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Interchangeability between methane measurements in dairy cows assessed by comparing precision and agreement of two non-invasive infrared methods

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ABSTRACT

In this study we assess the interchangeability and statistical agreement of two prevalent instruments from the non-invasive "sniffer" method and compare their precision. Furthermore, we develop and validate an effective algorithm for aligning time series data from multiple instruments to remove the effects of variable and fixed time shifts from the instrument comparison. The CH_4 and CO_2 gas concentrations for both instruments were found to differ for population means ($P < 0.05$) and intra-cow variation (precision) ($P < 0.05$) and for inter-cow variation ($P < 0.05$). The CH_4 and CO_2 gas concentrations from both instruments can be used interchangeably to increase statistical power for example, in genetic evaluations, provided sources of disagreement are corrected through calibration and standardisation. Additionally, averaging readings of cows over a longer period of time (one week) is an effective noise reduction technique which provides phenotypes with considerable inter-cow variation.

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1. Introduction

Methane (CH_4) is an abundant and potent greenhouse gas with a global warming potential substantially larger than that of carbon dioxide (CO_2) (IPCC, 2014). Dairy cattle through enteric methanogenesis contribute up to 20% of global livestock greenhouse gas emissions (Gerber et al., 2013). Research into mitigation strategies such as nutritional additives, housing, vaccination and genetic improvement has gained impetus in recent years. The assessment of strategies requires accurate and repeatable individual measurements under commercial conditions. Multiple instruments and or techniques have been developed to measure enteric CH_4 intensity and emissions from cattle and other ruminants, each with their own scope of applications, merits and demerits (Hill et al., 2016). No single method is perfect in all aspects and thus in many instances a reference method from which to make comparisons is lacking.

An emerging method for the measurement of CH_4 and CO_2 concentrations in the breath of dairy cattle, which is high throughput, non-invasive and viable in commercial conditions, is the "sniffer" method (Lassen et al., 2012; Garnsworthy et al., 2012a). Air is continuously sampled from the concentrate bin of automated milking systems (AMS) during individual milking and sample gas concentrations recorded. Two prevalent instruments are the Gasmet DX-4000 (Gasmet; Gasmet Technologies Oy, Helsinki, Finland) (Lassen et al., 2012; Haque et al., 2014) and the Guardian NG/Gascard (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK) (Garnsworthy et al., 2012a,b; Bell et al., 2014a,b). While the techniques and calculations differ, with the former employing a prediction equation based on the ratio of the two gas concentrations and production traits (Madsen et al., 2010) and the latter utilising a scaling factor and methane emission rate (Garnsworthy et al., 2012a), both methods rely on gas concentration readings. The cost of non-invasiveness is restricting the animal to instrument interface and introducing sources of error and imprecision between readings due to air turbulence within the AMS and movement of the cows head in the AMS concentrate bin (Huhtanen et al., 2015). Repeating spot samples over a number of days to obtain a phenotype e.g. average gas concentrations over a week, reduces sources of error by a function of $1 + r(n - 1)/n$ where r is the intra-class correlation and n the number of records; thus obtaining a representative value capable of ranking animals (Hegarty, 2013; Hill et al., 2016). No comparative studies have been conducted on the two instruments to determine their equivalence or lack thereof.

Assessing the statistical agreement between instruments is crucial to informing the manner in which information from multiple

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instruments can be combined, for example, towards genetic evaluations. When measurements from both instruments on large-scale numbers of individuals are available, a genetic correlation between methods exceeding 0.8 is suitable to ascertain equivalence for genetic evaluations (Robertson, 1959). However, one may wish to establish agreement or lack thereof prior to measuring large numbers of individuals. According to Barnhart et al. (2007a), methods may disagree due to different population means, differing between-subject variances and differing within-subject variances. Population means can be corrected through calibrations, but different variances either require the more cumbersome instrument variance reduction or standardisation techniques (Barnhart et al., 2007a). As Bland and Altman (1999) pointed out, the partitioning of the random error variance into within-subject variances (imprecision) cannot be done without replicate measurements per subject per instrument. Analysing replicate measures on cows from AMS is challenging as the number of visits per cow to the AMS (replicates) is variable. Furthermore, time has elapsed between measures and thus the underlying biology has changed between measures due to factors such as diurnal variation patterns of CH_4 and CO_2 concentrations (Lassen et al., 2012). Thus replicate measures per cow must be taken simultaneously with each instrument and treated as paired observations i.e. "linked" replicates (Carstensen, 2011).

Choosing the correct indices to assess agreement must be done with care, for instance, despite having been discouraged for decades as being irrelevant and misleading, some authors still compute Pearson's correlation coefficient in method comparison studies (Altman and Bland, 1983; Bland and Altman, 1986; Carstensen, 2011). Even if one of the methods is perfect, it will correlate poorly to a second less precise method (Barnhart et al., 2007a). Likewise, unscaled agreement indices such as the coefficients of variation within- and between-animals, as well as scaled agreement indices such as Lin's three forms of concordance correlation coefficients (CCC) and intra-class correlations coefficients (ICC), are reliant on between-animal variance. Therefore, imprecise methods recorded on heterogeneous populations will still appear to agree favourably (Barnhart et al., 2007a). An agreement index suited to repeated measures with large errors and less reliance on population heterogeneity is the coefficient of individual agreement (CIA) (Barnhart et al., 2007b). Methods are regarded as interchangeable only if individual measurements between instruments are similar to replicated measures within instrument (Barnhart et al., 2007a).

