

A universal equation to predict methane production of forage-fed cattle in Australia

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Abstract. The methods for estimating methane emissions from cattle as used in the Australian national inventory are based on older data that have now been superseded by a large amount of more recent data. Recent data suggested that the current inventory emissions estimates can be improved. To address this issue, a total of 1034 individual animal records of daily methane production (MP) was used to reassess the relationship between MP and each of dry matter intake (DMI) and gross energy intake (GEI). Data were restricted to trials conducted in the past 10 years using open-circuit respiration chambers, with cattle fed forage-based diets (forage >70%). Results from diets considered to inhibit methanogenesis were omitted from the dataset. Records were obtained from dairy cattle fed temperate forages (220 records), beef cattle fed temperate forages (680 records) and beef cattle fed tropical forages (133 records). Relationships were very similar for all three production categories and single relationships for MP on a DMI or GEI basis were proposed for national inventory purposes. These relationships were $MP \text{ (g/day)} = 20.7 (\pm 0.28) \times DMI \text{ (kg/day)}$ ($R^2 = 0.92$, $P < 0.001$) and $MP \text{ (MJ/day)} = 0.063 (\pm 0.008) \times GEI \text{ (MJ/day)}$ ($R^2 = 0.93$, $P < 0.001$). If the revised MP (g/day) approach is used to calculate Australia's national inventory, it will reduce estimates of emissions of forage-fed cattle by 24%. Assuming a global warming potential of 25 for methane, this represents a 12.6 Mt CO₂-e reduction in calculated annual emissions from Australian cattle.

Additional keywords: beef, dairy, emissions intensity, tropical.

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Introduction

Australia reports its national greenhouse accounts using country-specific Tier 2 methods (Department of the Environment 2014), as recommended by the IPCC (2000). Enteric fermentation by cattle is responsible for ~52% of total agricultural emissions of greenhouse gases (total was 87.4 million t CO₂-e in 2012) and 8.4% of the total national emissions (Australian Greenhouse Emissions Information System 2014). In the Australian Inventory of Greenhouse Gases, methane emissions from forage-fed cattle currently rely on two methods. The method for dairy cattle and temperate beef cattle is based on an equation reported by Blaxter and Clapperton (1965) and subsequently corrected by Wilkerson *et al.* (1995). The method for tropical beef cattle is based on an equation originally reported by Kurihara *et al.* (1999), but subsequently modified by Hunter (2007). Both methods rely on an estimation of feed intake. For dairy cattle,

feed intake is based on the equation of Minson and McDonald (1987) corrected for milk production (Standing Committee on Agriculture 1990). For beef cattle, dry matter intake (DMI) is derived from bodyweight (BW) and BW gain (Minson and McDonald 1987). In the most comprehensive Australian study of tropical diets, Kennedy and Charmley (2012) concluded that the Kurihara *et al.* (1999) method for estimating methane emissions from tropical cattle overestimated methane production by ~30%. Consequently, the Australian Department of Environment commissioned a re-analysis of all recent, available Australian data collected from open-circuit respiration chambers to determine whether it was appropriate to reconsider the Tier 2 method used by Australia for estimating enteric methane emissions from forage-fed cattle.

In the present study, data were collated with the objective of deriving a relationship or relationships involving DMI or gross

energy intake (GEI) for estimating methane production (MP) from all classes of cattle in Australia fed or grazing on a wide range of diets and diet qualities.

Materials and methods

All experiments that contributed to the dataset were approved under the Australian code of practice for the care and use of animals for scientific purposes (NHMRC 2004), according to the respective State in which the experiments were conducted. Data for the analysis were primarily sourced from published datasets involving only data collected using open-circuit respiration chambers from individual cattle that were fed >70% forage in the ration, on diets which were not expected to reduce methane emissions or yield. Data were sourced from the following four sites: Department of Economic Development, Jobs, Transport and Resources Victoria for the dairy data (DAIRY); New South Wales Department of Primary Industries and the University of New England for the temperate (southern) beef data (S.BEEF); and CSIRO Rockhampton and Townsville for the tropical (northern) beef data (N.BEEF; Table 1). To maintain intellectual-property rights of contributing sources, the data provided comprised only sex, liveweight, DMI, MP (g/day, MJ/day), treatment design and an experimental designation, which included some information on diet composition. For

Table 1. Description of the data used in the analysis for dairy (DAIRY), southern beef (S.BEEF) and northern beef (N.BEEF) cattle

Parameter	DAIRY	S.BEEF	N.BEEF
Number of observations	220	680	133
Female	220	313	0
Entire male	0	323	0
Steer	0	39	133
<i>Description of livestock</i>			
Subspecies	<i>Bos taurus</i>	<i>Bos taurus</i>	<i>Bos indicus</i>
Holstein–Friesian	220	0	0
Angus	0	675	0
Brahman	0	0	133
Shorthorn	0	5	0
<i>Bodyweight (kg)</i>			
Mean	575	365	309
Range	458–706	156–640	214–425
<i>Dry matter intake (kg/day)</i>			
Mean	19.8	5.94	4.90
Range	11.8–27.7	3.55–11.6	2.18–8.02
<i>Methane production (g/day)</i>			
Mean	421	133	94.7
Range	237–623	78.9–241	32.2–184

tropical diets, gross energy (GE) concentration of the diets was also provided. This was necessitated as the mean GE concentration of tropical diets used by Kennedy and Charmley (2012) was lower than the standard value of 18.4 MJ/kg DM used for temperate forages in the present paper and in current accounting methods. For all data, a constant energy value for methane of 55.22 MJ/kg methane (Brouwer 1965) was adopted. There was a total of 1034 observations, including 220 dairy observations, 680 temperate beef observations and 133 tropical beef observations (Table 1). The northern beef data comprised 114 observations on Brahman steers at Rockhampton and 19 observations on Brahman steers at Townsville.

Description of the feeds

The dairy data were taken from five published experiments using lactating cows (Grainger *et al.* 2008, 2010; Moate *et al.* 2013; Williams *et al.* 2013; Deighton *et al.* 2014) and five unpublished experiments. The cows were at different stages of lactation and were fed on a wide variety of diets containing in excess of 70% forage (pasture, pasture hay, pasture silage, or lucerne hay) and between 0 and 30% concentrate (barley, triticale, or wheat). None of these diets contained any known methane mitigants (Table 2).

The temperate beef data were taken from a project designed to evaluate the phenotypic and genetic variation in methane production, described by Donoghue *et al.* (2013) and Herd *et al.* (2014). Growing Angus heifers, bulls and steers were fed a commercial lucerne and oaten hay chaff ration at ~1.2 times maintenance (Table 2). A small number of observations (15) were also incorporated from two separate studies conducted at the University of New England with Shorthorn and Angus cattle (J. Velazco, unpubl. data).

