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Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements

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*Spot measurements of methane emission rate (*n *= 18 700) by 24 Angus steers fed mixed rations from GrowSafe feeders were made over 3- to 6-min periods by a GreenFeed emission monitoring (GEM) unit. The data were analysed to estimate daily methane production (DMP; g/day) and derived methane yield (MY; g/kg dry matter intake (DMI)). A one-compartment dose model of spot emission rate* v. *time since the preceding meal was compared with the models of Wood (1967) and Dijkstra* et al. *(1997) and the average of spot measures. Fitted values for DMP were calculated from the area under the curves. Two methods of relating methane and feed intakes were then studied: the classical calculation of MY as DMP/DMI (kg/day); and a novel method of estimating DMP from time and size of preceding meals using either the data for only the two meals preceding a spot measurement, or all meals for 3 days prior. Two approaches were also used to estimate DMP from spot measurements: fitting of splines on a 'per-animal per-day' basis and an alternate approach of modelling DMP after each feed event by least squares (using Solver), summing (for each animal) the contributions from each feed event by best-fitting a one-compartment model. Time since the preceding meal was of limited value in estimating DMP. Even when the meal sizes and time intervals between a spot measurement and all feeding events in the previous 72 h were assessed, only 16.9% of the variance in spot emission rate measured by GEM was explained by this feeding information. While using the preceding meal alone gave a biased (underestimate) of DMP, allowing for a longer feed history removed this bias. A power analysis taking into account the sources of variation in DMP indicated that to obtain an estimate of DMP with a 95% confidence interval within 5% of the observed 64 days mean of spot measures would require 40 animals measured over 45 days (two spot measurements per day) or 30 animals measured over 55 days. These numbers suggest that spot measurements could be made in association with feed efficiency tests made over 70 days. Spot measurements of enteric emissions can be used to define DMP but the number of animals and samples are larger than are needed when day-long measures are made.*

Keywords: models, splines, GreenFeed

Implications

Short-term (spot) measurements are being used to verify on-farm mitigation of livestock enteric methane but their accuracy and precision are poorly defined. Modelling in this study showed the spot emission rate was poorly correlated with feeding pattern in beef cattle, even allowing for all feeding events in the previous 72 h ($r = 0.41$). However, study of the sources of variation by a power analysis provided a basis for design of future experiments with spot measurements, showing detection of a 10% treatment difference in emission rates is possible in a spot sampling programme made in association with feed efficiency tests over 70 days.

Introduction

Measurement of enteric emissions from ruminants in their production environments is increasing (Hegarty, 2013). The simplicity of obtaining short-term (spot) measurements of enteric methane production has caused these methods to be developed for verifying mitigation on-farm (DoE, 2013) and for development of genetic parameters for methane

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production (Pickering *et al.*, 2013). Typically, the arithmetic average of spot measures is used as the estimate of daily methane production (DMP; g CH_4/day) yet the accuracy and precision of this approach has not been studied. Emission rates are known to change over momentary, diurnal and longer seasonal patterns (Ulyatt *et al.*, 2002; Munger and Kreuzer, 2008; Crompton *et al.*, 2011), requiring representative sampling. If the protocol does not incorporate sampling of emissions at least over the diurnal feeding and activity cycle, a scaling-up coefficient (as used by Garnsworthy *et al.*, 2012) or adjustment factors (such as for animal activity and time spent in each activity as used by Chagunda *et al.*, 2009) may be required to avoid bias in estimating DMP.

On-farm measurement of livestock DMP is likely to occur without knowledge of the dry matter intake (DMI) although herd intake may be determined (Jones *et al.*, 2011). In more controlled animal experiments, where individual animals have their feed intake patterns measured and/or controlled, it is possible to attempt to relate methane measurements to intake patterns (e.g. Jonker *et al.*, 2014). Velazco *et al.* (2014) supplemented cattle with nitrate and reported unexpected DMP results using the GreenFeed emission monitoring (GEM) system that coincided with differences in time interval between feeding and GEM measurement, which may have skewed the estimates of DMP. This finding stimulated a more intensive examination of the relationship between DMP and feeding history of the animal. Post-feeding emission curves have a similar shape to lactation *v.* time curves, with a relatively fast build up to peak production followed by a slow decline (Wood, 1967; Djikstra *et al.*, 1997). Alternately, a non-linear, pharmacokinetic, one-compartment oral dose model can be fitted (three parameters reflect the area under the curve, elimination rate and absorption rate) to estimate daily flux (JMP, 2014).

The work reported addressed three objectives relating to understanding the dynamics of methane production and its relationship to recent feeding events. Specific objectives were to determine; (1) whether the simple arithmetic mean is the best way to estimate DMP from multiple spot emission measures; (2) how much DMP variation is explained by the timing and size of recent meals; and (3) the number and distribution of short-term methane measures required to detect between group differences in emissions when no feed intake data is available.

