**Equations to predict methane emissions from cows fed at maintenance energy level in
pasture-based systems**

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ABSTRACT

Ruminant production is a vital part of food industry but it raises environmental concerns, partly due to the associated methane outputs. Efficient methane mitigation and estimation of emissions from ruminants, requires accurate prediction tools. Equations recommended by international organizations or scientific studies have been developed with animals fed conserved forages and concentrates and may be used with caution for grazing cattle. The aim of the current study was to develop prediction equations with animals fed fresh grass in order to be more suitable to pasture-based systems and for animals at lower feeding level. A study with 25 nonpregnant nonlactating cows fed solely fresh-cut grass at maintenance energy level was performed over two consecutive grazing seasons. Grass of broad feeding quality, due to contrasting harvest dates, maturity, fertilisation and grass varieties, from eight swards was offered. Cows were offered the experimental diets for at least 2 weeks before housed in calorimetric chambers over 3 consecutive days with feed intake measurements and total urine and feces collections performed daily. Methane emissions were measured over the last 2 days. Prediction models were developed from 100 3-day averaged records. Internal validation of these equations, and those recommended in literature, was performed. The existing in greenhouse gas inventories models under-estimated methane emissions from animals fed fresh-cut grass at maintenance while the new models, using the same predictors, improved prediction accuracy. Error in methane outputs prediction was decreased when grass nutrient, metabolisable energy and digestible organic matter concentrations were added as predictors to equations already containing dry matter or energy intakes, possibly because they explain feed digestibility and the type of energy-supplying nutrients more efficiently. Predictions based on readily available farm-level data, such as liveweight and grass nutrient concentrations were also generated and performed satisfactorily. New models may be recommended for predictions of methane emissions from grazing cattle at maintenance or low feeding levels.

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49 **Keywords:** Grazing, methane, prediction, mitigation, cattle, greenhouse gases, maintenance,

50 validation

1. Introduction

Ruminants have a special role in food production industry because of their exclusive ability to transform non-edible material (e.g. cellulose in grass) to foods of unique nutritional quality that provide a range of beneficial nutrients to humans, such as proteins of high biological value, fatty acids, vitamins, antioxidants and minerals (Cottle *et al.*, 2011; MacRae *et al.*, 2005). In addition, ruminants fed at pasture do not directly compete with humans for food (Buddle *et al.*, 2011). However, in previous publications from Food and Agriculture Organization of the United Nations (FAO) and Intergovernmental Panel on Climate Change (IPCC) livestock production, and in particular ruminants, have been associated with an undesirable increase of greenhouse gases (GHG), such as carbon dioxide, methane and nitrous oxide (FAO, 2010; IPCC, 2006). At global scale, milk production contributes 2.7% of total anthropogenic GHG emissions while total emissions attributed to dairy herds including transport activities, meat production from old or young fattened stock and draught power are estimated to about 4.0% of total anthropogenic GHG (FAO, 2010). Methane and nitrous oxide are the main GHG emitted from the dairy sector, representing over 50% and about 30-40% of total emissions respectively (FAO, 2010). In the United Kingdom (UK), emissions from agriculture sector represented 9.6% of total GHG emissions in 2011, thus showing a slightly higher contribution when compared with 1990 (8.4%), although emissions from agriculture were overall decreased by 19.7% during the same period (Salisbury *et al.*, 2013). A recent report showed that 30% of UK agricultural emissions (or 2.7% of total GHG emissions in UK) may be attributed to rumen enteric fermentation (Webb *et al.*, 2014). Methane is a by-product of fermentation of organic matter (OM; starch, cellulose, hemicelluloses, protein and other minor materials) by anaerobic bacteria, protozoa and fungi in the rumen, and secondarily in hindgut, while methanogenesis results on a 2-12% loss of gross energy intake (GEI) (Johnson and Johnson, 1995). The latter highlights methanogenesis' relevance not only

to the environmental impact of dairy herds, but also to cow feeding efficiency and farm profitability.

Previous reviews (Cottle *et al.*, 2011; Moss *et al.*, 2000; Pacheco *et al.*, 2014; Ulyatt and Lassey, 2001) reported that ruminant enteric methane production depends on dry matter intake (DMI), forage type, diet composition, individual feed components, animal physiological differences, genetic parameters and grazing and herd management. The accurate assessment of methane emissions from cattle is challenging because these are highly dependent not only on DMI and GEI but also on the type of energy-supplying nutrients. For example, grain supplementation or offering highly digestible grass may result on lower methane emissions per unit of GEI than a more fibrous forage-based diet (Cottle *et al.*, 2011). This implies accuracy of predicting methane emissions may be improved if different equations were developed for each production level (e.g. maintenance, growth, lactation) and type of diet (e.g. total mixed ration, pasture-based diets with high concentrate allowance, low-input grazing diets). The latter practice may also serve for the development of Tier 3 predictions, which is recommended by IPCC to substitute Tier 2 currently using GEI, which is calculated from standard models, and a standard methane conversion factor (methane energy output:GEI) (IPCC, 2006). For these modifications, any future proposed equations should be internationally peer-reviewed and shown to improve prediction accuracy (IPCC, 2006).

A number of studies have presented prediction equations for methane emissions from beef and dairy cattle using DMI, GEI, grass nutrient concentrations and digestibility, dietary components, physiological state, rumen volatile fatty acid concentrations, animal liveweight (LW) and milk composition as predictors (Blaxter and Clapperton, 1965; Ellis *et al.*, 2009; Moraes *et al.*, 2014; Ramin and Huhtanen, 2013; Yan *et al.*, 2000; Yan *et al.*, 2009). However, these models, as well as the equations recommended by IPCC (2006) and FAO (2010), have been developed using data collected with animals fed mainly conserved forage

and concentrates and, given the influence of the nature of feed ingredients on methane emissions, may be used with caution for grazing cattle. Pasture-based ruminant production is the most common management system in some cool and moist areas of the world capable of long grazing seasons, such as New Zealand, Ireland and parts of UK, United States of America and Australia, and the contribution of grazing animals to methane emissions from the agricultural sector in these countries is distinctively important (Ferris, 2007; Pacheco *et al.*, 2014). Therefore, there is a necessity for additional prediction tools which may be more suitable for grazing cattle, and within the different production levels, than the existing models. The main objectives of this study were therefore to (i) to examine relationships of methane emissions with grass nutrient content and digestibility parameters, energy concentrations and LW, using a broad range of fresh-cut grass quality, (ii) develop prediction equations for methane emissions from nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level, using feed, nutrient and energy intakes, LW and grass nutrient/energy concentrations and digestibility parameters as predictors either in single or multiple linear relationships, and (iii) to validate the existing and new equations using data from animals fed solely fresh-cut grass diets at maintenance feeding levels.

2. Materials and methods

The present study was performed under the regulations of Department of Health, Social Services, and Public Safety of Northern Ireland, in line with the Animal (Scientific Procedures) Act 1986 (Home Office, 1986).

2.1 Abbreviations

A, actual value; ADF, acid-detergent fibre; ADFI, acid-detergent fibre intake; CH₄, methane emissions; CH₄-E, methane energy output; DDMI, digestible dry matter intake; DEI, digestible energy intake; DM, dry matter; DMd, dry matter digestibility; DMI, dry matter intake; DOMD, digestible organic matter in dry matter; DOMI, digestible organic matter

intake; EE, ether extract; Eq., equation; FAO, Food and Agriculture Organization of the United Nations; FL, feeding level; GE, gross energy; GE_d, gross energy digestibility; GEI, gross energy intake; GHG, greenhouse gases; IPCC, Intergovernmental Panel on Climate Change; LW, animal liveweight; Max, maximum value observed; ME, metabolisable energy; MEI, metabolisable energy intake; Min, minimum value observed; MPE, mean prediction error; MSPE, mean-square prediction error; N, nitrogen; n, number of samples; NDF, neutral-detergent fibre; NDFI, neutral-detergent fibre intake; OM, organic matter; OMI, organic matter intake; P, predicted value; R², pseudo correlation coefficient; SD, standard deviation; UK, United Kingdom; WSC, water-soluble carbohydrates.

2.2 Experimental design

The current study presents results from two metabolism experiments of 300 daily measurements of dry matter (DM) and nutrient intakes and output in faeces and urine, and 200 daily measurements of gas emissions, which were carried out in two consecutive grazing seasons (2007 and 2008) and included 25 nonpregnant nonlactating Holstein cows fed solely fresh-cut grass at maintenance energy level. All experiments were carried out in the calorimetric chambers of Agri-Food and Biosciences Institute, Hillsborough, UK, which are designed for accurate recording of gas exchanges (methane and carbon dioxide emissions and oxygen consumption) as well as total daily feed intake and faeces and urine outputs. Animals were between 5 and 8 years old and their LW and body condition score are presented in Table 1. Maintenance ME requirements were estimated as 0.65 MJ per kg of metabolic body size (Agnew *et al.*, 2004). Grass feeding quality for accurately feeding animals at maintenance energy requirements was assessed daily by (i) measuring grass DM contents by microwaving at full power for 3-5 minutes and (ii) predicting grass ME contents by near-infrared spectroscopy (Agnew *et al.*, 2000), using a NIRSTTM 5000/6500 Feed and Forage Analyser (FOSS, Hillerød, Denmark). Daily grass ME concentrations were estimated as the average

value of the samples collected over the previous two consecutive days. For each individual cow, data on DM and nutrient intakes and outputs in feces and urine were average values of a 3-day (continuous) period and data on gas emissions were average values of the last two days of the same period; thus, a total of 100 records were used to develop prediction equations for methane emissions. A broad range of fresh-cut grass quality was offered to cows, as a result of harvesting eight perennial ryegrass swards of contrasting management practices, such as harvest season, fertiliser input, maturity stage and grass variety, which are highly influential to grass nutritive value.

