

IMPACTS OF CFI METHODOLOGIES ON WHOLE FARM SYSTEMS

Draft report

EXECUTIVE SUMMARY (200 word limit)

The project has integrated sheep and beef rumen models with nitrate and lipid models to run within AusFarm: Outputs include: VFA production and microbial protein outflow. International collaboration with New Zealand and California has been critical in developing and evaluating the rumen models. Case studies across 5 sites for sheep and beef production evaluated long-term effects of mitigation strategies. The results indicate that a lipidxNO₃ mitigation strategy simulated across 30 years of variable climate reduced CH₄ production per product from 40 to 42% and total GHG emissions from 14 to 33%. For a wether production system (8.2 wethers/ha) CH₄ production (kg/kg wool) was reduced by 42% and wool productivity increased by 9%. A self-replacing ewe flock (5.3 ewes/ha) showed reduced CH₄ production (kg/kg wool) by 42%, increased wool productivity by 9% and LWG by 7%. A steer production system (1.5 steers/ha) reduced CH₄ production (kg/ kg LWG) by 40% and increased LWG by 32%; and a steer production system (1 steer/ha) reduced CH₄ production (kg/ kg LWG) by 39% and increased LWG by 24%. It is concluded that nitrate and oil in combination offer an effective and financially desirable mitigation strategy for both beef and sheep enterprises.

1. BACKGROUND

This project aims to determine the long-term stocking rates and productivity of livestock farming following the implementation of a range of possible methane mitigation strategies under the Carbon Farming Initiative (CFI). It is intended that this will facilitate the development and uptake of methane-reducing CFI methodologies. The project will provide productivity and economic assessments of mitigation strategies to provide producers with an assessment of the economic impact on their enterprise.

These aims will be achieved by:

- Providing a facility to estimate the long-term impact of mitigation tools on livestock enterprise productivity and emissions across the full range of seasonal conditions likely to be experienced in any Australian agricultural region. This will be achieved by modifying the existing AusBeef (Figure 1.1) rumen model that will then be used in Australia's leading grazing animal system model (AusFarm) to reflect animal production changes resulting from mitigation strategies and simulating the effects of mitigation on production across 30 years of local climate data.
- Evaluating the impacts of potential CFI methodologies (nitrate, oils, alone and in combination) on whole-farm systems across three sites in Australia (Northern NSW, Victoria, and WA) and 1 site in New Zealand (NZ) and North America. This will further test the robustness of the estimates based on systems at extremes of pasture quality (NZ v Australia) and supplement intake (North America v Australia).
- On-farm evaluations of long-term productivity and emission responses to methane mitigation and nitrous oxide emissions at an enterprise level.
- Enhancing the objectives of the Global Research Alliance on Agricultural Greenhouse Gases and complementing the Australian domestic modelling research efforts to reduce waste and increase production by reducing emissions intensity of beef and lamb production.

Methanogenesis is a consequence of hydrogen accumulation in the rumen. While our current understanding of hydrogen production/use is adequate for normal situations, when methane production is inhibited (mitigated), up to 50 per cent of the hydrogen produced cannot be accounted for. This means there is a large error, not just in estimating mitigation (if not measured) but in estimating the quantity of useful nutrients supplied by other ruminal processes [e.g. microbial protein and volatile fatty acids (VFA)]. Consequently, it is necessary to 'add up' the balance of hydrogen producing and utilising reactions in the rumen to estimate residual hydrogen that will go into methane production. While this project is not seeking a rumen model of extreme accuracy or extreme complexity, it is seeking to model that reflects the changes in microbial processes that occur when methane is suppressed.

