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## Invited review: Large-scale indirect measurements for enteric methane emissions in dairy cattle: A review of proxies and their potential for use in management and breeding decisions

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### ABSTRACT

Efforts to reduce the carbon footprint of milk production through selection and management of low-emitting cows require accurate and large-scale measurements of methane (CH<sub>4</sub>) emissions from individual cows. Several techniques have been developed to measure CH<sub>4</sub> in a research setting but most are not suitable for large-scale recording on farm. Several groups have explored proxies (i.e., indicators or indirect traits) for CH<sub>4</sub>; ideally these should be accurate, inexpensive, and amenable to being recorded individually on a large scale. This review (1) systematically describes the biological basis of current potential CH<sub>4</sub> proxies for dairy cattle; (2) assesses the accuracy and predictive power of single proxies and determines the added value of combining proxies; (3) provides a critical evaluation of the relative merit of the main proxies in terms of their simplicity, cost, accuracy, invasiveness, and throughput; and (4) discusses their suitability as selection traits. The proxies range from simple and low-cost measurements such as body weight and high-throughput milk mid-infrared spectroscopy (MIR) to more challenging measures such as rumen morphology, rumen metabolites, or microbiome profiling. Proxies based on rumen samples are generally poor to moderately accurate predictors of CH<sub>4</sub>, and are costly and difficult to measure routinely on-farm. Proxies related to body weight or milk yield and composition, on the other hand, are relatively simple,

inexpensive, and high throughput, and are easier to implement in practice. In particular, milk MIR, along with covariates such as lactation stage, are a promising option for prediction of CH<sub>4</sub> emission in dairy cows. No single proxy was found to accurately predict CH<sub>4</sub>, and combinations of 2 or more proxies are likely to be a better solution. Combining proxies can increase the accuracy of predictions by 15 to 35%, mainly because different proxies describe independent sources of variation in CH<sub>4</sub> and one proxy can correct for shortcomings in the other(s). The most important applications of CH<sub>4</sub> proxies are in dairy cattle management and breeding for lower environmental impact. When breeding for traits of lower environmental impact, single or multiple proxies can be used as indirect criteria for the breeding objective, but care should be taken to avoid unfavorable correlated responses. Finally, although combinations of proxies appear to provide the most accurate estimates of CH<sub>4</sub>, the greatest limitation today is the lack of robustness in their general applicability. Future efforts should therefore be directed toward developing combinations of proxies that are robust and applicable across diverse production systems and environments.

**Key words:** enteric methane, dairy cattle, proxy, breeding, management

### INTRODUCTION

Increases in milk production through management and genetics have substantially improved feed efficiency and decreased costs per unit of product over recent decades. However, dairy systems are also associated with environmental costs (Baskaran et al., 2009), with

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the methane ( $\text{CH}_4$ ) emissions associated with rumen microbial fermentation being both an important contributor to global greenhouse gas (GHG) emissions and an avoidable loss of energy that could otherwise be directed into milk production. The livestock sector is responsible for 14.5% of the global anthropogenic GHG emissions (Gerber et al., 2013). Dairy cattle account for 18.9% of these emissions, mainly in the form of enteric  $\text{CH}_4$  emissions (van Middelaar et al., 2014b).

Several  $\text{CH}_4$  phenotypes have been defined (Herd et al., 2013); the most widely used is  $\text{CH}_4$  production (MeP) in liters or grams per day. The problem in selecting for this trait is that it is highly correlated with feed intake and, consequently, with the ultimate breeding goal in dairy cattle: milk production. Alternative phenotypes are  $\text{CH}_4$  intensity (MeI), which is defined as liters or grams of  $\text{CH}_4$  per kilogram of milk, and  $\text{CH}_4$  yield (MeY), which is defined as liters or grams of  $\text{CH}_4$  per kilogram of DMI. Residual  $\text{CH}_4$  production (RMP) is calculated as observed minus predicted  $\text{CH}_4$  production (Herd et al., 2014; Berry et al., 2015), with predicted values based on factors such as milk production, BW, and feed intake. At the moment, it is not obvious which of these phenotypes to use but it is important to monitor associations between the chosen  $\text{CH}_4$  phenotype and the other important traits in the breeding goal (e.g., production, fertility, longevity) to avoid unfavorable consequences.