In 2007, the trial consisted of two 7-week experimental periods, one period for a first regrowth sward, fertilised with 8 kg N ha⁻¹, and one period for a second regrowth sward, fertilised with 54 kg N ha⁻¹. In 2008, the trial consisted of six 7-week experimental periods for two primary growth, two first regrowth and two second regrowth swards, which were fertilised with medium or high amounts of nitrogen (45-40-35 kg N ha⁻¹ or 70-65-60 kg N ha⁻¹, respectively for each harvest date). In both years, primary growth was harvested between April and May, first regrowth between June and July and second regrowth between August and September; however, the second-regrowth late-maturity grass from one medium-fertilised sward in 2007 and one medium-fertilised sward in 2008 was assessed in early October. Swards were sown with five different mixtures of ryegrass varieties, including Merlinda, Spelga, Fetione, Aberstar, Aberzest and Magella. Details on the sward characteristics and management have been previously reported (Stergiadis *et al.*, 2015b).

Two groups of four cows (8 animals in total) were used per experimental period in 2007, with one group allocated in a low-fertilised sward and one group in a high-fertilised sward, and four groups of four cows in 2008 (17 animals in total due to one replacer cow used in one occasion), with two groups allocated in medium-fertilised swards and two groups in high-fertilised swards. Animals between the two groups used in 2007 and the four groups used in

2008 were balanced for age and LW. Cows had free access to water throughout the probationary and experimental periods. Each sward was initially harvested at a residual height of 4 cm and allowed to grow for 2-3 weeks prior to experimental grass feeding. When grass height was suitable to zero-graze (about 15 cm; week 3), the first group of four cows, which were under restricted grazing with no concentrate supplementation over the previous 2 weeks, was introduced to the experimental diet (solely fresh-cut grass). Between the 2nd and 4th week after commencing the experimental feeding animals were taken, in one or two different occasions, into the calorimetric chambers for consecutive 3-day periods, for (i) daily assessments of feed intake, and total feces and urine outputs and (ii) gas exchange measurements over the last 2 days. During the last week of this period, a second group of four cows, which was also under restricted grazing with no concentrate supplementation over the previous 2 weeks, was introduced to the experimental diet (fresh-cut grass of the same sward) and followed the same feeding/measurements pattern as the first group during the 5th to 7th week of the experimental period. Consequently, for each sward the first group of cows was offered grass of early growth (4-6 weeks mature) and the second group was offered grass of late growth (7-9 weeks mature). The individual daily data for each cow and 3-day periods into the calorimetric chambers were averaged on (i) a 3-day basis for feed intakes and faeces and urine outputs parameters and (ii) 2-day basis for gas exchange measurements, thus producing one or two data points (depending on whether the animal went into the chambers once or twice) for each cow per sward and year. Grass harvesting and feeding management practices, including methodologies to feed animals at maintenance energy levels, were described in detail by Stergiadis et al. (2015b; 2015c).

2.3 Measurements

The same data collection protocols were used across 2007 and 2008. Liveweight and body condition score were recorded before morning feeding at the first and last day in the

chambers. Assessment of body condition score was performed on a 1 (thin) to 5 (very fat) scale, as described by Mulvany (1977). Daily total feed intake was assessed by weighing offered grass in the feeders and grass refusals within 24 h. Grass DM content was assessed daily. Sample collection of grass, feces and urine, and sample preparation and chemical analyses has been carried out as described by Stergiadis et al. (2015c) and Mayne and Gordon (1984). Assessments on grass, faeces and urine nutrient concentrations were performed on a 3-d bulked sample for each chamber period. Calorimetric chamber design, operation and methane measurements were performed using techniques previously described by Munoz et al. (2012). Nutrient and energy digestibility was also measured with the same nonpregnant nonlactating dairy cows fed at maintenance energy level, during the chamber period. Grass ME concentrations were calculated by extracting energy output in feces, urine and methane from grass gross energy (GE) content. Methane energy output was calculated using the following equation: $\text{CH}_4\text{-E (MJ d}^{-1}\text{)} = 39.54 \times \text{CH}_4 \text{ (L d}^{-1}\text{)}$, assuming molar mass and gas volume for methane to be 16.04 g and 22.4 L mol⁻¹ respectively (Ramin and Huhtanen, 2013).

2.4 Statistical analysis

Analyses of data were carried out in GenStat (VSN International, 2013). Regression equations were developed using residual maximum likelihood (Robinson, 1987; Searle *et al.*, 1992) so that the potential random effects of cow, field, year, harvest period, fertiliser input, maturity stage and grass variety could be accounted for. Linear regression relationships, where the response variables were methane emissions (CH₄; g d⁻¹), methane energy output (CH₄-E; MJ d⁻¹) and ratios of CH₄-E per kg of DMI (CH₄:DMI) and CH₄-E per MJ of GEI (CH₄-E:GEI) and the explanatory variables were (i) DMI, OM intake (OMI), digestible DMI (DDMI), digestible OMI (DOMI), GEI, digestible energy intake (DEI), and metabolisable energy intake (MEI) in single linear relationships (part of which are graphically presented in Fig. 1), and (ii) DMI, GEI, DEI, MEI, LW, grass contents of nitrogen (N), water-soluble

carbohydrates (WSC), ether extract (EE), neutral-detergent fibre (NDF), acid-detergent fibre (ADF), ash, metabolisable energy (ME) and digestible OM in DM (DOMD) in multiple linear relationships, were developed (Tables 2, 3, 4 and 5). The first step was undertaken in order to produce easy-to-use models with only one predictor, relevant to feed intake and digestibility, while the second step offered the opportunity to improve prediction accuracy but in expense of model complexity, because models would also include energy and nutrient concentrations of fresh grass. For each equation developed in the current study, the optimum random model for each response variable was identified by fitting the same fixed effect model and the candidate models of the random variation, always including cow because it was the experimental unit, by using the change in deviance. The optimum random models identified from the linear regression analysis were used when developing multiple linear regression equations for the response variables. The significance of the explanatory variables fitted in the multiple linear regressions was assessed using the Wald statistic. All predictors used in the equations showed a significant effect according to the Wald statistic. The residual diagnostics of the final model were assessed using normality plots. An approximate R^2 , referred to as the pseudo correlation coefficient, was derived to represent the amount of variability explained. This was defined to be the squared correlation of the response and the fitted values. In the presented equations, a different combination of predictors were used, in order to improve applicability of equations to commercial and research scale, depending on the available data. An internal evaluation, according to previously described methods (Stergiadis *et al.*, 2015a, b), was carried out to validate prediction equations which were developed in the current study. For this, the whole dataset ($n = 100$) was split into two sub-datasets of $n = 67$ (two-thirds of total data) and $n = 33$ (one-third of total data), according to harvesting year, variety, harvest date and maturity stage. The first dataset was used to develop prediction equations for methane emissions and methane energy output by using the exact same models in terms of

random and fixed variables (Appendix; Tables A1 and A2), as those produced by using the whole dataset. The new equations, generated by this procedure, were validated against the remaining one-third of the main dataset to evaluate their prediction accuracy (Table 6). This evaluation was performed by the mean-square prediction error (MSPE) technique (equation I).

$$MSPE = \frac{1}{n} \sum (P-A)^2 \quad [I]$$

where P and A represent the predicted and actual values, respectively, and n is the number of pairs of P and A values compared. Mean prediction error (MPE) was calculated, to describe the prediction accuracy, using the following formula (equation II):

$$MPE = \sqrt{MSPE} / (\sum A/n) \quad [II]$$

The same one-third of the current dataset was also used to validate external equations (Appendix, Table A3) for the prediction of methane emissions and methane energy output, as presented by other authors (Blaxter and Clapperton, 1965; Ellis *et al.*, 2007; Mills *et al.*, 2003; Moraes *et al.*, 2014; Wilkerson *et al.*, 1995) or international organizations (FAO, 2010; IPCC, 2006) (Table 6). In order to quantify the agreement between actual (measured in calorimetric chambers) and predicted methane emissions and methane energy outputs, the Lin's concordance correlation coefficient was generated for all equations developed in the current study and those previously presented (Blaxter and Clapperton, 1965; Ellis *et al.*, 2007; FAO, 2010; IPCC, 2006; Mills *et al.*, 2003; Moraes *et al.*, 2014; Wilkerson *et al.*, 1995) and Bland-Altman plots were developed to depict part of this analysis (Fig. 2, 3 and 4).

