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Variation in enteric methane emissions among cows on commercial dairy farms

M. J. Bell, S. L. Potterton, J. Craigon, N. Saunders, R. Wilcox, M. Hunter, J. R Goodman and P. C. Garnsworthy†

School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

† Corresponding author: Phil Garnsworthy. E-mail: Phil.Garnsworthy@nottingham.ac.uk

Running Head: Variation in enteric methane emissions

Abstract

Methane (CH_4) emissions by dairy cows vary with feed intake and diet composition. Even when fed on the same diet at the same intake, however, variation between cows in CH_4 emissions can be substantial. The extent of variation in CH_4 emissions among dairy cows on commercial farms is unknown, but developments in methodology now permit quantification of CH_4 emissions by individual cows under commercial conditions. The aim of this research was to assess variation among cows in emissions of eructed CH_4 during milking on commercial dairy farms. Enteric CH_4 emissions from 1,964 individual cows across 21 farms were measured for at least 7 days per cow using CH_4 analysers at robotic milking stations. Cows were predominantly of Holstein Friesian breed and remained on the same feeding systems during sampling. Effects of explanatory variables on average CH_4 emissions per individual cow were assessed by fitting a linear mixed model. Significant effects were found for week of lactation, daily milk yield and farm. The effect of milk yield on CH_4 emissions varied among farms. Considerable variation in CH_4 emissions was observed among cows after adjusting for fixed and random effects, with the coefficient of variation ranging from 22 to 67% within farms. This study confirms that enteric CH_4 emissions vary among cows on commercial farms, suggesting that there is considerable scope for selecting individual cows and management systems with reduced emissions.

Keywords: Dairy cows, enteric methane, variation, commercial farms

Implications

Abatement of enteric methane emissions from livestock has gained interest due to the association between greenhouse gas concentrations in the atmosphere and climate change. New technologies offer a low cost and repeatable means of assessing variation in enteric methane emissions among individual animals under commercial conditions. This study provides, for the first time, an assessment of phenotypic variation in enteric methane emissions among dairy cows on commercial farms. Variation was explained largely by animal and farm factors, but considerable residual variation remained which suggests opportunities may exist for selection of animals and systems with lower emissions.

Introduction

Enteric methane (CH_4) is produced in the digestive tract by microorganisms called A*rchaea* as a by-product of anaerobic fermentation (methanogenesis). Enteric CH_4 is a significant source of greenhouse gas (**GHG**) emissions from ruminant livestock, accounting for >50% of greenhouse gas emissions from milk production (FAO, 2010). Interest in measuring enteric CH_4 emissions has moved from a focus on nutritional inefficiency (Blaxter and Clapperton, 1965) to one of contributing to GHG concentrations in the atmosphere and climate change (Johnson and Johnson, 1995; IPCC, 2007). Quantifying CH_4 emissions accurately is important for national inventories of GHG emissions and also for evaluating mitigation strategies.

Although a large proportion of the variation in enteric CH_4 emissions from dairy cows can be explained by diet composition and dry matter (DM) intake (Beauchemin *et al.* 2008; Bell and Eckard, 2012), there is additional variation in enteric CH_4 emissions among animals (de Haas *et al.,* 2011; Garnsworthy *et al.,* 2012a). Manipulation of the diet can alter potential production of CH_4 emissions immediately (Martin *et al*., 2010), whereas other mitigation options, such as breeding, may have potential to reduce CH_4 emissions in the medium to long-term. The extent of variation in CH_4 emissions among dairy cows on commercial farms is unknown, but such information would be invaluable for calculating uncertainties associated with GHG inventories and for identifying systems with potential to mitigate CH_4 emissions. Until recently, it has not been possible to measure CH_4 emissions on commercial farms. Garnsworthy *et al.* (2012b), however, developed a mobile and repeatable technique that can be used to measure enteric CH_4 emissions from individual dairy cows during milking on commercial farms.

The objective of this study was to assess variation in enteric CH_4 emissions among cows on commercial dairy farms and to identify major influences.

Material and methods

Data

Measurements of eructed CH_4 emissions during milking were obtained from 43,820 milkings of 1,964 individual cows on 21 commercial dairy farms between September 2011 and March 2013. Cows were milked individually at automatic (robotic) milking stations. During milking, cows consumed concentrates from an integral feed bin within the milking station. Methane released by the cow through eructation and breathing altered CH_4 concentration in the feed bin and a CH_4 analyser (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK) in each milking station recorded CH_4 concentration continuously for at least 7 days. For a full description of the technique see Garnsworthy *et al*. (2012b). The technique is briefly described below.

The CH_4 concentration measured by the analyser was logged at 1 second intervals on data loggers (Simex SRD-99; Simex Sp. z o.o., Gdańsk, Poland) and visualised using logging software (Loggy Soft; Simex Sp. z o.o.). The CH_4 analyser was calibrated using standard mixtures of CH_4 in nitrogen (0.0, 0.25, 0.50, 0.75 and 1.0% CH_4, Thames Restek UK Ltd., Saunderton, UK). A custom-designed program was used to identify and quantify peaks for eructed CH_4 concentrations during milking from raw logger data (Figure 1). For each milking, frequency of CH_4 peaks (eructation rate) was multiplied by area under the curve (integral) of CH_4 peaks to calculate a CH_4 emission index in milligrams per litre of ambient air sampled by the analyser. The CH_4 emission index was converted to concentration of CH_4 emitted by the cow by an estimate of the dilution of eructed air. The dilution factor was determined once at the end of each sampling period for each robotic milking station and varied from 11.2 to 43.7. A fixed volume (2.7 l) of 1.0% CH_4 in nitrogen was released at 2 locations in the feed bin of the milking station, which were: at the base of the trough and at the centre of the feed bin level with the sample tube. Release of CH_4 was replicated 3 times at each location, with the dilution factor being the mean ratio of 6 values of CH_4 concentrations in released and sampled gas. The CH_4 emissions were calculated by equation [1]:

$$CH_4 \text{ (mg/l)} = (\text{average integral of } CH_4 \text{ per eructation x frequency of eructations}) \text{ x dilution factor} \quad [1]$$

Milkings with less than 3 eructations were excluded from the analysis.

