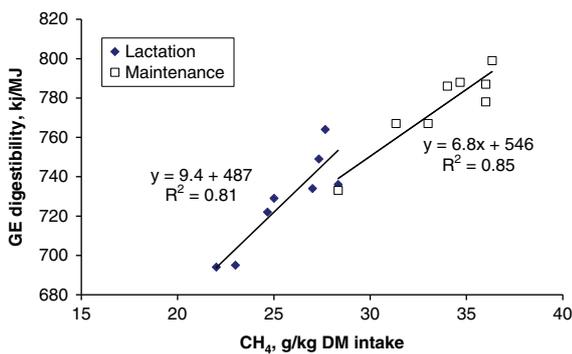
Figure 4 Relationship between methane (CH_4) yield and gross energy (GE) digestibility in dairy cows fed at maintenance and lactation production levels. Adapted from Schiemann *et al.* (1970a and 1970b). DM = dry matter.

maintain more optimal conditions for cell wall digestion. However, Cabezas-Garcia *et al.* (2017) found that there was no relationship between rumen pH and diet digestibility when the variations due to the diet and period effects were removed. Rumen pH was found to be a moderately repeatable trait ($t = 0.46$).

Interactions between emissions and efficiency traits

The current paradox over whether selection for either feed efficiency or limited CH_4 emission produces a favourable correlated response in the other trait is related, at least partly, to trait definitions. Comparisons of divergent feed efficiency groups from numerous precision studies in which RFI data were recorded from a small number of animals have been inconsistent in identifying the relationships between RFI and CH_4. For example, favourable relationships between RFI and CH_4 production predicted from energy intake and the CH_4/CO_2 concentration ratio from cows maintained on concentrate feeders were reported for lactating Nordic red cattle (Negussie *et al.*, 2014). Meanwhile, no relationships were detected in lactating Holstein, Simmental or Jersey cows fed forage-based diets (Münger and Kreuzer, 2008), non-lactating Holstein-Friesians fed alfalfa cubes or lactating Holstein-Friesians maintained on pasture grazing (Waghorn and Hegarty, 2011). One genetics study reported a favourable genetic correlation between RFI and CH_4 production, but CH_4 production was predicted from DMI, which may invalidate the result (de Haas *et al.*, 2011).

It has been claimed that animals with a favourable feed efficiency eat less than their contemporaries for the same level of production and that because CH_4 production is proportional to DMI, so that improving RFI should reduce CH_4 production (Yan *et al.*, 2010; Waghorn and Hegarty, 2011). However, empirical digestibility and digesta passage rate data from dairy cattle have shown that reduced CH_4 yield is associated with reduced diet and cell wall digestion, such that those animals that are inefficient at digesting (extracting energy from) fibre produce less CH_4 (Cabezas-Garcia *et al.*, 2017).

This discordance in results, and thus interpretation of the relationship between feed efficiency and CH_4 emissions, can be attributed, in part, to differing trait definitions, such as the definition of RFI. Often researchers and geneticists want to evaluate treatments and animals independent of scaling (i.e. large animal, high DMI, high milk and high CH_4) and thus express efficiency as FCE or RFI. Similarly, CH_4 emission can be expressed as a ratio (CH_4 yield or CH_4 intensity), or as residual CH_4 production, corrected for DMI or ECM. Both approaches have their advantages, but the largest difference between them is their assumption of the independence of their component traits. For example, in models (1) to (3), the definition of RFI omits phenotypic covariance between DMI and production trait 'sinks', bringing their phenotypic correlations close to zero, whereas genetic correlations can differ from zero. A more closely aligned method to actual breeding practices is to define DMI as genetically independent of breeding values for ECM, MBW and ΔLW, a concept we refer to as genetic RFI (RFIg). In this way, the genetic covariance between DMI and production traits is used to define RFIg as being genetically uncorrelated to production traits but still correlated to DMI. Notably, although RFIg is considered to be genetically uncorrelated to production traits, it remains phenotypically correlated to production traits. Both approaches require large numbers of records to estimate parameters among traits accurately.