3. Results

3.1 Description of the data used for the development of prediction equations

The mean, standard deviation, minimum and maximum measured values for animal data, grass nutrient concentrations, intakes and digestibilities, grass energy concentrations and

intakes and methane outputs are presented in Table 1. Variation for variables used in the development of prediction equations for methane emissions and methane energy output were relatively high. For example, the heaviest animal used was 292 kg heavier than the lightest animal used. Maximum measured grass nutrient concentrations were 1.4 (for NDF) to 4.5 (for WSC) times higher, with grass maximum contents of DM, N and EE being also over 3 times higher, when compared with the minimum values. Highest concentrations of GE, digestible energy and ME in grass were 9.6%, 35.1% and 42.2% higher than lowest concentrations, respectively. Maximum OMI and DOMI, and intakes of GE, digestible energy and ME were over 2 times higher than lowest intakes while this difference was marginally lower for intakes of DM (1.96) and DDMI (1.99). Maximum recorded DOMD and digestibility of DM (DMd) were about 30% higher than lowest recorded values. Methane outputs, depending on the unit they were expressed at, varied by 79.6-103.4% between the lowest and highest measured emissions.

3.2 Prediction of methane emissions and methane energy output from feed or energy intakes

When single linear predictions were developed for methane emissions using DMI, OMI, DDMI or DOMI (Table 2; Eq. 1a-1d) and for methane energy output using GEI, DEI or MEI as sole predictors (Table 2; Eq. 2a-2c), the effect of all explanatory variables was significant according to the Wald statistic. Methane emissions and methane energy output were positively correlated with all feed and energy intake parameters. The relationships between methane emissions and DMI or OMI and those between methane energy output and GEI are presented in Fig. 1. Lower MPE for the prediction of methane emissions was observed when gross intakes (of DM and OM) were used instead of digestible intakes and when OMI was used instead of DMI. Prediction of methane energy output showed lower MPE when GEI was used instead of DEI or MEI as sole explanatory variables while predictions based on DEI had lower MPE than predictions based on MEI.

3.3 Prediction of methane emissions and methane energy output from feed or energy intakes and LW

When multiple linear predictions were developed for (i) methane emissions using DMI, OMI, DDMI or DOMI as primary predictors and LW as secondary predictor (Table 3; Eq. 1e-1h) and (ii) methane energy output using GEI, DEI or MEI as primary predictors and LW as secondary predictor (Table 3; Eq. 2d-2f), the effect of all explanatory variables was significant according to the Wald statistic. Correlations between methane emissions and methane energy output with feed and energy intake parameters and LW were positive. Adding LW as a predictor in a prediction equation of methane outputs that already contains feed intake parameters did not improve or slightly increased MPE. Using gross intakes or OMI improved MPE when compared with using digestible intakes or DMI respectively. When LW was added as an explanatory variable in predictions of methane energy output, MPE was slightly improved when GEI or MEI were used as primary predictors but showed no change when DEI was the primary predictor. Similarly to the equations using energy intakes as sole explanatory variables to predict methane energy output, the lowest MPE was observed for the equation using GEI and LW, the highest for the combination MEI and LW, while using DEI and LW showed intermediate MPE.

3.4 Prediction of methane emissions and methane energy output from DMI or GEI, respectively, LW and grass nutrient, ME and DOMD contents

When multiple linear predictions were developed for methane emissions using DMI, LW and grass nutrient, ME and DOMD contents, (Table 4; Eq. 1i-1n) and (ii) methane energy output using GEI, LW and grass nutrient, ME and DOMD or DMd contents (Table 4; Eq. 2g-2k), the effect of DMI, GEI, LW and grass contents of NDF, WSC, ash, EE, ME and DOMD were significant in various equations according to the Wald statistic. Positive correlations between methane emissions and methane energy output with DMI, GEI, LW and grass WSC and

DOMD contents were observed but the two correlations with grass NDF, ash, EE and ME contents and DM digestibility were negative. Prediction accuracy was improved when grass nutrient, ME and DOMD contents were added as predictors in equations which already include LW and DMI or GEI. When DMI or GEI were used in combination with grass ME to predict methane emissions or methane energy output respectively, the addition of grass nutrient and DOMD contents improved MPE. The combination of DMI or GEI, with grass ME and DOMD showed the best prediction accuracy, followed by the equation in which DOMD was substituted with grass WSC contents. Addition of LW as predictor to the latter equation did not improve prediction accuracy.

When multiple linear predictions were developed for methane emissions using LW as primary predictor and grass nutrient contents as secondary predictors (Table 4; Eq. 1o-1r), grass WSC, EE and ADF concentrations were identified as significant according to the Wald statistic. Methane emissions were positively correlated to grass WSC and NDF and negatively correlated to grass EE. Prediction accuracy was improved by using grass ADF or EE or WSC as additional to LW explanatory variables, and the lowest MPE was observed when LW, and grass WSC and EE contents were used in the same equation.

3.5 Prediction of methane emissions or methane energy output from grass nutrient, ME and DOMD contents

When methane output or energy emissions, expressed per kg of DMI or MJ of GEI respectively, were predicted by multiple linear equations using grass contents of nutrients, ME and DOMD (Table 5; Eq. 3a-3c and 4a-4c), the effect of grass N, WSC, ash, NDF, ME and DOMD concentrations was significant according to the Wald statistic. Both response variables were positively correlated to grass N, WSC, ash and negatively correlated to grass ME and NDF. Prediction accuracy was improved by using grass ME and DOMD in combination with grass N or NDF for the prediction of methane emissions or methane energy

output respectively. For the prediction of methane emissions per kg DMI, excluding grass N slightly increased MPE but substituting ME and DOMD with WSC contents in grass greatly reduced prediction accuracy. For the prediction of methane energy output per MJ GEI, excluding grass NDF would slightly increase MPE but substituting NDF with ash contents in grass considerably reduced prediction accuracy.

3.6 Internal validation of prediction equations

Thirty five new equations, one for each equation produced in the present study by using the whole dataset, were developed from the two-thirds of data by using the exact same random and fixed model and are presented in the Appendix in (1) Table A1 (Eq. A1-A18 and B1-B11), for the prediction of methane emissions and methane energy output per day, and (2) Table A2 (Eq. C1-C3 and D1-D3) for the prediction of methane emissions and methane energy output per kg of DMI and MJ of GEI respectively. These new equations as well as previously published equations, shown in the Appendix (Table A3), for the prediction of methane energy output per day (Eq. E1-E5; Ellis *et al.*, 2007; FAO, 2010; IPCC, 2006; Mills *et al.*, 2003; Moraes *et al.*, 2014) or per MJ of GEI (Eq. E6; published by Blaxter & Clapperton (1965) and corrected by Wilkerson *et al.* (1995)) were also validated against the same one-third of data. In total, thirty external equations were validated including those showing the best prediction accuracy within each study, as well as those recommended by international organizations for the assessment of methane energy output. The results of the validation of the new and previously published equations are summarised on Table 6.

Regarding the equations developed in the current study, mean predicted values were very similar to actual values for all assessed response variables. Mean differences between predicted and actual values represented a difference less than 1.4%, 1.6%, 2.0% and 7.8% of the actual values for methane emissions, methane energy output, methane emissions per kg DMI and methane energy output per MJ GEI, respectively, with mean differences for certain

375 equations representing as low as 0.03%, 0.1%, 0.8% and 0.6% difference from the actual
376 value, respectively. Similarly, standard errors of the equations were less than 9.4%, 9.7%,
377 4.3% and 4.7% of the actual values for methane emissions, methane energy output, methane
378 emissions per kg DMI and methane energy output per MJ GEI, respectively, with certain
379 equations showing standard errors as low as 7.3%, 6.9%, 4.2% and 4.5% of the actual values,
380 respectively. Mean prediction errors for all equations for each predicted variable averaged to
381 0.129 (0.094-0.180) for methane emissions, 0.133 (0.091-0.162) for methane energy output,
382 0.114 (0.096-0.147) for methane emissions per kg DMI and 0.111 (0.095-0.123) for methane
383 energy output per MJ GEI.