Nagorcka Model

Confidential CSIRO

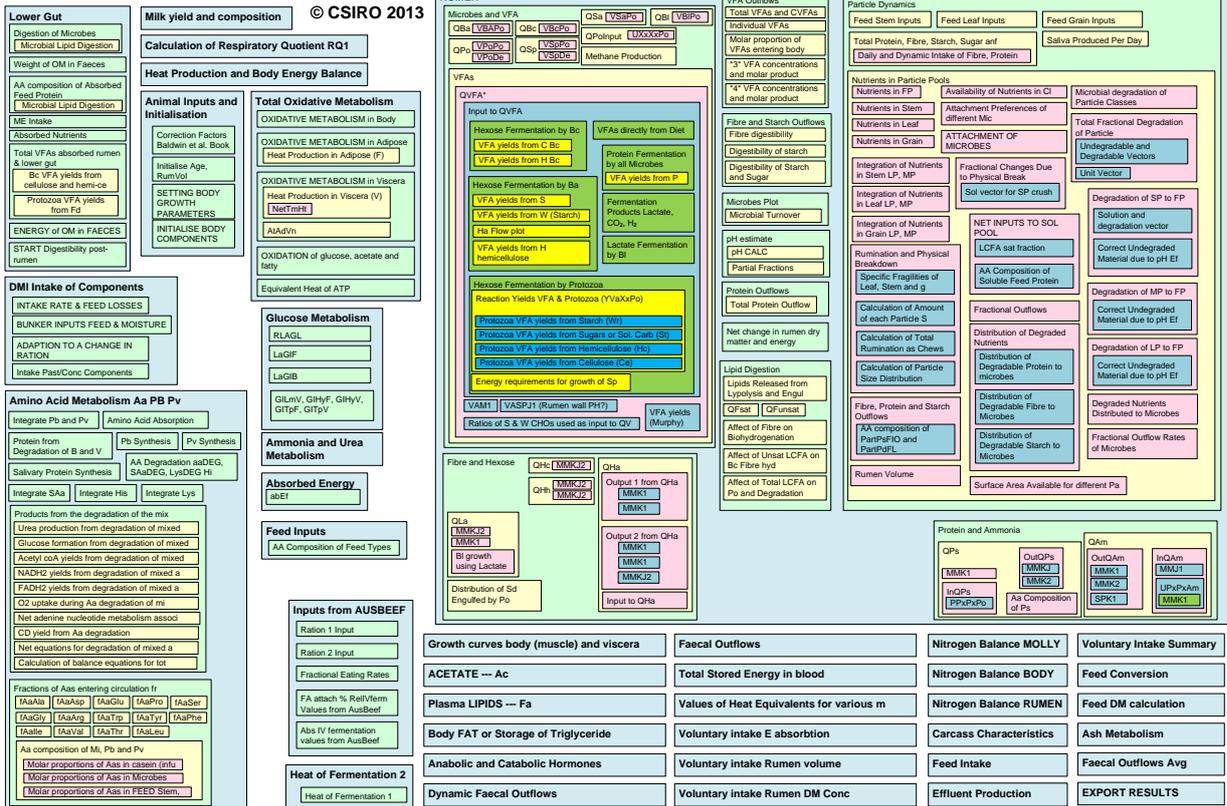


Figure 1.1. Flow chart of the AusBeef rumen model.

Table 1.1. provides a glossary of terms used throughout the main body of this report. Additional tables for terms used to describe the models are included where necessary.

Table 1.1. Glossary terms in the main body of this report

Term	Meaning
Ac	Acetate
Bu	Butyrate
CH ₄	Methane
CS	Condition score
DLL	Dynamic-link library
DM	Dry matter
DMI	Dry matter intake
LCFA	Long Chain Fatty Acids
LW	Live weight
N	Nitrogen
NH ₄	Ammonia
N ₂ O	Nitrous oxide
NO ₂	Nitrite
NO ₃	Nitrate
MCFA	Medium Chain Fatty Acids
Molly	Dairy model
Molly14	Sheep rumen model converted from Molly
Molly15	Nitrate and lipid models added to Molly14
P	Phosphorus
Pr	Propionate
SE	Standard error
SEM	Standard error of the mean
VFA	Volatile fatty acids

2. METHODOLOGY

The methods applied to achieve the activities are as follows:

2.1 AusBeef conversion to modular C++ code

The rumen model from AusBeef (Nagorcka et al. 2000) was extracted from the AusBeef source code. The original source code for AusBeef was written in VisSim, a graphical language for simulation and model-based embedded development, (PTV 2004). The rumen model of AusBeef was re-engineered into modular C++ code so that a rumen module could be linked with the AusFarm decision support tool. The re-engineered C++ code was imported into the Delphi C++ programming environment and configured as a new AusFarm module. Variable names were renamed where appropriate to represent a common modelling protocol. Programming code from CSIRO was provided to develop linkages with the AusBeef rumen model and the GrassGro animal growth, stock and pasture production modules (Figure 2.1).

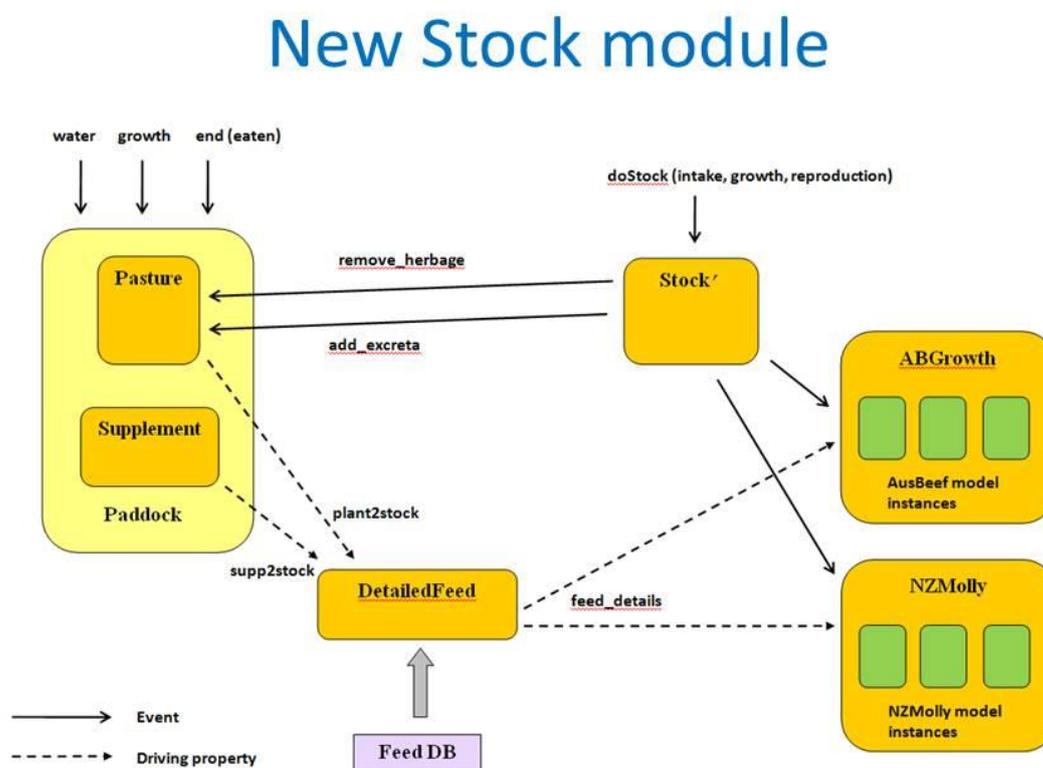


Figure 2.1. New stock module for AusFarm that integrates AusBeef and New Zealand sheep rumen model.