Cows were predominantly of Holstein Friesian breed and remained on the same feeding system during the sampling period. A summary for each farm of the number of cows, diet, average milk yield, live weight and CH_4 records is shown in Table 1. Cows were fed *ad libitum* and diets fed were classified as either a partial mixed ration (i.e. conserved forage and concentrate feed; PMR) or a PMR with grazed pasture. All cows received concentrate feed during milking with the amount dependent on the cow's daily milk yield. At farm I, CH_4 emissions of cows were measured during two consecutive periods; in the first period cows were fed on a PMR whilst housed, and in the second period the same cows were fed on grazed pasture with free access to a PMR indoors.

Ambient temperature was recorded in each milking station every 5 minutes using a data logger (MSR145-B51010; Omni Instruments Ltd., Dundee, UK).

Statistical analysis

Data were analysed using Genstat Version 15.1 (Lawes Agricultural Trust, 2012). A linear mixed model was used to assess effects of explanatory variables on average CH_4 emissions (mg/l) per individual cow. Each variable was analysed in a univariate analysis. The most significant variables from the univariate analyses were added first to a multivariate model and only those variables that made a significant ($P<0.05$) additional contribution when fitted last were retained. Robotic milking station within farm was included as a random effect and covariates were centred to a zero mean. Variables that had confounding effects between each other were tested by running the model with and without each variable; any variable showing a significant effect when fitted last was retained. The explanatory variables assessed were: farm, season (1 = October to March, 2 = April to September), average number of milkings per day, lactation number (1, 2, 3, 4 and 5+), week of lactation at start of sampling (1, 2, 3,..., 50), age at calving (months), average daily concentrate DM intake, live weight, daily milk yield, diet (PMR or PMR plus grazed pasture) and average ambient temperature during milking. At farm I only, cows had CH_4 emissions measured while being fed on a PMR and on grazed pasture with PMR.

Of the explanatory variables assessed, the model that best described the average CH_4 emissions ($Y_{ijk,}$ mg/l) of individual cows was equation [2]:

$$Y_{ijk} = \mu + W_i + b_1M \times F_j + F_j.R_k + E_{ijk} \qquad [2]$$

where, μ = overall mean; W_i = fixed effect of week of lactation; b_1M = linear regression of Y on daily milk yield; F_j = fixed effect of farm; $F_j.R_k$ = random effect of milking station within farm; E_{ijk} = random error term. To account for the random effect of multiple milking stations within farms ($F_j.R_k$) on CH_4 emissions, milking stations were numbered and the station visited at each milking was recorded. The milking station each cow visited most frequently was determined and included in the model as a factor. In the multivariate model, residual variance estimates were allowed to differ among farms. Using the data for farm I only, differences between cows and diets, and repeatability of CH_4 emission phenotype, were assessed using equation 2 with individual cow added as a random effect and without the effect of farm fitted in the model. The repeatability of the CH_4 emission phenotype was assessed by σ^2 animal / σ^2 animal + σ^2 residual, where σ^2 is the variance component. The residual coefficient of variation was calculated from variance components as root mean square error divided by the estimated mean.

Spearman's rank correlation was used to assess repeatability and ranking of CH_4 emissions from the same cows at farm I when fed on a PMR only or PMR with grazed pasture.

Results and Discussion

Across the 21 farms studied, cows averaged 624 ± 78 kg live weight and cows were milked 2.3 ± 0.7 times per day, producing 27.9 ± 10.1 kg/day of milk (mean ± s.d.; Table 1). Eructed CH_4 emissions during milking were measured when cows were fed a PMR at 8 of the 21 farms, with a PMR with grazed pasture being fed at the remaining 14 farms; at farm I there were 74 cows fed both a PMR and PMR with grazed pasture during consecutive periods. The number of eructations per cow averaged 0.9 ± 0.1 per minute across farms.

Predicted mean CH_4 emissions ranged from 0.6 mg/l from cows at farm M to 4.5 mg/l for cows at farm F (Figure 2). The coefficient of variation estimated from variance components was on average slightly lower among cows fed a PMR (36.5%) compared to cows fed a PMR with grazed pasture (39.0%) (Figure 2). This is in agreement with Vlaming *et al.* (2005), who found lower variation in enteric CH_4 emissions measured using the SF_6 measurement technique among individual housed dairy cows (21%) compared to grazing cows (31%). Generally, the current study found a greater coefficient of variation in CH_4 emissions (ranging from 21.8 to 66.8%) among lactating dairy cows on commercial farms (Figure 2) compared to the range of 3 to 34% in coefficient of variation found in studies using respiration chambers to measure emissions in research herds (Grainger *et al.*, 2007; Ellis *et al.*, 2007; Yan *et al.*, 2010). In contrast to enteric CH_4 measured in chambers, the current study used a technique that takes repeated measurements of enteric CH_4 emissions from individual animals in their normal environment. It is to be expected that the controlled conditions imposed on cows in respiration chambers will reduce variation (Garnsworthy *et al.,* 2012a).

Effect of week of lactation, milk yield and farm on CH_4 emissions

Significant effects on CH_4 emissions were found for week of lactation, daily milk yield, farm, and the interaction between daily milk yield and farm (all $P<0.001$) (Table 2).

