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HOW PRECISE ARE ENTERIC METHANE EMISSION PHENOTYPES OR BREEDING VALUES ESTIMATED FROM SPOT FLUX MEASURES?

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SUMMARY

This study was conducted to determine the precision or confidence interval of phenotypes and estimated breeding values for enteric methane (CH_4) emissions of individual animals estimated from spot measurements of methane flux. Components of variance of daily methane production (DMP; gCH_4/d) and methane yield (MY; gCH_4/kg DMI) from trial data were used to estimate the precision for assumed heritability values. The precision was relatively insensitive to number of measures per animal per day and to the number of days of measurement. The values of the residual components of variance (between measures, within-animals, within-days) are high compared to between animal and between day variance but the confidence intervals for EBVs for DMP and MY estimated from spot flux measures are about 20% each side of the mean, which should be adequate for industry implementation in breeding schemes.

INTRODUCTION

Cattle breeders can select for lower methane (CH_4) production directly via the use of methane measuring equipment, such as respiration chambers (Herd *et al.* 2014), Greenfeed emission monitoring units (GEM; Velazco *et al.* 2014) or indirectly via pasture feed intake (Cottle 2011, 2013). Indirect selection is only superior to direct selection if the indirect measurement is easier to make, has a high genetic correlation with the direct trait and has moderate to high heritability. At present measuring pasture intake of large numbers of cattle is no easier than measuring CH_4 production. On-farm measurement of DMP is likely to occur without knowledge of the dry matter intake, although herd intake may be determined (Jones *et al.* 2011). The simplicity of obtaining short-term (spot) measurements of enteric CH_4 production rate is causing these methods to be evaluated for their use in estimating genetic parameters for CH_4 production (Pickering *et al.* 2013). Typically, the arithmetic average of spot measures is used to estimate daily CH_4 production (DMP; g CH_4/d) yet the precision of this approach has not been reported (Cottle *et al.* 2015). Emission rates are known to change over momentary, diurnal and longer seasonal patterns (Crompton *et al.*, 2011; Ulyatt *et al.*, 2002; Munger and Kreuzer, 2008), requiring representative sampling.

This study aimed to determine the precision or 95% confidence interval of individual phenotypes and EBVs for CH_4 emissions estimated from ~3-5 minute, spot measurements of enteric methane flux.

METHODS

Two data sets (grazing and feedlot; Cottle *et al.* 2015) were used to calculate the minimum number of spot flux measures needed to phenotype the true average CH_4 emissions of an animal as required to develop DMP estimated breeding values (EBVs). DMP was estimated from multiple 3-5 min spot measures of methane flux made by the GEM system using 24 cattle. The analysis was based on an acceptable margin of error (MoE) for sampling, a level of confidence to be associated with the final estimates, and an estimated coefficient of variation for each particular sample. MoE is the maximum permitted deviation of the estimate from the true mean. These calculations assume

the confidence level for sampling would be 90% (i.e. the measured value of DMP should be within 10% of the true value). DMP estimates from cattle were estimated by GEM while cattle grazed pastures (173 gCH_4/d) then again when they were feedlot finished (DMP = 142 gCH_4/d). The MoE for each individual methane measurement was chosen as ± 5-10 gCH_4/d. Measurement errors expressed as a percentage of the means, when MoE = 10 gCH_4/d were therefore, 100*(10/142) = 7%, and 100*(10/173) = 6% for feedlot and pasture respectively. For desired margins of error and levels of confidence, sample sizes were calculated as follows:

Sample size (N) = $(z^2 * CV^2)/(MoE/\mu)^2$

where:

z is the value associated with the chosen confidence interval;
CV was 40% (feedlot) or 30% (pasture); and
MoE/μ is the ratio between the margin of error and the mean.

To determine the optimum number of days and measures per day to achieve desired precisions of phenotype estimates and EBVs for DMP and MY, the 95% two-tailed confidence interval was estimated from the feedlot variance estimates reported by Cottle *et al*. (2015). The standard error was calculated using the formulae in Cox and Solomon (2003) as shown below:

Standard error (mean) = sqrt $[\sigma^2 / (n_a.n_d.n_r) + \tau_a / n_a + \tau_d / n_d]$,

where:

σ^2 is the residual variance;
n_a, n_d and n_r are respectively the numbers of animals, days and samples per day, and
τ_a and τ_d are the variance components for animals and days respectively.

The confidence intervals for EBVs were estimated as $\pm 1.96 * \sqrt{((\sqrt{(1-h^2)}*\sqrt{V_A}) + V_E)}$,

where:

h^2 is heritability,
V_A is additive genetic variance, and
V_E = environmental variance $((1-h^2)*V_P)$.

RESULTS AND DISCUSSION

To be 90% confident of the DMP phenotype estimate being within 7.5% of the true mean 68 spot measures were needed from an animal in a feedlot situation and 60 spot measures under grazing conditions (Table 1). CV is lower at pasture for a given absolute MoE as DMP is higher.

Table 1. Number of short-term GEM measures required to estimate the DMP phenotype of an individual animal (g CH_4/day) with a specified margin of error and with a defined confidence using feedlot and grazing data sets

MoE (gCH_4/d)	Confidence interval (%)			
	70	80	90	95
Feedlot data set				
5	61	93	153	217
7.5	27	41	68	97
10	15	23	38	54
Grazing data set				
5	54	81	134	190
7.5	24	36	60	85
10	13	20	34	48

The relationships between 95% confidence intervals and days of measurement and measurements per day are shown for DMP and MY EBVs in Figure 1.

