Final report

Assessment of the Australian Feedlot Enteric Methane Inventory equation

Project code: B.FLT.5013

Prepared by: Amelia de Almeida and Fran Cowley
University of New England

Date published: 21 June 2024

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

The aim of this study was to evaluate equations in the literature for predicting methane (CH_4) emissions of beef cattle when fed tempered barley-based diets typical of the Australian feedlot industry. A large database of methane measurements performed in respiratory calorimeters' taken from beef cattle fed a range of barley-based feedlot diets was assembled and analysed. This database with 384 individual measurements of 53 animals from 4 studies included a wide range of factors that are known to impact methane production such as dry matter intake, ether extract, crude protein, cell wall components, amongst others. The current methodology used by the Australian Government to report CH_4 emissions from grain-fed beef cattle was evaluated against observed CH_4 emissions, along with 7 alternative predictive equations, but all displayed significant mean and system bias in prediction of CH_4 production. The current Moe & Tyrell (1979) equation was found to overpredict CH_4 production, such that the average CH_4 emissions produced by Australian feedlot cattle was predicted by Moe & Tyrell (1979) to be 2.44 times the observed emissions measured in respiratory calorimeters. Two new enteric methane prediction equations were developed; a methane yield equation based on DMI: CH_4 g/d = 9.89 ± 1.54 × DMI of (n = 384; $P < 0.01$; RMSE = 32.6 g/d; $r^2 = 0.85$); and an equation based on DMI, neutral detergent fibre (NDF) and dietary fat (EE): CH_4 (g/d) = 5.11 ± 1.58 × DMI - 4.00 ± 0.821 × EE + 2.26 ± 0.125 × NDF (n = 384; $P < 0.05$; RMSE = 22.2 g/d; $r^2 = 0.91$). The results of this evaluation will inform the most appropriate methodologies for accurately predicting the methane emissions of the Australian feedlot cattle industry, at a country and international reporting level.

Executive Summary

Background

The United Nations Framework Convention on Climate Change (UNFCCC) mandates that industrialized nations report their greenhouse gas (GHG) emissions estimations and uncertainties using the Intergovernmental Panel on Climate Change (IPCC, 2006; IPCC, 2019) guidelines. The Australian government currently employs country specific Tier 2 methods (Australian Government, 2023), as per IPCC guidelines, to calculate the methane emissions stemming from Australian feedlot cattle. The Moe and Tyrrell (1979) equation is used in the Australian National Inventory (Australian Government, 2023) to predict methane (CH_4) emissions from beef cattle in feedlots, considering variables related to cell wall carbohydrates [hemicellulose (HC) and cellulose (CEL)] and soluble residue (SR; non-fibrous carbohydrates and starch). However, the Moe and Tyrrell (1979) equation was originally developed using data from dairy cattle fed dairy cattle diets in the late 1970s, and it may not be suitable for estimation of the methane emissions from modern grain-fed beef cattle.

Objectives

The aims of this study were to;

1) Undertake a large respiration chamber calorimeter experiment to measure the enteric methane emissions of Australian feedlot cattle fed tempered barley diets containing different levels of dietary fat and roughage (NDF).
2) Assemble a database of methane emissions from Australian feedlot cattle including the results from the study specified in Objective (1), as well as other data sets of control methane emissions from previous MLA funded respiration calorimeter experiments.
3) evaluate equations in the literature for predicting CH_4 emissions of beef cattle when fed tempered barley-based diets typical of the Australian feedlot industry. The results of this evaluation will inform the most appropriate methodologies for accurately predicting the methane emissions of the Australian feedlot cattle industry sector, at a country and international reporting level.
4) Develop new and more accurate ways to predict enteric methane from Australian lot fed cattle.
5) Provide these results to the Department of Climate Change, Energy, the Environment and Water.

Methodology

A large database of methane measurements performed in respiratory calorimeters' taken from beef cattle fed a range of barley-based feedlot diets was assembled and analysed. This database with 384 individual measurements of 53 animals from 4 studies included a wide range of factors that are known to impact methane production such as dry matter intake, ether extract, crude protein, cell wall components, amongst others.

Results

The Moe and Tyrrell (1979) equation currently utilized by the Australian National Inventory report had poor accuracy with mean bias overprediction of 115 g CH_4/d, such that the predicted CH_4 production (mean of 194.9 g CH_4/d) was 2.44 × observed CH_4 production (mean of 79.9 g CH_4/d), along with significant linear bias ($P < 0.01$), and poor precision ($r^2 = 0.05$). Methane was overpredicted by 55.0 and 163 g/d at minimum, and maximum predicted values, respectively.

All other evaluated equations lacked accuracy and precision in predicting methane emissions of feedlot cattle. Two new equations for predicting enteric methane emissions from feedlot cattle were developed;

1) CH_4 (g/d) = 5.11 ± 1.58 × DMI - 4.00 ± 0.821 × EE + 2.26 ± 0.125 × NDF (n = 384; P < 0.05; RMSE = 22.2; g/d; σ^2_s = 8.02; r^2 = 0.91)
2) CH_4 (g/d) = 9.89 ± 1.54 × DMI (n = 384; P < 0.01; RMSE = 32.6 g/d; r^2 = 0.85)

Where:

DMI = dry matter intake, kg/day;

EE = ether extract (dietary fat), % dietary DM; and

NDF = neutral detergent fibre, % dietary DM.

Benefits to industry

This study contributes to developing accurate estimations of enteric methane emissions for the Australian beef feedlot sector. Applied to the 2021 national feedlot activity data, the new equation (2) would result in a 43.5 % reduction of total emissions from the Australian beef feedlot sector, equivalent to 992,296 tonnes of CO_2-equivalents. Considering all scope 1 emissions from the Australian beef feedlot section, equation (2) would result in a 30 % reduction in total scope 1 emissions. Modelling of new equation (1) will require additional data (EE and NDF composition of typical domestic, mid- and long-fed diets). A dossier will be developed using a peer-reviewed publication from this project, and results from previous MLA projects demonstrating observed methane emissions are lower than predicted by the current methodology. This will be submitted to the Australian Government to support a change in Australia's tier 2 IPCC National Inventory reporting methodology for enteric methane emissions emanating from Australian feedlot cattle to a more accurate equation.

Future research and recommendations

This research has proposed an Australian-specific methodology for predicting enteric methane emissions from feedlot cattle, using barley-based diets, representative of the Australian feedlot industry. This equation should now be validated with a different dataset of methane emissions, potentially extended also to wheat-based diets as the other main grain source used in Australian feedlots.

Table of contents

1. Background

The United Nations Framework Convention on Climate Change (UNFCCC) mandates that industrialized nations report their greenhouse gas (GHG) emissions estimations and uncertainties using the Intergovernmental Panel on Climate Change (IPCC, 2006; IPCC, 2019) guidelines. Additionally, the Australian government is required to report on climate change policies and measures, including emissions, progress towards targets, projections, and mitigation actions. The Australian National GHG Inventory report, published annually, categorizes emissions by economic sector, namely, energy, industrial processes and product use, land use, waste, and agriculture, within each state. In 2022, agriculture contributed 17.9% of Australia's GHG emissions, with enteric methane (CH_4) production accounting for 71% of that total (DCCEEW, 2024). This aligns with the global trend of enteric CH_4 being a major contributor to GHG emissions in industrialized countries.

The Australian government currently employs country specific Tier 2 methods (Australian Government, 2023), as per IPCC guidelines, to calculate the methane emissions stemming from Australian feedlot cattle. The Moe and Tyrrell (1979) equation is used in the Australian National Inventory (Australian Government, 2023) to predict CH_4 emissions from beef cattle in feedlots, considering variables related to cell wall carbohydrates [hemicellulose (HC) and cellulose (CEL)] and soluble residue (SR; non-fibrous carbohydrates and starch). However, the Moe and Tyrrell (1979) equation was originally developed using data from dairy cattle fed dairy cattle diets in the late 1970s, and it may not be suitable for estimation of the methane emissions from modern grain-fed beef cattle.

We hypothesized that although dairy and beef cattle are both same species, the interactions of their distinct diets and digestive physiologies, arising from selection, should be considered. Factors such as the digestive tract volume, mean retention time of digesta, the digestibility of the feed offered, and digestion and fermentation characteristics may affect the accuracy of the Moe and Tyrrell (1979) equation in predicting CH_4 emissions from feedlot cattle. Furthermore, recent research from the Netherlands (Van Gastelen et al., 2019) has suggested that developments in management and breeding of ruminant animals in the past few decades may have altered their digestive physiology (i.e., greater intake capacity, increased passage rate and decreased digestibility). Therefore, studies from the 1980s or earlier may not be applicable in describing today's animals' physiology.

The present study was designed to develop a world leading data base of enteric methane emissions measurements from feedlot cattle, measured using respiratory calorimeters to 1) evaluate existing equations that predict methane in the literature and identify their adequacy for predicting CH_4 production from feedlot cattle, specifically under conditions similar to those used in the Australian feedlot industry and 2) develop new approaches for predicting enteric methane from feedlot cattle. The present study will contribute towards an adequate country-specific estimation of enteric CH_4 emissions for feedlot cattle in Australia.

2. Project objectives

1. Results from an *in-vivo* study to determine the effect of dietary fat and roughage levels on baseline emissions of feedlot cattle under conditions representative of the Australian feedlot industry.
2. Validation of the available methods for predicting methane emissions from feedlot cattle in Australia using data from the in-vivo study as part of this work and other data sets available from previously MLA funded projects.
3. Through the involvement (sub-contract) of Integrity Ag & Environment – make a submission to DISER National inventory team with the data from this project providing evidence of the best equation to predict methane emissions from Australian lot fed cattle.