The tropical beef data collected on growing *Bos indicus* steers were previously published by Kennedy and Charmley (2012). In total, 23 diets were evaluated that included the tropical grasses black speargrass (*Heteropogon contortus*), buffel grass (*Cenchrus ciliaris*) bisset creeping bluegrass (*Bothriochloa insculpta*), mitchell grass (mixture of *Astrelba lappacea*, *Astrelba elymoides*) and rhodes grass (*Chloris gayana*). Speargrass, buffel grass and bluegrass were given at contrasting levels of maturity and speargrass was also fed with or without urea. Grasses were fed alone or in combination with the legumes dolichos (*Dolichos lablab*), burgundy bean, (*Macroptilium bracteatum*), stylo cv. verano (*Stylosanthes hamata*) and lucerne (*Medicago sativa*). All diets were fed *ad libitum* as hays and no concentrate feeds were given. Data for diets containing leucaena, presented in the original paper (Kennedy and Charmley 2012) were excluded because leucaena was shown to reduce methane emissions. Data from a further three grasses (mitchell grass hay, rhodes grass hay and

Table 2. Description of the feeds given to dairy (DAIRY), southern beef (S.BEEF) and northern beef (N.BEEF) cattle used in the data analysis

Parameter	DAIRY	S.BEEF	N.BEEF
Forage species	Temperate pasture, pasture hay, grass silage, lucerne hay	Lucerne–oaten hay fed as a chaff	Tropical grass hays, tropical legume hays, lucerne hay
Concentrate type	Barley, triticale, wheat	n.a.	n.a.
Crude protein (% DM)	15–30	6–14	3–22
Gross energy (MJ/kg DM)	18.4	18.4	17.7

a mixed *Urochloa*, rhodes, buffel pasture) from trials conducted in Townsville were included to expand the dataset (N. W. Tomkins, unpubl. data).

Methane measurement

All measurements were made in open-circuit respiration chambers from which methane concentration was measured. However, the procedures at the four sites differed to some degree and the methods are summarised below.

Dairy data: Ellinbank, Victoria

The physical dimensions and major characteristics of the two open-circuit respiration chambers and emission calculations have been described previously (Grainger *et al.* 2007). Operation of the chambers was as described by Williams *et al.* (2013). Briefly, cows were fed and milked twice a day and methane measurements were made for 22 h per day on two consecutive days. During the two 1 h periods when the doors were open for milking, it was assumed that the rate of methane production was the same as during the hour before door opening. Methane was detected using an infrared sensor (GFx 0–500 mg/kg, Servomex Group, Crowborough, UK). Temperature and humidity were controlled and intake and exhaust air were sequenced to the analyser in a 12-min cycle (4 min outside air, 4 min exhaust air Chamber 1, 4 min exhaust air Chamber 2). Methane measurements taken in the last 2 min of each 4 min sampling period were used to determine emissions. The methane concentrations, exhaust air flow rates, relative humidity, temperature and gas pressure at the inlet and exhaust were recorded at 10 s intervals. Each chamber was calibrated at the beginning and end of the experiments, with additional calibrations between experiment cycles as scheduling permitted. Calibration consisted of injecting individual gases at controlled rates while running the regular data-collection process.

Temperate (southern) beef data: Armidale, New South Wales (NSW)

A full description of the methane testing facility located on the University of New England campus in Armidale, NSW, Australia, was given by Hegarty *et al.* (2014) and Herd *et al.* (2014). It comprises 10 open-circuit respiration chambers, each of 20 m³ internal volume and with an air flow rate of 1.6 m³/min. The respiration-chamber ambient air flow is reliant on negative pressure in the system, achieved by 2 × Aerovent HPE400 3-phase fans (Aerovent Australia, Melbourne, Vic., Australia) placed in parallel at the exhaust of the system. Exhaust air from each chamber flows directly into a flow control manifold composed of 10 mass-flow meters (Model ST75V, Fluid Components International, San Marcos, CA, USA). A continuous subsample of gas is drawn from each gas line immediately after each flow meter, dried through a custom refrigerated drier (AZCO Instruments, Auckland, NZ) and methane concentrations are measured by a Servomex analyser Model 4100C1 (Servomex Group) fitted with an infrared detector for methane (GFx1210, 0–500 ppm) over 10 s after a 40 s purge time. Gas production was determined every 9 min per chamber for a period of 20 s. The Servomex was calibrated for methane with two high-purity gas standards (low: 0 mg/kg, and high:

97.5 mg/kg methane). Methane recovery through the chambers was measured between studies by a continuous release of methane standard. Methane production was measured over 2 × 24 h consecutive periods, with animals fed once a day in the morning.

Tropical (northern) beef data: Rockhampton, Queensland

Full details were provided by Kennedy and Charmley (2012) and Tomkins *et al.* (2011). Briefly, in each of two chambers, animals were fed once daily in the morning at a level equivalent to the feeding level established in the previous 5 days during pen feeding. Methane emissions were measured over one 24 h period. Air was sampled from alternate chambers every 3 min and methane output was calculated from air sampled over the last 2 min of each 3 min sampling cycle. Calibrations of the methane analyser were checked every 2 h and system recoveries were assessed after each experimental period. If feed intake on the day of measurement was less than 90% that of the previous day, the measurement was repeated several days later.

Tropical (northern) beef data: Townsville, Queensland

Four open-circuit chambers were used with methane emissions collected over two consecutive days. Each chamber was 4.0 × 2.4 × 2.4 m and was constructed of a tubular galvanised steel frame over which 4.5 mm clear polycarbonate was attached providing full visibility for each animal. A modified squeeze crush within each chamber defined a confinement area that accommodated cattle of different sizes. Each chamber was fitted with a door (1050 × 2100 mm) at either end for entry and exit of the animal. Animals were fed at *ad libitum* levels established in the days before animals entered chambers. Measurements were taken over 23 h and extrapolated to a 24 h methane production. Intake air was sourced external to the animal building, with chamber air vented through the roof line. Inline fans (TD800/200N, Fantech, Melbourne, Vic., Australia) fitted with variable speed controllers maintained flow rates of 100 L/s and a slight negative pressure within each chamber. Relative humidity and temperature (HMT 330, Vaisala, Melbourne, Vic., Australia) and pressure (QBM75-1U/C, Siemens, Zurich, Switzerland) sensors installed in each chamber permitted air flow to be corrected to standard temperature and pressure. Air flow was measured on the exhaust with thermal flow sensors (SS20.500 SCHMIDT[®] Flow sensor, St Georgen, Germany). Air for gas analysis was drawn from a point in the exhaust duct through polyurethane tubing at 4.5 L/min, using a micro diaphragm pump located between a multiport gas-switching unit (SW & WS Burrage, Ashford, Kent, UK) and membrane drier (Perma Pure LLC, Toms River, NJ, USA). Following particulate filtering and dehumidifying using a four pot refrigerated drier (AF30-02, SMC Pneumatics Australia, Sydney, NSW, Australia), air samples entered the multiport gas-switching unit that sampled each chamber and two outside air ports for 180 s. Air samples then passed through the membrane drier and were metered through independent rotameters before analysis for methane (GFx Infrared, 0–500 mg/kg, Servomex 4100, Servomex Group). Sampling events, internal monitoring of chamber conditions and data management were handled by Innotech[®] processors (Genesis II, Innotech[®], Brisbane, Australia) using digital I/O at 4–20 mA. All data were compiled in a dedicated computer by using a structured query

language database. Daily (24 h) methane emissions were calculated by averaging the last 90 s of each sampling period. System recoveries were assessed by releasing methane (99.9% purity) at known rates (g/min) and regressed against chamber readings between each experimental period.