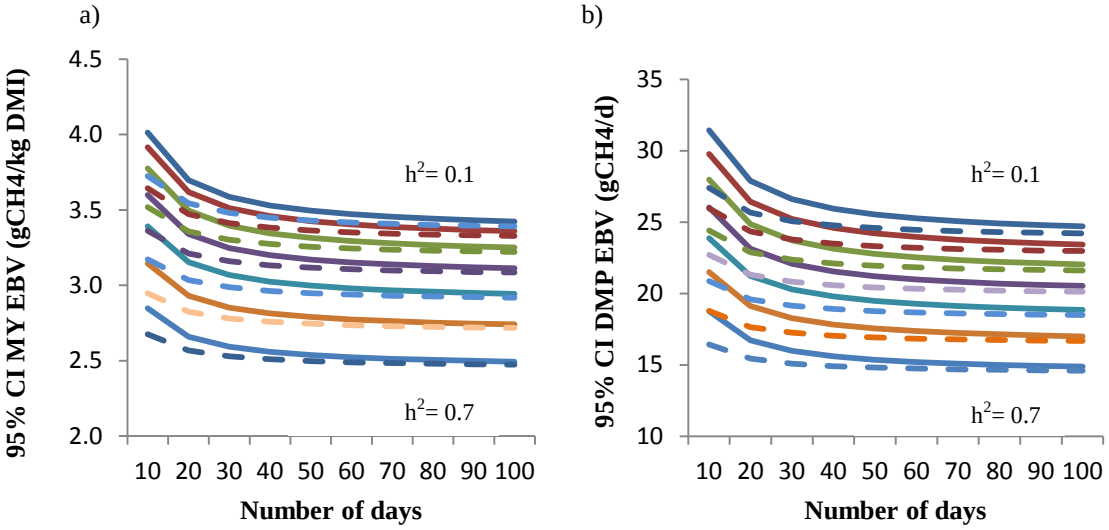

Figure 1. Estimated width of 95% confidence intervals of a) DMP EBVs and b) MY EBVs (either side of mean) vs. numbers of days with 2 measurements / animal / day (solid line) or 10 measurements / animal / day (dashed line). Heritabilites from top to bottom: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7.

The number of spot measures per animal per day (n_r) is dependent on the frequency of supplement delivery by the GEM and voluntary visitation by cattle which is largely outside the control of the researcher. Visitation had a minor effect on the number of days required to achieve a target precision for phenotypes. It would seem prudent to assume $n_r = 2$, knowing that a higher number will slightly improve precision. Measuring animals less than 50 times will probably not achieve desired phenotype MoE and confidence intervals.

The power analyses suggested that spot measurements would result in a precision in the DMP estimate of <10% deviation from true DMP value if they are made over a 70d period as routinely used with RFI tests in a feedlot. Spot measurements of enteric emissions can be used to define DMP but the number of animals and samples are larger compared to measurements made in respiration chambers with a lower CV (Hegarty 2013).

Regarding establishing a precise estimate of the long-term emission phenotype, in a feedlot an animal needed 54 spot emission measurements to be 95% confident that the estimated mean is within 10% of the true DMP phenotype (Table 1). If MoE is 7.5 g/d, the required minimum number of measures (n=60) to describe a grazing animal's phenotype within 10% of the true mean DMP, can be achieved by sampling an animal twice a day over 30 days, or 5 times a day over 12 days. The more intense sampling schedules could confound the estimates under grazing conditions because a higher amount of supplement per day is required to attract the animals into the GEM unit. Within those ranges, all combinations of sampling regimes should deliver estimates within 10% of the true phenotype. Less intense sampling regimes may increase the number of animals utilising a GEM unit.

There is a minimum data requirement for all EBV traits so the optimization of the CH_4 test duration will seek to provide the data at the lowest cost. A 35 day test was suggested by Archer *et*

al. (1997) to be sufficient to phenotype an animal's feed intake (critical for the calculation of MY). In that case, 2 flux measures per day would enable the phenotype of DMP for a specific age and animal class to be used to calculate MY. If DMP is to be related to growth rate, a minimum 70 day test length with cattle weighed every two weeks is suggested (Exton 2001) so a 70 day test for growth rate can easily be run concurrently with the CH_4 determinations.

From Figure 1, the 95% confidence interval for DMP EBV estimates was ±25 gCH_4/day and for MY EBV estimates was ±3.5 gCH_4/kg DMI, assuming a heritability of 0.26 for DMP and 0.23 for MY (Lassen and Lovendahl 2013). Increasing the number of measurements / animal / day or number of days of measurement, (i.e. total number of measurements), had little impact on the precision of EBVs. The confidence intervals are about 20% of the mean values for DMP (~150g CH_4/day) and MY (~13g CH_4/kg DMI) each side of the mean, which is a relatively wider confidence interval than most traits, but should be of adequate precision for use in industry via breeding schemes such as Breedplan. The design of future enteric CH_4 experiments will usually depend on the available budget and logistic limitations. Our formula and results can be used as a guide for any future experimental designs.

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