3. Methodology

3.1 Database description

3.1.1 Respiration calorimeter study

3.1.1.1 Experimental design

A 40 head respiration calorimeter study evaluated the effect of increasing dietary fat and roughage levels on baseline emissions of feedlot cattle under conditions representative of the Australian feedlot industry. All procedures were approved by the University of New England Animal Ethics Committee (Authority number ARA22-013). The experiment was conducted at the University of New England Centre for Animal Research and Teaching (CART), Armidale, NSW, Australia. Forty Angus steers (15 – 18 months of age) of initial liveweight (LW) of 338 ± 23.5 kg. After transport to the research facility, the steers were inducted with visual identification (eartag), vaccinated (Ultravac® 7-in-1, Zoetis, Melbourne Australia and Bovilis MH+IBR, Coopers Animal Health, Macquarie Park, NSW, Australia) and received an oral anthelmintic ("Flukazole + Selenium": Virbac, Milperra NSW, Australia). Throughout the experiment, the steers were housed in individual indoor pens. During an initial 7-day acclimation period before the trial, the steers were fed a 100% forage diet, ad libitum, to permit acclimation to the housing. Initial LW was recorded (Gallagher W310 (Gallagher, Hamilton, NZ) and used to split the steers into four blocks (light and heavy LW) of 10 steers each. Throughout the experiment, LW was recorded on days 20, 27, 48, 55, 76, 83, 104 and 111, before feeding and on days 21, 28, 49, 56, 77, 84, 105 and 112 4 hours after feeding. Prior to each weighing procedure, the scale was calibrated with a known weight of 300 to 400 kg. Blocks 1 to 4 commenced the experiment one day apart, so that all procedures were staggered by one day for each subsequent block. The purpose of this staggered design was to account for the number of respiration chambers, which allowed 10 animals to be measured per day. The steers in each block completed a 112-day experimental feeding period.

The in vivo study consisted of a randomised block design with 4 groups of 10 animals (n=40). During 112 d (four 28-d runs) steers were housed in the UNE CART facilities and were subjected to 8 measurement periods in methane chambers (Fig. 1). The trial was carried out from 6th July of 2022 and 27th October 2022. The treatments (fat level and NDF content) were defined in consultation with industry veterinarians and nutritionists in prior to study commencement, considering standard practice in feedlots.

Total NDF %	Oil level (10 steers per Oil level)				Period
	3% DM	4.3% DM	5.6% DM	(7% DM)	
35%	Starter/no oil	Starter/Low oil	Starter/Medium oil	Starter/High oil	28 d
30%	T1/no oil	T1/Low oil	T1/Medium oil	T1/High oil	28 d
25%	T2/no oil	T2/Low oil	T2/Medium oil	T2/High oil	28 d
20%	Finisher/no oil	Finisher/Low oil	Finisher/Medium oil	Finisher/High oil	28 d

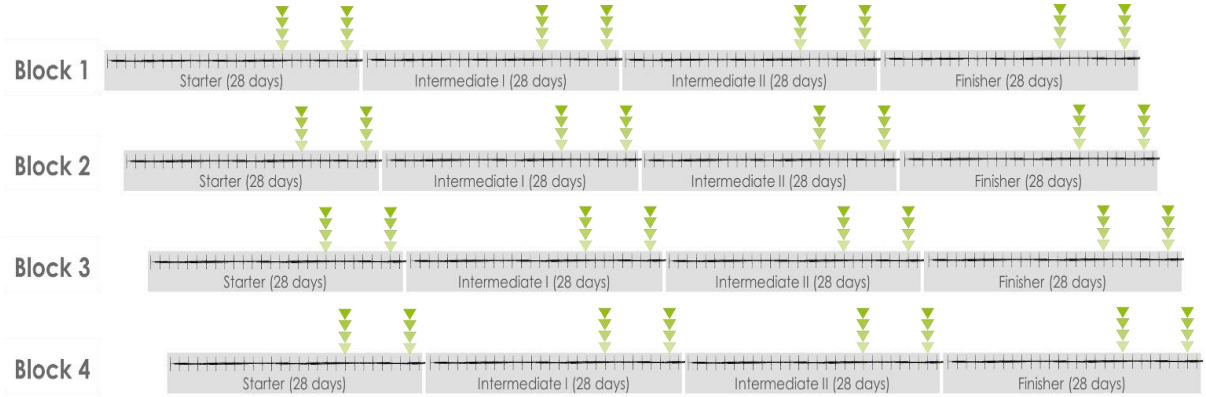


Figure 1. Experimental design depicting diets and respiration chambers measurements.

3.1.1.2 Measurement of methane emissions

The CH_4 production was estimated by confining each steer in an individual open circuit respiration chamber (Hegarty et al., 2012) for 24 hours on days 20, 27, 48, 55, 76, 83, 104 and 111. The period of confinement commenced at the usual time of feeding. A Servomex analyser was used to quantify production of CH_4, O_2, and CO_2. The CH_4 production data were corrected for recovery of a known quantity of pure CH_4, which was introduced via a mass flow controller (Smart Trak 2 Series 100, Sierra Instruments, Monterey, CA, USA) and measured by the Servomex analyser before and after each CH_4 measurement-period (Hegarty et al., 2012). Mean CH_4 recovery was 101 % ± 3.28%, recoveries were performed on the 28th June 2022, 18th August 2022 and 13th October 2022.

Total methane production (g of CH_4/d) and methane yield (g of CH_4/kg DMI) by day were reported.

3.1.1.3 Diet composition and analysis

Steers were fed once daily, at 0900 h (block 1), 0945 h (block 2), 1030 h (block 3) 1115 h (block 4). Feed offered was adjusted daily to maintain refusals of 0.5 – 1.0 kg (as-fed). Feed offered and refusals were recorded daily, and sampled for DM and proximate analysis. All steers had ad libitum access to clean, fresh water. There were 16 different diets formulated and mixed as part of this study: four levels of ether extract (3.0, 4.3, 5.6 and 7.0%) and four levels of total NDF (20, 25, 30, and 35 %) were examined (Fig.1, and Table 1).

The feeding progression to the finisher diet used step adjustments with increasing levels of tempered barley and total oil (Table 1). Barley was tempered at Tullimba feedlot for 16h at approximately 20 % moisture prior to rolling in an 18” x 36” mill (R & R Machine Works, Dalhart, Texas), with dry matter (DM) percentage and flake weight targeted to 20 % moisture and 44 kg/hL flake density (monitored daily). DM of feed offered for each diet, and refusals for individual steers were measured daily. Grab samples (500 g) were collected from each mixer

load of the main diet and bulked weekly and analysed for content of crude protein (AOAC Method 2001.11), acid detergent fibre (ADF, AAFCO Method 008.08), neutral detergent fibre (NDF, NFTA Method 2.2.2.5), organic matter (ISO 5984:2002(E)), ether extract (AFIA Method 1.14R), starch (AOAC Method 996.11), water soluble carbohydrates (AFIA Method 1.11A), digestible dry and organic matter (AFIA Method 1.7R) (NSW DPI Laboratory Services - Wagga Wagga Chemistry Services Laboratory, PMB Pine Gully Road, Wagga Wagga NSW 2650 (Table 2)). Metabolisable energy (ME) content was calculated according to the equations for grains and concentrates of AFIA Method 2.2R (MAFF, 1990). Please refer to Appendix 1 for wet chemistry results.

Table 1. Formulated feedlot total mixed rations feed to Angus steers during the 112-d feeding period.

	Diet (feeding days)[1]			
Fat level (ether extract)	**3.0 % DM**	**4.3 % DM**	**5.6 % DM**	**7.0 % DM**
	Starter (0-27) NDF = 39.5% DM			
Ingredient, % DM				
Tempered Barley	53.0	53.0	53.0	36.7
Oaten hay	16.3	17.2	18.1	26.5
Wheat Straw	7.77	8.18	8.59	12.6
Whole cottonseed	6.71	6.70	6.69	7.96
Mill run	11.1	8.55	5.94	7.14
Molasses	2.60	2.56	2.56	2.64
Canola Oil	0.00	1.31	2.68	3.88
Mineral Premix	2.52	2.51	2.51	2.51
	Transition I (28-56) NDF = 33.0% DM			
Ingredient, % DM				
Tempered Barley	53.0	53.0	53.0	53.0
Oaten hay	16.3	17.2	18.1	19.1
Wheat Straw	7.77	8.18	8.59	9.10
Whole cottonseed	6.71	6.70	6.69	6.67
Mill run	11.1	8.55	5.94	2.96
Molasses	2.60	2.56	2.56	2.55
Canola Oil	0.00	1.31	2.68	4.09
Mineral Premix	2.52	2.52	2.51	2.51
	Transition II (57-83) NDF = 26.5.5% DM			
Ingredient, % DM				
Tempered Barley	69.5	69.5	69.4	67.8
Oaten hay	8.70	9.72	10.7	11.0
Wheat Straw	4.17	4.64	5.10	5.24
Whole cottonseed	6.87	6.85	6.84	6.71
Mill run	5.66	2.88	0.11	0.00
Molasses	2.62	2.62	2.61	2.61
Canola Oil	0.00	1.34	2.74	4.12
Mineral Premix	2.52	2.51	2.51	2.50
	Finisher (84-112) NDF = 20.0% DM			
Ingredient, % DM				
Tempered Barley	85.7	84.1	82.1	80.1
Oaten hay	2.22	2.51	3.14	3.82
Whole cottonseed	6.90	6.89	6.85	6.82
Molasses	2.68	2.68	2.66	2.65
Canola Oil	0.00	1.43	2.73	4.13
Mineral Premix	2.51	2.51	2.49	2.48

[1]Diets were formulated using the Concept 5 software.

For each respiration chamber event, feed offered was sampled for each diet at feeding (~ 500 g grab samples), and analysed for proximate analysis and Van Soest fibre fractions, for use in CH_4 prediction equations. The samples were sealed and frozen at -20 °C before later analysis. Gross energy was determined by using an adiabatic calorimetric bomb (Parr Instrument Co., Moline, Il). Fat content (hexane soxtec extract LMOP Method 2-1122; AOAC, 1990, method 930.15), protein content by N analysis with Dumas combustion by using Leco FP-528LC (Etheridge et al., 1998) were also analysed. The NDF was determined with amylase and without sodium sulphate (Van Soest et al., 1991), and ADF and ADL was determined (Goering and Van Soest, 1970) in an ANKOM 2000 Fibre Analyser (ANKOM Technology, Macedon NY, USA). Hemicellulose was calculated by difference (NDF-ADF), cellulose was calculated as the difference between ADF and ADL, soluble residue was the sum of crude fat, crude protein and soluble carbohydrates.