Defining feed efficiency and CH_4 emission traits as ratios is a useful metric for describing groups of animals, such as by treatment group, herd, breed or species. However, typically, ratio traits violate two statistical assumptions in a manner that can have consequences for defining the linear relationship (correlation or regression) between sets of traits, and thus make them less suitable for use in genetic selection (Zetouni *et al.*, 2017). The first assumption violated is the assumption that a ratio is independent or uncorrelated to its numerator or denominator and the second is 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, and further showed that the severity of non-linearity 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 trait(s) and the relationship between the traits is linear. The inclusion of a biased correlation in a selection index results in suboptimal index weights and may lead to unpredictable genetic responses (Gunsett, 1984). The implications are that the correlation estimates, and thus the relationship between feed efficiency and CH_4 emissions, when either or both are expressed as ratios, are likely to be a biased reflection of trait relationships. Therefore, it would be prudent to include the components of the ratios, each associated with its own appropriate weighting, in a selection index.

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Impact and interaction with microbiome

Linking the rumen microbiome to feed efficiency and methane emission

A critical challenge in rumen microbiology has been to link mircobiome composition, in terms of the functional roles of individual species, with cow phenotypes (Bickhart and Weimer, 2017). In the case of feed efficiency, small studies have been inconsistent in identifying operational taxonomic units (OTUs) in dairy cattle (Jami and Mizrahi, 2012; Jewell et al., 2015) and sheep (Shi et al., 2014). Grouping of OTUs or meta-transcriptomics at the species/genus level, or higher levels, has been variable across studies, often with opposing (positive v. negative) correlation values (Jami and Mizrahi, 2012; Shi et al., 2014). A notable example of this challenge can be seen with respect to OTUs belonging to the genus Prevotella, a predominant rumen bacterial genera with a large variety of metabolic functions but few cultured representatives (Stevenson and Weimer, 2007). In Holstein cattle divergent for RFI as well as Holstein and Swedish red cattle divergent for CH_4 emission, a number of significant associations of Prevotella OTUs have been found across both sets of divergent groups (Jewell et al., 2015). Prevotella species need to be better characterised before it can be concluded that this genus underlies high and low divergent groups for CH_4 production and RFI.

The known methanogenic capabilities of Archaea spurred research into Archaea associations with CH_4 production (Wallace et al., 2014). It has been postulated that Archaea biomass yield should be proportional to CH_4 production because Archaea rely almost solely on methanogenesis for ATP synthesis (Wallace et al., 2014). However, reviews of recent studies have suggested that production of hydrogen and other substrates of methanogenesis drive CH_4 production (Tapio et al., 2017; Wallace et al., 2017). Thus, further research into other groups of rumen microbes, such as bacteria, protozoa and anaerobic fungi, is required to better characterise how the rumen microbiome relates to CH_4 production.

Potential diet–microbe interactions

The rumen can be seen as a flow-through batch fermenter wherein the substrate and flow rate impact CH_4 emissions and nutrient availability to the host through effects on the rumen microbiome. An extreme illustration of this notion can be seen in in vitro batch culture rumen fermentation studies, in which rumen fluid samples are pooled (to reduce host effects on microbes) and direct effects of additives on CH_4 production and efficiency of VFA production are assessed (Yáñez-Ruiz et al., 2016). Such in vitro studies are often followed up with in vivo studies to validate the observed effects of screened compounds. This approach has resulted in numerous dietary strategies for CH_4 mitigation based on substrate rumen microbiome interactions; this line of research is well reviewed and will not be discussed here (Hristov et al., 2013; Knapp et al., 2014; Patra, 2016). In general, these methods inhibit protozoa and, thus,

associated abundant archaea serve as electron or hydrogen sinks, or are toxic to methanogens or inhibit methanogenesis directly (Patra, 2016). These strategies have aimed mostly to reduce CH_4 emission, but in some instances have also aimed to improve feed efficiency (Hristov et al., 2013).

Interestingly, many of these strategies appear to be temporary and not cumulative, or to have negative influences on milk production (de Haas et al., 2014). Microbial adaption is the primary cause of effect waning (Wallace et al., 2002). However, these limitations can, in principle, be overcome by rotating and combining complementary additives (Klop et al., 2017).