384 Regarding previously published equations, prediction accuracy was lower while standard
385 errors and proportional differences between predicted and actual values were higher when
386 compared with models developed in the current study. For the prediction of methane energy
387 output, difference between predicted and actual values represented 5.8% to 32.2% of the
388 actual values, standard errors represented 6.7% to 8.8% of the actual values while average
389 MPE for all equations was 0.225, ranging from 0.146 to 0.347. The model proposed by Ellis
390 et al. (2007) showed the lowest prediction error while lower, but still satisfactory, prediction
391 accuracy was observed by using the equation recommended by Moraes et al. (2014); the other
392 external models evaluated in the current study (FAO, 2010; IPCC, 2006; Mills *et al.*, 2003)
393 showed relatively low prediction accuracy. The equation from Blaxter and Clapperton (1965),
394 as corrected by Wilkerson et al. (1995), for the prediction of methane energy output per MJ
395 GEI showed relatively good accuracy compared to the other external equations assessed in the
396 current study, showing higher MPE only when compared to the equation of Ellis et al. (2007).
397 For the assessment of the agreement between predicted and actual values for the equations
398 developed in the present study or previously recommended (Blaxter and Clapperton, 1965;
399 Ellis *et al.*, 2007; FAO, 2010; IPCC, 2006; Mills *et al.*, 2003; Moraes *et al.*, 2014; Wilkerson

et al., 1995), an agreement analysis using Lin's concordance correlation coefficient was carried out and the outcome is presented in Table 6. In order to graphically represent the agreement between predicted and actual values of methane energy output, Bland-Altman plots were developed for the equations which were (i) recommended by IPCC and FAO and the updated models developed in the current study by using the same predictors (Fig. 2), (ii) previously published by other authors (Ellis *et al.*, 2007; Mills *et al.*, 2003; Moraes *et al.*, 2014) and those of the current study that showed the lowest MPE among the sets that used DMI, GEI or LW in combination with grass contents of nutrients, ME and DOMD (Fig. 2) and (iii) presented by Blaxter and Clapperton (1965), as corrected by Wilkerson *et al.* (1995), for methane energy output per MJ GEI prediction and the equation that showed the best prediction accuracy for this response variable in the current study (Fig. 3). Lin's concordance correlation coefficients with 95% confidence interval in brackets are also shown in the same graphs; for all equations some agreement between predicted and actual values was observed with Rc being higher for the equations developed in the current study. All external equations generally under-estimated methane energy output from animals fed solely fresh-cut grass at maintenance energy levels, and this observation was more apparent for the equations recommended by IPCC (2006) and FAO (2010). When the equation from Blaxter & Clapperton (1965), as corrected by Wilkerson *et al.* (1995), was used to predict methane energy output per MJ GEI, a similar under-prediction was observed when actual values were over 0.080.

4. Discussion

The data used for generating models in the current study have been collected from nonpregnant nonlactating cows fed solely fresh-cut grass at maintenance energy levels, in contrast with previous studies which developed methane predictions from animals fed rations based on conserved forage and concentrates (Blaxter and Clapperton, 1965; Ellis *et al.*, 2007;

Mills *et al.*, 2003; Moraes *et al.*, 2014; Yan *et al.*, 2000; Yan *et al.*, 2009). Recent studies have raised concerns about applying equations developed from animals fed total mixed rations to grazing livestock, recommending this should be done with caution (Moraes *et al.*, 2014), as a result of two main reasons. First, high-grain diets are expected to generate less methane in the rumen as a result of the production of propionate instead of acetate, which would be promoted under high-forage diets (Ellis *et al.*, 2009; Ramin and Huhtanen, 2013). Second, Ramin & Huhtanen (2013) described that the proportion of energy intake released as methane may be decreased by increasing feed intake through changes in rumen fermentation patterns, feed particles passage rates and microbial population methanogenesis efficiency. A recent study recommended that, physiological stage, alongside with dietary information, should be taken into consideration when predicting methane emissions from beef and dairy cattle (Ricci *et al.*, 2013). In the present work, an average of 8.6% of GEI was released in methane, a proportion which is higher than the proportion reported for animals fed total mixed rations at production levels (Johnson and Johnson, 1995) or used for the development of prediction models for dairy and beef cattle (Ellis *et al.*, 2009; Moraes *et al.*, 2014; Ramin and Huhtanen, 2013; Yan *et al.*, 2000; Yan *et al.*, 2009); the revised value may be more representative to grazing animals fed at restricted feed intakes. New equations may therefore be complimentary to the models previously developed with animals fed conserved forages and concentrates, because they may be more suitable to use in case of grazing animals at near maintenance levels. Nonlactating cows may compose about 15% of the dairy herd and different prediction equations are necessary because they differ on methane emissions and energy utilisation efficiency when compared with animals in production stage (Ramin and Huhtanen, 2013; Stergiadis *et al.*, 2015c). However, the equations presented in the current study may be used in lactating grazing cows, fed at production energy levels, after corrections related to feeding level because the methane emissions and methane energy outputs per kg

DMI are influenced by DMI (Ramin and Huhtanen, 2013). Equations characterised by the simplicity of the predictors used, in order to be applicable at commercial level, as well as more detailed ones using a number of nutritional parameters, in order to be used at research and policy-making level, were developed.

4.1 Assessing predictors for methane emissions from animals fed solely fresh-cut grass

4.1.1 Feed and energy intakes

The single linear equations developed in the present study showed an increase in DMI or OMI (either expressed as gross or digestible amounts) and GEI would increase methane emissions and methane energy output. This strong positive relation may be explained by the fact that higher feed and energy intake increases the supply of substrate (dietary OM) for methane production in rumen, where over 50% of the digestible OM is fermented by anaerobic microorganisms (Cottle *et al.*, 2011; Moss *et al.*, 2000). However, methane emissions per kg of DMI decreases with increasing DMI possibly due to the lower rumen retention times with higher DMI, which reduces digestibility of feed (Buddle *et al.*, 2011). Another possible explanation for this observation could be that greater DMI also increases acid production per unit of time, which downgrades living conditions for cellulolytic and methanogen microorganisms but favours lactic bacteria (Moss *et al.*, 2000); hence, there could be a proportionately higher production of propionate, which serves as a hydrogen sink (Moss *et al.*, 2000). However, rumen pH was not assessed in the present study and animals were fed at maintenance energy level. The positive relation of feed and energy intake with methane emissions has been previously reported for animals fed conserved forages and concentrates (Cottle *et al.*, 2011; Ellis *et al.*, 2007; Ramin and Huhtanen, 2013) while DMI and MEI have been identified as the best predictors for methane energy output in linear or non-linear equations (Ellis *et al.*, 2007; Johnson and Johnson, 1995; Mills *et al.*, 2003).

4.1.2 Animal liveweight

In the present study, slopes observed for animal liveweight were positive, which means that bigger animals produce more methane. This relationship has been previously shown by other authors (Moraes *et al.*, 2014) and may be explained by the higher DMI in heavier animals. The significance of LW as a predictor for methane emissions and methane energy output has been attributed to differences in gut capacity, ruminal weight and feed kinetics between animals of contrasting LW, which would influence the extent of ruminal fermentation of feeds and production of volatile fatty acids; thus, fermentation of the same amount of feed may be different between animals of different LW (Moraes *et al.*, 2014).

4.1.3 Grass basic nutrient composition

In the current study, grass WSC, NDF and ADF concentrations were among the main predictors in a number of equations, thus confirming the links and the positive relation, between dietary carbohydrates, and their type, with methane emissions from animals that are fed solely fresh-cut grass at low feeding levels. The fact that grass soluble carbohydrates and fibre may be significant predictors for methane emissions has been discussed by Pacheco *et al.* (2014) for sheep fed fresh-cut ryegrass. Profile of volatile fatty acids in the rumen, which determines microbial population and methane production, is dependent not only to the amount of carbohydrates but also to their type, with more acetic acid and methane being produced by the digestion of cell wall fibre (NDF, ADF) than digestion of more fermentable carbohydrates (e.g. WSC) (Buddle *et al.*, 2011; Johnson and Johnson, 1995). Dietary fibre also influences DMI and digesta dynamics, by mainly increasing rumen retention time and, eventually, methane production (Allen and Mertens, 1988; Pacheco *et al.*, 2014). Feeding grass with less fibre has been proposed as a methane mitigation strategy for pasture-based systems (Pacheco *et al.*, 2014; Ulyatt and Lassey, 2001) but the effectiveness of this method has yet to be established (Buddle *et al.*, 2011; Pacheco *et al.*, 2014).

In contrast to the findings of the current work, in the study of Moraes et al. (2014) the effect of carbohydrate type on methane emissions from nonlactating animals fed conserved forage and concentrate diets was not significant. This may be explained by the higher dietary NDF content between the current study (51.2% of DM) and that of Moraes et al. (2014; 36.3% of DM), which was expected for diets based on fresh-cut grass. Also, fermentation of soluble carbohydrates, which are the main readily available carbohydrate in grass, have been associated with higher methane emissions than fermentation of starch, which is the main carbohydrate in total mixed rations (Johnson and Johnson, 1995). The latter may not directly originate from differences on the methane produced from the fermentation of these two types of carbohydrates; soluble sugars have been found to promote butyric acid production and increase digestibility of dietary fibre, thus resulting in higher methane production through additional pathways (Knapp *et al.*, 2014; Miller *et al.*, 2001).

The negative correlations of grass NDF concentrations with methane emissions in one equation may be explained by the confounding effect of DMI, that had a positive correlation to grass NDF ($r = 0.432$; data not shown), in the same equation; the individual correlation between grass NDF concentrations and methane emissions was positive ($r = 0.170$; data not shown). Negative correlations between grass NDF and methane energy output in lactating cows have been reported by Ellis et al. (2007) and were attributed to differences on the relative proportions of NDF fractions (cellulose, hemicellulose, lignin).