2.1.1 Backup methodology

A backup approach to achieving the outcomes of the project was developed because of a delay in obtaining the source code of the AusFarm Stock module from CSIRO. The approach was to implement an AusFarm module based on the greenhouse gas equations from the Australian National Greenhouse Gas Inventory method (Dept. of the Environment, 2012) as implemented in the spreadsheet GHG calculation tools of the Primary Industries Climate Challenges Centre (available at: <http://www.greenhouse.unimelb.edu.au/Tools.htm>).

In this new AusFarm module (EckardGHG), greenhouse gas calculations are performed on a daily basis (as opposed to the season-by-season approach of the spreadsheet tools) and cattle numbers, feed values and weather information are obtained from other AusFarm modules as illustrated in Fig. 2.1

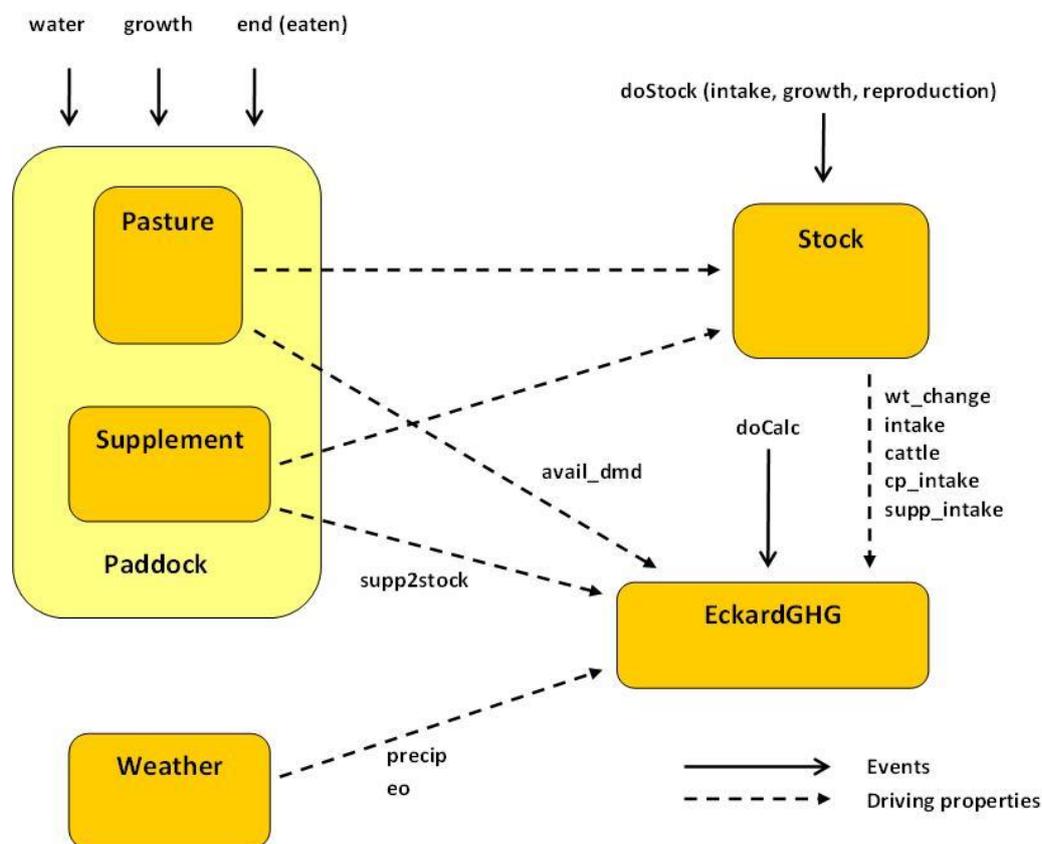


Fig. 2.1 EckardGHG module interactions within AusFarm

An initialisation screen allows information concerning electricity use, fertilisers and tree planting to be entered into the module. Results available for output include estimates of the emissions of carbon dioxide and enteric methane as well as nitrous oxide from excreta and from leaching, runoff and atmospheric deposition.

2.2 Sensitivity analysis

Four outputs of interest were chosen for the sensitivity analysis. These were: (i) The ratio of acetate production rate to propionate production rate (Ac:Pr), (ii) Energy (MJ/day) from absorbed volatile fatty acids (EVFA), (iii) Microbial protein outflow rate (g/day) (MiPrt), (iv) Methane production rate (mol/day) (CH₄). The analysis involved parameters in the rumen sub-model of the AusBeef model which are internal model parameters rather than feed or animal inputs. Software was written to interface an executable C++ routine with R. The analysis was conducted using 305 rumen model parameters that have values different from 0 or 1. For the global and local analysis, model parameters were assumed to be distributed uniformly with boundaries of baseline parameter value $\pm 10\%$.

Data input for simulation. Three diets were used to perform this sensitivity analysis: a barley-based feedlot diet representative of Australian systems (AUS; Greenwood *et al.* 2015), a corn-based feedlot diet representative of northern California systems (US; unpublished), and a New Zealand mature ryegrass pasture diet (NZ; Jonker *et al.* 2015). The feed compositions are given in Table 2.1. All animals were Angus steers, with no implant and an initial age of 18 months. Initial live weight was 400 kg, assuming 4% shrunk at arrival, with a frame score of 6.5 and a body condition score of 3.5. Each simulation was run until a final market weight of 550 kg was reached, assuming *ad lib.* feeding. The model was run for each diet using baseline values. The model was then run 1000 times for each feed,

using random values for each parameter. Parameters values were drawn from a uniform distribution with boundaries $\pm 10\%$ of the initial baseline parameter value, and the output values were averaged over the last 10 days of the simulation.