Emissions of CH_4 increased over the first 20 weeks of lactation, but were relatively constant from week 21 to week 50 of lactation (Figure 3); ranging from 2.2 mg/l at week 1 to 3.2 mg/l at week 50. This is in agreement with the findings of Garnsworthy *et al.* (2012a). The effect of week of lactation on CH_4 emissions may be explained by changes in amount and composition of feed consumed; CH_4 emissions are positively related to DM intake and negatively related to proportion of concentrates in the diet (Beauchemin *et al.*, 2009); DM intake is typically lower, and contains a higher proportion of concentrate feed in early lactation than later in lactation.

On average, mean CH_4 emissions increased with increasing daily milk yield ($b_1 = 0.02$, $P < 0.001$; Table 2), reflecting the positive relationship between milk yield and DM intake. However, there was a significant interaction between effects of daily milk yield and farm ($P < 0.001$; Table 2, Figure 4); CH_4 emissions increased with increasing daily milk yield at most farms, but decreased with increasing daily milk yield at farms M, P, S and U. Different responses among farms in CH_4 emissions with increased milk yield may reflect differences in feeding regimes and energy utilisation by cows. For example, the greatest positive association between CH_4 emissions and milk yield was found for Farm R and the greatest negative association at Farm S. On both farms the feeding regime was a PMR plus concentrates fed in the milking station according to milk yield. Concentrates with high starch or fat concentrations can reduce CH_4 emissions, particularly at high feeding levels (Beauchemin *et al.*, 2009); concentrates at both farms had similar starch concentrations (205 g/kg DM), but concentrates at Farm R had a lower fat concentration (50 g/kg DM) than concentrates at Farm S (62 g/kg DM). Furthermore, the PMR at Farm R consisted of grass silage and whole-crop wheat silage, whereas the PMR at Farm S was based mostly on maize silage, but also contained whole linseed meal, which is known to suppress CH_4 emissions (Beauchemin *et al.*, 2009). Thus, increased milk yield at Farm S would result in increased DM intake, but each incremental kg of feed would contain a greater proportion of concentrates, and total intake of a CH_4 inhibitor (whole linseed) would increase. Differences between farms may also be due to the observation that effects of feeding level and energy efficiency on enteric CH_4 emissions are independent (Yan *et al.*, 2010).

Although CH_4 emissions increased overall with increasing milk yield, emission per kg of milk decreased as milk yield increased. The reduction in emissions per unit milk may be due to a combination of a higher proportion of concentrate feed in the diet reducing methane per unit of feed intake (Beauchemin *et al.*, 2008; Bell *et al.*, 2010) and an increased efficiency of energy utilisation by dilution of maintenance energy requirements. Dillon (2006) found that if cows are to meet their genetic potential for milk production, they need to maximise feed intake, which can be achieved using a more digestible total mixed ration (conserved forage and blended concentrate mix) rather than pasture. In the present study, cows were fed concentrate feed during milking in addition to the non-forage component in the PMR. The amount of concentrate fed during milking depended on the cow's milk production. Concentrate feed is known to have a curvilinear effect on fibre digestion, resulting in a depression in CH_4 emissions per unit intake (Reynolds *et al*., 2011).

Overall, after adjusting CH_4 emissions for significant fixed effects (equation [2]) there was considerable residual variation in CH_4 emissions among cows within farms (Figure 5). Extent of within-farm residual variation varied between farms; notably, farms C to J and P to T were associated with a greater range of residual values compared to other farms. Because many unquantified factors vary between farms, such as management practices, building design, feed sources and cow genetics, it is not possible to explain differences in residual variation. Further research is needed to explore some of these factors and thereby improve predictions of on-farm CH_4 emissions.

Repeatability and effect of diet on CH_4 emissions

Mean CH_4 emissions for 74 cows at Farm I were highly repeatable, whereby cows fed on a PMR had a high and positive rank correlation ($r = 0.86$, $P<0.001$) with CH_4 emissions measured when the same cows were fed on a PMR with grazed pasture (Figure 6). Repeatability of the CH_4 emission phenotype from variance components was high at 0.89 and the coefficient of variation was 27.3%. No significant effect of diet, when classified as PMR or PMR with grazed pasture, on CH_4 emissions was found between all farms or within Farm I. In the study of Garnsworthy *et al.* (2012b), approximately 50% of variation in emission rate per unit intake was explained by differences between diets (effects of DM intake and diet composition). It is well recognised that DM intake and diet composition (digestibility, fat, energy and carbohydrate content) have large effects on enteric CH_4 emissions (Mills *et al.*, 2003; Ellis *et al.*, 2007) and hence these are common variables in empirical prediction equations (Bell and Eckard, 2012). The lack of an effect of diet type on CH_4 emissions in the current study would suggest that more detailed information on diets was needed. Or it might be that the diets fed were of high quality and that variation in CH_4 emissions was largely explained by the effect of feed intake level (described by week of lactation and milk yield).

The average CH_4 concentration across farms in the current study was 2.9 mg/l (Figure 2) which, estimated by the equation of Garnsworthy *et al.* (2012b), would equate to 418 g/day of eructed CH_4 (CH_4 g/day = 252 + 57.2 × 2.9 mg/l). This value is within the range reported for dairy cows by Grainger *et al.* (2007) of 220 to 480 g/day.

In conclusion, this study demonstrates that there is considerable variation in CH_4 emissions among commercial dairy cows. Differences within farms in CH_4 emissions can be explained by week of lactation, daily milk yield, farm, and the interaction between milk yield and farm. Differences between farms in mean CH_4 emissions, and in variation within farm, are inevitably confounded by factors such as location, diet, management, genotype, and instrument installation. Nevertheless, the findings of this study suggest that there is scope for selecting individual cows and systems that have the potential to produce lower enteric CH_4 emissions at any level of milk output.