Table 2. Chemical Composition feedlot total mixed rations (average ± standard deviation) fed to Angus steers during the 112-d feeding period.

	Diet (feeding days)[1]			
Fat level (ether extract)	**3.0 % DM**	**4.3 % DM**	**5.6 % DM**	**7.0 % DM**
		Starter (0-27) NDF = 39.5% DM		
Dry Matter (DM), % as-fed	81.8 ± 1.10	81.4 ± 0.521	83.4 ± 0.302	81.9 ± 0.662
Crude Protein, % DM	12.2 ± 0.240	11.6 ± 0.504	11.3 ± 0.646	11.2 ± 0.424
Fat, % DM	3.07 ± 0.106	4.30 ± 0.308	5.22 ± 0.474	6.36 ± 0.362
Gross energy, MJ/kg	17.7 ± 0.206	17.9 ± 0.104	18.1 ± 0.113	18.2 ± 0.169
NDF, % DM	32.7 ± 1.33	35.6 ± 2.07	33.5 ± 2.59	34.4 ± 0.548
ADF, % DM	14.3 ± 3.61	18.0 ± 1.60	16.6 ± 1.91	17.5 ± 17.5
ADL, % DM	2.35 ± 0.298	2.48 ± 0.239	2.16 ± 0.283	2.29 ± 0.209
Soluble residue, % DM	52.0 ± 1.24	48.4 ± 1.82	0.282 ± 2.67	48.0 ± 0.854
		Transition I (28-56) NDF = 33.0% DM		
Dry Matter (DM)	80.3 ± 1.99	80.6 ± 1.93	80.2 ± 2.01	81.1 ± 1.78
Crude Protein, % DM	11.9 ± 0.468	11.4 ± 0.622	10.9 ± 0.980	10.5 ± 0.789
Fat, % DM	2.99 ± 0.266	4.07± 0.242	5.38 ± 0.491	6.72 ± 0.308
Gross energy, MJ/kg	17.9 ± 0.241	17.9 ± 0.251	18.2 ± 0.170	18.5 ± 0.173
NDF, % DM	29.8 ± 1.22	29.1 ± 0.820	30.2 ± 0.790	30.2 ± 2.06
ADF, % DM	13.2 ± 1.11	13.2 ± 0.422	13.8 ± 1.00	14.1 ± 1.20
ADL, % DM	1.89 ± 0.200	1.66 ± 0.165	1.86 ± 1.86	1.73 ± 0.247
Soluble residue, % DM	55.2 ± 1.35	0.165 ± 0.472	53.5 ± 0.425	52.4 ± 2.05
		Transition II (57-83) NDF = 26.5.5% DM		
Dry Matter (DM)	79.8 ± 0.776	80.6 ± 0.851	80.5 ± 0.754	82.6 ± 0.535
Crude Protein, % DM	11.4 ± 0.891	11.2 ± 0.462	10.5 ± 0.551	10.6 ± 0.738
Fat, % DM	3.19 ± 0.360	4.05 ± 0.400	5.04 ± 0.359	6.55 ± 0.181
Gross energy, MJ/kg	18.0 ± 0.091	18.1 ± 0.099	18.3 ± 0.067	18.6 ± 0.138
NDF, % DM	25.4 ± 1.76	24.8 ± 1.91	24.5 ± 1.41	24.4 ± 0.983
ADF, % DM	11.1 ± 1.86	10.6 ± 1.58	10.8 ± 0.803	11.0 ± 0.696
ADL, % DM	1.73 ± 0.402	1.58 ± 0.523	1.52 ± 0.296	1.53 ± 0.309
Soluble residue, % DM	59.8 ± 1.75	59.8 ± 1.86	59.9 ± 1.17	58.2 ± 1.32
		Finisher (84-112) NDF = 20.0% DM		
Dry Matter (DM)	82.6 ± 0.115	83.1 ± 0.776	82.9 ± 0.102	82.9 ± 0.802
Crude Protein, % DM	11.8 ± 1.34	11.7 ± 1.24	11.2 ± 0.974	11.3 ± 1.26
Fat, % DM	3.39 ± 0.431	4.58 ± 0.212	5.55 ± 0.247	7.07 ± 0.601
Gross energy, MJ/kg	17.9 ± 0.214	18.0 ± 0.170	18.3 ± 0.146	18.5 ± 0.128
NDF, % DM	19.8 ± 2.14	19.8 ± 0.906	19.2 ± 2.30	18.9 ± 2.13
ADF, % DM	8.16 ± 1.40	7.86 ± 0.473	7.64 ± 1.50	7.53 ± 1.19
ADL, % DM	1.29 ± 0.531	1.27 ± 0.243	1.06 ± 0.431	1.09 ± 0.388
Soluble residue, % DM	64.8 ± 3.44	63.8 ± 1.99	63.9 ± 1.98	62.6 ± 2.10

[1]Analysis conducted at the Ruminant nutrition laboratory at the University of New England

3.1.1.4 VFA concentrations, rumen ammonia and protozoal enumeration

Post-feeding rumen-fluid samples (~70 mL) were collected oro-gastrically from each animal 4 hours after feed was offered on days 21, 28, 49, 56, 77, 84, 105 and 112. The rumen fluid was tested for pH (EcoScan Portable pH/ORP meter with TPS pH Sensor) and redox potential (Mettler Toldeo SevenEasy S20 pH meter with TPS Intermediate Junction Redox Sensor) immediately after sampling, and then subsampled for measurement of volatile fatty acid (VFA) profiles, rumen ammonia, and rumen protozoa enumeration.

The method used VFA profiles was based on the method of GC Separation of VFA C2 – C5 (Supelco Bulletin no.749D). Briefly, 1mL of rumen fluid is centrifuge at 13000 rpm for 10 minutes. 100uL of sample supernatant was added to 1mL internal standard (0.9mM 3-Methyl Valeric acid in 2.24 % (w/v) Phosphoric acid), mixed and then quantified gas chromatography with flame ionization detection. The column used is a capillary column HP-FFAP (30 m x 0.53 mm x 1.0 micron) with hydrogen as the carrier gas. Rumen ammonia-N was determined by a modified direct enzymatic method using Ammonia Reagent (Cat No.OSR61154 supplied by Beckman Coulter Australia) on the Olympus AU480 Autoanalyser (Beckman Coulter Australia Pty Ltd, Mount Waverley, Victoria) (Henry et al. 1964). The modification consisted in the use of with In-House calibrator $(NH_4)_2SO_4$ used at 150 mg/L and 600 mg/L and acidified to pH 2.0 using concentrated sulphuric acid. For protozoa enumeration, 4 mL of rumen fluid was suspended in 16 mL of an isotonic formaldehyde-saline of 4 %, then subsampled and stained with brilliant green (Nguyen and Hegarty, 2016) before microscopic enumeration of ciliate protozoa on a Fuchs – Rosenthal optical counting chamber (0.0625 mm^2, 0.2 mm depth) by using a technique adapted from Dehority (1984).

3.1.1.5 Statistical analysis

To determine the effect of increasing dietary fat and roughage levels on rumen fermentation and methane emissions during the 112-d feedlot period in beef cattle, the data from 40 steers were analysed as a completely randomized block design including block as a random effect (n = 4). Fat content (n = 4) was considered as a fixed effect and analysed within each NDF Level.

3.1.2 Database design

A database of stored diet samples and historical records of diet composition and methane emissions 384 individual records from 53 feedlot cattle was assembled from 3 previous MLA feedlot cattle research projects and the fourth *in vivo* experiment conducted as part of this project. These projects all used diets representative of Australian feedlot industry, based on tempered barley rations, including a range of fat (ether extract, EE) and neutral detergent fibre (NDF) inclusion rates. The 3 historical projects were designed to test the effect of different rumen modifiers (B.FLT.0244), increasing doses of 3-nitrooxypropanol (B.FLT.5010, negative control group only), and increasing doses of *Asparagopsis taxiformis* extract in a canola-oil carrier (P.PSH.1351, negative control group only). These were supplemented with data from a matrix experiment which tested 16 diet formulations, using combinations of 4 levels of NDF and 4 levels of EE content. The diets of all studies contained 25 mg/kg DM of monensin. Study cattle were managed as close as possible to commercial feedlot conditions. No study cattle were treated with hormonal growth promotants or fed other additives to stimulate or alter growth, feed efficiency or methane production. The matrix experiment formed the largest proportion of the database (82 % of records), followed by P.PSH.1351 (8.3 % of records), B.FLT.5010 (7.8 % of records), and B.FLT.0244 (1.8 % of records).

3.1.3 Measurement of methane emissions

All experiments were conducted in the same facility, at the University of New England, Armidale, NSW. For each experiment, CH_4 production was estimated by confining each animal in an individual open circuit respiration calorimeter (Hegarty et al., 2012) for 24 hours after at least 7 days adaptation to a particular diet. The facility at the University of New England comprises of 10 individual animal respiration calorimeters. Briefly, calorimeters were sealed in the morning, when cattle were fed. When in calorimeters, the cattle had *ad libitum* access to feed and water. Air temperature was controlled centrally and kept at ~21 ºC for all calorimeters. Air temperature and relative humidity were measured in each calorimeter using sensors (BME280, Bosch Sensortec, Gerlingen, Germany). Air flow through each calorimeter (mean = 1.6 m^3/min) was controlled using a flow meter (Model ST75V, Fluid Components International, San Marcos, CA, USA). The concentration of CH_4 (parts per million per volume) was measured in the calorimeter incoming (ambient) and exhaust air streams using a Servomex Multigas Analyzer (Servomex 4100 Gas Purity Analyzer) calibrated for CH_4, CO_2 and O_2, before each measurement day. Moisture was removed by a drying column before a multiplexer was used to direct the dried sample air from each calorimeter and the ambient air into the analyzer in turn. CH_4, CO_2 and O_2 concentrations were measured over 10 s after a 40 s purge time, by the Servomex analyzer. Air flow was corrected to standard temperature and pressure, accounting for altitude, and hourly variation in temperature and barometric air pressure. Air flow and gas concentration data from the sampled air were loaded directly into a daily workbook with separate Excel spreadsheets for each chamber every 9 minutes and used to calculate g of CH_4/L air. CH_4 production was averaged hourly and daily methane production estimated by the area under the curve by the approximate integral using the trapezoidal rule. Recovery of CH_4 through the calorimeters was assessed pre-measurement and post-measurement by introducing pure CH_4 at a known rate via a mass flow controller (Smart Trak 2 Series 100, Sierra Instruments, Monterey, CA, USA) and the Servomex analyzer was used to quantify CH_4 concentration. The CH_4-production data were corrected for recovery of a known quantity of pure CH4, which was introduced via a mass flow controller (Smart Trak 2 Series 100, Sierra Instruments, Monterey, CA, USA) and measured by the Servomex analyser before and after each CH_4 measurement-period (Hegarty et al., 2012). Mean CH_4 recovery was 96.8 ± 4.48 % (B.FLT.0244), 92.8 ± 1.36% (B.FLT.5010), 97.5 ± 2.05% (P.PSH.1351), and 102.4 ± 2.03 % (B.FLT.5013).