Material and methods

Experimental design

This experiment was approved by the University of New England Animal Ethics Committee (AEC 14/002). Angus cattle lines divergently selected for low or high residual feed intake (RFI) established at the Trangie Agricultural Research Center, New South Wales, Australia (Arthur *et al.*, 1996 and 2001) provided 20-month old steers ($n = 30$) and heifers ($n = 34$) of starting live weight 406.9 kg (±35.8 SD) generated by ~ 2.5 generations of divergent selection. Steers and heifers were allocated in two separate feedlot pens. Three heifers and one steer were removed from the study before measurements commenced due to initial inappetence, so that 29 steers and 31 heifers were available for RFI and potentially for methane emission measurement. The total duration of the test (excluding the induction to the feedlot ration) was 70 days and heifers and steers were swapped between pens on day 35. A total of 24 animals having the most methane data (>85 or more measures of >3 min length) were chosen from these 64 animals for intensive study of the effects of feeding pattern on DMP (17 heifers and seven steers; nine high RFI and 15 low RFI genetic merit animals).

Animals and feeding

Over 14 days cattle were adjusted to a total mixed ration (TMR) based on barley, cottonseed and hay (Table 1) provided for *ad libitum* consumption, with the ration being dispensed through GrowSafe automatic feeders (GrowSafe Systems Ltd., Airdrie, AB, Canada). Each pen had four individual automatic feeders, enough to provide *ad libitum* feeding for the animals' BW (Bindon, 2001). These feeders recorded the number of feeding events and the duration and weight of feed consumed at each feeding session (called a 'feed event') and were activated by radio-frequency identification (RFID) whenever an animal entered the feeding stall. A meal was defined as the period from which a new animal was detected in the automatic feeder and continued until the animal left. Weekly subsamples of the feed were frozen and pooled for later analysis of nutrient content (Table 1). Chemical analysis of the feed was conducted by Wagga Wagga Agricultural Institute (New South Wales Department of Primary Industries, Australia).

Methane measurements

DMP was estimated from multiple short-term breath measurements using the GEM (manufactured by C-Lock Inc.,

Table 1 *Chemical analysis of the finisher feedlot ration fed to steers and heifers during the experimental period as their main ration and as supplement pellets supplied by the GEM unit*

	Ration	
	Finisher ration	GEM pellets
DM (%)	90.2	93.1
NDF (% DM)	18	13
ADF (% DM)	8	5
CP (% DM)	12.3	17.7
DOM (%)	86	84
Ash (% DM)	5	9
Organic matter (% DM)	95	91
Metabolisable energy (MJ/kg DM)	13.5	12.9
Crude fat (% DM)	3.8	2.5

GEM = GreenFeed emission monitoring; DM = dry matter; DOM = digestible organic matter.
Finisher ration composed of barley (73%), cottonseed (10%), hay (8%), liquid supplement (5%), water (4%) as mixed.

Rapid City, SD, USA). The GEM is a feeding station where pelleted supplement is provided to cattle in a controlled manner (quantity/feed event and number of feed events/day) based on their identity detected by an RFID ear-tag. To access the supplement, cattle placed their head in an open shroud into which pellets are dispensed from a hopper. Air was continuously drawn through the shroud and past the neck of the feeding animal at a precisely measured rate. The concentrations of CH_4 and CO_2 were measured in the exhaust gas. Background gas concentrations were measured when no cattle were present and periodic calibrations and recovery tests performed to define sensor responses to known concentrations of methane and CO_2 and to ensure that >96% of the CO_2 test gas released into the GEM shroud was recovered in the exhaust gas stream. A spot measurement period of 3 to 5 min typically detected several eructation events and is called a 'spot sample' hereafter. To avoid data when animals stepped away from the shroud during methane measurement, a proximity sensor in the shroud, that monitors head-position of the animal throughout each feeding event, was used to identify this happening with such data being excluded. The emission rates over all useful feeding events (at least 3 min length with head in position) during a day were averaged to provide a mean DMP for that day. The pellets delivered in the GEM unit were 6 mm in diameter and the system was programmed to deliver up to four drops, each of 53 g DM, separated by 45 s and then wait at least 3 h to allow a new supplement session for an individual animal. GEM pellets were formulated to closely match the ingredients and nutrient content of the main diet (Table 1) and also contained 0.075% aniseed XL flavoring (Fluidarom 1957, Norel Spain) to increase pellet palatability.

Data processing

The data from the GrowSafe feeders and GEM units were transferred to an Excel spreadsheet with the following columns: tag number, pen/GEM unit, date and time of feed event, length of feed event, CO_2 (g/day), CH_4 (g/day) and TMR intake (g). The GEM emission data were interspersed with GrowSafe feeder data, with data rows arranged in chronological order for each animal. Visual basic (VB) routines were written to calculate the time interval before each spot methane measurement and each recent feed event and then associated with the amount eaten at each feed event. The VB code calculated the time intervals and meal size of all feed events for up to 3 days before each spot methane measurement. The maximum number of feed events in the 3 days before a GEM spot measurement for any animal was 71. Methane measurements within the first 3 days of feeding the finisher ration were omitted from the analyses.

Data analysis

Relationships between spot emission rate and intake were studied using different approaches reliant on increasing levels of feed information inputs as described below.