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Table 1 *Mean (s.d.) live weight, milk yield, number of milkings per day, and methane concentr*
(A to U) for cows fed on diets consisting of partial mixed rations (PMR), or PMR with grazed p

Farm	Diet	No. of cows	Live weight	Milk yield	Milkings per day	Methan
			kg	kg/d	No. per cow	
A	PMR	53	-	27.8 (8.9)	2.5 (0.6)	
B	PMR + grazing	66	576 (77)	23.1 (7.4)	1.9 (0.6)	
C	PMR + grazing	51	642 (59)	28.1 (8.1)	2.0 (0.4)	
D	PMR + grazing	47	607 (62)	27.3 (10.0)	2.0 (0.6)	
E	PMR + grazing	66	626 (55)	28.3 (7.4)	2.5 (0.6)	
F	PMR + grazing	45	596 (71)	26.5 (7.5)	2.5 (0.5)	
G	PMR	116	624 (74)	25.5 (7.9)	2.3 (0.7)	
H	PMR + grazing	148	667 (65)	28.7 (8.2)	2.2 (0.6)	
I	PMR	77	-	26.8 (10.5)	2.9 (1.0)	
I	PMR + grazing	76	-	23.8 (9.0)	2.3 (0.9)	
J	PMR	96	594 (73)	26.8 (7.9)	2.1 (0.6)	
K	PMR and grazing	36		17.5 (5.6)	1.8 (0.5)	
L	PMR	222	665 (63)	29.5 (9.2)	2.4 (0.8)	
M	PMR	46	546 (44)	25.1 (4.6)	3.1 (0.7)	
N	PMR	156		24.7 (7.2)	2.2 (0.6)	
O	PMR	55	691 (62)	28.7 (7.9)	3.0 (0.8)	
P	PMR	110	603 (72)	35.3 (9.1)	2.4 (0.6)	
Q	PMR	104	597 (84)	23.5 (9.1)	2.8 (0.9)	
R	PMR	80	577 (72)	18.8 (6.8)	2.6 (0.7)	
S	PMR	28	-	29.8 (10.5)	2.3 (0.7)	
T	PMR	253	-	34.7 (13.3)	2.0 (0.5)	
U	PMR	33	664 (78)	35.5 (8.0)	2.4 (0.6)	
All		1964	624 (78)	27.9 (10.1)	2.3 (0.7)	

[1] Methane emission during milking is the mean product of eructation frequency, integral of methane concent factor of eructed gas.

Table 2 *Significant explanatory variables for CH_4 emissions during milking (mg/l) among lactating dairy cows from multivariate analysis*[1]

Variable	Effect (s.e.)	df	F statistic	s.e.d.	P value
Week of lactation[2]		49	3.9	0.2	<0.001
Milk yield (kg/day)	0.02 (0.01)	1	56.8		<0.001
Farm[3]		20	5.3	0.8	<0.001
Milk yield × farm		20	2.49	0.03	<0.001

[1] Linear mixed model with milking station within farm added as a random effect and covariates centred to a zero mean.

[2] Weeks 1 to 50 of lactation, with predicted means presented in Figure 3.

[3] Farms A to U, with predicted means presented in Figure 2.

Figure 1 Concentration of CH_4 in parts per million visualised by the data logging software during a 40 minute sampling period at farm B showing eructations during milking for three cows which milked sequentially.

Figure 2 Predicted mean (with s.d. bars) CH_4 emissions during milking for cows at farms A to U after adjusting for effects of week of lactation, daily milk yield and farm.