An additional challenge when comparing instruments with time-stamped measurements, is the clock synchronisation problem, where clocks can have fixed and variables shifts in time (Ridoux and Veitch, 2007). In the absence of synchronised time stamping, as is often the case when comparing readings from multiple instruments, it is possible to obtain a misleading result. Even the most precise instrument will compare poorly when timestamped by an inaccurate clock.

The objectives of this paper were: (1) Demonstrate a fast method for detecting fixed and variable shifts in time series. (2) Conduct a method comparison analysis in the presence of linked and variable number of replicates from each instrument. (3) Standardise instrument recordings to achieve satisfactory agreement for joint analysis.

2. Materials and methods

2.1. Design animals and feeding

Data was recorded over a three week period from end of April to mid May 2015 at the Danish Cattle Research Centre (DCRC, Foulum, Denmark). A total of 56 Holstein cows, average body weight 686.6 ± 86.5 kg (mean ± sd), milk production 38.4 ± 0.34 kg/day roughage dry matter 20.47 ± 4.43 kg /day and concentrates 2.5 ± 0.28 kg/day were recorded during the experimental period. Cows were of mixed parity 44% 1st parity, 35% 2nd parity, 21% 3rd parity at mixed stages of lactation 36% early, 27% mid and 38% late (14–100 DIM early, 100–200 DIM mid, 200–305 late). The DCRC barn is a free stall housing system with cubicles. Cows had access to an AMS (DeLaval International AB, Tumba, Sweden) where they were provided up to 3 kg of concentrate a day within the concentrate bin. Cows were offered a TMR consisting of corn silage, rapeseed meal and soybean meal *ad libitum* in individualised feeding troughs (RIC-system, Insentec, Marknesse, The Netherlands). Data on feed intake (concentrate and roughage), weight and milk production are recorded continuously at the DCRC. The study was conducted without altering management protocols or feeding schemes conducted at the research centre. Cows had free access to AMS with a minimum visit cycle limitation of 4 h, except during the two daily automated cleaning cycles. Cows presented for milking on average 2.4 ± 0.86 visits/day (mean ± sd) during the trial period. The data in this study is generated on cows performing under typical commercial conditions which are representative of a general dairy cattle population in Denmark.

2.2. Breath sampling analysis

CH_4 and CO_2 gas concentrations are routinely analysed at DCRC by using infrared gas analysers installed within each AMS (Guardian NG/Gascard, Edinburgh Instruments Ltd, Livingston, UK) with a range of 0–1% CH_4 and 0–5% CO_2 and logged with NOVUS FIELD LOGGER software (NOVUS Automation, www.fieldlogger.net). The air inlet was custom installed in the upper left rear side of the AMS feed bin so as to be aligned with the nostrils of a feeding cow as per the second experiment described by Garnsworthy et al. (2012a). Air is sampled continuously at a rate of 1 L/min through a 4 mm polyurethane tube approximately 3 m in length with an inline particulate filter to remove dust and a permeable tube with pressurised dehumidified air to remove water vapour before reaching the sensors. The exhaust port of the analyser is vented a minimum of 3 m clear of any sampling point. Data is logged at 1 s intervals and stored perpetually though the use of remote access. Sensors were calibrated prior to the experiment by flushing the sensor inlet with a calibration gas containing 0.0% CH_4 and 0.0% CO_2 to set the lower range and then flushed with a calibration gas containing 1.0% CH_4 and 3.0% CO_2 to set the upper range (both gases in synthetic air HiQ 4.0; AGA, Fredericia, Denmark). Sensors were installed for the recording of entry and exit times within the same time series as the continuous gas concentrations.

The portable Fourier transformed infrared analyser FTIR (Gasmet DX 4000, Gasmet Technologies Oy, Helsinki, Finland) was installed at DCRC as per methods used for sampling through-out Denmark (Lassen and Løvendahl, 2016). The inlet was installed within the feed bin of the AMS in a manner analogous to the Guardian with the exception that the location was in the upper right rear of the bin to mirror and the inlet of the Guardian, in order to prevent the differential pumping rates from creating turbulence at the inlets. Air was sampled continuously through the integral pump at a rate of 4 L/min, starting with an inline particulate filter at the inlet via a 5 m long hose heated to 180 °C before entering the sensor unit. The exhaust gases were vented more than 3 m away from any sampling points. Data was logged continuously at 5 s intervals using Calcmet Software and stored on an integral laptop, thus the Guardian and Gasmet data was timestamped by different data logger software on different servers. The analyser provides reading for the multiple gases as well as water vapour, external

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