Data analyses

The relationships between MP (g/day or MJ/day) and DMI (kg/day) or GEI (MJ/day) were analysed for the combined data in a meta-analysis using linear mixed-effect models (Galwey 2006) by REML in GENSTAT (17th edn, 2014, VSN International, Hemel Hempstead, UK). The mixed-effect models consisted of fixed effects to characterise the relationships between MP and intake, and random effects to characterise the variance structure of the data.

The fixed effects included a linear term in DMI (or GEI) by factorial effects of cattle class (DAIRY, S.BEEF or N.BEEF), cattle breed (Shorthorn versus Angus) within S.BEEF, and sex (female, male or steer). Also included were quadratic terms in DMI by cattle class.

The random effects comprised variance components for experiment, period within experiment, treatment within experiment, and residuals. The variance component for experiment accounted for variance among experiments within each class. The period-within-experiment variance component was relevant only to two short-term, dairy crossover trials. The periods in some longer-term dairy experiments related to measurements taken in autumn and spring, over 2 years. These long-term periods were considered as separate experiments in the present analysis. The treatment-within-experiment variance component accounted for feed-type effects. In addition to these, separate residual variances for the four locations from which the data were sourced, were included in the model. Finally, an additional variance for dairy experiment was included, allowing for larger variance among dairy experiments than among beef experiments. This random-effect structure was employed in all analyses.

The overall aim of the present paper was to present the simplest satisfactory relationship between MP and intake. Accordingly, the fixed-effects model was progressively simplified by removing terms that were not statistically significant or that were physically implausible. The initial full model was as follows:

$$MP_{li} = \mu + \beta_0 x_{li} + \delta_0 x_{li}^2 + c_k + \beta_k x_{li} + \delta_k x_{li}^2 + s_j + \phi_j x_{li} + b_q + \gamma_q x_{li} + E_e + D_{ke} + P_{kp} + T_{et} + \varepsilon_{li},$$

where x is either DMI or GEI, μ is a constant (intercept). Other Greek letters indicate coefficients for linear and quadratic effects in DMI. Lower-case Latin letters are factorial fixed effects. Upper-case Latin letters are random effects, assumed to be normally distributed, and ε is a normally distributed residual error. Subscript $l = 1 \dots 4$ indicates the location of experiment, e denotes the experiment from which the data were derived, t indexes the treatment within experiment, p denotes the period (within dairy crossover experiments), and i identifies the observation within levels of location, experiment, treatment and period. Subscript $k = 1 \dots 3$, indicates the class of cattle (DAIRY, S.BEEF, N.BEEF), $j = 1 \dots 3$, the cattle sex (female, male, steer), and $q = 1 \dots 3$, the cattle breed (Shorthorn, Angus,

other). The variances of the random effects are variance among experiments,

$$\text{Var}(E_e) = \sigma_E^2,$$

extra variance among dairy experiments,

$$\text{Var}(D_{ke}) = \begin{cases} \sigma_D^2, & \text{if } k = \text{dairy} \\ 0, & \text{otherwise} \end{cases},$$

variance among periods,

$$\text{Var}(P_{kp}) = \begin{cases} \sigma_P^2, & \text{if } k = \text{dairy} \\ 0, & \text{otherwise} \end{cases},$$

variance among fodder treatments,

$$\text{Var}(T_{et}) = \sigma_T^2,$$

and residual error variances for the four locations,

$$\text{Var}(\varepsilon_{l\text{etpi}}) = \sigma_l^2, l = 1 \dots 4.$$

Models were tested with and without intercepts. The minimal model, that included just a linear term in DMI through the origin, and random (noise) effects, was as follows:

$$MP_{li} = \beta_0 x_{li} + E_e + D_{ke} + P_{kp} + T_{et} + \varepsilon_{li}.$$

A generalised R^2 statistic (Nakagawa and Schieizeth 2013), suitable for use in mixed-effect models, was calculated for each model. Fixed effects were tested using F -tests from accumulated analysis of deviance, and contrast Student's t -tests derived from coefficient estimates and their variance-covariance matrix. Distributional assumptions of normality and constant variance were checked graphically using histograms, normal quantile plots and plots of residuals versus fitted values.

The predictive capability of the final model was assessed by cross validation in which each of the 29 experiments was successively excluded from the calibration data, to which the model was refitted, with the excluded data being used as test data. The root mean square of percentage discrepancies between the cross-validation predicted and observed test data MP for all 29 experiments was used to summarise precision of prediction. Percentage, rather than absolute, discrepancy was used because of its stability for these data, consistent with variance increasing with mean, but coefficient of variation remaining constant.

Results

Methane production and DM intake

A graph of MP versus DMI data indicated a linear relationship, possibly through the origin, with the spread of data observed to increase with increasing DMI (Fig. 1).

In the initial meta-analysis model, Shorthorn did not differ significantly (breed effects $P > 0.05$) from Angus in intercept or slope of DMI. Accordingly, breed was excluded from all subsequent models. However, there were significant effects of cattle class ($P = 0.03$) and sex ($P < 0.001$) that remained significant in the model with breed effects excluded. Slope and intercept estimates for these are shown in Table 3. Some of the intercepts were significantly different from zero (S.BEEF (male), $P = 0.032$, and S.BEEF (steer), $P < 0.001$). The estimated slopes were not

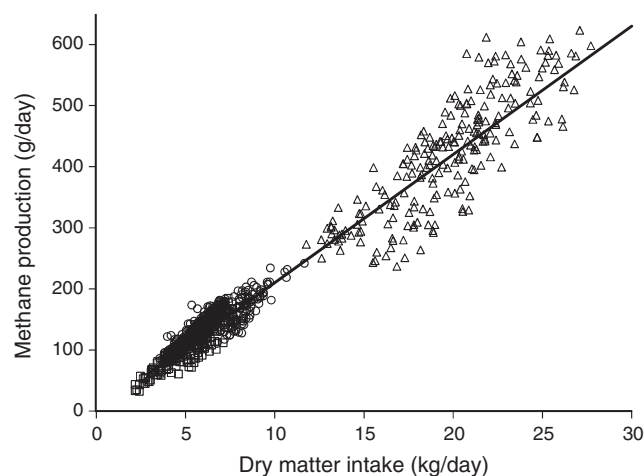


Fig. 1. The combined relationship between methane (CH₄) production and dry matter intake for dairy (DAIRY, open triangles), southern beef (S.BEEF, open circles) and northern beef (N.BEEF, open squares). The solid line represents the relationship $MY = 20.7 (\pm 0.28) \text{ g CH}_4/\text{kg DMI}$.