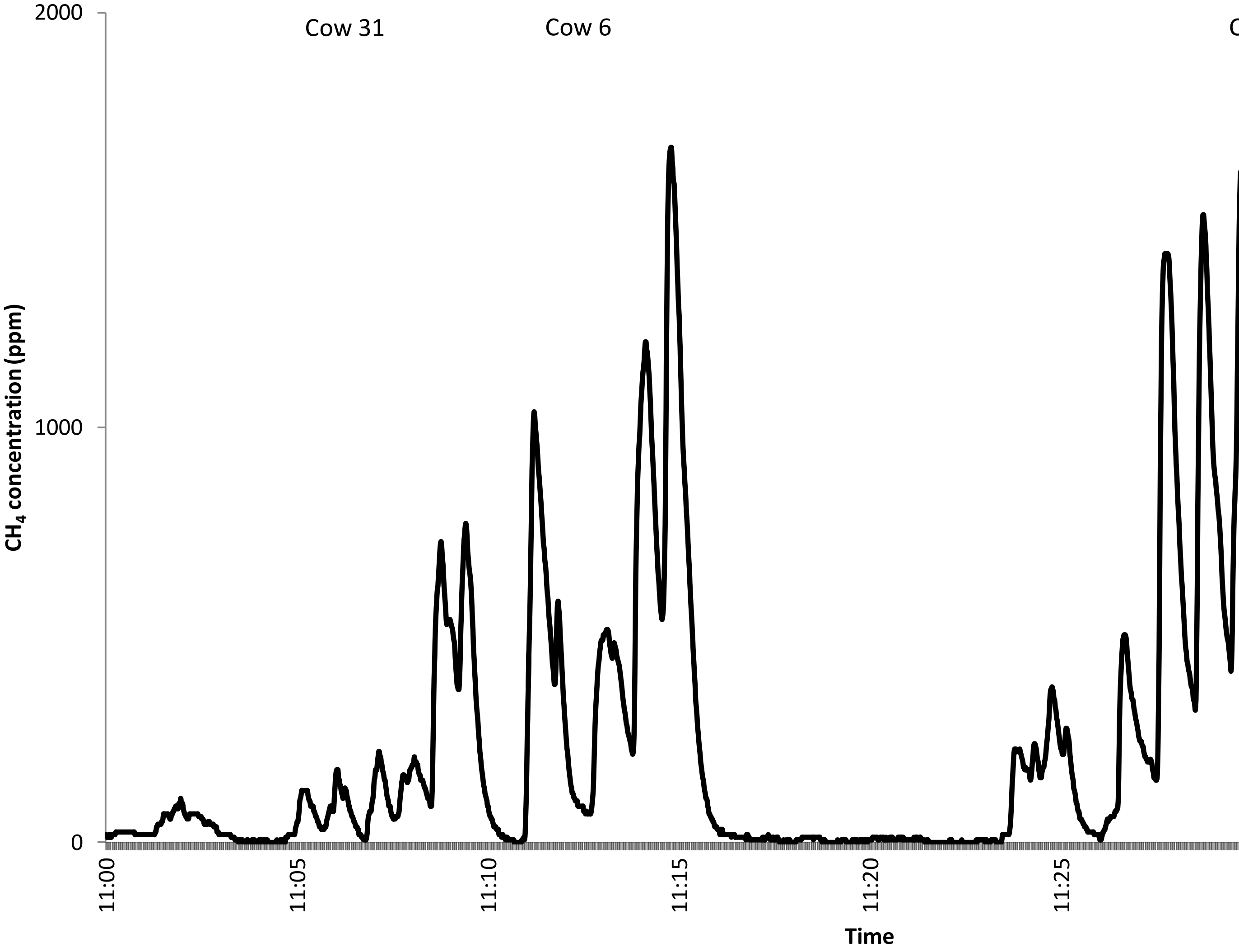
Figure 3 Change in predicted mean (with s.e. bars) CH_4 emissions during milking with week of lactation after adjusting for effects of week of lactation, daily milk yield and farm; the line of best fit shown by the solid line: CH_4 (mg/l) = 3.0 – 1.02 × $(0.81^{\text{week of lactation}})$.

Figure 4 Regression coefficient (with s.e. bars) for effect of daily milk yield on CH_4 emissions during milking among individual cows at farms A to U after adjusting for the effects of week of lactation, daily milk yield and farm.

Figure 5 A box and whisker diagram showing the minimum, lower quartile, median, upper quartile and maximum residual CH_4 emissions during milking for individual cows after adjusting for effects of week of lactation, daily milk yield and farm at farms A to U.

Figure 6 Mean CH_4 emissions during milking for the same individual cows at farm I fed on PMR and subsequently on PMR with grazed pasture. The rank correlation (r) is shown with the line of best-fit.