Although diet changes and feed additives can be effective mitigation strategies for  $\text{CH}_4$  emissions (e.g., Beauchemin et al., 2009; Martin et al., 2010; Hristov et al., 2013b), their effects depend on continued use of the diet or additive and the rumen microbiome can adapt to additives. In contrast, breeding for reduced  $\text{CH}_4$  emissions would have a permanent and cumulative effect (Wall et al., 2010). Several studies have shown that  $\text{CH}_4$  emissions by ruminants have a genetic component, with heritability in the range of 0.20 to 0.30 (e.g., de Haas et al., 2011; Donoghue et al., 2013; Pinares-Patiño et al., 2013; Kandel et al., 2014; Lassen and Løvendahl, 2016). Breeding for reduced  $\text{CH}_4$  emissions, alone or together with other mitigation strategies, could therefore be effective in reducing the environmental impact of cattle farming and, possibly, also in increasing feed efficiency. Such breeding scheme would require, as a fundamental starting point, accurate measures of individual  $\text{CH}_4$  emissions on a large scale.

Several techniques have been developed for the measurement of  $\text{CH}_4$  emissions from ruminants, with varying degrees of accuracy (see review by Hammond et al., 2016), but routine individual measurements on a large scale (a requisite for genetic selection) have proven difficult to obtain and expensive (Pickering et al., 2015; Negussie et al., 2016). Therefore, identifying proxies

(i.e., indicators or indirect traits) that are correlated with  $\text{CH}_4$  emission but that are easy and relatively low cost to record on a large scale is a much-needed alternative. Proxies might be less accurate but could be measured repeatedly to reduce random noise.

Previous studies have reported a large array of  $\text{CH}_4$  proxies; for example, milk MIR (Dehareng et al., 2012), rumen metagenome profiles (Ross et al., 2013b), and milk fatty acid profiles (Chilliard et al., 2009; Dijkstra et al., 2016), differing widely in accuracy and applicability under different conditions. The ideal proxy would be phenotypically and genetically highly correlated with  $\text{CH}_4$  emissions and cost effective, and it should be easily, and potentially repeatedly, measured on a large scale. A systematic summary and assessment of existing knowledge is needed for the identification of robust and accurate  $\text{CH}_4$  proxies for future use. To fill this gap, this review aims to (1) systematically describe the biological basis of current potential  $\text{CH}_4$  proxies for dairy cattle; (2) assess the accuracy and predictive power of single proxies and determine the added value of combining proxies; (3) provide a critical evaluation of the relative merit of the main proxies in terms of their simplicity, cost, invasiveness and throughput; and (4) discuss their suitability as selection traits.

## DESCRIPTION OF PROXIES FOR $\text{CH}_4$ EMISSIONS

This section presents a systematic review of available methane proxies, with measurement/sampling sites arranged according to the chronological progression of nutrients through the animal: (1) feed intake and feeding behavior; (2) rumen function, metabolites, and microbiome; (3) milk production and composition; (4) hindgut and feces; and (5) measurements at the level of the whole animal.

### Feed Intake

Enteric  $\text{CH}_4$  is a by-product of microbial fermentation in the rumen (principally) and hindgut of animals. Feed intake is therefore one of the major drivers of MeP (Mills et al., 2003; Ellis et al., 2007; Moraes et al., 2014), along with the fermentability of feed (Hristov et al., 2013b). Scientific evidence has shown that DMI (kg/d) and metabolizable energy (ME) intake (MJ/d) appear to be reasonably adequate predictors of MeP. According to Ellis et al. (2007), DMI predicted MeP with an  $R^2$  of 0.64, and ME intake (MJ/d) predicted MeP with an  $R^2$  of 0.53 for dairy cattle. This is in agreement with previous literature reports. For instance, Mills et al. (2003) showed that DMI predicted MeP with an  $R^2$  of 0.60, and ME intake predicted MeP with an  $R^2$  of 0.55. More recently, Moraes et al. (2014)

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