Table 3. Meta-analysis slope and intercept estimates for methane production versus dry matter intake (DMI) and gross energy intake (GEI) by animal class

Fixed-effect models were $MP = \alpha_i + \beta_i \times DMI$, $R^2 = 91.5$, and $MP = \alpha_i + \beta_i \times GEI$, $R^2 = 91.5$, for class and sex combination i

Class	Slope	s.e.	Intercept	s.e.
<i>DMI</i>				
DAIRY (female)	19.22	1.404	38.0	29.03
S.BEEF (female)	19.35	0.668	12.82	6.79
S.BEEF (male)	24.74	0.733	-15.29	7.12
S.BEEF (steer)	13.5	1.421	68.51	12.38
N.BEEF (steer)	20.64	1.185	-6.1	6.46
<i>GEI</i>				
DAIRY (female)	0.058	0.004	2.140	1.614
S.BEEF (female)	0.059	0.002	0.714	0.382
S.BEEF (male)	0.075	0.002	-0.853	0.400
S.BEEF (steer)	0.041	0.004	3.824	0.691
N.BEEF (steer)	0.066	0.004	-0.380	0.363

significantly different by class ($P = 0.74$), but were different by sex ($P < 0.001$).

When the lines were constrained to pass through the origin, slope estimates were significantly different among classes ($P = 0.042$) and sexes ($P < 0.001$; Table 4, Fig. 2). Pairwise tests of slope estimates between class by sex groups are given in Table 5. These showed significant differences between N.BEEF and each other class by sex group, except for S.BEEF (female). Within S.BEEF, there were significant differences between females and each of males and steers.

Further simplification of the fixed effects, to a single line-through-the-origin, gave the combined estimate of slope as

$$MP = 20.7 (\pm 0.28) \times DMI, \quad (1)$$

where MP is in g/day and DMI in kg/day (Table 4, Figs 1, 2). Despite appearances, results of Tables 4 and 5 are not

Table 4. Estimates of fixed-effect slopes for methane production (MP) versus dry matter intake (DMI) and gross energy intake (GEI) straight line through the origin

Data shown are by cattle class and sex (DMI, $R^2 = 0.924$; GEI, $R^2 = 0.925$), and combined, single line through the origin (DMI, $R^2 = 0.922$; GEI $R^2 = 0.927$)

Cattle class	Slope	s.e.	95% confidence limit	
			Lower	Upper
<i>MP vs DMI (g/kg)</i>				
DAIRY (female)	20.99	0.45	20.09	21.88
S.BEEF (female)	20.48	0.43	19.62	21.33
S.BEEF (male)	20.99	0.42	20.15	21.82
S.BEEF (steer)	21.53	0.45	20.64	22.43
N.BEEF (steer)	19.60	0.49	18.61	20.58
Combined	20.74	0.28	20.18	21.30
<i>MP vs GEI (KJ/MJ)</i>				
DAIRY (female)	0.0635	0.0013	0.0608	0.0662
S.BEEF (female)	0.0619	0.0013	0.0592	0.0645
S.BEEF (male)	0.0634	0.0013	0.0609	0.0660
S.BEEF (steer)	0.0651	0.0014	0.0623	0.0678
N.BEEF (steer)	0.0619	0.0017	0.0587	0.0652
Combined	0.0634	0.0008	0.0618	0.0650

inconsistent. Unlike the confidence intervals for the individual slopes given in Table 4 and Fig. 2, the pairwise tests in Table 5 are designed for testing differences and, therefore, take into account the shared structure in the experimental and sampling design. For example, for S.BEEF, female and male, or female and steer, sometimes occurred together within the same experiment. This resulted in a more precise comparison of their slopes that is not reflected in their individual confidence intervals.

Estimates of the variance components for MP, expressed in Table 6 as standard deviations for ease of interpretation, were calculated under the mixed model having minimal fixed-effects consisting of a common single straight line through the origin. The largest components of variance all pertained exclusively to dairy data. These were, in a decreasing order, the dairy residual variance, dairy experiment variance and dairy period variance. The residual standard deviation for dairy was approximately four-fold the residual standard deviations for the beef classes. The variance component for beef experiment was of an order of magnitude similar to the residual variances. The variance component for treatment was the smallest component, consistent with anti-methanogenic treatments having been excluded from the data. While components have been tabulated in Table 6 only for the simplest fixed-effect model, the estimates derived from other models were very similar and have not been presented here.

Methane production and GE intake

For N.BEEF data, individual GE concentrations were measured and used for each feed. The mean GE concentration for the N.BEEF diets was 17.7 MJ/kg DM. As a consequence of the relativity between mass and energy, results expressed on an energy basis were essentially the same as those expressed on a DM basis. The one exception was for N.BEEF data, where there was a 5% increase in methane yield (MY) on an energy basis versus a DM basis. This is most apparent in Fig. 2 where the mean MP values are given for the five class by sex groups.

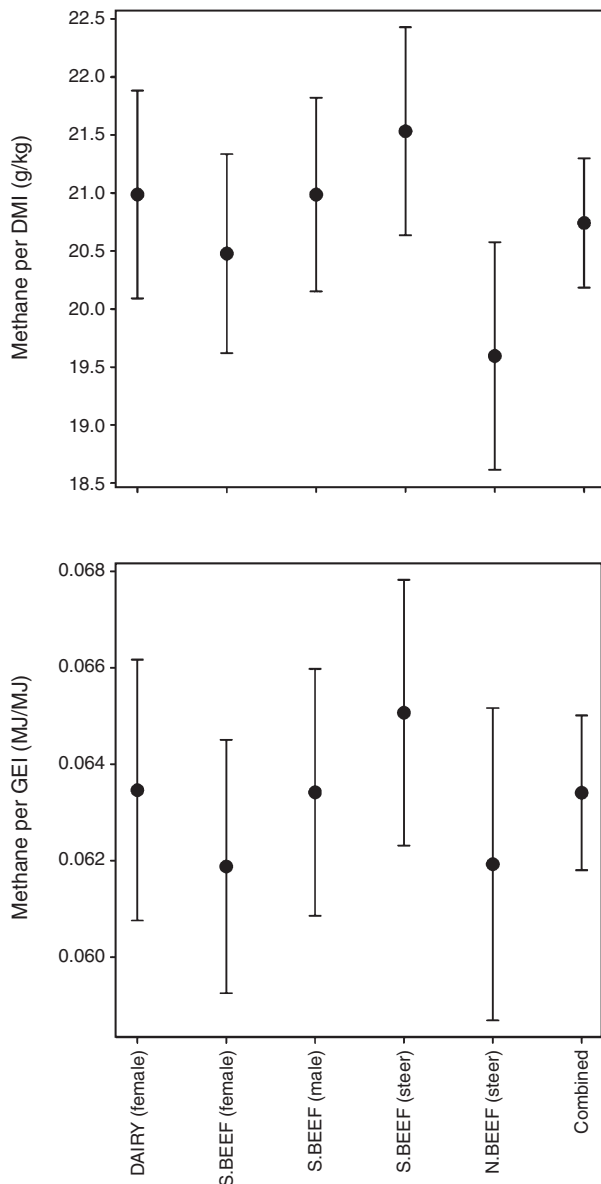


Fig. 2. Estimates of methane yields (dry matter (DM) and gross energy (GE) basis) with 95% confidence intervals by cattle class and sex, and combined. Methane yields (g/kg DM or MJ/MJ GE intake (GEI)) were derived as slopes for methane production (MP) versus DM intake (DMI) or GEI as straight lines through the origin, in a meta-analysis using a linear mixed-effect model.