Figure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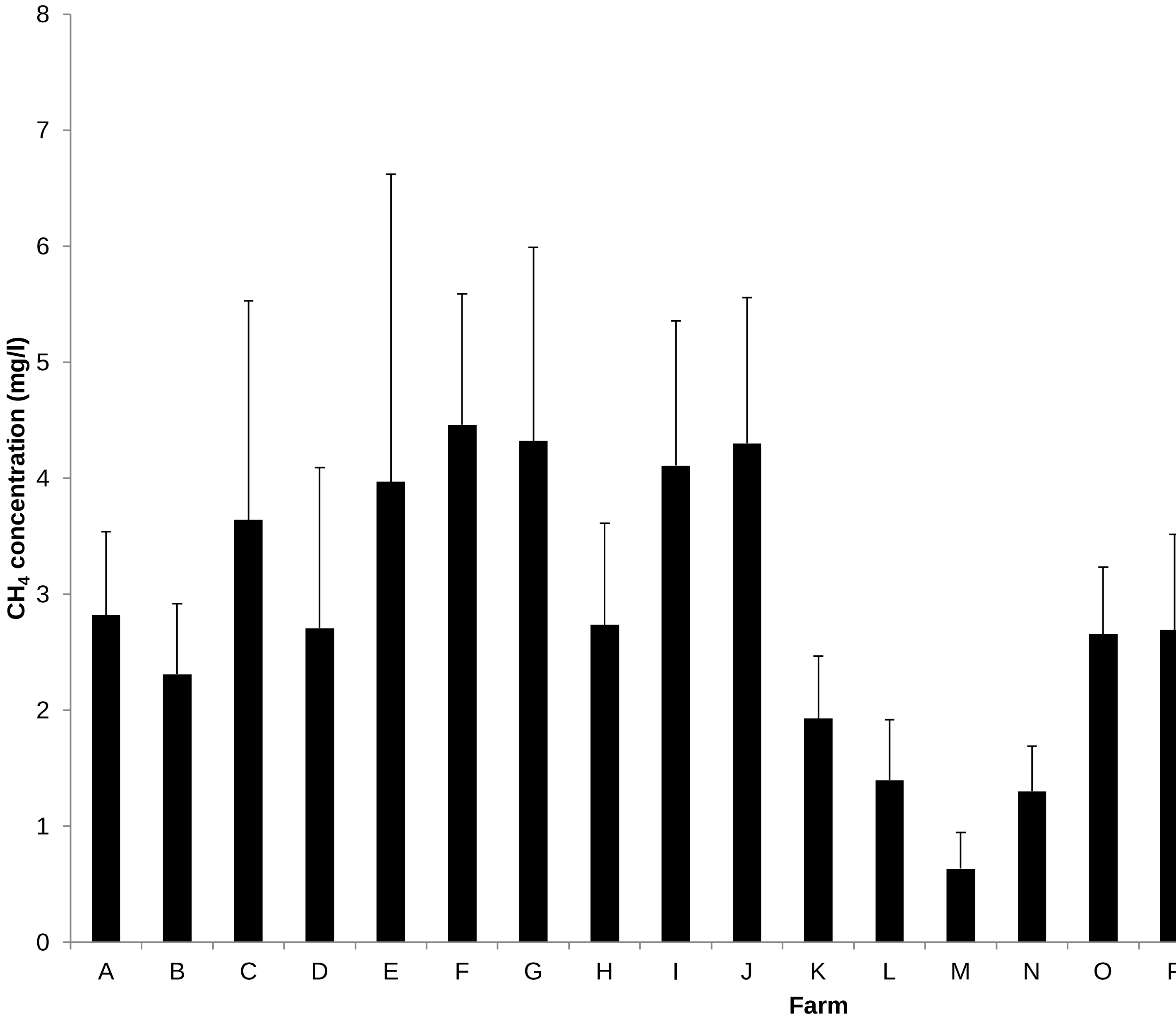
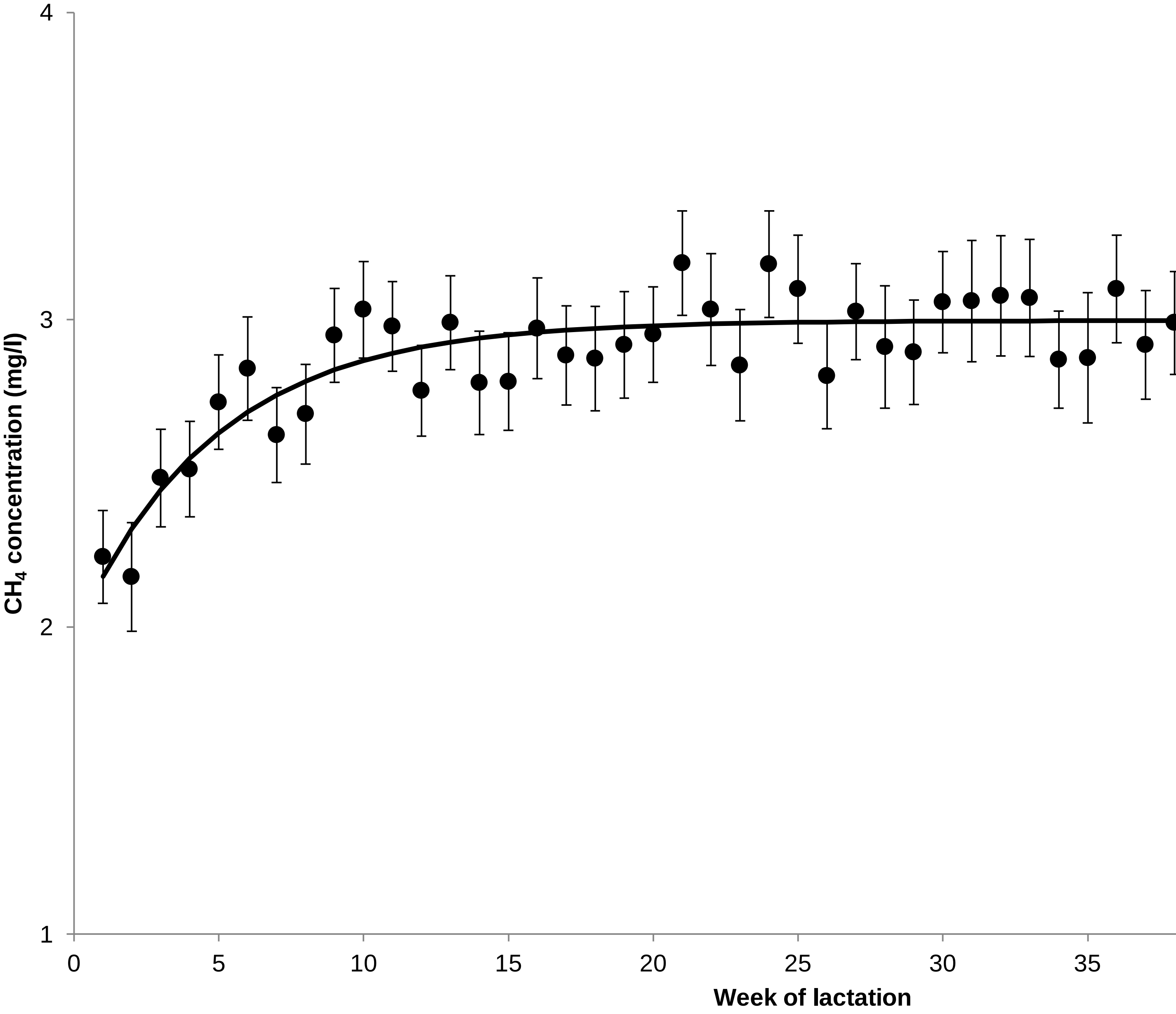
1000
0
Cow 31
Cow 6
C
CH$_4$ concentration (ppm)
11:00
11:05
11:10
11:15
11:20
11:25
Time