There are exceptions, including nitrate and the patented compound 3-nitrooxypropanol, a methyl coenzyme M reductase inhibitor, which decreases CH_4 emissions by up to 30% in lactating cows without compromising feed intake or milk production (Hristov et al., 2015). Its persistence is likely related to its narrow specificity, which reduces the risk of target microbes acquiring resistance. Thus, diet formulation, digestion rate and substrate passage rate have direct effects on rumen microbiome composition, feed efficiency and CH_4 emissions. Rumen microbes may simply be indicators of digestibility and substrate availability. Notwithstanding, the rumen microbiome can adapt to some feed additives, demonstrating that substrate–microbe interactions are not unidirectional.

Potential host–rumen interactions

Increasing evidence suggests that microbes affect the development, health and metabolism of their hosts, and that the host itself affects its own rumen microbiome. Weimer et al. (2010) exchanged ~ 95% of the rumen contents between cows and found that the rumen microbiome reverted almost completely back to pre-exchange characteristics, suggesting some level of host specificity of the rumen microbiome. Similar archaea:bacteria ratio differences were found among the progeny of eight animals from different breeds, suggesting that this ratio may be heritable (Roehe et al., 2016), in agreement with a large study of 750 Danish Holsteins in which 5% ~10% of rumen 16s rRNA OTU counts were found to have significant heritability (Difford et al., 2016b). Such findings suggest that it may be possible to breed for the relative abundance of certain rumen microbes associated with favourable phenotypes. However, while ruminants' genomes are inherited, they are not born with a functional rumen microbiome but rather acquire it from their mothers and other animals in the environment (Jewell et al., 2015). Large cohort studies are needed to characterise genetic influences on host–microbe interactions, the results of which would also be useful for characterising and annotating rumen microbial species.

Holobionts – a holistic view of the host and its microbiome

Complex digestive system phenotypes, such as CH_4 emission or feed efficiency, are an expression of animal genetic, microbial community and environmental factors (Bickhart and Weimer, 2017). Holobiont theory holds that where the

holobiont is the collective of host and associated micro-biomes, it is the holobiont that is subjected to joint selection for the best performance in an environment (Rosenberg and Zilber-Rosenberg, 2011; Bordenstein and Theis, 2015). Consequently, breeding or dietary management directed at traits like feed efficiency and CH_4 emission without consideration of microbial factors are likely to be suboptimal. Alternatively, incorporating knowledge of the rumen microbiome in the selection for animals with higher than typical feed efficiency on particular diets may be key to future improvements in ruminant production.

Conclusion

The present comparative examination of methods used to express feed efficiency and greenhouse gas emission in dairy cattle indicates that feed efficiency assessment requires accurate measurement of feed intake as well as of related output traits, and that emissions should be measured in commercial herd environments. However, sufficient accuracy for estimation of breeding value may require examination of feed intake over short time periods. Emissions can be assessed accurately with relatively low-cost, high-capacity instruments. It is important that covariate traits in any model are accurately recorded, especially LW and BCS. Emission and efficiency data obtained during the relatively strong-mobilisation, low-feed intake period of early lactation correlate weakly with data obtained during other phases of lactation, but should be included in recording protocols with records from other lactation stages. Because the relationship between feed efficiency and CH_4 emission are likely to be a biased reflection of the relationship between the traits when either or both are expressed as ratios, the components of the ratios should be included, with appropriate weightings, in the selection index. The rumen microbiome plays a dual role in digestion, facilitating nutrient extraction from fibre-rich feed while also generating CH_4. There are clear indications of interactions between bovine hosts and their microbiomes, and both show genetic variation and covariation. The strategy of preventing energy loss via eructed CH_4 to make more energy available for lactation needs further study to clarify its feasibility and true potential, not least because the alternative is that energy can be lost as eructed hydrogen. The availability of low-cost, high-capacity recording methods can facilitate the development of genetic- and nutrition-based improvements in feed efficiency and reduction of emissions.

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Declaration of interest
The authors declare no conflict of interest.

Ethics statement
All handling of animals was conducted according to a protocol approved by The Animal Experiments Inspectorate, Danish Veterinary and Food Administration, Ministry of Environment and Food of Denmark (Approval number 2016-15-0201-00959).

Software and data repository resources
None of the data were deposited in an official repository.

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