3.1.4 Diet composition and analysis

Dry matter (DM) of feed offered and refusals were measured and feed offered was analysed for proximate analysis and van Soest fibre fractions (Table 3). Grab samples (~500 g) were collected at mixing of each batch of the diet. The samples were sealed and frozen at -20 °C before later analysis. Gross energy was determined by using an adiabatic calorimetric bomb (Parr Instrument Co., Moline, Il). Fat content (hexane soxtec extract LMOP Method 2-1122 [B.FLT.0244 & P.PSH.1351]; AOAC, 1990, method 930.15 [B.FLT.5010]), protein content by N analysis with Dumas combustion by using Leco FP-528LC (Etheridge et al., 1998) were also analysed. The NDF was determined with amylase and without sodium sulphate (Van Soest et al., 1991), and ADF and ADL was determined (Goering and Van Soest, 1970) in an ANKOM 2000 Fibre Analyser (ANKOM Technology, Macedon NY, USA). Hemicellulose was calculated by difference (NDF-ADF), cellulose was calculated as the difference between ADF and ADL, soluble residue (SR) was calculated by subtracting CP and EE from the neutral-detergent solubles.

Table 3. Summary of descriptive statistics of all data used in the evaluation database

Trait	N	Median	SD[2]	Minimum	Maximum
Dry matter intake (kg/d)	384	9.07	2.08	3.50	14.1
Body weight (kg)	384	412	71.0	163	737
Diet composition (% of DM)					
Crude protein	25	11.6	0.896	10.5	14.6
Ether extract	25	4.90	1.28	2.97	7.30
Neutral detergent fibre	25	27.1	5.70	18.9	44.2
Acid detergent fibre	25	11.9	3.44	7.32	19.9
GE (MJ/kg DM)	25	18.1	0.327	17.4	19.5
Hemicellulose	25	15.2	3.00	11.4	29.3
Cellulose	25	10.2	3.06	5.37	16.8
Starch	25	34.9	9.63	20.0	51.0
CH_4 emissions					
CH_4 production (g/d)	384	79.9	27.5	20.9	179
CH_4 yield (g/kg DMI)	384	9.32	3.74	1.97	21.1
Y_m (% of GE intake)	384	2.86	1.16	0.595	6.61

3.1.4.1 *Statistical analysis of in vivo experiment*

All data were analysed with a mixed model linear regression, with block and animal as random effects, and including the interaction of dietary fat inclusion with sampling day within an NDF-level diet, using the *lme4* (Bates *et al.*, 2015) package of R (R Core Team, 2016). Dietary fat levels were compared within each NDF level. Least-squares means and linear contrasts were computed with the *emmeans* package (Lenth, 2020), using the Holm-Bonferroni adjustment for multiple treatment groups.

3.2 Evaluation of methane emissions prediction equations

Presently, the Australian Government reports CH_4 emissions from Australian feedlot cattle in its national inventory predicted using the equation of Moe & Tyrrell (1979) (Australian Government 2023, Equation 1, Table 4). To test its accuracy, we evaluated the predictions from this equation against the observed CH_4 emissions from the database. We also evaluated 7 other equations for prediction of CH_4 from grain-fed beef cattle, from 4 other sources (IPCC (2006), IPCC (2019), Escobar-Bahamondes et al. (2016), and Galyean and Hales (2022), Table 4). These equations were selected because they required input variables that were available in our database and included predictor variables that can be easily determined by commercial feed analysis laboratories.

Each equation was assessed by regressing residual (observed – predicted) values on the predicted values centered on their mean values (St-Pierre, 2003), this procedure makes the intercept and slope estimates independent. We performed the analysis using the PROC MIXED of SAS 9.4 (SAS Systems Inc., Cary, NC), (St-Pierre, 2001). The slopes and intercepts of each equation were estimated using the ESTIMATE statement of the MIXED procedure in SAS, along with the root mean square error (RMSE) of this regression. The intercepts of the regression equations were the mean biases, whereas the slopes of such regression equations were the linear biases. When linear bias was significant ($P \leq 0.05$) the bias and minimum and maximum predicted values were calculated. Additionally, observed CH4 was regressed on predicted CH_4 for each equation. The coefficient of determination (r^2) was obtained as a measure of the strength of the relationship between observed and predicted CH4. To evaluate model precision, several commonly used measures of adequacy were employed, including mean absolute error (MAE), mean square prediction error (MSPE), as described by McMeniman *et al*. (2009). The

MSPE was decomposed into mean bias, systematic bias and random variation to assess sources of variation (McMeniman et al., 2009).

Pearson's correlation coefficients were calculated using the CORR procedure to determine the strength of the linear relationship between the input variables and CH_4 emissions in feedlot cattle as well as between the input variables themselves. Then sensitivity analysis was performed to evaluate the variability of existing empirical equations to predict feedlot cattle CH_4 emission predictions using Monte Carlo simulations. This involved varying the input of independent variables within each equation using the minimum and maximum values of each input variable in the database to simulate the local sensitivity of CH_4 predictions while the other input variables within a given equation was kept at its mean value. The resulting range of CH_4 predictions for each equation was then illustrated using tornado plots.

Table 4. Evaluated literature equations used to predict feedlot cattle CH4 production (g/d)

Equation	Source	Description[1]
(1)	Moe and Tyrrell (1979)[2]	This equation takes into account the intake of cell wall carbohydrates and cell contents: $\boldsymbol{CH_4} = [3.406 + 0.510 \times \boldsymbol{SR} + 1.736 \times \boldsymbol{HC} + 2.648 \times \boldsymbol{CEL}]/0.05522$
(2)	IPCC (2006)[3]	This equation uses an emission conversion factor (Ym) based on daily gross energy intake. For diets containing 90% or more concentrate (typical of feedlot cattle), the Y_m is 3.0% ± 1.0% of GEI. For all other diets and cattle categories, the Y_m is 6.5 % ± 1.0 %: $\boldsymbol{CH_4} = [(\frac{Y_m}{100}) \times \boldsymbol{GEI})]/0.05565$
(3a)	IPCC (2019)	This equation uses CH_4 yield (MY) of 21.0 g CH4/kg dry matter intake (DMI, for total mixed rations with 15 to 75% of high-quality forage), or MY = 13.6 g CH_4/kg DMI (for non-steam-flaked corn-based diets, forage from 0-15%): $\boldsymbol{CH_4} = (MY \times \boldsymbol{DMI})$
(3b)	IPCC (2019)	This equation uses an emission conversion factor (Ym) based on daily gross energy intake. For total mixed rations with 15 to 75% of high-quality forage, the Y_m is 6.3 ± 1.0 %, and for non-steam-flaked corn-based diets, forage from 0-15%, the Y_m is 4.0 ± 1.0%: $\boldsymbol{CH_4} = [(\frac{Y_m}{100}) \times \boldsymbol{GEI})]/0.05565$
(4a)	Escobar-Bahamondes et al. (2016)[4]	These equations include as inputs BW, crude protein intake, CP:NDF and dietary starch:NDF ratios, and polynomial effects of fat[2], DMI[2] and (NDF-ADF)[3]: $\boldsymbol{CH_4} = -26.4 + 0.21 \times \boldsymbol{BW} + 30.1 \times \boldsymbol{CP} - 70.5 \times \boldsymbol{fat^2} + 10.1 \times (\boldsymbol{NDF} - \boldsymbol{ADF})^3$
(4b)		$\boldsymbol{CH_4} = -10.1 + 0.21 \times \boldsymbol{BW} + 0.36 \times \boldsymbol{DMI^2} - 69.2 \times \boldsymbol{fat^3} + 13.0 \times \frac{\boldsymbol{CP}}{\boldsymbol{NDF}} - 4.90 \times \left(\frac{\boldsymbol{STARCH}}{\boldsymbol{NDF}}\right)$
(5a)	Galyean and Hales (2022)[5]	These equations use modified Ellis et al. (2009) equations that considered DMI and dietary starch:NDF ratio with or without dietary ether extract (EE) concentration: $\boldsymbol{CH_4} = 0.2883 - 0.03474 \times \left(\frac{\mathbf{STARCH}}{\mathbf{NDF}}\right)$
(5b)		$\boldsymbol{CH_4} = 0.3227 - 0.0334 \times \left(\frac{\mathbf{STARCH}}{\mathbf{NDF}}\right) - 0.00868 \times \mathbf{EE}$

[1] CH_4 = Methane production in g/day; [2] conversion of original Moe & Terrell (1979) equation assuming 4.184 megajoule per megacalorie and 55.22 MJ/kg CH_4 (Brouwer 1965) as utilized by the Australian Government (DCCEEW, 2021). [3] GEI = Gross energy intake in MJ/day. [4] ADF, acid detergent fiber (kg/d); BW, body weight (kg/d); CP, crude protein (kg/d); DMI, dry matter intake (kg/d); NDF, neutral detergent fiber (kg/d); Fat is expressed as kg/d. [5]DMI, dry matter intake in kg/d; BW = body weight in kg; STARCH, starch, % DM; NDF, neutral detergent fiber, % DM; EE, ether extract, % DM.