As with MP on a DM basis, a single line-through-the-origin gave the combined estimate of slope as

$$MP = 0.0634 (\pm 0.0008) \times GEI, \quad (2)$$

where MP and GEI are in MJ/day (Table 4, Fig. 2). Slope did not differ significantly between N.BEEF steers and other class-by-sex groups, as it did on the basis of DM intake (Table 5).

Discussion

Methane and DMI

There is a single, strong, linear relationship between MP and DMI across all classes of forage-fed cattle in Australia (Eqn 1).

Table 5. *P*-values for pairwise Student's *t*-test contrasts between slope coefficients of methane production (MP) versus dry matter intake (DMI) and gross energy intake (GEI) straight line through the origin, by cattle class and sex

Note that some pairwise tests were more sensitive than might be expected given their 95% confidence intervals. This is because these combinations of class and sex sometimes occurred together in the same experiments

	DAIRY (female)	S.BEEF (female)	S.BEEF (male)	S.BEEF (steer)
<i>DMI</i>				
S.BEEF (female)	0.41			
S.BEEF (male)	0.99	0.001		
S.BEEF (steer)	0.39	0.001	0.11	
N.BEEF (steer)	0.04	0.18	0.04	0.005
<i>GEI</i>				
S.BEEF (female)	0.404			
S.BEEF (male)	0.979	0.001		
S.BEEF (steer)	0.408	0.001	0.109	
N.BEEF (steer)	0.469	0.98	0.472	0.144

Table 6. Variance components, expressed as standard deviations (square root of variance component) corresponding to the meta-analysis mixed-model random effects for methane (CH₄) production under the simplest fixed-effect (line through the origin) model: $MP = \beta \times DMI$

Source	Standard deviation (g CH ₄ /day)	
	DMI	GEI
Beef experiment	10.3	0.49
Dairy experiment	24.8	1.21
Treatments	4.8	0.26
Period, dairy	17.0	1.01
Residual, dairy	44.7	2.49
Residual, beef Rockhampton	10.4	0.58
Residual, beef Townsville	13.5	0.74
Residual, beef southern	9.5	0.54

This is despite the data being collated from experiments conducted at four different research sites, using a wide range of diets typical of those fed to cattle on Australian farms, and involving 220 lactating Holstein dairy cows, 680 Angus beef cattle, including yearling heifers, 2-year-old bulls and steers and 134 Brahman steers. Equation 1 was developed from the most comprehensive dataset relevant to the Australian cattle industries and is strong ($P < 0.001$, $R^2 = 0.92$). In addition, the methane-emission measurements in this dataset were all made using respiration chambers, a method generally accepted as the most accurate of those currently available (Storm *et al.* 2012). For these reasons, we propose Eqn 1 as a universal equation to describe methane emissions from all forage-fed cattle in Australia.

A linear relationship between MP and DMI has also been shown by other researchers. Ricci *et al.* (2013) reported that DMI for beef cattle accounted for 70% of the variation in MP in a meta-analysis of 38 published international studies on dairy and beef

cattle. Their data included methane measurements made by both respiration chambers and by the SF₆ technique of Johnson *et al.* (1994), and this may account for some of the variance in their dataset. Hristov *et al.* (2013a, 2013b) also demonstrated a simple relationship between MP and DMI in a meta-analysis of dairy data that included DMI over a range similar to that in the current analysis ($\text{MP (g/day)} = 19.14 \times \text{DMI} + 2.54$). Similarly, Dijkstra *et al.* (2011) reported that methane yield (MY, g CH₄/kg DMI) for dairy cows in The Netherlands was 23.1, suggesting a linear relationship between MP and DMI, with an intercept of zero.

In contrast, a curvilinear relationship between MP and DMI was developed by Blaxter and Clapperton (1965), and subsequently corrected by Wilkerson *et al.* (1995). Recently, Moate *et al.* (2014) listed six reasons why the equation of Blaxter and Clapperton (1965) should not be used for Australian cattle, including that the work was undertaken predominantly using sheep and feeds that are qualitatively different from the forage-based diets typically used in Australia. A curvilinear relationship based on the chemical composition of the diet is used to estimate emissions from dairy cattle in the United States (Rotz *et al.* 2011). Reported curvilinearity between MP and DMI is most likely due to an increasing proportion of concentrate in the diet (Rotz *et al.* 2011). The purpose of our analysis was to develop a universal equation for forage-fed cattle in Australia, which represents a substantial proportion of the Australian beef and dairy industries that are pasture or forage-based. The equation neither uses data from cattle fed over 30% concentrate in the diet, nor is it intended to be used for estimating emissions from such cattle. Constraining our dataset to low concentrate-inclusion levels may explain the lack of curvilinearity at higher intakes observed in our analysis.

Methane yield

The slope of the proposed universal relationship between methane and DMI was similar to previously published values. For dairy cattle alone, the MY was 21.0 ± 0.45 g/kg DMI, which compares well with values reported in the scientific literature that range from ~17 g methane per kg DMI to a maximum of ~25 g/kg DMI. Grainger *et al.* (2007) summarised data from Australian dairy cows and found a low yield of methane of 17.1 g/kg DMI. While some of the Grainger data were included in the present analysis, the current dairy dataset excludes all data where the concentrate proportion of the diet was over 30% and also includes data obtained since 2007 (Moate *et al.* 2014). The dairy MY is a little below that of 23.1 g CH₄/kg DMI reported by Dijkstra *et al.* (2011) for dairy cows in The Netherlands and slightly higher than the 19.1 g CH₄/kg DMI reported by Hristov *et al.* (2013a, 2013b). The analysis by Hristov *et al.* (2013a, 2013b) included high-concentrate diets and this probably contributed to the slightly lower MY than in our analysis.