Table 2.1. Ingredients and nutritional value of Australian, USA, and New Zealand diets offered to Angus steers to establish parameter sensitivities

Diet/Measure		USA		New Zealand	
Australian					
Simulation Length (d)	75		67		127
Ingredient (% as fed)					
Barley	77.5	Steam-Flaked Corn	70	Mature Ryegrass Pasture (30:70 Leaf: Stem)	100
Grassy Lucerne Hay (30:70 Leaf:Stem)	10	DDG + Solubles	8.7		
Cottonseed Meal	2.5	Cane Molasses	6		
Molasses	8	Sorghum-Sudangrass Hay (30:70 Leaf:Stem)	4		
Sodium Bicarbonate	0.5	Wheat Stem Hay (30% leaf, 70% stem)	4		
Ammonium Sulfate	0.5	High Quality Tallow	2.5		
Lime (Calcium Hydroxide)	1	Calcium Carbonate	1.5		
		Trace Mineral Salt	1.5		
		Urea (42% Nitrogen)	0.75		
		Monosodium Phosphate	0.5		
		Potassium Chloride	0.4		
		Magnesium Oxide	0.1		
Nutritional Value					
Dry Matter (% as fed)	87.2		89.3		20.4
NDF (% DM)	22.7		15.1		59.1
ADF (%DM)	8.8		7.03		31.7
Ash (%DM)	7.0		5.5		7.2
Crude Protein (%DM)	13.7		12.5		10.6
Lipid (%DM)	2.12		6.83		1.9

Statistical analysis. Local sensitivity analysis was performed in R using the sensFun function for local sensitivity analysis of dynamic models (Soetaert & Petzoldt 2015). Sensitivities were calculated from average daily outputs for each day of the simulations (75 days for Aus, 67 days for US, and 127 days for NZ diets), and the function was run using a perturbation of $\text{tiny} = 1e-03$. The last 10 days of each simulation run were used to calculate sensitivities, and sensitivities of interest were non-zero mean absolute values. Sensitivities were normalized as part of the function's run, and normalized sensitivities >0 were ranked by diet and measure, and compared to find commonalities across diets and measures.

Global sensitivity analysis using differential equation-based models was run using the fast99 function (Pujol et al. 2015) for global sensitivity. Parameters were assumed to be distributed uniformly with boundaries of $\pm 10\%$ of the baseline, and the model was run 100 times per diet/output combination. The function outputs the first order sensitivity (main effect), the total global sensitivity (total effect), and any interactions, if present. The function was limited to running one diet/output combination at a time.

2.3 Development of Nitrate and Lipid models for sheep and cattle production

Nitrate and lipid models for sheep

Nitrate model. The model of Vetharanim et al. (2015) was used and modifications to the hydrogen sink equation were made. Matlab and excel were used to develop up the modified model.

MollyRum14 (Vetharanim et al., 2015) introduced rumen hydrogen (H₂) as an explicit pool, (RH2, in moles) with a governing differential equation:

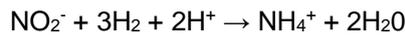
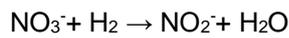
$$DRH2 = DTHy (1 - k_1 * DMI/EBW^{0.75}) - KWAP * RH2 - 4.0 * DTCH4, \quad (1)$$

Here DRH2 is the time derivative of RH2. DTHy is the nett flux of hydrogen from various fermentation processes, the factor $(1 - k_1 * DMI/EBW^{0.75})$ empirically adjusts for unaccounted changes in stoichiometry with DMI and KWAP where KWAP is the rate of water passage (d⁻¹). DTCH4 is the rate of methane production, given by

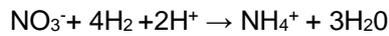
$$DTCH4 = m_f * Mi * RH2/RLV \quad (2)$$

where Mi is the microbial mass, RLV is rumen liquid volume, and m_f is a rate constant which can be fitted to data.

The introduction of the hydrogen pool facilitates modelling of nitrate supplementation in a dynamic way, since nitrate reduction to ammonia consumes H₂ that could otherwise be used by methanogens via the following reactions:



A pool, RNO3 (moles), representing rumen nitrate was introduced in MollyRum15. On the bases that nitrite would reduce faster than nitrate and be a much smaller pool, and that rate determination is likely to occur in the reduction of nitrate, a similar rumen nitrite pool was not introduced, but rather the above two reactions were represented as the net reaction:



The following equation was used for the rate of this reaction:

$$NO3_NH4_rate = kNO3 * 1e6 * Mi * RNO3 * RH2 / (RLV * RLV) \quad (3)$$

where kNO3 is an adjustable parameter and 1e6 is simply a scaling factor. This equation assumes no pH affects and since Molly has an empirical estimation of pH, based on VFA concentrations, and does not account for H⁺ flux and buffering, the effect of this reaction on pH was ignored. A linear relationship between hydrogen concentration and the reaction rate was assumed although 4 moles of H₂ are consumed per mole of nitrate.

Equation (1) was then modified to account for this hydrogen sink, which will automatically reduce the hydrogen pool in the presence of nitrate and thus reduced the methane production predicted by Equation (2):

$$DRH2 = DTHy (1 - k_1 * DMI/EBW^{0.75}) - 4.0 * DTCH4 - KWAP * RH2 - 4.0 * NO3_NH4_rate \quad (4)$$

MollyRum15 makes a distinction between supplementation with ammonium nitrate and with other forms of nitrate. Ammonium nitrate supplied is represented in the model by an input AmNitSup and other forms of nitrate by another input NitSup, both specified in terms of kg of NO₃ supplied per kg of feed.