3.3 New equation parametrisation

Based on the correlation analysis and graphical exploration on the input variables in the database, we identified variables that exhibited a strong association with CH_4 production, had low correlation among themselves, and could be easily measured on the farm. To assess the potential for adjusting coefficients in predicting daily CH_4 production in g/d, we conducted stepwise regression analyses using SAS software (STEPWISE procedure; SAS Systems Inc., Cary, NC). Variables tested included: DMI, CP, EE, starch, and NDF content. Significance was declared at $P < 0.05$. Statistical analysis in all models was performed using the MIXED procedure of SAS software. Linear mixed model regressions were fitted assuming the effect of study as a random effect. The slopes and intercepts of each equation were estimated using the ESTIMATE statement of the MIXED procedure in SAS.

The general statistical model used was as follows:

$$CH_{4ijk} = a_i + b_i \times X_{ij} + s_j + e_{ijk} \quad [6]$$

CH_{4ijk} = is the dependent variable for the j^{th} animal of the i^{th} study,

X_{ij} is the independent variable for the j^{th} animal of the i^{th} study,

a_j and b_j are the parameters to be estimated,

s_j is the random effect of the i^{th} study ~ $N(0, \sigma_s^2)$,

e_{ijk} is residual error ~ $N(0, \sigma_e^2)$.

Multiple mixed model equations (Eq. [2]) were also fitted considering study as random effect. The slopes and intercepts of each equation were estimated using the ESTIMATE statement of the MIXED procedure in SAS.

$$CH_{4ijk} = a_i + b_{1i} \times X1_{ij} + b_{2i} \times X2_{ij} + s_j + e_{ijk} \quad [7]$$

CH_{4ijk} = is the dependent variable for the j^{th} animal of the i^{th} study,

$X1_{ij}$ and $X2_{ij}$ are significant independent variables for the j^{th} animal of the i^{th} study,

a_j, b_{1i} and b_{2i} are the parameters to be estimated,

s_j is the random effect of the i^{th} study ~ $N(0, \sigma_s^2)$,

e_{ijk} is residual error ~ $N(0, \sigma_e^2)$.

4. Results

4.1 Results of *in-vivo* study

4.1.1 Animal performance

The *in vivo* study was not powered to address differences in performance traits. There was little detectable effect on animal performance of any dietary fat level within NDF level on live weight (LW), average daily gain (ADG), dry matter intake (DMI) and gain to feed (G:F) (Table 5). DMI increased with progression of the experiment in all dietary fat treatments, as liveweight increased and NDF inclusion decreased.

Table 5. Responses of live weight (LW), average daily gain (ADG), dry matter intake (DMI) and gain to feed ratio (G:F) in Angus steers fed different dietary fat and roughage levels during the overall feeding period (day 0 to 112).

	Dietary fat (ether extract %DM)					
Item	3.0	4.3	5.6	7.0	SE[1]	*P-value*[2]
Initial LW, kg	337	336	341	338	12.9	0.74
d 27 LW, kg	349	350	351	340	12.5	0.59
d 55 LW, kg	387	403	388	387	13.3	0.25
d 83 LW, kg	442	462	445	439	12.6	0.24
Final LW, kg	491	516	494	495	12.2	0.22
ADG, kg						
d 0 to 27	0.409	0.449	0.336	0.0576	0.177	0.41
d 28 to 55	1.35[b]	1.93[a]	1.34 b[b]	1.69[ab]	0.0982	<0.01
d 56 to 83	1.97	2.14	2.03	1.83	0.180	0.64
d 84 to 112	1.75	1.93	1.77	2.02	0.123	0.24
Overall d 0 to 112	1.36	1.59	1.36	1.38	0.0820	0.15
DMI, kg/d						
d 0 to 27	7.00	6.27	6.86	6.49	0.441	0.26
d 28 to 55	8.81	8.89	8.81	8.50	0.394	0.83
d 56 to 83	9.52	10.2	9.72	9.77	0.349	0.59
d 84 to 112	9.99	11.0	10.2	10.6	0.381	0.18
Overall d 0 to 112	8.83	9.08	8.90	8.85	0.357	0.92
G:F						
d 0 to 27	0.0485	0.0700	0.0451	0.00380	0.0299	0.49
d 28 to 55	0.157[b]	0.215[a]	0.152[b]	0.199[a]	0.0115	<0.01
d 56 to 83	0.205	0.214	0.208	0.188	0.0181	0.70
d 84 to 112	0.174	0.176	0.174	0.191	0.00980	0.46
Overall d 0 to 112	0.152	0.176	0.152	0.157	0.0103	0.049

[1]Standard error of the mean [2]When P-value for fat content was significant (≤ 0.05) pairwise comparison was performed using Tukey's test, in that case means within a row showing different superscripts depict significant effects of fat content within NDF level (starter, T1, T2, finisher).

4.1.2 Methane production

The DMI during chamber measurements day was not affected by dietary fat level at any NDF level (Table 6). Unsurprisingly, CH_4 production and yield reduced as NDF content decreased in the diet (Table 6), which provides an invaluable dataset for further methane model evaluations. The *in vivo* study alone was unable to detect a significant effect of dietary fat on CH_4 production or yield at any NDF level. However, it is noteworthy that at all NDF levels, the highest dietary fat level (7 % DM) consistently provided the lowest level of CH_4 production or yield, which can be explored in the larger model evaluation.

Table 6. Least squared means of methane (CH_4) emissions of Angus steers fed different dietary fat and roughage levels.

		Dietary fat (% DM)				
Neutral detergent fibre (% DM)		3.0	4.3	5.6	7.0	*P-value*[2]
DMI, kg/d						
	35	7.51 ± 0.379	6.78 ± 0.379	7.28 ± 0.346	7.18 ± 0.265	0.735
	30	8.93 ± 0.454	9.08 ± 0.454	8.07 ± 0.454	8.02 ± 0.454	0.116
	25	10.34 ± 0.389	10.79 ± 0.407	10.41 ± 0.389	10.52 ± 0.389	1.000
	20	10.26 ± 0.462	11.64 ± 0.484	9.96 ± 0.462	11.37 ± 0.462	0.813
CH_4, g/d						
	35	82.66 ± 4.452	75.90 ± 4.452	82.54 ± 4.162	72.36 ± 4.541	0.434
	30	81.26 ± 5.162	73.90 ± 5.162	78.43 ± 5.162	60.31 ± 5.162	0.049
	25	64.93 ± 4.995	75.99 ± 5.194	69.82 ± 4.995	61.58 ± 4.995	0.818
	20	50.87 ± 3.921	52.08 ± 4.127	47.24 ± 3.921	45.69 ± 3.921	0.762
CH_4, g/kg DMI						
	35	11.20 ± 0.632	11.22 ± 0.632	11.45 ± 0.598	10.23 ± 0.645	0.773
	30	9.15 ± 0.638	8.24 ± 0.638	9.84 ± 0.638	7.72 ± 0.638	0.536
	25	6.33 ± 0.393	6.95 ± 0.412	6.71 ± 0.393	5.86 ± 0.393	0.656
	20	4.86 ± 0.271	4.46 ± 0.286	4.60 ± 0.271	4.05 ± 0.271	0.199
CH_4, g/kg LW						
	35	0.214 ± 0.0131	0.223 ± 0.013	0.245 ± 0.012	0.214 ± 0.013	0.462
	30	0.214 ± 0.015	0.192 ± 0.015	0.202 ± 0.015	0.161 ± 0.015	0.087
	25	0.151 ± 0.010	0.169 ± 0.101	0.161 ± 0.010	0.144 ± 0.101	0.893
	20	0.105 ± 0.007	0.103 ± 0.008	0.098 ± 0.007	0.093 ± 0.007	0.668

[1]Standard error of the mean [2]Multiple pairwise comparisons adjusted by the Holm-Bonferroni method.

4.1.3 Rumen function

No differences in rumen fermentation parameters were detected as a result of varying dietary fat inclusion (Table 5). Although this *in vivo* study is not able to compare the effect of NDF level as it is confounded with time, it is noteworthy, and unsurprising, that at all fat levels, total VFA concentration was highest in the lowest NDF (20 % DM) diet, propionate proportion of VFA consistently increased inverse to NDF content, and acetate and butyrate proportions of VFA consistently increased with increasing NDF content (Table 7). There was no effect of dietary fat detected on rumen pH or reduction-oxidation potential (Table 8).

Table 7. Post-feeding ammonium, volatile fatty acid (VFA) amounts and profile in the rumen fluid of Angus steers fed different dietary fat and roughage levels during a 112-d feeding period (day 0 to 112).