Relationship between spot methane production rate and time since last feeding event. The relationships between spot methane production rate (g CH_4/day) and time since just the preceding feeding event (intake 1) were analysed for each animal by including all spot measurements in three curve fitting functions as follows: (1) one-compartment oral dose model $((abc)/(c-b)) \times (e^{-bt} - e^{-ct})$, (2) Wood (1967) model with $b > 0$ $(a \times t_1^b \times e^{-ct})$ and (3) Dijkstra *et al.* (1997): Crompton *et al.* (2011) model $(a \times e[(b \times (1 - e^{(-c \times t1)})/c - dt])$ where a, b, c and d are the best fit curve parameters and t is time (minutes) since last GrowSafe feed event. In addition, a spline was fitted through spot data (*v.* time since last feed) over all days for each animal to provide an alternate mean of averaging spot emission rates.

Relationship between spot methane production rate and time and weight of last two feeding events (two intake quadratic model). The spot methane data was analysed as $y = a + b1 \times \text{intake1 (g)} + b2 \times \text{intake2 (g)} + c1 \times t1\text{(min)} + c2 \times t2\text{(min)} + d1 \times t^2 + d2 \times t^2$

The areas under the emission rate (g/day) curves from 0 to 1440 min post-feeding (divided by 1440 min) gave the estimated average DMP after a feed event. The areas were estimated by calculating the trapezoids under the curves.

Relationship between spot methane production rate or methane yield (MY) and time and weight of all feeding events in the preceding 72 h (3 day intake models). Preliminary analyses (not presented) indicated that the DMP and MY (g/kg DMI) effects of each individual GrowSafe feed event extended for >2 days, as found for sheep (Robinson *et al.*, 2011). Hence, for each MY estimation, all feed events from the previous 3 days were identified and aligned. The one-compartment dose model (JMP, 2014) was coded into Excel, summing the fitted MY from each individual feed event accounting for the time between a given methane measurement and the quantity of, and time delay since, each meal in the previous 72 h. The model allowed different MY patterns to be fitted for each animal. Solver (MSExcel) was used to fit the model coefficients, by minimising the residual sums of squares. The area under this curve to infinity estimated total MY (total methane produced from each kg of intake). The area after 3 days was very small, justifying the decision to only account for feeds in the past 3 days. There were marked variations in DMP and MY values, so outliers over 2.5 SD (on the log-scale) from the mean were culled. This resulted in only 103 readings being omitted (<3%; less-so because the original standard deviation was inflated by these outliers).

GLM

Daily intakes, measured DMP and MY were plotted over the entire feeding period revealing notable patterns over time in each trait. For each measured DMP, the average daily intake for the 3 previous days was used to calculate MY. Splines over time (with 3 DF) were fitted for the diurnal effect and days. Animals were fitted either as random effects, or as a

fixed effect and the interaction estimated (i.e. allowing the animals to have different patterns over time). The predicted DMP and MY values for the animals were also estimated using the equations used in the Australian National Greenhouse Gas Inventory Report for feedlot beef cattle (Department of Environment, 2014; Moe and Tyrell, 1979). Concentrations of lignin (1.1%) and silica (0.1%) were assumed in estimating the cellulose content in the diet (cellulose = ADF – lignin – silica) to solve the Moe and Tyrell (1979) equation.

Results and Discussion

Raw data, feed events and diurnal variation in emissions

There were 18 700 rows of raw data, being individual methane production measures of 24 cattle over 64 days during the 70 days test. The number of feed events in the 3 days before a methane measurement was normally distributed with a mean of 32 ± 9.6 (median 31, mode 33) and a range from 8 to 71. As meal frequency increased the average size of each meal diminished from 1228 g (±1006) at the first meal in the 3 days before each methane measurement to 190 g (±180) for the 71st meal in the preceding 3 days for the animals with 71 feeds. For DMP, day (as a spline term) was the dominant effect, with R^2 of 23.3%. The diurnal effect (Figure 1) lifted this to 27.7%, and then adding 'animal' as a fixed effect gave a final R^2 of 36.1%.

Curve fitting

Simpler models. The simple models of spot DMP *v.* time since the most recent feed event explained little variance in spot DMP, reflecting the high variability in spot measures and that these models did not include the size of the feed event or time and size of earlier feed events. For example, the R^2 of the one-compartment dose model varied from 0.01 to 0.23 for the 24 cattle, averaging 0.10. Daily intake is recognised as a good predictor of DMP (Kennedy and Charmley, 2012), but association of spot emission rate with the most recent feed event or events was poor.

When the arithmetic mean spot emission rate for the trial for each animal was regressed against the fitted DMP, estimated as the area under the curve from models relating emission rate to time since last feed, the proportion of average spot rate variance explained (Figure 2), was $R^2 = 0.54$ for the one dose curve fit. The estimated areas under the curves equating to average DMP for the time curves are given in Table 2.