The negative relations found between methane emissions and methane energy output and grass EE and ash contents were expected. Previous studies have reviewed that dietary lipid, which is not digested in rumen, may reduce methanogenesis in the rumen by (1) reducing fibre and OM fermentation, (2) increasing propionic acid in expense of acetic and butyric acid production, (3) inhibiting protozoa and being toxic to methanogens and, to a lesser extent, (4) shifting hydrogen usage towards the hydrogenation of unsaturated fatty acids (Ellis *et al.*,

2007; Johnson and Johnson, 1995; Ramin and Huhtanen, 2013). The current study showed that using grass EE contents as a predictor for methane emissions or methane energy outputs may not improve prediction accuracy when DMI or GEI are known. However, when these parameters are not available in practice and LW is used as the primary predictor, using grass EE as additional predictor may be recommended. Ellis et al. (2009) reported that equations excluding dietary lipid content may be inaccurate to predict methane emissions. The fact that methane emissions are reduced when grass contains more ash may be explained by the inorganic nature of ash which does not deliver any fermentable energy (McDonald *et al.*, 2011); its occurrence in grass simply dilutes the energy-yielding organic compounds such as carbohydrates and proteins.

The positive slope between grass N contents and methane energy output per unit of DMI or GEI may be explained by the confounding effect of grass ME and DOMD concentrations which were significant predictors in the same equations. Correlation between grass N and methane emissions and methane energy output per day, DMI or GEI were negative (-0.491, -0.491, -0.082 and -0.165 respectively; data not shown). Ramin et al. (2013) reviewed that grass with higher N content may decrease methane emissions because fermentation of N in the rumen produce less methane than carbohydrates, although the effect was relatively small. In other reviews, grass N has been also found among the required predictors to increase the explained variation in methane emissions from sheep fed ryegrass (Pacheco *et al.*, 2014).

4.1.4 Grass digestibility and metabolisable energy contents

In the current study, grass nutrients, ME and DOMD concentrations were identified as significant predictors of methane emissions and methane energy output in various equations, most likely because methane production is related to the chemical composition of the feed (Ellis *et al.*, 2009; Ellis *et al.*, 2007; Mills *et al.*, 2003; Moraes *et al.*, 2014). For example, offering diets with less fibre and more digestible forage or supplementing pasture-based diets

with highly fermentable grains may reduce methane outputs, as a result of modifications in rumen ecology and function (Cottle *et al.*, 2011; Moss *et al.*, 2000; Ulyatt and Lassey, 2001). Diets with less fibre and more digestible carbohydrates would (i) reduce populations of protozoa and other methanogens by decreasing rumen pH and (ii) increase propionic acid production which competes the pathway for the production of methane (Cottle *et al.*, 2011; Moss *et al.*, 2000). Indeed, in the present study concentrations of DOMD in grass were found among the significant predictors in equations that showed the best prediction accuracy. Its positive relation to methane emissions and methane energy output may be a result of confounding effects between grass DOMD and the other predictors in the same equations, possibly grass ME contents, rather than explaining a physiological function. The latter explanation may be supported by the relatively high positive correlation between grass ME and DOMD concentrations ($r = 0.736$; data not shown). When grass DOMD was independently correlated to methane emissions and methane energy output, the slope was negative ($r = -0.206$; data not shown). In contrast, the positive relation between methane energy output expressed per MJ of GEI and feed energy digestibility, strongly related to grass DOMD ($r = 0.911$; data not shown), has been previously described for animals at maintenance or productive levels (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013) and may explain the positive slope for grass DOMD when methane energy output expressed per MJ of GEI was predicted.

4.2 Implications for potential methane mitigations strategies

Based on the fact that grazing contribution to DMI of cows in pasture-based systems are usually between 50% and over 95%, depending on the production intensity, the effect of grass quality on methane emissions from pasture-based systems is critical. The negative relation between grass ME concentrations and methane emissions and energy outputs, expressed per day or per unit of DMI or GEI, reinforce the opinion that feeding good

quality forage and breeding improved pasture-plants may reduce methane emissions from pasture-based systems (Pacheco *et al.*, 2014; Ulyatt and Lassey, 2001). A possible explanation for this is that grass with higher ME has less fibre, which is the most methanogenic among grass nutrients (Buddle *et al.*, 2011; Johnson and Johnson, 1995). In addition, lower dietary fibre concentration may reduce rumen retention times (Allen and Mertens, 1988) and then the activity of methanogenic microorganisms, which are less competent under these conditions (Moss *et al.*, 2000; Ramin and Huhtanen, 2013).

The findings of the current study underline that, in pasture-based systems, animals fed at maintenance energy levels on improved feeding value pastures (with more ME, DOMD and lipid and less NDF and ADF) may show reduced methane emissions. This practice can be considered for implementation at commercial level in conjunction with other methane mitigation strategies for grazing animals such as dietary supplementation of concentrate feeds and lipid, although the latter treatments may show practical difficulties in extensive grazing herds in which animals are not daily fed by producers (Cottle *et al.*, 2011; Pacheco *et al.*, 2014; Ulyatt and Lassey, 2001). On that basis, modifying grass nutrient composition can be an alternative approach (Buddle *et al.*, 2011; Pacheco *et al.*, 2014). However, cattle fed grass containing more N may excrete more N in faeces and urine, and thus increasing nitrate pollution to the ground water and emissions of ammonia and nitrous oxide to the atmosphere (Hristov *et al.*, 2013; Stergiadis *et al.*, 2015b). This should be taken into consideration when assessing mitigation strategies for the nitrate and ammonia pollution and overall GHG emissions from the dairy herd. The fact that grass with more ash would reduce methane production cannot serve as a mitigation option because higher ash contents reduce grass nutritive value, and this would be undesirable at both maintenance and production level.

A combination of different mitigation strategies, rather than solely nutritional changes, have been recommended because grass chemical composition did not explain much of the variation

in methane emissions in other studies (Buddle *et al.*, 2011; Pacheco *et al.*, 2014); this was also confirmed in the current study with R^2 being lowest for equations based only on grass chemical composition.

4.3 Validation of methane emissions predictions

The highest prediction accuracy in the current study was seen for equations that included DMI or GEI as primary predictors and ME and DOMD as additional predictors and may be recommended in practice when these variables are available. The strong relations of grass nutrient, ME and DOMD contents with rumen microbial population and methane production, as described in the previous sections, may explain the improvement of prediction accuracy when these predictors were added in equations already containing feed intake parameters because they describe the nature of the compounds available to methanogens rather than just ingested amounts. This was observed in other studies, in which animals were fed at conserved forage and concentrate diets, for beef cattle (Ellis *et al.*, 2007; Moraes *et al.*, 2014), but not for nonlactating cows because prediction accuracy in the study of Moraes *et al.* (2014) was better when GEI was used as a sole predictor. In case of lactating cows, improvement on prediction accuracy by adding dietary variables as predictors has been observed when the primary predictor was GEI (Moraes *et al.*, 2014; Ricci *et al.*, 2013) but not when the primary predictor was DMI (Ellis *et al.*, 2007).

Interestingly, predictions based on GEI outperformed those based on MEI, when used as sole predictors in the current study, and may be recommended for grazing animals fed at low feeding levels, although MEI has been extensively used in equations developed from data collected from dairy and beef cattle fed conserved forages and concentrates (Ellis *et al.*, 2007; Mills *et al.*, 2003; Moraes *et al.*, 2014). This finding has been previously seen for beef and dairy cattle (Ricci *et al.*, 2013) and may be attributed to the more complicated techniques of assessing MEI than GEI, which may impose higher errors in their measurement in practice.

Equations using more readily available and accurately measured data, such as DMI, have also outperformed equations using MEI in other studies (Ellis *et al.*, 2009; Ellis *et al.*, 2007). Other authors suggested non-linear models may better describe physiological relations and should be preferred when methane emissions are predicted from equations including DMI or GEI as primary predictors (Ellis *et al.*, 2009; Mills *et al.*, 2003). However, the prediction accuracy of an external non-linear model published by Mills *et al.* (2003) and validated for animals fed fresh-cut grass at maintenance energy levels was lower than that of external, and also developed in the present study, linear models.