	Dietary fat (ether extract %DM)				
Neutral detergent fibre (% DM)	3.0	4.3	5.6	7.0	*P-value*[2]
		Ammonium-N, ug/mL			
35	31.66 ± 100.7	121.80 ± 98.73	50.99 ± 96.58	173.92 ± 104.70	1.000
30	201.01 ± 123.4	114.90 ± 123.4	21.35 ± 123.4	133.63 ± 123.4	0.967
25	10.03 ± 3.844	8.01 ± 4.018	7.54 ± 3.844	6.03 ± 3.844	1.000
20	11.77 ± 2.484	9.57 ± 2.621	10.40 ± 2.484	9.56 ± 2.484	1.000
		Total VFA, mmol/L			
35	64.54 ± 3.758	62.86 ± 3.676	61.75 ± 3.586	61.72 ± 3.929	1.000
30	68.01 ± 5.679	76.75 ± 5.679	68.40 ± 5.679	63.75 ± 5.679	0.682
25	67.27 ± 6.070	69.31 ± 6.301	67.78 ± 6.070	57.46 ± 6.070	0.576
20	73.65 ± 4.523	80.41 ± 4.730	61.80 ± 4.422	65.18 ± 4.422	0.052
		Acetate, %			
35	60.01 ± 0.894	60.00 ± 0.880	59.95 ± 0.834	58.44 ± 0.925	0.744
30	53.04 ± 1.675	52.34 ± 1.675	53.93 ± 1.675	54.32 ± 1.675	0.681
25	46.91 ± 0.833	47.16 ± 0.863	47.22 ± 0.833	47.44 ± 0.833	1.000
20	47.18 ± 1.323	48.48 ± 1.372	49.05 ± 1.294	49.54 ± 1.294	0.358
		Propionate, %			
35	24.93 ± 1.405	26.01 ± 1.381	23.56 ± 1.314	25.37 ± 1.459	1.000
30	29.72 ± 1.374	27.92 ± 1.374	28.66 ± 1.374	29.38 ± 1.374	1.000
25	33.95 ± 1.134	33.48 ± 1.194	31.53 ± 1.134	33.68 ± 1.134	0.685
20	42.02 ± 1.592	40.77 ± 1.677	40.67 ± 1.542	39.51 ± 1.542	0.843
		Iso-butyrate, %			
35	0.73 ± 0.698	0.63 ± 0.069	0.69 ± 0.064	0.65 ± 0.070	1.000
30	0.57 ± 0.052	0.52 ± 0.052	0.60 ± 0.052	9.56 ± 0.052	1.000
25	0.62 ± 0.057	0.56 ± 0.060	0.59 ± 0.057	0.61 ± 0.057	1.000
20	0.52 ± 0.042	0.43 ± 0.043	0.51 ± 0.041	0.48 ± 0.041	0.833
		Butyrate, %			
35	10.70 ± 0.936	9.54 ± 0.920	11.86 ± 0.878	11.94 ± 0.971	0.465
30	12.99 ± 0.968	14.69 ± 0.068	13.25 ± 0.968	11.77 ± 0.968	0.461
25	13.27 ± 0.671	13.23 ± 0.707	14.71 ± 0.671	12.43 ± 0.671	0.734
20	6.49 ± 0.685	6.62 ± 0.723	6.41 ± 6.662	6.83 ± 0.662	1.00
		Iso-valerate, %			
35	1.34 ± 0.298	1.42 ± 0.294	2.31 ± 0.277	1.80 ± 0.307	0.292
30	1.000 ± 0.236	0.786 ± 0.236	1.234 ± 0.236	1.112 ± 0.236	0.8935
25	0.61 ± 0.168	0.64 ± 0.176	0.885 ± 0.168	0.727 ± 0.168	1.000
20	0.67 ± 0.052	0.56 ± 0.054	0.70 ± 0.050	0.64 ± 0.050	1.000
		Valerate, %			
35	1.73 ± 0.324	1.98 ± 0.321	1.39 ± 0.293	1.46 ± 0.32	0.880
30	2.31 ± 0.401	3.19 ± 0.401	1.88 ± 0.401	2.39 ± 0.401	1.000
25	4.18 ± 0.372	4.63 ± 0.388	4.54 ± 0.372	4.52 ± 0.372	1.000
20	2.64 ± 0.243	2.73 ± 0.255	2.33 ± 0.239	2.88 ± 0.239	0.745
		Caproate, %			
35	0.54 ± 0.180	0.43 ± 0.178	0.29 ± 0.161	0.26 ± 0.177	0.688
30	0.29 ± 0.108	0.63 ± 0.108	0.37 ± 0.108	0.54 ± 0.108	0.680
25	0.37 ± 0.068	0.40 ± 0.071	0.44 ± 0.068	0.50 ± 0.068	0.530
20	0.32 ± 0.096	0.26 ± 0.100	0.25 ± 0.093	0.19 ± 0.093	0.754

[1]Standard error of the mean [2] Multiple pairwise comparisons adjusted by the Holm-Bonferroni method.

Table 8. Post-feeding pH and redox potential in the rumen fluid of Angus steers fed different dietary fat and roughage levels during a 112-d feeding period (day 0 to 112).

Neutral detergent fibre (% DM)	Dietary fat (ether extract %DM)				*P-value*[2]
	3.0	4.3	5.6	7.0	
pH					
35	6.44 ± 0.117	6.67 ± 0.111	6.78 ± 0.108	6.83 ± 0.120	0.056
30	6.87 ± 0.121	6.83 ± 0.114	6.96 ± 0.116	7.06 ± 0.114	0.818
25	6.70 ± 0.138	6.70 ± 0.144	6.84 ± 0.148	7.12 ± 0.138	0.055
20	6.78 ± 0.142	6.84 ± 0.150	7.02 ± 0.142	6.94 ±0.142	0.558
Redox potential					
35	-208 ± 30.08	-183 ± 28.57	-206 ± 27.48	-196 ± 30.77	1.000
30	-145 ± 25.33	-134 ± 35.13	-133 ± 35.13	-140 ± 35.13	1.000
25	-149 ± 60.76	-173 ± 61.37	-181 ± 60.76	-174 ± 60.76	1.000
20	-262 ± 64.66	-310 ± 68.72	-314 ± 64.66	-318 ± 64.66	1.000

[1]Standard error of the mean [2] Multiple pairwise comparisons adjusted by the Holm-Bonferroni method.

4.2 Evaluation of methane emissions prediction equations

Without exceptions, all existing equations evaluated exhibited both mean bias and systematic bias (Fig. 2). The equation currently used by the Australian Government in reporting its national inventory (i.e. that proposed by Moe and Tyrrell (1979; Eq. 1)) overestimated enteric CH_4 emissions by a mean of 115 ± 1.37 g/d ($P < 0.01$) or 144 %, from the observed mean of 79.9 ± 25.4 g/d. However, all other tested equations also overestimated CH_4 emissions. Eq. 2 (IPCC, 2006) overestimated CH_4 by 75.9 ± 1.34, or 94 %. The prediction equations 3a and 3b from the IPCC (2019), overestimated CH_4 by 86.4 ± 1.36 (108 %) and 81.9 ± 1.36 g/d (102 %), respectively. The equation proposed Escobar-Bahamondes et al. (2016; Eq. 4a), that required as inputs BW, CP, and polynomial effects of hemicellulose and fat, overpredicted CH_4 by 24.0 ± 1.40 g/d (30 %) whereas this was reduced to an overestimate of 17.8 ± 1.41 g/d (22 %) when BW, CP:NDF, STARCH:NDF ratios, and polynomial effects of DMI and fat were used as inputs (Eq. 4b). The equations proposed by Galyean and Hales (2022) that considered DMI and ratio of dietary starch to NDF concentrations alone or in combination with EE concentration overestimated CH_4 predictions by 80.9 ± 1.38 (101 %) and 76.7 ± 1.37 g/d (99 %), respectively (Eq. 5a-b, Table 9).

All equation displayed significant systematic (linear) bias in CH_4 prediction (Table 9), with the overestimation of CH_4 increasing as CH_4 prediction increased. Type of diet (finisher or transition) did not affect the mean or systematic bias.

Table 9. Statistics from regressions of residual methane production (CH4, g/d) on CH4 predicted by existing empirical equations centred on their mean value.

Equation[1]	Mean bias[2]		Linear bias[3]		Bias at minimum predicted CH_4 (g/d)	Bias at maximum predicted CH_4 (g/d)	RMSE[4]
	Estimate ± SE	*P*-value	Estimate ± SE	*P*-value			
(1) Moe and Tyrrell (1979)	-115 ± 1.37	<0.01	-0.744 ± 0.063	<0.01	-55.0	-163	26.8
(2) IPCC (2006)	-75.9 ± 1.34	<0.01	-0.848 ± 0.0257	<0.01	24.3	-186	26.4
(3a) IPCC (2019) MY	-86.4 ± 1.36	<0.01	-0.827 ± 0.0322	<0.01	10.47	-184	26.6
(3b) IPCC (2019) Y_m	-81.8 ± 1.36	<0.01	-0.836 ± 0.0326	<0.01	11.4	-178	26.7
(4a) Escobar-Bahamondes et al. (2016)	-24.0 ± 1.40	<0.01	-0.938 ± 0.0474	<0.01	54.1	-123	28.0
(4b) Escobar-Bahamondes et al. (2016)	-17.8 ± 1.41	<0.01	-1.00 ± 0.0653	<0.01	56.0	-101	27.5
(5a) Galyean and Hales (2022)	-80.9 ± 1.38	<0.01	-0.834 ± 0.0456	<0.01	2.21	-147	27.1
(5b) Galyean and Hales (2022)	-76.7 ± 1.37	<0.01	-0.797 ± 0.0439	<0.01	4.14	-151	26.8

[1]Empirical equations described in the material and methods.

[2]Mean bias is estimated as the intercept of the regression of the residuals (observed–predicted) on the predicted values centered at their means (g/d).

[3]Linear bias is estimated by the slope of the regression of the residuals (observed–predicted) on the predicted values. It represents the change in the bias of the prediction (g/d) per unit change in the prediction (i.e., per g/d in predicted CH_4). Therefore, it is unitless.

[4]Root mean square of error.

Tornado plots were used to investigate the relative importance of each equation's inputs on the uncertainty of CH_4 emission prediction (Figure 2). For those equations relying on more than one input, the most influential inputs for prediction of CH_4 emissions were dietary solubles content, BW and DMI, for Moe and Tyrrell (1979), Escobar-Bahamondes et al (2016) and Gaylean and Hales (2022), respectively.

Several equations were revealed to have high correlations between inputs, raising concerns regard potential collinearity issues, which can lead to unstable and unreliable estimates of the regression coefficients (Harrell, 2017). For example, in the Moe and Tyrell (1979) equation (Eq. 1) there is a high correlation between SR and HC (0.43) as well as CEL and HC (0.43); in Escobar-Bahamondes et al. (2016) equations, there was a high correlation between BW and DMI, and HC and DMI; in Galyean and Hales (2022) equations, there was high correlation between DMI, NDF, and starch (Figure 3).

Figure 2. Tornado plot depicting low (blue zone) and high (red zone) input variables of existing equations to predict daily CH_4 emission in feedlot cattle. GEI = gross energy intake, SR = soluble, CEL= cellulose, HC = hemicellulose, DMI = dry matter intake, BW = body weight, NDF = neutral detergent fibre, ADF = acid detergent fibre, CP = crude protein.