For the classes of beef cattle used, MY ranged from 19.6 ± 0.49 to 21.5 ± 0.45 g/kg DMI, which is within previously published values. Irish beef data suggested a higher MY of ~25 g/kg DMI (Yan *et al.* 2009), with a reasonable relationship between MP and DMI ($R^2 = 0.68$). Their analysis included trials with growing beef cattle-fed diets containing 0–70% concentrate. This discrepancy between Irish and Australian data may be attributed to the

predominance of extensively fermented grass silages in many of the Irish studies. Ensiling is known to increase GE concentration as some fermentation products have a higher energy value than has non-fermented carbohydrate. However, this effect would still be too small to account for all of the difference in MP. Recent research from the United Kingdom (Hammond *et al.* 2014) supports Irish research (Yan *et al.* 2000) showing that MY is higher for ensiled forages. Hammond *et al.* (2014) found MY to be between 28.0 and 28.9 g/kg DMI for ryegrass and ryegrass–legume silages. However, when similar forages were grazed, MY was <23 g/kg DMI. It should be noted that Hammond *et al.* (2014) used respiration chambers for the silage study, while the SF₆ technique was used for the grazing study. In the United States, calculations for estimating national emissions rely on an empirical approach for beef cattle based on Tier 2 methods for feedlots (USDA 2011). With a baseline methane yield on an energy basis of 3% GE, the value is modified on the basis of the nature of the diet being fed. The nearest relationship to a high-forage diet is a MY of 4.2% for diets with less than 45% concentrate. Using this relationship, US estimates are markedly below those we propose for Australian growing–finishing beef cattle (Fig. 3).

It has been reported that MY decreases with increasing concentrate in the diet (Tyrrell and Moe 1972) and can be as low as 3% of GEI (Johnson and Johnson 1995) for diets with a high proportion (>60%) of concentrate. Many forage-based dairy feeding systems typically include a low level of concentrate feeding to reduce possible deficiencies in ruminally available nitrogen or soluble carbohydrate (Doyle *et al.* 2005). In the current analysis, we selected a maximum concentrate percentage of 30% on the basis of the assumption that rumen fermentation and MY are not significantly affected up to this concentration (Sauvant *et al.* 2011).

Limitations of the dataset

The current dataset is potentially confounded in several ways that could influence the validity of the relationship.

Animals of different ages were used in the DAIRY and BEEF datasets, with correspondingly different DMI and MP values. Measurements in the DAIRY dataset were from

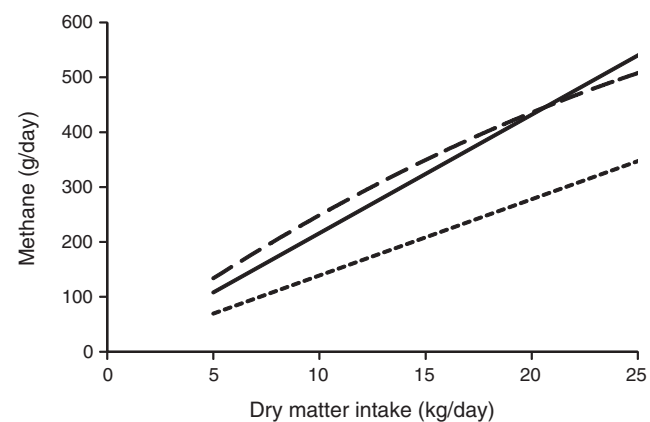


Fig. 3. Comparison of the proposed universal relationship (solid line) for Australian cattle with US relationships for dairy (dashes) and beef (dots) cattle (USDA 2011).

lactating Holstein–Friesian dairy cows with a high DMI and high MP, while measurements in the BEEF dataset were from growing young stock with a low DMI and low MP. The analysis did not include any data for young growing dairy heifers or mature beef breeder cattle.

A sex effect was detected for the S.BEEF dataset, where males, heifers and steers were evaluated. All measurements in the N.BEEF dataset were taken on steers and those in the DAIRY dataset were from lactating cows, so a possible sex effect in *Bos indicus* and dairy was not able to be tested.

Different diets were used at different experimental sites and diet is well known to affect methane production (e.g. Hristov *et al.* 2013a). The DAIRY and N.BEEF data are based on a wide variety of diets, with generally few observations per diet, while the S.BEEF data were mainly derived from a very large dataset where the diet quality was deliberately standardised. Site of digestion can be influenced by the form in which the forage is fed. Fresh and grazed forages are more extensively degraded in the rumen than are hays and dried forages (Holden *et al.* 1994). This could have influenced MP in the DAIRY data versus S.BEEF and N.BEEF data, since the DAIRY data included fresh forages, whereas the majority of BEEF data were collected from cattle fed dried forages. Furthermore, within the beef breeds, all data for tropical forages were generated from *Bos indicus* cattle, while temperate forage data were generated from *Bos taurus* cattle. It cannot be discounted that the 5% lower MY on an energy basis in N.BEEF data may have been due to genetic differences. *Bos indicus* × *Bos taurus* steers have been shown to produce ~10% less methane than *Bos taurus* steers of comparable weight and age (Vercoe 1970). Generally, it is considered that tropical grasses are associated with higher methane emissions per unit of DMI than are temperate grasses (Kurihara *et al.* 1999; Ulyatt *et al.* 2002) due to higher lignification and subsequent lower rate of passage of feed. However, direct comparisons between tropical and temperate grasses are scarce (e.g. Margan *et al.* 1988; Archimède *et al.* 2013). Archimède *et al.* (2011) conducted a meta-analysis of 22 studies and concluded that methane emissions were 12% higher for ruminants fed C4 (tropical) grasses than for those fed C3 (temperate) grasses. Their analysis employed a range of techniques, including the SF₆ technique, and a range of livestock species, including sheep and goats. Our data, which are restricted to cattle and measurements made in respiration chambers, clearly showed that MY was no higher for *Bos indicus* cattle fed C4 (tropical) grasses, than from *Bos taurus* cattle fed C3 (temperate) grasses.

In an analysis of this type, it is impossible to eliminate all factors that may have contributed to some of the statistical ‘noise’. The apparent positive relationship of residual variance with MP may have been an artefact of experimental conditions at different sites and the observation of a sex effect in the S.BEEF data may have been an artefact of data measurement or selection. Nevertheless, we believe that the effects of these factors, if present, were too small to markedly influence the results, and this is borne out by the strong regression statistic ($R^2 = 0.92$) of the universal relationship. In addition, the current dataset, with over a thousand individual measurements of MP from cattle in open-circuit respiration chambers, is very much larger than the datasets used to establish the current Tier 2 estimates of methane emissions used in the Australian inventory.

Choice of statistical approach

The discussion around the statistical approach is confined to MP on a DM basis, because the same arguments and conclusions would also apply to MP expressed on an energy basis.

The data for the current meta-analysis were derived from experiments that were not planned as a whole series. Consequently, the data were both experimental and observational, involving a certain amount of imbalance and confounding due to differences between sites and the choices of experimental protocols, animal type and sex. The challenge for the present meta-analysis was to detect and represent the structure in the data in an efficient and realistic manner, so as to reliably select and estimate models descriptive of MP. A mixed-model framework was well suited to this end (St-Pierre 2001; Sauvant *et al.* 2008).