When the current models recommended by IPCC (2006) and FAO (2010) were compared with equations developed in the present study using the same explanatory variables (GEI and GEI in conjunction with DM digestibility, respectively), the new equations improved prediction accuracy. Equations from IPCC and FAO underestimated actual methane energy output by an average of 24% and 32% indicating a risk of considerable error in practice when these equations, developed with animals at total mixed rations at high feeding levels, are used for grazing animals at near maintenance levels. This finding may be explained by the facts that (i) a lower methane production at the same GEI level would be expected when the main source of energy is starch (diets rich in concentrates) rather than WSC and fibre (diets rich in fresh grass) (Johnson and Johnson, 1995; Ulyatt and Lassey, 2001), and (ii) a lesser proportion of gross energy would be released as methane energy with increasing feeding level (Johnson and Johnson, 1995). The error with IPCC (2006) equation probably derives from the inability to account for changes in digestibility between poor and high quality forage, thus showing a high error in the current dataset which composed of a wide range of grass quality. The revised equations may therefore substitute the existing models when methane emissions from grazing animals at maintenance or low feeding levels are assessed in GHG inventories or at commercial level, without increasing complexity on data collection because they are

based on the same predictors. Similar underestimation of methane energy output, although at a lower scale (averaged between 6% and 14% of the actual value), was observed when other external equations were validated against the one-third of the whole dataset. This indicates that equations developed in the current study may improve prediction accuracy when used for grazing animals at low feeding levels, even when compared with the most recent equations, which previously outperformed IPCC and FAO equations (Moraes *et al.*, 2014).

In the present work, the addition of LW as a predictor when DMI, GEI or their combination with grass nutrient, ME and DOMD concentrations were already used as predictors did not, or only marginally, improved accuracy and, therefore, LW may be excluded in practice when DMI or GEI are available. This finding was also reported for nonlactating cows fed conserved forage and concentrate diets (Moraes *et al.*, 2014) and may be explained by the fact that the importance of LW may be associated to intake capacity which is better described by intake-related variables, such as DMI and GEI. However, LW may be an important predictor at farm-level assessments because DMI or GEI at pasture is generally not available or poorly assessed (Buddle *et al.*, 2011; Stergiadis *et al.*, 2015c). Equations developed in the current study based on LW and grass nutrient concentrations, which are both routinely available in practice, showed satisfactory accuracy and outperformed previously published more complex models (which include DMI or GEI as predictors), thus being a relatively reliable prediction technique for methane emissions at farm-level for animals fed at near maintenance level in pasture-based systems.

Models developed in the current work showed that prediction accuracy of methane emissions may improve when model development reflects animal physiological stage and diet under assessment and the most appropriate combinations of predictors may be dependent on their availability in practice. Prediction accuracy of methane emissions from animals fed solely fresh-cut grass at maintenance energy level was improved when both grass intake and quality

parameters were taken into account. The new equations may serve in the development of methane mitigation strategies in pasture-based systems and the update of existing GHG inventories and models used for policy-making decisions.

5. Conclusions

Animals fed at maintenance or low feeding levels may compose up to 15% of the dairy herd. Using existing equations, developed from data collected with animals fed conserved forages and concentrates, may underestimate methane emissions from animals fed fresh-cut grass at maintenance energy levels. This is possibly because of the differences on nutrient digestibility due to contrasting (i) energy-supplying nutrient composition between pasture and total mixed rations and (ii) rumen digesta kinetics. Differences between studies highlight the importance of developing separate prediction models for each animal type, feeding level and production system in order to improve prediction accuracy in practice. The equations developed in the current study, using feed intakes, grass nutrient concentrations and digestibility, and liveweight or combinations of these parameters as predictors, may be recommended in practice (e.g. in GHG inventories or life-cycle assessments) for animals on pasture or zero-grazing diets at near maintenance energy levels. The current equations may be used in lactating grazing animals with correction for effects of feeding levels because differences in feeding levels can influence rumen ecology and function. Based on the relationships found between response and explanatory variables in the equations, mitigation strategies to reduce methane emissions from animals grazing at maintenance energy levels may include using grass with more ME, DOMD, N and EE and less NDF and ADF, although side-effects (e.g. increased outputs of manure N, nitrate pollution of ground water and emissions of ammonia and nitrous oxide in the atmosphere) from using highly digestible N-rich grass should also be considered on policy-making decisions.

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Figure captions

Fig. 1 Relationships between methane emissions (CH_4) or methane energy output ($\text{CH}_4\text{-E}$) and (i) dry matter intake (DMI; panel a) or organic matter intake (OMI; panel b), and (ii) gross energy intake (GEI; panel c), respectively, assessed in calorimetric chambers with nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level ($n = 100$). R^2 and MPE represent pseudo correlation coefficient and mean prediction error respectively. MPE derived from an internal validation with new equations, listed in the Appendix (Table A1; Eq. A1, A2, B1), which were developed from the two-thirds of the whole dataset and were validated against the remaining one-third of the whole dataset.

Fig 2. Bland-Altman plots showing the agreement between actual methane energy output ($\text{CH}_4\text{-E}$) and predicted from equations recommended by IPCC (2006) (panel a) and FAO (2010) (panel b) or developed in the current study by using the same explanatory variables (panels c and d, respectively). For panels a and c gross energy intake was used as sole predictor but for panels b and d digestible dry matter intake was also used as an additional predictor. Prediction equations are shown in the Appendix, in Table A1 (Eq. B1 and B11 for panels c and d, respectively) and Table A3 (Eq. E1 and E2 for panels a and b, respectively). Residual represents the difference between predicted minus actual value. Methane emissions were measured in calorimetric chambers with nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

Fig. 3 Bland-Altman plots showing the agreement between actual methane energy output ($\text{CH}_4\text{-E}$) and predicted from equations published by Moraes et al. (2014; panel a), Ellis et al. (2007; panel b) and Mills et al. (2003; panel c) or developed in the current study using intakes of dry matter or gross energy, liveweight and grass nutrient, metabolisable energy and digestible organic matter concentrations. Prediction equations are shown in the Appendix, in Table A1 (Eq. B9, A13 and A15 for panels d, e and f, respectively) and Table A3 (Eq. E3, E4 and E5 for panels a, b and c, respectively). In panels d and f predicted values as methane emissions in g d^{-1} were transformed to methane energy output in MJ d^{-1} using the following equation: $\text{CH}_4\text{-E (MJ d}^{-1}) = 39.54 \times \text{CH}_4 \text{ (L d}^{-1})$, assuming molar mass and gas volume for methane to be 16.04 g and 22.4 L mol^{-1} respectively (Ramin and Huhtanen, 2013). Residual represents the difference between predicted minus actual value. Methane emissions were measured in calorimetric chambers with nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

Fig. 4 Bland-Altman plots showing the agreement between actual methane energy output ($\text{CH}_4\text{-E}$) expressed per gross energy intake ($\text{CH}_4\text{-E:GEI}$) and predicted from an equation published by Blaxter and Clapperton (1965), as corrected by Wilkerson et al. (1995; panel a), or developed in the current study using grass neutral-detergent fibre, metabolisable energy and digestible organic matter concentrations (panel b). Prediction equations are shown in the Appendix, in Table A2 (Eq. D3 for panel b) and Table A3 (Eq. E6 for panel a). Residual represents the difference between predicted minus actual value. Methane emissions were measured in calorimetric chambers with nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level. Rc is Lin's concordance correlation coefficient with 95% confidence interval given in square brackets.

Tables

Table 1 Description of data collected from nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level over the two years of the experiment (n = 100 for all parameters except for grass nutrient contents, n = 57)

Parameters assessed	Mean $\pm$ SD	Min	Max
Animal data			
Liveweight (kg)	547 $\pm$ 63.1	423	715
Body condition score	2.6 $\pm$ 0.33	1.5	3.8
Grass nutrient contents (g kg ⁻¹ DM)			
DM (g kg ⁻¹ fresh)	179.8 $\pm$ 49.02	102.7	310.4
Nitrogen	22.7 $\pm$ 7.35	10.8	40.3
Water-soluble carbohydrates	154.9 $\pm$ 47.41	59.8	269.2
Ether extract	29.7 $\pm$ 7.75	16.6	51.0
Neutral-detergent fibre	511.8 $\pm$ 35.61	444.0	596.0
Acid-detergent fibre	274.8 $\pm$ 39.40	206.3	358.8
Ash	80.2 $\pm$ 18.81	47.5	124.0
Grass energy contents (MJ kg ⁻¹ DM)			
GE	18.3 $\pm$ 0.36	17.5	19.1
Digestible energy	14.4 $\pm$ 0.90	11.8	16.0
Metabolisable energy	12.0 $\pm$ 0.99	9.7	13.8
Nutrient (kg d ⁻¹) and energy (MJ d ⁻¹) intakes			
DMI	6.19 $\pm$ 0.882	4.54	8.91
Digestible DMI	4.92 $\pm$ 0.677	3.63	7.22
OM intake	5.70 $\pm$ 0.846	4.07	8.42
Digestible OM intake	4.68 $\pm$ 0.631	3.42	6.85
GEI	113.5 $\pm$ 16.01	80.8	162.9
Digestible energy intake	88.9 $\pm$ 12.10	64.0	128.0
Metabolisable energy intake	74.4 $\pm$ 12.39	46.9	112.4
Grass digestibility			
DM digestibility (kg kg ⁻¹)	0.796 $\pm$ 0.0427	0.678	0.880
Digestible OM in DM (kg kg ⁻¹ DM)	0.757 $\pm$ 0.0360	0.638	0.835
GE digestibility (MJ MJ ⁻¹)	0.785 $\pm$ 0.0439	0.645	0.870
Methane parameters			
CH ₄ (g d ⁻¹)	174.8 $\pm$ 24.85	123.2	250.6
CH ₄ :DMI (g kg ⁻¹)	28.4 $\pm$ 3.21	22.3	40.0
CH ₄ -E (MJ d ⁻¹)	9.65 $\pm$ 1.372	6.80	13.84
CH ₄ -E:GEI (MJ MJ ⁻¹)	0.086 $\pm$ 0.0101	0.067	0.123

SD = standard deviation; Min = minimum value observed; Max = maximum value observed; DM = dry matter; GE = gross energy; DMI = dry matter intake; OM = organic matter; GEI = gross energy intake; CH₄ = methane emissions; CH₄-E = methane energy output.