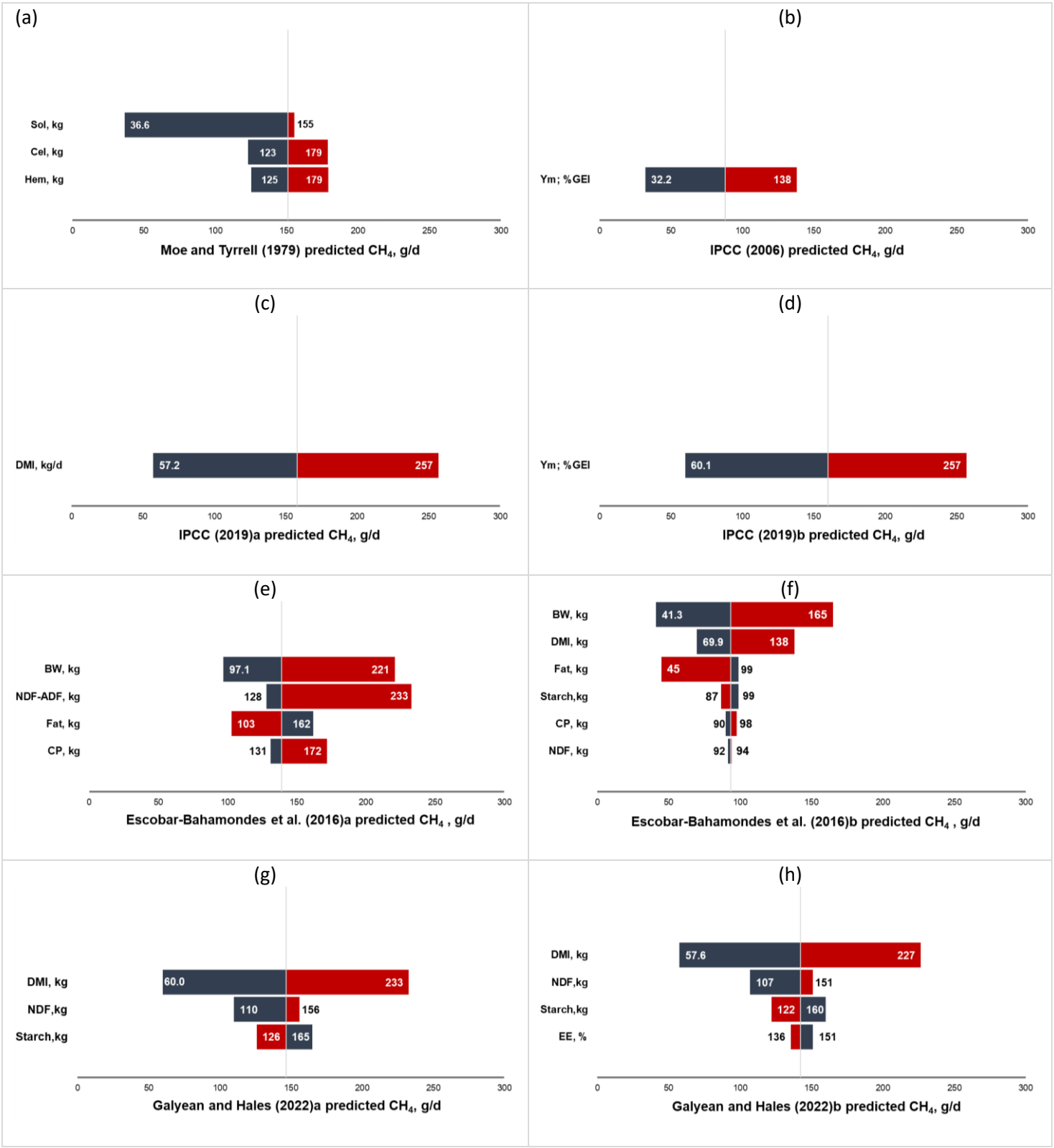
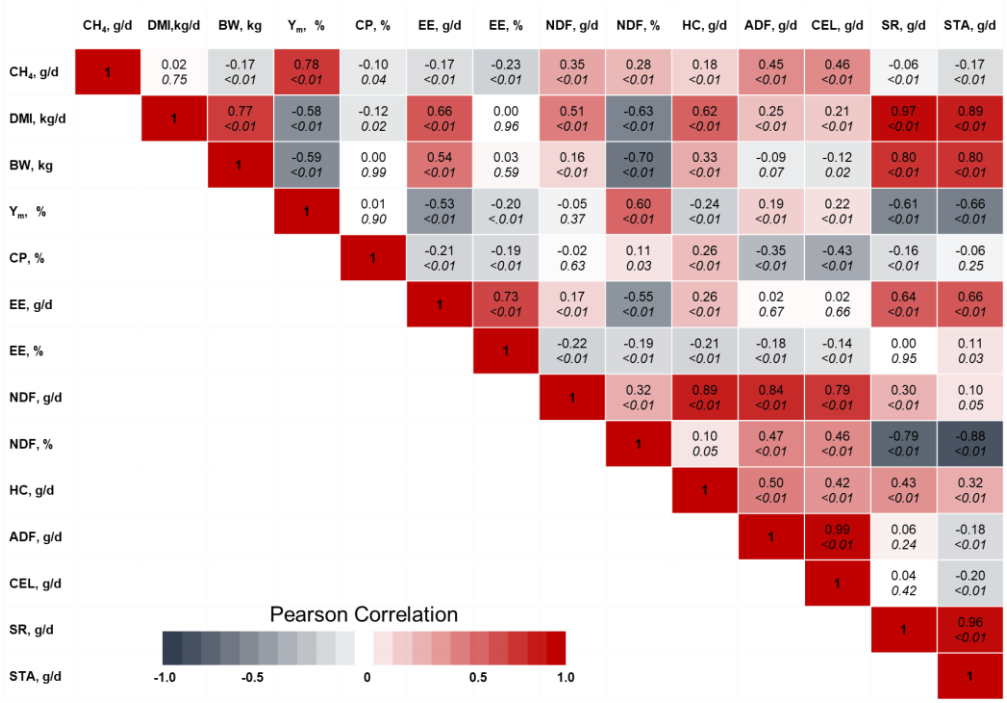


Figure 3. Linear relationship (Pearson correlation = r) of input variables required to predict methane in evaluated literature equations.



4.3 New equation parametrisation

We conducted a stepwise procedure to test the variables DMI, NDF, EE, starch, and CP to produce a regression equation that minimized the RMSE and maximized the adjusted-r^2 (Harrell, 2017). The stepwise regression analysis revealed that DMI, EE, and dietary NDF met the *P*-value threshold (≤ 0.05) for entry into the equation. NDF was the first variable selected in the stepwise analysis (r^2 = 0.892), followed by DMI (r^2 = 0.909) then EE (r^2 = 0.912). Thus, a multiple regression with intercept = 0 was fitted to predict CH_4 production from feedlot cattle (Eq. 2; n = 384; $P < 0.05$; RMSE = 22.2; g/d; σ^2_s = 8.02; r^2 = 0.91):

$$CH_4 \text{ (g/d)} = 5.11 \pm 1.58 \times \mathbf{DMI} - 4.00 \pm 0.821 \times \mathbf{EE} + 2.26 \pm 0.125 \times \mathbf{NDF} \quad [8]$$

These findings suggest that a 1% increase in dietary EE results in a 4.0 g reduction in CH_4 emissions, while each percentage point increase in dietary NDF contributes to a 2.3 g increase in CH_4 production. Alternatively, a simple linear equation with intercept = 0 was fitted between CH_4 production (g/d) and DMI (kg), in which the slope represented the CH_4 yield in g/kg DMI (Fig. 4; n = 384; $P < 0.01$; RMSE = 32.6 g/d; r^2 = 0.85):

$$\mathbf{CH_4} \text{ (g/d)} = 9.89 \pm 1.54 \times \mathbf{DMI} \text{ (kg/d)} \quad [9]$$

5. Discussion

The evaluation of these equations was facilitated by the inclusion of an evaluation dataset that encompassed a diverse range of NDF and EE levels. This dataset effectively captured variations found in both growing and finishing dietary programs for feedlot cattle in Australia, as well as various scenarios in between and encompassing diets fed to a broad range of cattle types in feedlots in Australia (short fed, medium fed and long fed cattle categories). The use of 53 individual animals, and 384 records in this project makes it possibly the largest database for modelling of CH_4 emissions in feedlot cattle globally. All records in this database were obtained from open circuit respiration chambers, which are considered the 'gold-standard' methodology to measure CH_4 emissions (Garnsworthy *et al* 2019). In comparison, of the 30 references cited in the development of the IPCC (2019) methodology, the median animal number was 12, the largest *in vivo* experiment used 76 respiration chamber records from 76 animals, and no other publication covering more than 20 animals was conducted in respiration chambers. Such comprehensive coverage of diets and extensive collection of records by gold-standard methodology allowed for a robust evaluation of multiple regression equations.

The current methodology used in Australian GHG reporting frameworks for estimating methane (CH_4) emissions from feedlot cattle relies on the Moe and Tyrell (1979) equation. This equation predicts daily CH_4 emissions based on the daily intake of HC, CEL, and SR. Analysis of the database as part of this study demonstrated that the Moe and Tyrell (1979) equation overpredicted methane of Australian lot fed cattle by 115 ± 1.37 g/d, resulting in an estimated mean methane emission prediction of 194 g/head.d, whereas the observed mean in the database was 79.9 g/head.d.

While in theory, the Moe and Tyrell (1979) equation offers an opportunity for emissions predictions to be 'customised' to individual feedlot producers based on their diet compositions, in the Australian context, the chemical analyses required to determine acid insoluble lignin fractions, and subsequently cellulose input into the Moe and Tyrell (1979) equation are not routinely performed, which makes usage of the equation difficult from a practical perspective. In practice, the Australian National Inventory commonly utilises industry average estimates for diet composition in its predictions.

It is important to note that this equation was derived from a meta-analysis of trials primarily conducted on Holstein dairy cattle, neglecting potential variations across different cattle breeds, production systems and diets. The evaluation of the Moe and Tyrell (1979) equation in predicting CH_4 revealed that although both dairy and beef cattle belong to the *Bos taurus* species, caution should be exercised when extrapolating results from one type of ruminant to another. This caution stems from their distinct digestive physiologies, which encompass factors such as mean retention time of digesta, digestibility of the feed provided, as well as digestion and fermentation characteristics (Bannink et al., 2008; Huhtanen et al., 2018). For instance, dairy cows, depending on physiological state, have a higher nutritional demand and can consume a large amount of feed to support milk production, typically ranging from 3.0 to 4.0% BW (NRC, 2001), whereas grain-fed beef cattle consume less feed on a DM-basis as a percentage of their BW (2.0-2.5%, NASEM, 2016). An additional point that has been raised is the impact of advancements in the management and breeding of ruminant animals over the past few decades on their digestive physiology. It is suggested that studies conducted in the 1980s or earlier may not be entirely applicable in present times due to greater intake capacity, increased passage rate and decreased digestibility, necessitating a cautious approach when extrapolating findings from older studies to contemporary conditions (Van Gastelen et al., 2019).