A differential equation for the change of the nitrate pool over time (DRNO3) was specified as:

$$DRNO3 = (NitSup + AmNitSup) * DMI * 1000 / MW_NO3 - NO3_NH4_rate - RNO3 * KWAP \quad (5)$$

Where the first term represents the contribution of nitrates from supplementation, with MW_NO3 being the molar weight of nitrate. The second term is the loss of nitrate due to reduction to ammonia, and the last term represents the loss of nitrate due to water passage.

If ammonium nitrate is used as a supplement, then this will provide an additional source of rumen ammonia. The pre-existing differential equation for rumen ammonia (Am, moles) was adjusted to:

$$DAm = fDNnAm + AaAm + SaNnAm + PUNAm - absRAM - AmMi + FdUrAm + AmNitSup * DMI * 1000.0 / MW_NO3 + NO3_NH4_rate \quad (6)$$

Where DAm is the time derivative of Am, fDNnAm, AaAm, SaNnAm, PUNAm and fdUrAm are respectively ammonia from NPN, amino acids, saliva and plasma urea transported across the rumen wall and urea supplements. The term absRAM is loss of ammonia due to absorption through the rumen wall, and AmMi is loss due to microbial utilisation of ammonia. The second to last term is a source of ammonia from ammonium nitrate supplementation, and the last term is from reduction of ammonia (Eq. 3).

MollyRum14 (Vetharaniem et al., 2015) required an estimate of total N intake to calculate plasma urea nitrogen. In MollyRum15, this equation was modified to the following to allow for supplying NO₃⁻:

$$\text{total_N_intake} = \left(\frac{0.16 * (fDPi + fDPs + fDNn)}{+ fDUr * 0.47MW_NH4 / MW_NO3 * AmNitSup} \right) * DMI * 1000 \quad (7)$$

where fDPi, fDPs, fDNn and fDUr are respectively insoluble protein, soluble protein, NPN and urea in the feed. This equation assumes crude protein is 16% N by weight and that urea is 47% N by weight. This term MW_NH4 / MW_NO3 * AmNitSup accounts for NH₄ from ammonium nitrate supplement, where MW_NH4 is the molar weight of ammonium.

Lipid model. The two main mechanisms by which lipids lower methane production are inhibition of fibre degradation and inhibition of methanogens (Rasmussen and Harrison, 2011). This is highly dependent on the type of lipid, and may not occur with some lipids. The most effective lipids at reducing methane are MCFAs and some LCFAs (Rasmussen and Harrison, 2011). This presents a rather complex set of relationships, and rather than capture these mechanistically, an empirical approach was used as an initial step, with the intention that the effect of different lipid supplements be represented by different parameter values.

Like its predecessors, MollyRum14 (Vetharaniem et al., 2015) allows for providing a lipid supplement but does not specify characteristics such as chain length or level of saturation. Rather it is assumed that a predetermined proportion of all lipids in the diet contribute to an unsaturated long-chain fatty acid pool (FI), though these proportions differ depending on whether the fat was in the feed or provided as a supplement. There are no other lipid pools represented, though some rates in the model affecting other pools are influenced by lipids. The FI pool has two sinks: bio-hydrogenation (which acts as a hydrogen sink) and passage out of the rumen at the same rate as water.

MollyRum14 has relationships that allow lipid supplements to affect the efficiency with which starch and fibre are digested, however the parameter values associated with that are fixed and give small effects. These relationships are based on the ratio of lipid supplement to lipid content of the feed. While large increases in the corresponding parameter values could give observed effects, this mechanism did not seem satisfactory, since lipids naturally in the diet could conceivably have similar inhibitory effects to supplementary lipids. Thus it was decided to relate the inhibitory effects to the unsaturated LCFA pool in MollyRum15 (latest version including the modifications to the model of nitrate and lipid).

To do this, the digestion rate parameter for hemicellulose and cellulose, KCeCs, was modified in MollyRum15 as follows:

$$KCeCs \rightarrow KCeCs / (1.0 + kLipDeg * FI /RLV) \quad (8)$$

where kLipDeg is a parameter introduced in MollyRum15 which can be adjusted according the type of lipid.

To account for methanogen suppression, Eq. (2) was modified to:

$$DTCH_4 = m_f * Mi / (1.0 + kLipDeg * FI / RLV) * RH_2 / RLV \quad (9)$$

where $kLipDeg$ is a parameter that can be fitted to reflect the effect of the lipid pool.

For the simulations in this study, the lipid-related parameters were adjusted so an additional lipid supplement of 3% produced a 15% reduction in CH_4 production.

The GrazPlan animal model (Freer et al. 1997) requires rumen degradable and undegradable protein (RDPi and UDPi) as inputs. In order to interface MollyRum15 with the GrazPlan animal model, equations were developed to estimate RDPi and UDPi from the predicted outflows of various forms of protein from the rumen. The following ordinary differential equation (ODE) was constructed to approximate DRDPi, the derivative of RDPi:

$$DRDPi = RAaP / (1000.0 * MWRAa) + (MiP + HaMiP + HbMiP) * 0.475 + absRAm * (14.0 / 1000.0 / 0.16) \quad (10)$$

where this equation includes the outflows of amino acids (RAaP, moles/day) and microbial protein (from the microbe pool, MiP, and the outflows from additional pools of microbes associated with starch (alpha-hexose), HaMiP, and microbes associated with cellulose and hemicellulose (HbMiP)). All microbial outflows are in kg/d. MWRAa is the nominal molar weight of amino acids and used to convert from moles to weight. The factor 0.475 assumes that microbial protein is typically 45 to 50% of microbial mass. The variable absRAm is the rate of ammonia absorption through the rumen wall; multiplying by 14 converts from moles to g, and dividing by 0.16 converting to a protein equivalence, assuming protein is 16% nitrogen by weight.