The spot average (and spline) DMP estimates were lower and dissimilar to DoE (2014) estimates, which use Moe and Tyrell's (1979) equation, and are the basis of Australia's Greenhouse Gas Inventory reporting. However, DoE (2014) estimates appear too high (e.g. Benchaar *et al*., 1998) and therefore are of limited use as a benchmark DMP value. The correlations of DoE (2014) estimates of DMP to the spot average, one dose, Wood, Djikstra and spline models were 0.29, 0.37, 0.31, 0.02 and 0.52, respectively. The correlations between the average of spot estimates of methane and the curve estimates in Table 2 are shown in Figure 2. The spot average was more highly correlated to areas under fitted curves than to DoE (2014) estimates but the values were about 40 g/day higher than estimated from the areas under the various curves. The one-compartment dose model using only time since the immediately preceding feed event fitted the data in this study better than the two intake quadratic model and had the highest correlation to the average spot estimates ($r = 0.74$). This was the model therefore used for additional analyses.

Two intake models. The intake and time of eating since measurement regressions against spot DMP measures were also not good fits, when only one or two intakes were included in the regression. The two intake quadratic regressions and Djikstra curves of spot methane *v.* time since feeding had shapes that did not resemble the expected

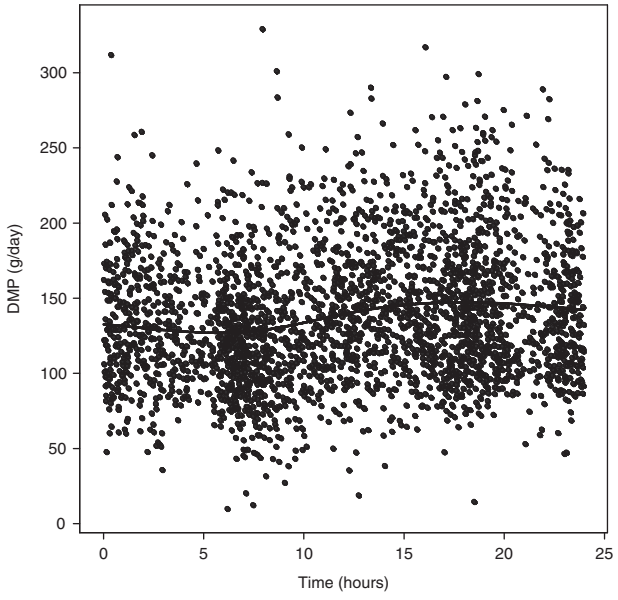

Figure 1 Diurnal spline fit for DMP based on spot emission rates of methane for 24 Angus cattle on a feedlot total mixed ration. DMP = daily methane production.

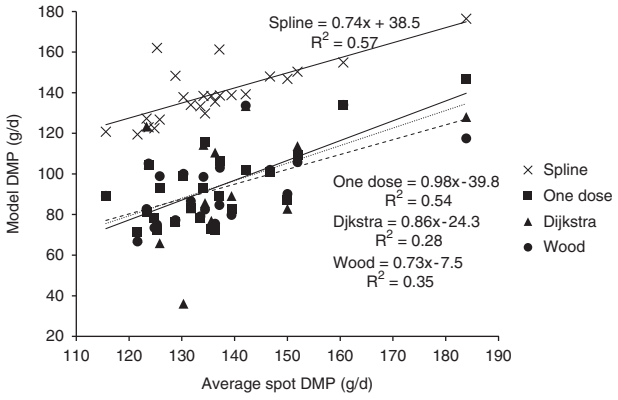

Figure 2 Estimates of DMP based on the integrated areas under curves derived from four models plotted relative to the average spot emission from each of the 24 Angus cattle over 64 days on a feedlot total mixed ration. DMP = daily methane production.

Table 2 *Estimated daily methane production (DMP: g/d) calculated as the arithmetic mean of all individual measurements (spot average) for individual cattle on feedlot rations (tag) or derived from the areas under various curves or as predicted by DoE (2014) equations*

			DMP					
Tag	Sex	NFI line	Spot average	One dose	Wood	Dijkstra	Spline	DoE (2014)
H077	H	Low NFI	123.3	81.4	82.7	123.3	127.3	196.9
H094	H	High NFI	128.8	76.2	77.3	76.9	148.3	208.0
H119	H	Low NFI	130.3	99.0	100.1	36.1	137.8	206.8
H146	H	High NFI	134.1	93.2	98.6	114.3	138.4	200.1
H164	H	High NFI	137.1	89.3	84.7	89.4	161.3	230.5
H187	H	High NFI	131.7	83.2	86.6	83.1	134.0	197.5
H190	H	Low NFI	136.3	72.3	75.5	110.5	135.6	143.2
H191	H	Low NFI	115.6	89.2	89.2	89.2	120.8	184.5
H199	H	Low NFI	183.9	146.9	117.5	128	176.4	209.0
H237	H	Low NFI	125.8	93.2	98.9	65.9	126.8	192.5
H242	H	High NFI	134.4	115.6	82.5	85.7	129.8	192.2
H249	H	High NFI	125.3	72.4	75.0	75.0	162.0	195.0
H251	H	Low NFI	123.7	104.6	105.1	105.1	123.0	183.2
H277	H	Low NFI	146.7	101.1	102	102.0	148.0	201.7
H281	H	Low NFI	133.5	78.5	79.1	78.5	133.3	190.5
H283	H	High NFI	142.1	102.2	133.7	133.4	139.2	191.4
H285	H	Low NFI	121.6	71.5	66.7	68.4	119.4	190.8
H129	S	Low NFI	124.8	78.3	73.5	78.5	122.5	169.4
H222	S	Low NFI	135.6	72.8	73.0	76.9	138.4	202.3
H255	S	Low NFI	137.2	106.1	103.0	106.1	138.6	183.4
H260	S	High NFI	151.9	109.5	105.8	113.7	150.3	212.6
H262	S	Low NFI	160.6	134.0	134.0	134.0	154.9	212.3
H279	S	High NFI	150.0	86.9	90.1	82.8	146.8	177.1
H300	S	Low NFI	139.4	82.6	79.8	89.1	138.8	178.4