Contrary to previous reports (Beauchemin and McGinn, 2005), the basal grain type used in feedlot diets (i.e., corn *vs.* barley) may not be the main driver of methane production as our results showed methane production comparable to that expected for beef cattle feed high corn diets (steam flaked) with added ionophores (IPCC, 2019).

In contrast to its relative simplicity, the IPCC (2006) methodology for predicting methane emissions is found to be effective for high-forage diets (as observed in Escobar-Bahamondes et al., 2017). However, it falls short in delivering accurate methane predictions. Equations using more input variables did not result in better predictions in CH_4, as shown in Table 3 and Figure 1. The complex nature of CH_4 formation, influenced by various factors including diet, microbial populations, and animal-specific characteristics, can contribute to a high degree of variability in methane emissions. It noteworthy that these complex equations may result in collinearity which can pose problems in regression analysis because it can make it difficult to determine the individual effects of the correlated variables on the dependent variable.

Dry matter intake is a crucial factor influencing CH_4 production in ruminants (Congio et al., 2022), and it is also closely associated with production traits. In the evaluation of CH_4 prediction equations, all the assessed equations had DMI explicitly or implicitly included as an input variable. This finding supports the validity of the Parsimony Law, which suggests that when multiple explanations exist for a phenomenon, the simplest explanation is often the correct one. This principle encourages the selection of simpler models that strike a balance between capturing essential patterns and minimizing unnecessary complexity. Bearing this in mind, we tailored the IPCC (2019) equation to create a tier 2 emission factor relevant to the Australian feedlot sector. This approach uses the MY represented by the slope of Eq. 7 (i.e., g CH_4/kg of DMI), 9.89 ± 1.54 multiplied by the DMI to estimate the CH_4 production for all Australian lot fed cattle. This equation explained 85 % of the variation in CH_4 production observed in the dataset. This tailored IPCC (2019) approach results in a new baseline for the total enteric CH_4 emissions of the Australian feedlot sector of 1.29 Mt CO_2eq. This is a 43.45 % (0.99 Mt CO_2eq) reduction from the current baseline of 2.28 Mt CO_2 eq that is estimated by the current approach used in the national inventory (Moe and Tyrrell, 1979; activity data available at https://www.dcceew.gov.au/sites/default/files/documents/ageis-activity-table-1990-2020-agriculture-livestock-national.xlsx).

Alternatively, we also fitted an equation that recognises the impacts of dietary fat and fibre composition on methane emissions (Eq. 6). For several decades, the dietary EE suppression on CH_4 production in ruminants has been recognized (Blaxter and Czerkawski, 1966). EE percentage was a relevant input variable in the new proposed equation, nevertheless, the inclusion of EE as a predictor in the equations did not result in improved predictions of CH_4 production, and the effect of additional EE on CH_4 emissions was small (4 g per 1 % inclusion increase). Dietary fibre (e.g. NDF) represents a primary source linked to the production of volatile fatty acids (VFA), which, in turn, serves as the principal supplier of hydrogen ions (H^+) for the methanogenesis in the rumen (Ungerfeld, 2015). The impact of increasing NDF content on CH_4 emissions was even smaller than for EE: for each one-percentage point increase in dietary NDF, there was a corresponding 2.3 g increase in CH_4 production. This equation explained 91 % of the variation in enteric CH_4 emissions in the dataset, although it was not significantly different from Eq. 7. A benefit of this equation is that it provides a pathway for mitigation via diet composition, although the small impact of EE and NDF on CH_4 emissions may not justify the additional complexity of Eq. 6 for national inventory purposes. The diet composition inputs for Eq. 7 (EE and NDF composition of typical domestic, mid- and long-fed diets) are not currently collected as part of the national inventory, so modelling of the impact of this equation on sectoral emissions is not possible at this stage.

6. Conclusion

6.1 Key Findings

This project assembled one of the world's largest data base on enteric methane emissions from feedlot cattle measured using gold standard respiration calorimeters. This research project has found that the current methodology employed by the Australian Government for calculating CH_4 emissions from the grain-fed beef sector for reporting in its National Inventory of greenhouse gasses (Moe & Tyrrell, 2019) overestimates enteric methane emissions. Average CH_4 emissions predicted by the Moe & Tyrell (1979) equation were found to be 2.44 times the observed emissions measured in respiratory calorimeters. A number of alternative, previously published predictive equations were also evaluated, and all displayed significant mean and system bias in prediction of CH_4 production. Two new methods are proposed to estimate CH_4 emissions from Australian feedlot cattle. Firstly, a new, Australian-specific emission factor (9.75 ± 1.34 × DMI) in line with IPCC (2019). This simplified methodology conforms to the Law of Parsimony, and shows that variation in DMI explains 85 % of the variation in CH_4 production, across a range of diet compositions typical of the Australian feedlot industry. Secondly, an equation that incorporates the effect of EE and NDF, in addition to DMI, on CH_4 emissions, and which explains 91 % of the variation in CH_4 production, across a range of EE and NDF levels.

6.2 Benefits to industry

On this basis of this report and a peer-reviewed publication detailing this research (Appendix), a dossier will be compiled and submitted to the Australian Government, advocating the evidence to support a change in the Australian Government's national inventory methodology for calculation of feedlot cattle enteric methane emissions.

7. Future research and recommendations

The emission factor proposed for Australian feedlot cattle has been derived using a database of 384 individual measurements of Australian cattle fed tempered barley-based feedlot diets. Other research has suggested that basal grain type (i.e. corn v barley) may affect CH_4 production, but this research found CH_4 emissions from barley-based diets are comparable to those fed high corn diets (steam flaked) with added ionophores (IPCC, 2019; Beauchemin and McGinn, 2005). Future research should validate this equation across both barley- and wheat-based diets, as the most common grain types used in the Australian feedlot industry.

8. References

Beauchemin K, McGinn S 2005. Methane emissions from feedlot cattle fed barley or corn diets. Journal of Animal Science 83(3): 653–661.

Blaxter KL and Czerkawski J 1966. Modification of the methane production of the sheep by supplementation of ITS diet. Journal of the Science of Food and Agriculture 17, 417–421.

Bannink A, Van Lingen HJ, Ellis JL, France J, Dijkstra J 2016. The contribution of mathematical modeling to understanding dynamic aspects of rumen metabolism. Frontiers in Microbiology 7: 1820.

Congio GF, Bannink A, Mayorga OL, Rodrigues JP, Bougouin A, Kebreab E, Silva RR, Maurício RM, da Silva SC, Oliveira PP 2022. Prediction of enteric methane production and yield in dairy cattle using a Latin America and Caribbean database. Science of the Total Environment 825: 153982.

Department of Climate Change, Energy, the Environment and Water (DCCEEW) (2019) National inventory by economic sector: 2019 emissions. Accessed: https://www.dcceew.gov.au/climate-change/publications/national-greenhouse-accounts-2019/national-inventory-by-economic-sector-2019-emissions.

Escobar-Bahamondes P, Oba M, Beauchemin K 2016. Universally applicable methane prediction equations for beef cattle fed high-or low-forage diets. Canadian Journal of Animal Science 97(1): 83–94.

Escobar-Bahamondes P, Oba M, Beauchemin K 2017. An evaluation of the accuracy and precision of methane prediction equations for beef cattle fed high-forage and high-grain diets. Animal, 11(1):68–77.

Galyean ML, Hales KE 2022. Prediction of methane per unit of dry matter intake in growing and finishing cattle from the ratio of dietary concentrations of starch to neutral detergent fiber alone or in combination with dietary concentration of ether extract. Journal of animal science 100(9): skac243.

Garnsworthy, PC, Difford, GF, Bell, MJ, Bayat, AR, Huhtanen, P, Kuhla, B, Lassen, J, Peiren, N, Pszczola, M, Sorg, D and Visker, MH (2019). Comparison of methods to measure methane for use in genetic evaluation of dairy cattle. Animals, 9(10): 837.

Goering HK, Van Soest PJ 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications), US Agricultural Research Service.

Harrell FE 2017. Regression modeling strategies. Bios 330(2018): 14.

Hegarty RS, Bird S, Woodgate R. 2014. Cattle respiration facility, Armidale. New South Wales, Australia. Chapter 2 C. Pinares, G. Waghorn (Eds.), 'Technical Manual on Respiration Chamber Designs', pp. 31–44

IPCC (2006). 2006 IPCC guidelines for national greenhouse gas inventories. Agriculture, Forestry and Other Land Use. Institute for Global Environmental Strategies, Hayama, Kanagawa, Japan. H. S. Eggleston, L. Buendia, K. Miwa, T. Ngara and K. Tanabe. Hayama, Japan, Institute for Global Environmental Strategies (IGES). 4: 30–32.

IPCC (2019) '2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Vol 4. Agriculture, Forestry and Other Land Use.'

Lenth, R. V. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means R package version 1.5.3.

McMeniman J, Defoor P, Galyean M 2009. Evaluation of the National Research Council (1996) dry matter intake prediction equations and relationships between intake and performance by feedlot cattle. Journal of Animal Science 87(3): 1138.

Moe, P. W. and H. F. Tyrrell (1979). "Methane Production in Dairy Cows." Journal of Dairy Science 62(10): 1583-1586.

NASEM (2016) 'Nutrient requirements of beef cattle.' (The National Academies Press: Washington DC)

National Research Council (2001). Nutrient Requirements of Dairy Cattle: Seventh Revised Edition.

Ungerfeld EM 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. Frontiers in Microbiology 6: 37.

van Gastelen S, Dijkstra J, Bannink A 2019. Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep? Journal of Dairy Science 102(7): 6109–6130.

Van Soest P, Robertson JB, Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science 74(10): 3583–3597