Figure



Figure



4
3
2
1
CH4 concentration (mg/l)
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5
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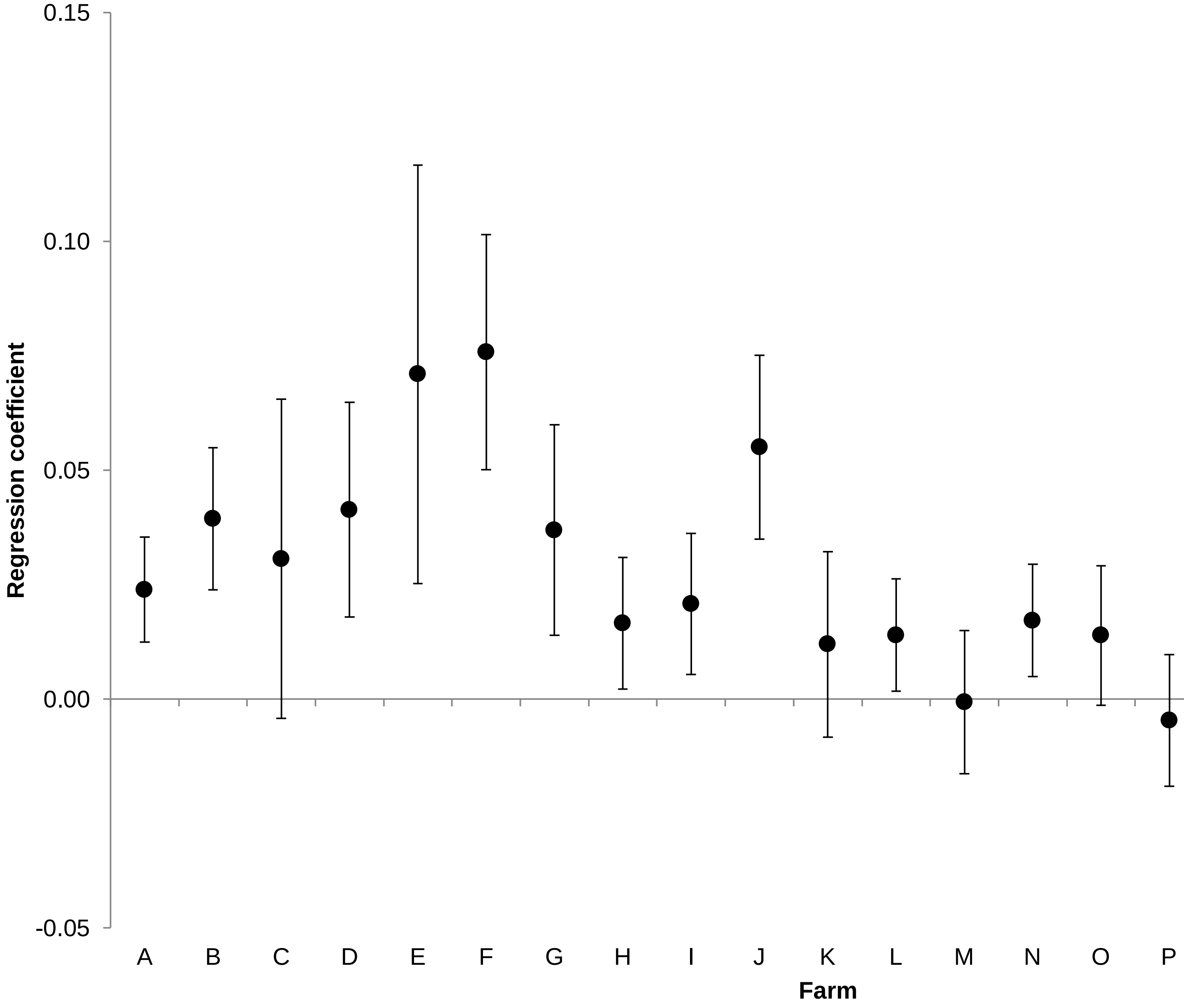
15
20
25
30
35
Week of lactation

Figure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Regression coefficient
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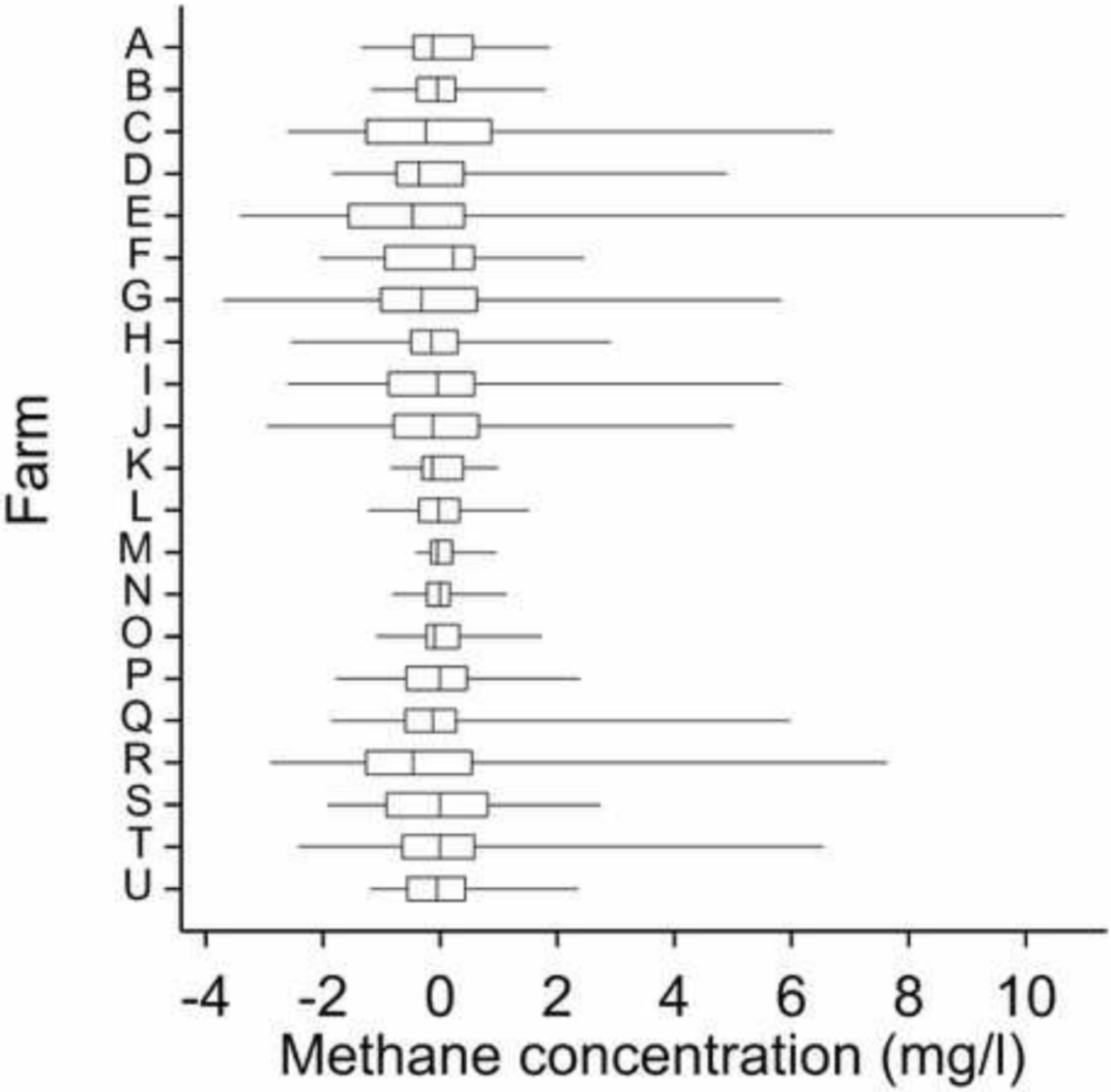
Farm