DMP = daily methane production; NFI = net feed intake; H = heifer; S = steer.

biological situation of a quick rise to peak and then steady decline. From time zero to 1 day post-feeding, the quadratic curves continued to rise while the Djikstra curves rose and fell twice (data not shown).

Three-day intake models. Solver was used to fit 'the average' (across-animals) one-dose model to DMP, scaled to a 1 kg feed event (so the feed amount was factored in and directly scaled). MY was the integral under this curve and was very close to the 'average' (on a per-animal per-day basis) estimated MY values.

The variation in the DMP data resulted in a fitted model in which only 16.9% of variation was explained by modelling GEM spot measurements in relation to the size of, and interval from, each meal in the previous 3 days. The fitted model allowed a lag period (from time of intake to start of methane production), but this always fitted as zero and rise to peak emissions was very rapid. Supplementary Figure S1 shows the instantaneous emission rate over time for 'the average animal' for 3 days, following a 1 kg feed event. The integral under this MY curve was 12.1 g/kg DMI.

This model identified marked animal differences about the mean MY value of 12.1, where MY was calculated as the ratio of DMP on a given day and the average DMI on that day and the previous 2 days. To better understand the changes in emission attributes over time, DMI, DMP and MY over the trial were plotted and splines were fitted. The R^2 for DMP fitted by splines was 24.1%, and Figure 3 shows steady increases in DMP calculated from spot measurements over

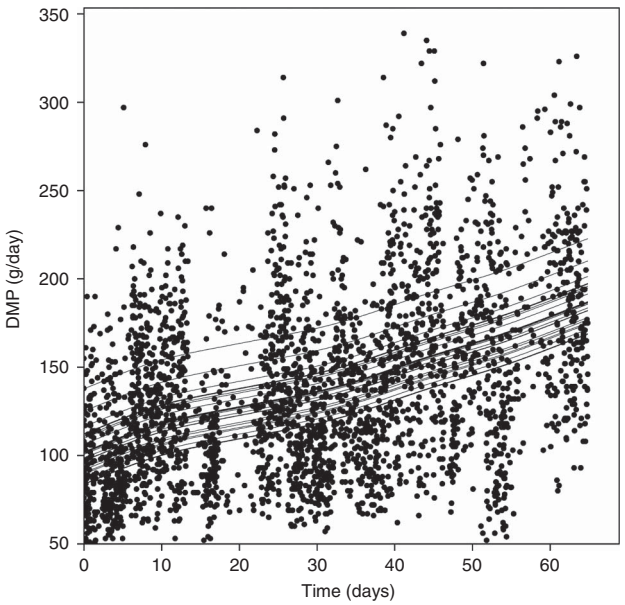

Figure 3 The fitted spines for estimated DMP of individual beef cattle ($n = 24$) on a feedlot total mixed ration with splines for individual animals. DMP = daily methane production.

time, which were consistent between animals. The average DMP values were 101.1 g/day at day 0, 136.4 g/day at day 30 and 190.1 g/day at day 65, so DMP increases were 1.53 g/d from days 30 to 65, or 1.37 g/d for the whole trial. This trend in increasing calculated DMP with time was mostly due to changes in daily intake over time, with the same spline model for DMI having R^2 of 41.1%, as shown in Figure 4. Taking the ratio of DMP on a given day and DMI (averaged over day of measurement and 2 previous days) to calculate MY, the same model gave an R^2 of 18.1%, as shown in Figure 5.