It was assumed that UDPi can be accounted for by rates of insoluble protein (PiP) and nitrate ($RNO_3 * KWAP$) exiting the rumen into the stomach. The derivative for UDPi (DUDPI) was thus written as

$$DUDPi = PiP + RNO_3 * KWAP * (14.0 / 1000.0 / 0.16) \quad (11)$$

where multiplying by 14/1000 converts the N in a mole of NO_3 to a weight in kg, and dividing by 0.16 converts to a protein equivalence, assuming protein is 16% nitrogen by weight. Molly does not have a flow of ammonia from the rumen into the stomach, hence the absence of such a term.

Integrating Eqs (10) and (11) on a daily time step yields a daily estimate of RDPi and UDPi. Figure 2.3.1 provides a schematic of the sheep rumen model where the digestion/urea/energy model is very simple and Table 2.3.1 provides a definition of terms used in Figure 2.3.1.

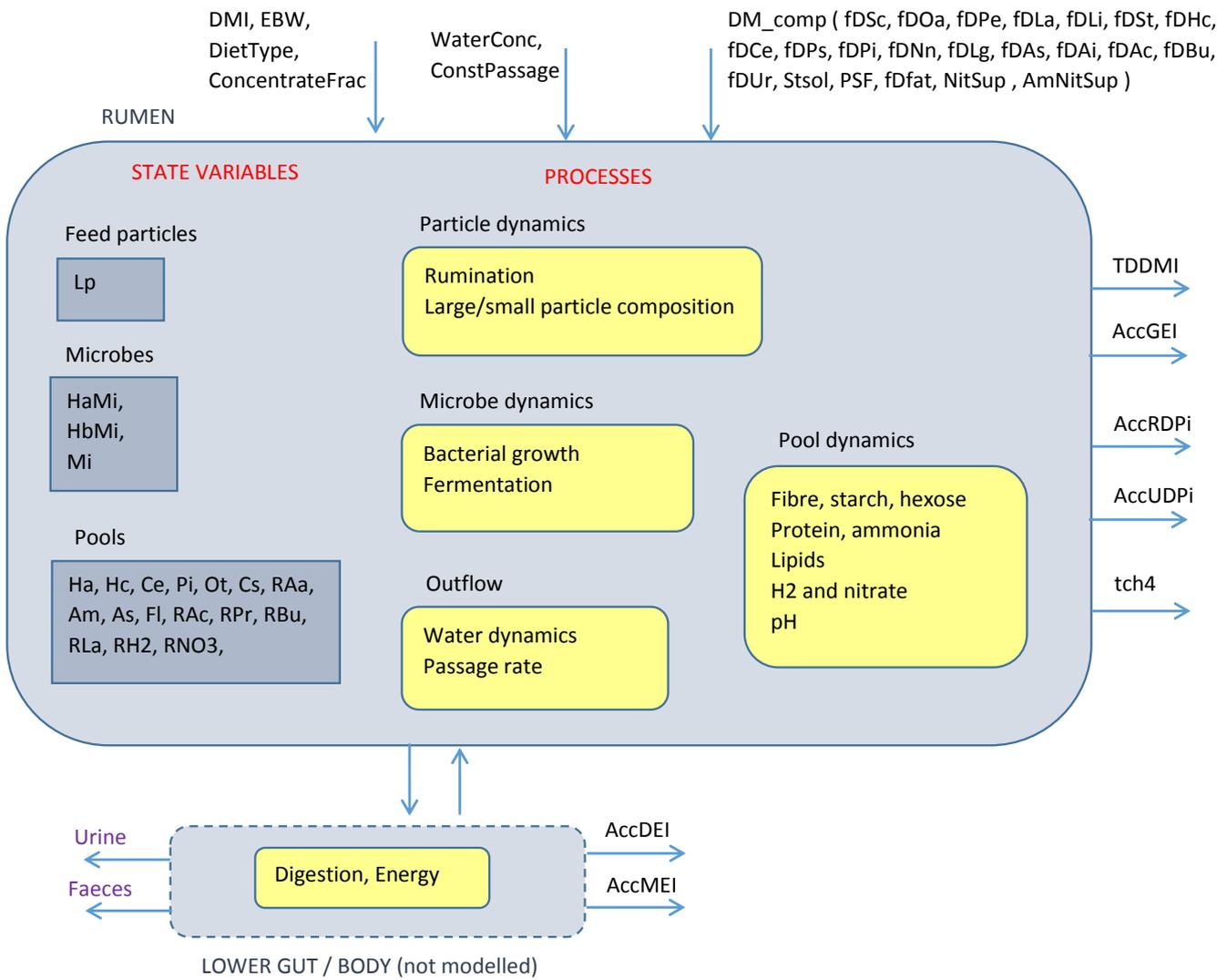


Figure 2.3.1. NZ Molly Rumen Model V1.5 Structure. Definitions in Table 2.3.1

Table 2.3.1. Name and definition of terms used in Figure 2.3.1.