Farm
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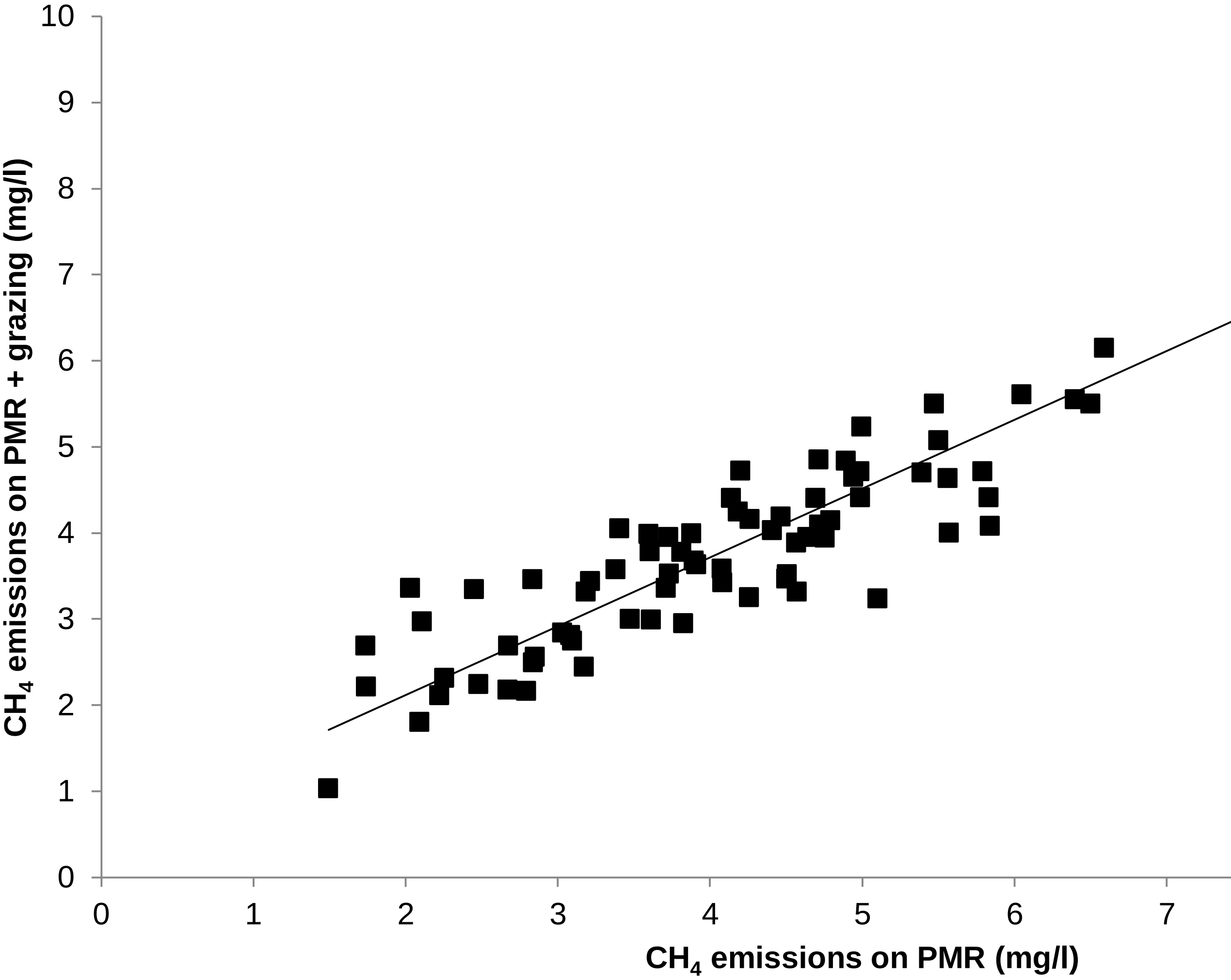
10
Methane concentration (mg/l)

Figure



10
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3
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7
CH_4 emissions on PMR + grazing (mg/l)
CH_4 emissions on PMR (mg/l)