Table 2 Single linear prediction of methane emissions and methane energy output from nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level (n = 100), using intakes of dry matter, organic matter or energy

	Equations ^a		R ²	MPE ^b	Eq.
CH ₄	=	50.67 _(14.03) + 19.95 _(2.16) DMI	0.67	0.134	(1a)
		50.85 _(13.52) + 21.63 _(2.28) OMI	0.68	0.126	(1b)
		73.15 _(16.01) + 20.56 _(3.10) DDMI	0.61	0.148	(1c)
		63.19 _(15.31) + 23.78 _(3.15) DOMI	0.62	0.139	(1d)
CH ₄ -E	=	2.727 _(0.807) + 0.061 _(0.007) GEI	0.68	0.138	(2a)
		4.341 _(0.887) + 0.060 _(0.009) DEI	0.63	0.155	(2b)
		6.110 _(0.805) + 0.047 _(0.010) MEI	0.62	0.162	(2c)

R² = pseudo correlation coefficient; MPE = mean prediction error; Eq. = equation; CH₄ = methane emissions; DMI = dry matter intake; OMI = organic matter intake; DDMI = digestible DMI; DOMI = digestible OMI; CH₄-E = methane energy output; GEI = gross energy intake; DEI = digestible energy intake; MEI = metabolisable energy intake.

^a Units: g d⁻¹ for CH₄; kg d⁻¹ for DMI, OMI, DDMI and DOMI; MJ d⁻¹ for CH₄-E, GEI, DEI and MEI. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). Numbers in parentheses represent standard errors. The random effects of cow and field were accounted for both predicted variables, according to changes in deviance during the development of the random model.

^b MPE derived from an internal validation with new equations, listed in the Appendix (Table A1; Eq. A1-A4, B1-B3), which were developed from the two-thirds of the whole dataset and by using the exact model presented in the current table; the new equations were validated against the remaining one-third of the whole dataset.

Table 3 Multiple linear prediction of methane emissions and methane energy output from nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level (n = 100), using liveweight and intakes of dry matter, organic matter or energy

	Equations ^a	R ²	MPE ^b	Eq.
CH ₄	= 20.63 _(16.10) + 16.25 _(2.39) DMI + 0.097 _(0.031) LW	0.68	0.134	(1e)
	20.22 _(15.94) + 17.89 _(2.50) OMI + 0.095 _(0.031) LW	0.69	0.127	(1f)
	25.07 _(17.87) + 15.52 _(3.18) DDMI + 0.134 _(0.032) LW	0.62	0.149	(1g)
	20.62 _(17.34) + 18.44 _(3.29) DOMI + 0.124 _(0.032) LW	0.64	0.142	(1h)
CH ₄ -E	= 1.049 _(0.918) + 0.049 _(0.007) GEI + 0.005 _(0.002) LW	0.69	0.137	(2d)
	1.410 _(1.010) + 0.045 _(0.010) DEI + 0.008 _(0.002) LW	0.63	0.155	(2e)
	2.049 _(1.017) + 0.036 _(0.009) MEI + 0.009 _(0.002) LW	0.61	0.161	(2f)

R² = pseudo correlation coefficient; MPE = mean prediction error; Eq. = equation; CH₄ = methane emissions; DMI = dry matter intake; LW = liveweight; OMI = organic matter intake; DDMI = digestible DMI; DOMI = digestible OMI; CH₄-E = methane energy output; GEI = gross energy intake; DEI = digestible energy intake; MEI = metabolisable energy intake.

^a Units: g d⁻¹ for CH₄; kg d⁻¹ for DMI, OMI, DDMI and DOMI; kg for LW; MJ d⁻¹ for CH₄-E, GEI, DEI and MEI. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). Numbers in parentheses represent standard errors. The random effects of cow and field were accounted for both predicted variables, according to changes in deviance during the development of the random model.

^b MPE derived from an internal validation with new equations, listed in the Appendix (Table A1; Eq. A5-A8, B4-B6), which were developed from the two-thirds of the whole dataset and by using the exact model presented in the current table; the new equations were validated against the remaining one-third of the whole dataset.

Table 4 Multiple linear prediction of methane emissions and methane energy output from nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level (n = 100), using intakes of dry matter or gross energy and/or liveweight and grass nutrient, metabolisable energy and digestible organic matter concentrations or dry matter digestibility.

	Equations ^a			R ²	MPE ^b	Eq.
CH ₄	=	119.8 _(23.6)	+ 20.45 _(2.10) DMI – 5.993 _(1.729) ME	0.71	0.121	(1i)
		201.7 _(41.5)	+ 22.49 _(2.23) DMI – 7.626 _(1.800) ME – 0.145 _(0.063) NDF	0.72	0.115	(1j)
		103.9 _(23.2)	+ 18.49 _(1.95) DMI – 5.661 _(1.677) ME + 0.156 _(0.037) WSC	0.73	0.101	(1k)
		154.4 _(25.4)	+ 19.46 _(2.10) DMI – 6.313 _(1.683) ME – 0.311 _(0.113) ASH	0.75	0.112	(1l)
		–74.28 _(39.43)	+ 25.72 _(1.89) DMI – 19.43 _(2.39) ME + 426.9 _(68.1) DOMD	0.73	0.094	(1m)
		54.45 _(27.33)	+ 15.38 _(2.11) DMI + 0.092 _(0.031) LW – 4.340 _(1.652) ME + 0.174 _(0.035) WSC	0.73	0.105	(1n)
		78.14 _(20.54)	+ 0.195 _(0.027) LW + 0.147 _(0.049) WSC – 1.091 _(0.274) EE	0.58	0.120	(1o)
		111.8 _(17.4)	+ 0.194 _(0.027) LW – 1.453 _(0.254) EE	0.59	0.132	(1p)
		25.52 _(18.50)	+ 0.200 _(0.031) LW + 0.260 _(0.053) WSC	0.59	0.137	(1q)
		16.58 _(25.50)	+ 0.205 _(0.032) LW + 0.169 _(0.067) ADF	0.60	0.180	(1r)
CH ₄ -E	=	6.946 _(1.272)	+ 0.064 _(0.007) GEI – 0.380 _(0.095) ME	0.73	0.122	(2g)
		5.970 _(1.262)	+ 0.058 _(0.006) GEI – 0.360 _(0.092) ME + 0.009 _(0.002) WSC	0.75	0.099	(2h)
		–3.913 _(2.143)	+ 0.080 _(0.006) GEI – 1.204 _(0.136) ME + 25.01 _(3.79) DOMD	0.76	0.091	(2i)
		3.433 _(1.509)	+ 0.048 _(0.006) GEI + 0.005 _(0.002) LW – 0.292 _(0.092) ME + 0.010 _(0.002) WSC	0.75	0.104	(2j)
		6.804 _(2.057)	+ 0.058 _(0.007) GEI – 4.688 _(2.228) DMd	0.71	0.134	(2k)

R² = pseudo correlation coefficient; MPE = mean prediction error; Eq. = equation; CH₄ = methane emissions; DMI = dry matter intake; ME = grass metabolisable energy content; NDF = grass neutral-detergent fibre content; WSC = grass water-soluble carbohydrates content; ASH = grass ash content; DOMD = grass digestible organic matter in dry matter; LW = liveweight; EE = grass ether extract content; ADF = grass acid-detergent fibre content; CH₄-E = methane energy output; GEI = gross energy intake; DMd = dry matter digestibility.

^a Units: g d^{–1} for CH₄; kg d^{–1} for DMI; MJ kg^{–1} dry matter for ME; g kg^{–1} dry matter for NDF, WSC, ASH, EE and ADF; kg kg^{–1} dry matter for DOMD; kg for LW; MJ d^{–1} for CH₄-E and GEI; kg kg^{–1} for DMd. The effect of all explanatory variables was significant according to the Wald statistic (F_{pr} < 0.05). Numbers in parentheses represent standard errors. The random effects of cow and field were accounted for both predicted variables, according to changes in deviance during the development of the random model.

^b MPE derived from an internal validation with new equations, listed in the Appendix (Table A1; A9-A18, B7-B11), which were developed from the two-thirds of the whole dataset and by using the exact model presented in the current table; the new equations were validated against the remaining one-third of the whole dataset.

Table 5 Multiple linear prediction of methane emissions or methane energy output, expressed per unit of dry matter intake or gross energy intake, respectively, from nonpregnant nonlactating dairy cows fed solely fresh-cut grass at maintenance energy level (n = 100), using grass nutrient, metabolisable energy and digestible organic matter concentrations.