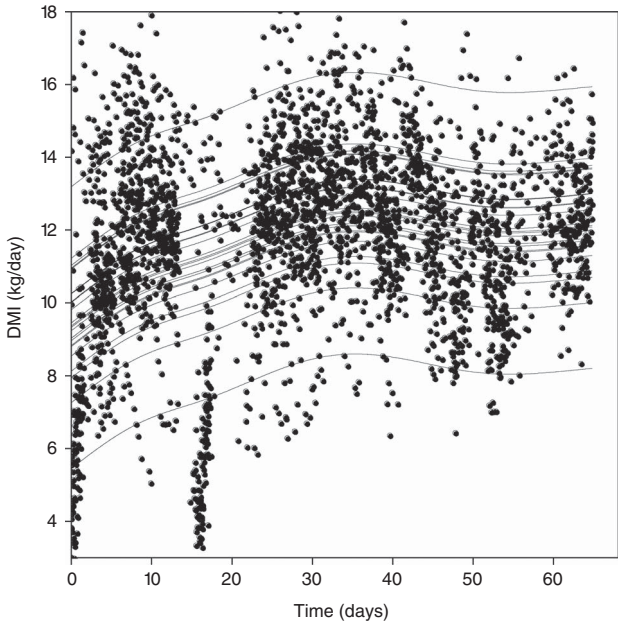

Figure 4 The fitted spines for estimated DMI of individual beef cattle animals ($n = 24$) on a feedlot total mixed ration with splines for individual animals. DMI = dry matter intake.

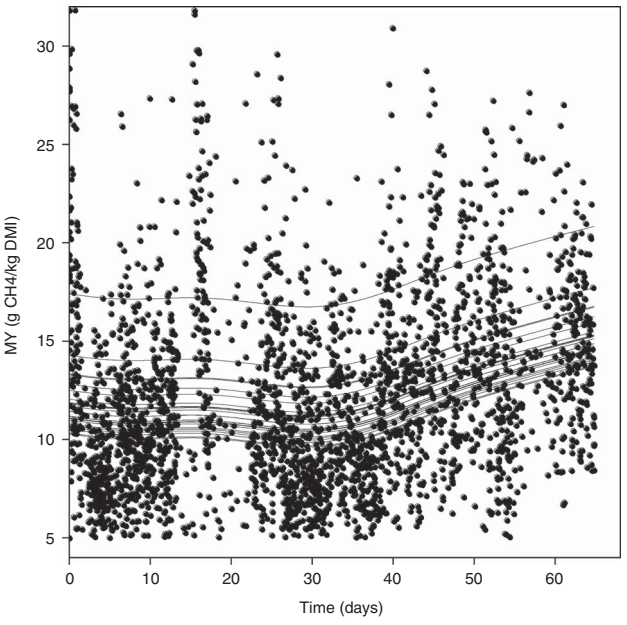

Figure 5 The fitted splines for estimated MY for individual beef cattle ($n = 24$) on a feedlot total mixed ration with splines for individual animals. MY = methane yield; DMI = dry matter intake.

MY was steady until around day 40. MY for the first 37 days averaged 11.5 g/kg DMI; for the last 26 days it showed a steady increase, with an average of 13.9 g/kg DMI. There was the same degree of between-animal variability as was noted with the 3-day model (a few animals had very high MY values). While these MY values are lower than other studies (e.g. Ramin and Huhtanen, 2013) they match observed data (MY 13.6 g/kg DMI) for similar concentrate diets (Hegarty *et al.*, 2007) and with these same 24 animals measured in respiration chambers on the same diet (MY 15.5 g/kg DMI; Herd *et al.*, unpublished data) 2 months later. The accuracy of using GEM has been shown (Velazco *et al.*, 2014) through comparison with respiration chambers, so there is no reason to doubt the accuracy of the GEM as the CO_2 recoveries were high (95.9% and 97.9% of the gravimetrically determined quantities of CO_2 released into the shroud were recovered by the two GEM units used in the experiment). All model methane estimates and the average of spot estimates were lower than those predicted for feedlot cattle using Moe and Tyrell (1979). As noted previously, the equation of Moe and Tyrell (1979) has been shown to have a very high error of prediction, a high general bias and explain a relatively low proportion of variance in methane production (Benchaar *et al.*, 1998; Ellis *et al.*, 2007).

Table 3 *Average methane yield (g CH_4/kg DM intake) of beef cattle on feedlot rations estimated by solver, splines and DoE (2014) equations*

		MY		
Tag	Sex	Solver	Spline	DoE (2014)
H077	H	10.4	11.4	18.0
H094	H	9.8	11.6	17.6
H119	H	9.5	11.0	17.6
H146	H	11.0	11.5	17.9
H164	H	9.3	11.0	16.9
H187	H	10.7	11.4	18.0
H190	H	17.8	17.8	21.7
H191	H	10.3	11.2	18.6
H199	H	12.8	13.8	17.6
H237	H	11.0	11.6	18.2
H242	H	11.6	12.1	18.2
H249	H	10.7	13.9	18.1
H251	H	11.3	11.7	18.7
H277	H	12.0	12.2	17.8
H281	H	11.6	12.4	18.3
H283	H	11.9	12.1	18.3
H285	H	10.6	10.6	18.3
H129	S	13.0	13.5	19.5
H222	S	10.7	11.5	17.8
H255	S	12.1	12.9	18.6
H260	S	11.1	11.9	17.4
H262	S	12.2	11.5	17.4
H279	S	14.2	14.7	19.0
H300	S	13.2	13.8	18.9

DM = dry matter; MY = methane yield; H = heifer; S = steer.