The purpose of the random effects was, first, to account for lack of independence arising by virtue of the data being grouped according to their site, experiment, time and experimental structure. Second, additional random effects were included to account for variance heterogeneity apparent in the data. This was achieved by the inclusion of multiple residual variances, one for each location from which the data were sourced. These four locations utilised different respiration chambers and experimental protocols that could have contributed to differing variances. Furthermore, these locations each measured just one class of animal, for which there were different ranges of DMI and thus also of MP. In particular, it was found that dairy, with its greater DMI and MP had greater variance, not only within, but also between experiments. Random effects were included specifically to account for this heterogeneity.

Variations of this approach to the meta-analysis were investigated. For example, log-transformation of MP before analysis was effective in removing much of variance heterogeneity, but it did not completely remove it. It did obviate the need for a special variance component for dairy experiments. Log-transformation, while maintaining the convenience of a linear model, implied a change in the form of the MP versus DMI relationship to that of a power curve. With this, a straight line through the origin occurs as a special case, when slope coefficient for log-DMI equals unity.

A third approach, noting that variance increases with both MP and DMI, was to analyse MY (i.e. the data formed by the ratio MP : DMI) directly. This too reduced variance heterogeneity, but not completely. It also simplified the fixed-effects model to one without DMI, or raised a perceived difficulty of dealing with DMI on both sides of the model equation.

All approaches tested produced similar estimates of slopes of the straight line between MP and DMI, through the origin. As all approaches require a reasonably complex random-effects structure, dictated by the grouped sampling structure, there appears to be no compelling reason to choose one above the other. We adopted the first approach, of analysing MP in terms of DMI by classifying factors, which is analogous to grouped regression, as being conceptually the simplest for the readership.

The case for adopting a single relationship

There was some evidence of non-zero intercepts in relationships between MP and DMI for males and steers within the

S.BEEF class. For males, the intercept was less than zero, whereas for steers, the intercept was greater than zero. The reasons for this remain obscure. It is possible that these were in fact Type I errors. Biologically, it makes sense that MP should be zero at zero DMI, so the relationship must eventually go through the origin. This implies that either the straight-line relationship does go through the origin and we were mistaken in allowing for non-zero intercept, or that the true relationship actually involved some

curvature. While we cannot exclude the possibility of curvature, it is unlikely that such curvature would manifest at the relatively low levels of DMI observed for these beef data, particularly when it was not apparent at the higher DMI observed for DAIRY. It is also apparent that the S.BEEF (male) and S.BEEF (steer) data do in fact not stray far from a straight line through the origin shared by all of the data (Fig. 4). We also note that a small change in slope can produce a large change in intercept, especially when

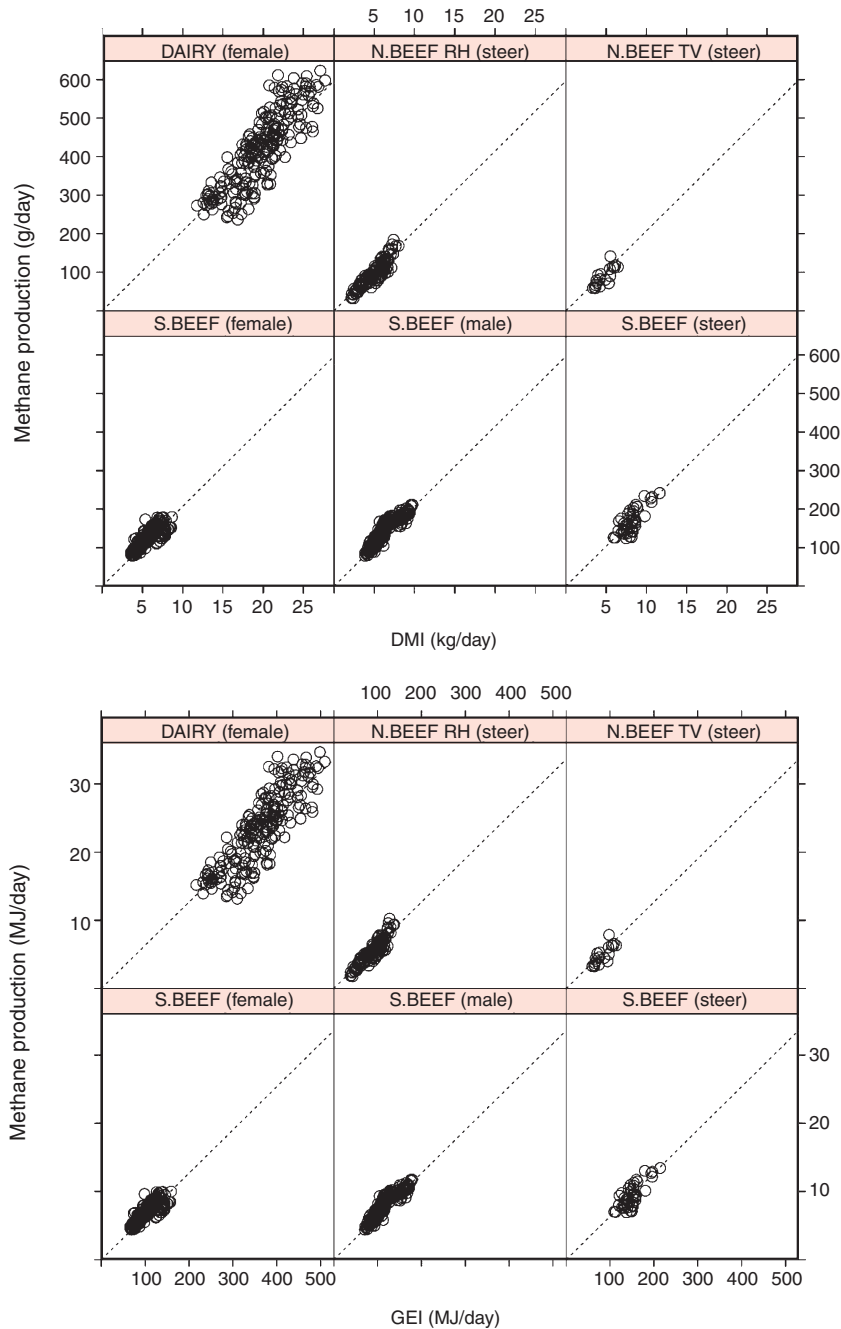


Fig. 4. Methane production plotted against dry matter intake (DMI) or gross energy intake (GEI), separately for each data source by sex combination that was present in the experimental data sourced from eastern Australia. The dashed line is the line through the origin (slope 20.7), fitted to the combined data using a mixed-effects meta-analysis.

DMI data are clumped well away from zero. For these reasons, we proceeded to include in our analysis straight-line models constrained to pass through the origin. When this was done, the generalised R^2 -value actually increased slightly (from 0.915 to 0.924; Tables 3, 4). Apart from its simplicity, the adoption of the straight line through the origin has the added advantage that the slope can be interpreted as the average MY.