Name	Definition
fDSc	soluble carbohydrate
fDOa	organic acids
fDPe	pectin
fDLa	lactate
fDLi	lipid
fDSt	starch
fDHc	hemicellulose
fDCe	cellulose
fDPs	soluble protein
fDPi	insoluble protein
fDNn	non-protein nitrogen
fDLg	lignin
fDAs	soluble ash
fDAi	insoluble ash
fDAc	acetate
fDBu	butyrate
fDUr	urea
Stsol	soluble starch
PSF	proportion small particle in diet
fDfat	fDfat is additional to fat in diet, and was specified in a procedure in Molly
NitSup*	nitrate salt supplement excluding ammonium nitrate (kg NO ₃ /kg DM)
AmNitSup*	ammonium nitrate sup (kg NO ₃ /kg DM)
Lp	Large particles in Rumen (kg)
HaMi	microbes associated with alpha-hexose (starch) (kg)
HbMi	microbes associated with hemicellulose (kg)
Ha	alpha-hexose (starch) in rumen (kg)
Hc	hemicellulose in feed (kg)
Ce	Cellulose (kg)
Pi	insoluble protein (kg)
Ot	lignin and insoluble ash (kg)
Cs	soluble carbohydrate in rumen
RAa	amino acids in rumen
Am	ammonia (mol)
As	soluble ash (kg)
FI	unsaturated long-chain fatty acids (mol)
RAc	acetate in rumen (mol)
RPr	propionate in rumen (mol)
RBu	butyrate in rumen (mol)
RLa	lactate in rumen (mol)
Mi	microorganism (kg)
RH2	rumen H ₂ (moles) -
RNO ₃ *	rumen nitrate pool (which implicitly contains nitrites) to interact with RH ₂
TDDMI	Total DM intake kg/day
AccGEI	Total gross energy intake over a simulation (MJ)
AccDEI	Total digestible energy intake over a simulation (MJ)

AccMEI	Total metabolisable energy intake over a simulation (MJ)
tch4	total (accumulated) methane over a simulation (mol)
AccRDPi*	Total Rumen degradable protein exiting rumen (kg) over a simulation.
AccUDPi*	Total Rumen undegraded protein exiting (kg) over a simulation .

* Parameters/variables introduced in MollyRum15.

Nitrate and lipid models for cattle

Nitrate model. The metabolism pathway of nitrate to ammonia was viewed as shown in Figure 2.3.2. The values to develop the models have been estimated from the literature (Lewis 1951; Wang et al., 1961; Ishigami and Inque, 1976). Excel was used to develop up the modified model.

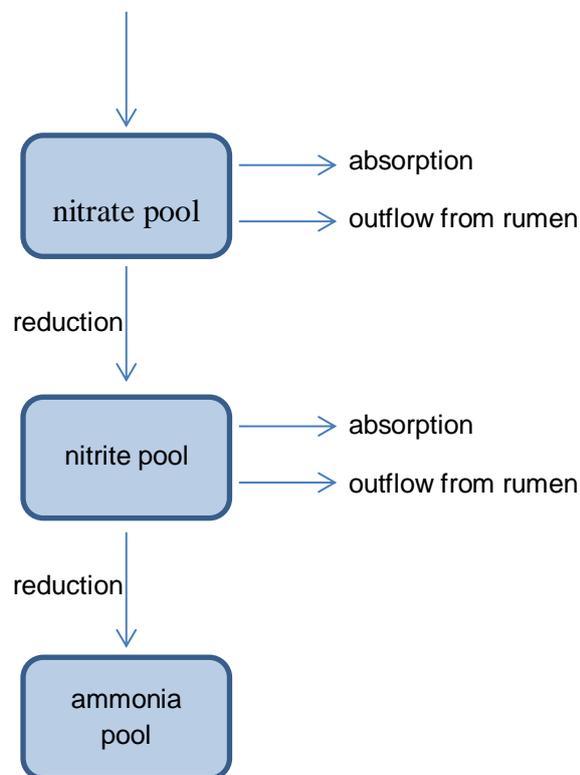


Figure 2.3.2. Schematic of nitrate to ammonia pool

Lipid model. Bio-hydrogenation was assumed to occur at a rate which is a linear function of the pool size of unsaturated fat in the rumen. The change in H₂ which gets consumed, occurs by adding to the rate of change of the saturated fat pool, and subtracting from the rate of change of the unsaturated fat pool. The fractional rate of bio-hydrogenation of unsaturated fats (*f_{biohydr}*) was determined by running a near steady-state simulation and aiming for an overall value of 85% of the unsaturated fat becoming saturated corresponding to a rate of 20% per hour. Excel was used to develop up the modified model.

2.4 Experimental study on nitrate and canola oil in cattle

This was the fourth animal study, following previous studies of mitigation by nitrate (de Raphelis 2016), defaunation (Nguyen et al., 2016) and NO₃ and defaunation together (Nguyen et al., 2015). The experiment was conducted between October 2015 and February 2016. Animals were handled in accordance with the University of New England Animal Ethics Committee. Four mature crossbreed cannulated steers (713 ± 20.5 kg liveweight) were used in a Latin square with 4 diet treatments offered over 4 periods and each steer was fed one of the four dietary treatments in each period.

Animals were housed individually in pens equipped with a feeder and water and were offered 7.5 kg of their experimental diet in 2 equal feeds/d. The basal diet was a blended chaff mixture (40% lucerne chaff; 60% rolled barley grain) fed alone (control; CON) or with inclusion of 2% nitrate (NO₃; provided as 3.14% calcium nitrate, 5Ca(NO₃)₂.NH₄NO₃.10H₂O, Bolifor CNF, Yara, Oslo, Norway). The third treatment (OIL) consisted of 5% canola oil inclusion in the chaff and the final treatment (NO₃-OIL) contained 2%NO₃ and 5% oil in combination, with all inclusions expressed as g/100g as fed.