A comparison of the solver, average spline and DoE (2014) estimates of MY for each animal are shown in Table 3. The correlation between solver and spline MY estimates was 0.91 and with DoE (2014) MY estimates was 0.83. The correlation of spline and DoE (2014) MY estimates was slightly lower at 0.78.

Power analysis

The precision of estimated DMP or MY values, with or without available feed intake data, depends on the inter-play between the different sources of variation and the numbers of replicates within each component. Taking our DMP estimate based on spot emission data as an example, we first fitted a 'base' mixed model with no fixed effects and 'animals' and 'days' as the random effects (to estimate their variance components). As listed in Table 4, the residual variances were large (*v.* their respective standard errors). As shown previously (Figure 4), however, 'days' do not appear to be a simple random effect, as there is an increasing trend in DMP over time. Table 4 also shows the variances for a second mixed model where the observed trend is accounted for by including a linear term as the fixed effect.

Table 4 *Variance components (SE) from the GLM models of DMP (g CH_4/day) and MY (g CH_4/kg DM intake) of beef cattle on feedlot rations*

	DMP		MY	
Fixed effect	Nil	Days (linear)	Nil	Days (linear)
Animal	160 (52)	164 (53)	2.69 (0.83)	2.67 (0.83)
Day	935 (176)	349 (71)	6.51 (1.23)	5.48 (1.05)
Residual (between-measures; within-animals and within-days)	1689 (45)	1687 (45)	14.84 (0.40)	14.84 (0.40)

DMP = daily methane production; MY = methane yield; DM = dry matter. Residual variance includes variance components other than between-animal and between-day variances.

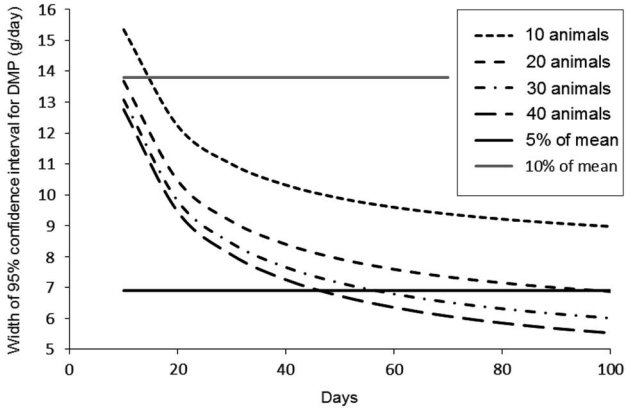

Figure 6 Estimated width of 95% confidence intervals (either side of mean) for DMP of beef cattle *v.* numbers of days for different numbers of animals (dashed lines) and the targeted 5% or 10% of the observed mean (solid black and grey lines, respectively). Two spot measures of methane production rate per day are assumed. DMP = daily methane production.

Removing the trend in days reduced the estimated variance components for days from 935 to 349 for DMP and 6.5 to 5.5 for MY. Even when de-trended, the variance components for days are notably larger than those for animal. Other cattle methane studies (e.g. Blaxter and Clapperton, 1965; Boadi *et al.*, 2002; Harper *et al.* 1999; Pinares-Patino *et al.*, 2003; Vlaming *et al.*, 2008) indicate that between-day variance in DMP is more likely to be closer to our lower value (i.e. when the effect of our linear trend is removed), so we used the between-day variance value of 349, rounded to 350, in a power analysis of experimental designs for DMP estimated from spot measurements.

For the power analyses of DMP, we rounded the linear trend values to 1700 for the residual variation and 160 for the variance components for animals. The power analysis investigated the precision of the estimated mean using the 95% two-tailed confidence interval, taking the variance formulae in Cox and Solomon (2003) as shown below:

$$\text{SE (mean)}\sqrt{[\sigma^2(n_a \times n_d \times n_r) + \tau_a / n_a + \tau_d / n_d]}$$

where σ^2 is the residual variance, n_a, n_d and n_r, respectively, the numbers of animals, days and replicates and τ_a and τ_d the variance components for animals and days, respectively.

The targeted precision was 5% of the estimated 64 days mean DMP or MY. Hence, we expect 95% power for any future experiments with combinations of numbers of animals, days and replicates where this target is met. Figures 6 and 7 show these patterns for DMP and MY, respectively, assuming two spot measures are made per-animal per-day (as occurred in this study).

As shown in Figure 6 it is realistically infeasible to achieve the targeted precision for DMP from a population of only 10 animals. With 20 animals 98 measurement days are needed. Doubling the number of animals to 40 results in 47 days being required (a time reduction of 52%).