The model suggested in the present paper can be used to predict the methane emissions from individual animals, from a herd of animals or emissions with respect to the national inventory. If the model is used to predict methane emissions from an individual animal, then, at a given DMI, the spread of data as shown in Fig. 1 provides an indication of the possible error of prediction. At any given DMI, the observed variation in methane emissions is due to several factors, including differences in diet composition (Johnson and Johnson 1995), variation in the day-to-day feed intake (Moate *et al.* 2012), genetic differences among animals (Hegarty *et al.* 2006) and measurement error (Gardiner *et al.* 2015). Our model indicates that all of these factors may collectively introduce an error of ~13.4% (root-mean-square percentage discrepancy between points and the fitted line) into the prediction of the methane emissions from an individual animal. The 'leave one experiment out at a time' cross-validation gave comparable root-mean-square percentage error of prediction of 14.4%. The variance component estimates presented in the paper can be used to estimate absolute prediction error for an animal, or for a herd, of a particular class. However, if the model is used to estimate the methane emissions for the national inventory, then, on the basis of Eqn 1, the error of prediction is likely to be ~1.4%.

Current inventory methods versus the universal equation

The current Australian national accounting system (Department of the Environment 2014) uses two distinct methods for estimating methane emissions. For dairy cattle and beef cattle in temperate regions, feed intake is based on the equation of Minson and McDonald (1987) corrected for milk production (Standing Committee on Agriculture 1990); then, the equation of Blaxter and Clapperton (1965) as corrected by Wilkerson *et al.* (1995) is used to predict the MY. For beef cattle in tropical regions, intake is calculated according to Minson and McDonald (1987), whereas MY is based on the equation of Kurihara *et al.* (1999) as modified by Hunter (2007). The following relationships between MP and DMI were derived using the methods of Australian Inventory of Greenhouse Gases (Department of the Environment 2014):

$$MP_{\text{DAIRY}} = -0.64 \times \text{DMI}^2 + 39.2 \times \text{DMI} - 66.0, \quad (3)$$

$$MP_{\text{S.BEEF}} = 26.4 \times \text{DMI} + 0.213, \quad \text{and} \quad (4)$$

$$MP_{\text{N.BEEF}} = 34.9 \times \text{DMI} - 30.8, \quad (5)$$

where MP is methane production (g/day) for dairy cattle and beef cattle in southern or northern Australia.

These relationships generally estimate a higher MP than does the new universal equation (Fig. 5). Estimates of MP for dairy cattle were reduced by ~10% at DMI up to 15 kg/day. Above this DMI, the curvilinearity of the current method used for accounting diminished the difference between the two relationships, such that

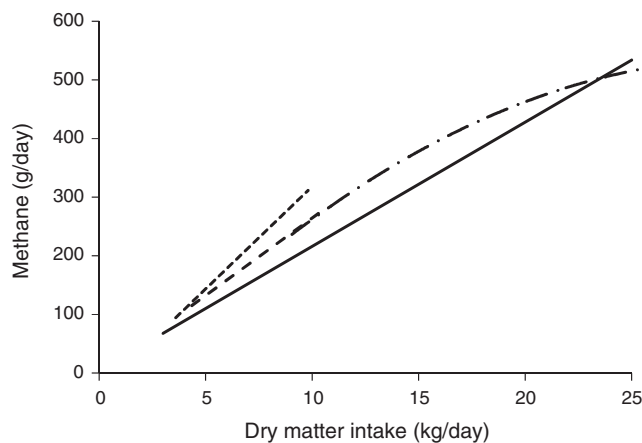


Fig. 5. Comparison of the relationships used in the Australian inventory for national greenhouse gas accounting for dairy (DAIRY, long dashes and dots), southern beef (S.BEEF, short dashes) and northern beef (N.BEEF, dots) with the proposed universal relationship (solid line).

at DMI above 23 kg/day, the proposed linear equation predicted marginally higher emissions. For beef cattle in temperate regions, estimates of MP were between 5% and 20% lower than the current inventory estimate across the likely intake range of growing beef cattle. For beef cattle in tropical regions, the reduction in estimated MP ranged from 25% to 30%.

Using the proposed linear equation dramatically reduces emission estimates from enteric fermentation of Australia's beef and dairy cattle (Table 7). By applying the universal equation to the 2012 inventory data (Department of the Environment 2014) and applying a global warming potential (GWP) of 21 from the 2012 inventory, emissions from cattle are reduced by 10.6 Mt (CO₂-e) or 24.1%. This would reduce the total contribution of agriculture from 16.1% to 14.1% of national GHG emissions. Under the proposed inventory methodology for the next accounting period, a GWP for methane of 25 will be used to recalculate the inventory back to 1990 (Department of the Environment 2015). If the current equations were used, the estimated total emissions from the cattle industries would be 52.1 Mt (CO₂-e), whereas by applying the new relationship, the emissions are calculated as 39.5 Mt (CO₂-e). Thus, the new universal equation predicts 12.6 Mt (CO₂-e) less emissions.

Compared with the 2102 inventory with a GWP of 21, the combined effect of the new universal equation and a GWP of 25 would result in estimates of 0.2 Mt CO₂-e more emission for DAIRY, no change for S.BEEF, 4.5 Mt CO₂-e less emissions for N.BEEF, and a net reduction in the total estimated inventory for cattle of 4.3 Mt CO₂-e annually.

Summary and conclusions

On the basis of the most comprehensive dataset for methane emissions measured in open-circuit respiration chambers in Australia, the case is made to revise the methods used for calculating the national inventory for forage-fed cattle. The results showed that a single relationship for dairy and all beef cattle is justified, considering the inconsequential differences found between the dairy and beef cattle in the tropical northern and temperate southern regions of Australia. It is proposed that

Table 7. Impact on Australia's calculated 2012 enteric-methane emissions (CO₂-e Mt/year) from cattle of applying the proposed relationship between methane production and dry matter intake for dairy (DAIRY), southern beef (S.BEEF) and northern beef (N.BEEF) cattle using a global warming potential (GWP) of either 21 or 25

Data and the old method used are from Department of the Environment (2014), the new method uses the relationship proposed in the current paper

Cattle class	Old method	New method	Change	Difference (%)
<i>GWP = 21</i>				
DAIRY	6.6	5.7	-0.9	-14
S.BEEF	14.0	11.8	-2.2	-16
N.BEEF	23.2	15.7	-7.5	-32
Total	43.8	33.2	-10.6	-24
<i>GWP = 25</i>				
DAIRY	7.9	6.8	-1.1	-14
S.BEEF	16.7	14.0	-2.7	-16
N.BEEF	27.6	18.7	-8.9	-32
Total	52.1	39.5	-12.6	-24

for the national inventory, a single relationship for forage-based diets (forage content of >70%) between MP and DMI of 20.7 g/kg DM should be adopted when GE content of the diet is unknown and assumed to be similar to the default value of 18.4 MJ/kg DM. If the GE concentration of the diet is known, then an energy loss as methane of 6.3% of GE is recommended. We do not support a more complex relationship that takes into account diet quality, rate of passage, class of cattle and physiological state on the presumption that the animal integrates these and other variables into the total feed eaten when consuming forage-based diets. Voluntary DMI is a product of both plant and animal characteristics affecting digestion and, as such, represents a suitable basis for estimating methane emissions.

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