	Equations ^a	R ²	MPE ^b	Eq.
CH ₄ :DMI	= 21.27 _(3.24) + 138.0 _(62.2) N + 28.86 _(11.66) WSC	0.44	0.147	(3a)
	11.68 _(5.08) - 3.437 _(0.352) ME + 76.62 _(9.73) DOMD	0.56	0.099	(3b)
	11.85 _(5.08) - 3.724 _(0.375) ME + 78.77 _(9.85) DOMD + 72.74 _(34.57) N	0.61	0.096	(3c)
CH ₄ -E:GEI	= 0.044 _(0.016) - 0.011 _(0.001) ME + 0.235 _(0.031) DOMD	0.64	0.116	(4a)
	0.028 _(0.017) - 0.012 _(0.001) ME + 0.260 _(0.031) DOMD + 0.081 _(0.038) ASH	0.64	0.123	(4b)
	0.083 _(0.025) - 0.011 _(0.001) ME + 0.205 _(0.034) DOMD - 0.044 _(0.022) NDF	0.66	0.095	(4c)

R² = pseudo correlation coefficient; MPE = mean prediction error; Eq. = equation; CH₄ = methane emissions; DMI = dry matter intake; N = grass nitrogen content; WSC = grass water-soluble carbohydrates content; ME = grass metabolisable energy content; DOMD = grass digestible organic matter in dry matter; CH₄-E = methane energy output; GEI = gross energy intake; ASH = grass ash content; NDF = grass neutral-detergent fibre content.

^a Units: g kg⁻¹ for CH₄:DMI; kg kg⁻¹ dry matter for N, WSC, DOMD, ASH and NDF; MJ kg⁻¹ dry matter for ME; MJ MJ⁻¹ for CH₄-E:GEI. The effect of all explanatory variables was significant according to the Wald statistic (Fpr < 0.05). Numbers in parentheses represent standard errors. The random effects of cow, field and year were accounted for both predicted variables, according to changes in deviance during the development of the random model.

^b MPE derived from an internal validation with new equations, listed in the Appendix (Table A2; Eq. C1-C3, D1-D3), which were developed from the two-thirds of the whole dataset and by using the exact model presented in the current table; the new equations were validated against the remaining one-third of the whole dataset.

Table 6. Internal validation using one-third of the whole dataset (n = 33) and equations developed from the remaining two-thirds of the whole dataset (n = 67)

Eq. ^a	Original Eq.	Predicted	Actual	r ²	MPE	SE	Rc	Predicted – Actual			
								Mean	SD	Min	Max
Methane emissions (g d ⁻¹)											
(A1)	(1a)	175.4	177.3	0.52	0.134	16.62	0.72	-1.9	17.27	-35.2	32.1
(A2)	(1b)	175.4	177.3	0.57	0.126	16.17	0.75	-1.9	16.48	-36.5	31.9
(A3)	(1c)	174.8	177.3	0.40	0.148	14.67	0.62	-2.5	17.96	-45.4	35.3
(A4)	(1d)	175.2	177.3	0.47	0.139	15.05	0.68	-2.1	17.14	-40.3	32.0
(A5)	(1e)	176.7	177.3	0.51	0.134	16.57	0.71	-0.6	17.31	-35.1	38.4
(A6)	(1f)	176.6	177.3	0.56	0.127	16.16	0.74	-0.7	16.56	-31.8	34.6
(A7)	(1g)	176.9	177.3	0.40	0.149	16.31	0.63	-0.4	18.59	-42.0	42.9
(A8)	(1h)	177.2	177.3	0.45	0.142	16.16	0.67	-0.1	17.78	-38.3	40.1
(A9)	(1i)	175.6	177.3	0.60	0.121	15.88	0.77	-1.7	15.97	-24.2	35.5
(A10)	(1j)	176.0	177.3	0.65	0.115	16.20	0.79	-1.3	15.96	-25.1	34.4
(A11)	(1k)	175.3	177.3	0.73	0.101	14.04	0.84	-2.0	13.82	-22.0	29.7
(A12)	(1l)	175.5	177.3	0.65	0.112	14.88	0.80	-1.8	14.84	-24.4	32.9
(A13)	(1m)	175.2	177.3	0.76	0.094	13.00	0.86	-2.1	12.80	-21.2	36.3
(A14)	(1n)	176.8	177.3	0.72	0.105	14.77	0.83	-0.5	14.56	-23.2	34.3
(A15)	(1o)	177.0	177.3	0.59	0.120	13.94	0.77	-0.3	14.97	-35.8	27.9
(A16)	(1p)	176.8	177.3	0.50	0.132	13.00	0.69	-0.4	15.99	-39.9	22.3
(A17)	(1q)	177.1	177.3	0.49	0.137	16.10	0.70	-0.1	17.34	-34.6	38.9
(A18)	(1r)	177.7	177.3	0.16	0.180	15.91	0.39	0.4	22.05	-38.2	54.9
Methane energy output (MJ d ⁻¹)											
(B1)	(2a)	9.696	9.789	0.49	0.138	0.9543	0.70	-0.094	0.9914	-2.123	2.026
(B2)	(2b)	9.706	9.789	0.34	0.155	0.8242	0.57	-0.083	1.0429	-2.511	2.264
(B3)	(2c)	9.651	9.789	0.26	0.162	0.6741	0.45	-0.138	1.0747	-2.581	2.178
(B4)	(2d)	9.666	9.789	0.49	0.137	0.9295	0.70	-0.123	0.9747	-2.117	2.212
(B5)	(2e)	9.934	9.789	0.36	0.155	0.9211	0.60	0.144	1.0620	-2.191	2.729
(B6)	(2f)	9.631	9.789	0.32	0.161	0.8714	0.55	-0.158	1.0808	-2.506	2.436
(B7)	(2g)	9.664	9.789	0.60	0.122	0.8920	0.76	-0.126	0.8946	-1.429	1.801
(B8)	(2h)	9.775	9.789	0.75	0.099	0.7686	0.85	-0.014	0.7589	-1.100	1.894
(B9)	(2i)	9.680	9.789	0.78	0.091	0.6915	0.87	-0.110	0.6820	-1.267	2.038
(B10)	(2j)	9.812	9.789	0.73	0.104	0.8071	0.84	0.023	0.7963	-1.246	1.983
(B11)	(2k)	9.740	9.789	0.51	0.134	0.9327	0.72	-0.050	0.9646	-1.977	1.983
(E1)	---	7.461	9.789	0.49	0.274	0.8615	0.25	-2.328	0.9405	-4.285	-0.212
(E2)	---	6.642	9.789	0.52	0.347	0.8076	0.16	-3.148	0.8946	-4.762	-1.156
(E3)	---	8.404	9.789	0.52	0.192	0.7438	0.42	-1.385	0.8719	-3.101	0.431
(E4)	---	9.219	9.789	0.50	0.146	0.8477	0.64	-0.570	0.9254	-2.240	1.176
(E5)	---	9.025	9.789	0.52	0.155	0.9307	0.61	-0.764	0.9617	-2.646	1.125
Methane emissions per dry matter intake (g kg ⁻¹)											
(C1)	(3a)	28.39	28.62	0.18	0.147	1.216	0.29	-0.228	2.9267	-8.020	5.652
(C2)	(3b)	28.10	28.62	0.68	0.099	1.195	0.74	-0.526	1.9166	-4.048	3.588
(C3)	(3c)	28.05	28.62	0.70	0.096	1.199	0.76	-0.571	1.8493	-3.987	2.687
Methane energy output per gross energy intake (MJ MJ ⁻¹)											
(D1)	(4a)	0.080	0.086	0.71	0.116	0.0038	0.65	-0.006	0.0056	-0.017	0.006
(D2)	(4b)	0.079	0.086	0.71	0.123	0.0041	0.62	-0.007	0.0055	-0.017	0.003
(D3)	(4c)	0.087	0.086	0.71	0.095	0.0040	0.80	0.001	0.0056	-0.010	0.012
(E6)	---	0.079	0.086	0.37	0.157	0.0028	0.27	-0.007	0.0084	-0.027	0.014

Eq. = equation; r² = correlation between predicted and actual values; MPE = mean prediction error; SE = standard error; Rc = Lin's concordance correlation coefficient; SD = standard deviation; Min = minimum value observed; Max = maximum value observed.

^a Equations developed in the internal validation are presented in the Appendix (Table A1, Eq. A1-A18, B1-B11; Table A2, Eq. C1-C3, D1-D3). External equations that were validated are also presented in the Appendix (Table A3, Eq. E1-E6). References for external equations are: E1, (IPCC, 2006); E2, (FAO, 2010); E3, (Moraes *et al.*, 2014); E4, (Ellis *et al.*, 2007); E5, (Mills *et al.*, 2003); E6, published by Blaxter & Clapperton (1965) and corrected by Wilkerson *et al.* (1995).

Figure 1

Figure 2

Figure 3

Figure 4