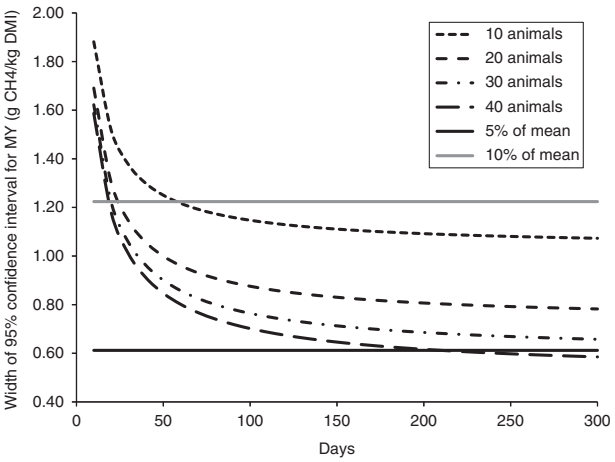

Figure 7 Estimated width of 95% confidence intervals (either side of mean) for daily MY *v.* numbers of days, for different numbers of beef cattle (dashed lines) and the targeted 5% or 10% of the observed mean (solid black and grey lines, respectively). Two spot measures of methane production rate per day are assumed. MY = methane yield; DMI = dry matter intake.

Conversely, for a 50-day trial 36 animals are needed to achieve the targeted precision; *v.* 20 animals for a 100-day trial (a 44% reduction in the number of animals). So while the between-day variation was higher than between animals, the overall differences in the required numbers of days or animals are not all that pronounced, so the numbers of animals and days are reasonably interchangeable in respect to power of the test. They both contribute equally to reducing the residual variance. The design of each future experiment will, of course, depend on the available budget and logistic limitations, but our formula and figures can be used as a guide for experimental design.

The maximum number of spot measures per-animal per-day (n_r) is set by the researcher but whether cattle utilise all the measuring opportunities is the animal's choice. However, increasing n_r only had a relatively minor effect here – for 20 animals, the required number of days to achieve target precision only reduces from 98 (for $n_r = 2$) to 91 for $n_r = 5$ and to 89 for $n_r = 10$. For 40 animals, the required numbers of days were 47, 45 and 44 for $n_r = 2$, 5 and 10 spot measures per day, respectively. It would seem prudent for future researchers to perhaps assume n_r of 2, knowing that if they do achieve a higher number this will improve their precision (but only slightly). Therefore, in summary, the precision of DMP and MY improves rapidly as the number of animals multiplied by days increases up to 30 and obtaining more measures per day (which is more difficult to control as it depends on animal behaviour) has little effect on precision.

Feed events known

In overview, a strong association between DMP and daily DMI was apparent in keeping with published assessments (Kennedy and Charmley, 2012; Ramin and Huhtanen, 2013), however, a key objective of this study was assessing the association of individual spot measures of enteric emissions with feeding history. Our analyses found that if only the time of the preceding feed event is recorded before spot methane measurements then taking the arithmetic average of all spot measures is not much inferior to fitting various time models, such as the Wood, Dijkstra or one dose models. Of those models tested, the one-compartment dose model fitted as well as any and had the advantages of the fitted curve parameters being more easily numerically integrated to estimate areas under the curve and having a shape consistent with the expected biochemistry.

Including data (time and amount) of all feed events in the 3 days before each spot measurement, which consisted of up to 71 feed events per 3 days and obtaining a least squares solution for DMP or MY reduced the (underestimation) bias of the data but still left most variance in DMP or MY unexplained by the model. It is suggested that when feed events are recorded, that use is made of the data for all feed events and not just those immediately before methane measurements. The fitting of splines further improved the goodness of fit and identified an increasing trend in MY as the trial progressed. Spines were fitted because of the variable nature of the methane emissions during the trial. All independent variables (DMP, DMI and MY) were converted to a 'per-animal per-day' basis. The fitted terms in the statistical model were 'animal' (notably different) and 'days'. DMP rose steadily following the patterns in DMI, while MY increased during the trial. The causes of this increase in MY are not known. One possibility is that while daily DMI increased over the first 30 days, periods of rumen acidification may have suppressed methanogenesis as methanogens are sensitive to acidity (Russell, 1998). Once daily feed intake stabilised, a more neutral pH could have been maintained allowing methanogens to increase and methanogenesis and MY to progressively rise.

The alternate (and novel) approach of modelling the measured patterns of DMP after each feed event using Solver was studied because the analysis was not a simply derived regression. Instead, the contributions from each feed event were summed (for each animal) by best-fitting the one-compartment dose model. The equation (Supplementary Figure S1) is based on each kg of intake, and the integration (over 3 days, which was the time period found necessary) resulted in an average MY value of 12.1 (g CH_4/kg of intake). The spline and solver approaches gave similar answers; but the more traditional spline method identified patterns in the measurements over time.

Feed events unknown

With grazing animals, the timing and size of feed events is typically unknown (Cottle, 2013). For this situation, we have provided the estimates of variance components and calculations for estimating how many animals and days of sampling would be needed to estimate sample means of DMP and MY with a desired precision. This data can then be used to calculate the sampling regime required to detect a desired percentage difference in emissions between treatment groups.

The power analyses suggested that spot measurements made over a 70-days period, as is used in RFI tests, would be sufficient to estimate treatment means of DMP and of MY to a precision of within 5% to 10% of the true mean. Spot measurements of enteric emissions can be used to define DMP but the number of animals and samples are larger than are needed when day-long measures are made, such as in respiration chambers.

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Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/10.1017/S1751731115001676

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