Filtered rumen fluid samples (14 per animal) were collected via the cannula for volatile fatty acid (VFA) determination after 14d of diet adaption. Each ten ml sample was taken and acidified with 0.3 mL (18M) H₂SO₄ and then frozen at -20°C. Volatile fatty acid concentrations were determined by gas chromatography using a Varian CP-8400 auto-sampler. Methane production (24 h) was measured by open circuit respiration chambers approximately after 13 d (except period 1) and again after 19 d of diet adaption, with data averaged for the 2 measures per animal per period. Digesta markers (Cr₂O₃, Yb-acetate and CoCl₃) were either fed or infused-intraruminally. Enriched Ammonium Chloride (¹⁵N-NH₄Cl) was also infused intraruminally to enable estimation of microbial protein outflow in association with sampling of reticular digesta and bacterial cells for ¹⁵N enrichment.

2.5 Case studies

Sheep production

Data

Holbrook wethers. In this scenario Merino wethers were purchased on 17th April each year at an age of 6 months with live weight 25 kg, CS 1.5 and stock were sold at 2 to 3 years of age on the 16th April. The simulation was modelled for a site in Holbrook (NSW, Australia) situated at Latitude 35°42'S and Longitude 147°18'E. A single 1000 ha paddock was simulated comprising red duplex soil type with Phalaris, Subterranean Clover (Seaton Park) and Annual Ryegrass pasture (Table 2.5.1a). A stocking rate of 8.2 wethers / ha was established. A lupins supplement (Tables 2.5.2 to 2.5.4) of 40 g/head/day was fed between 1st March and 15th May. No production feeding rules were set. Additional detail to run the simulation can be found in the appendices (Tables A.1 to A.3).

Holbrook ewes. In this scenario a self-replacing flock of medium Merino ewes were simulated where weaner ewes were added to the main flock on 1st Jan each year to maintain the stocking rate and cast for age stock were sold at 5 to 6 years of age on the 31st Dec. The single 1000 ha paddock was simulated, detail of site and pasture, as mentioned above. A stocking rate of 5.3 ewes/ha was established. A lupins supplement (Tables 2 to 4) of 50 g/head/day was fed between 15th Jan and 1st June. No production feeding rules were set. Additional detail to run the simulation can be found in the appendices (Tables A.1 to A.3, A.5 and A.6).

New Zealand ewes. In this scenario Suffolk ewes were purchased on 1st January at an age of 7 months with live weight 38kg and condition score 3 and stock were sold aged 6 to 7 years on 15th Jan. Note: this simulation was not a self-replacing simulation. Mating was on the 8th March with Dorset rams with a mature weight of 77 kg with a mating rate of 1 ram per 50 ewes. The simulation was modelled for a site in Whykikapaki New Zealand situated at Latitude 39°50'S and Longitude 176°38'E. A single 570 ha paddock was simulated comprising Calcareous Orthic Melanic soil with perennial ryegrass, annual ryegrass and white clover pasture (Tables 2.5.1a and 2.5.1b). A stocking rate of 10 ewes/ha was established. A rye grass supplement (Tables 2.5.2 to 2.5.4) of 73g/head/day was fed between 1st May and 31st July. No production feeding rules were set. Additional detail to run the simulation can be found in the appendices (Tables A.11 to A.15).

Nitrate and lipid treatments

For the nitrate treatment, a supplement was used to provide 2% nitrate on a dry-matter basis.

For the lipid treatment a supplement of canola seed (Tables 2.5.2 to 2.5.4) was used to raise the lipid component of the feed available to 5% on a dry-matter basis.

For the nitrate and lipid interaction treatment amounts as described above were used.

Table 2.5.1a. Feed evaluation of average (Avg) leaf and stem for phalaris, annual ryegrass, and subterranean clover of pasture availability for a site in Holbrook NSW, Australia (Latitude 35°42'S and Longitude 147°18'E). Data are expressed as proportions.

Component	Phalaris		Annual Ryegrass		Sub Clover - Seaton Park	
	Avg. Leaf	Avg. stem	Avg. leaf	Avg. stem	Avg. leaf	Avg. stem
solubleAsh	0.0589	0.0569	0.0376	0.0423	0.0825	0.0664
Starch	0.0354	0.0297	0.0277	0.0249	0.1059	0.0784
totalCHO	0.0988	0.0918	0.0642	0.0852	0.1685	0.1464
ADF	0.2803	0.3745	0.4073	0.4688	0.1969	0.3655
solubleCHO	0.0634	0.0621	0.0365	0.0603	0.0626	0.0680
Ash	0.0600	0.1017	0.0900	0.0700	0.0992	0.0927
Protein	0.1769	0.0881	0.1556	0.0594	0.3613	0.1975
ADL	0.0681	0.0900	0.0684	0.0900	0.0614	0.1200
Lipids	0.0222	0.0084	0.0202	0.0118	0.0324	0.0224
NDF	0.4439	0.6300	0.5893	0.7174	0.2176	0.3989

Table 2.5.1b. Feed evaluation of average (Avg) leaf and stem for phalaris, annual ryegrass, and subterranean clover of pasture availability for a site in New Zealand. Data are expressed as proportions.

Component	Perennial Ryegrass		White clover	
	Avg. leaf	Avg. stem	Avg. leaf	Avg. stem
solubleAsh	0.0687	0.0617	0.0825	0.0664
Starch	0.0298	0.0179	0.1059	0.0784
totalCHO	0.2204	0.1620	0.1685	0.1464
ADF	0.2499	0.3343	0.1969	0.3655
solubleCHO	0.1325	0.0861	0.0626	0.0680
Ash	0.1185	0.1056	0.0992	0.0927
Protein	0.2259	0.1912	0.3613	0.1975
ADL	0.0556	0.0724	0.0614	0.0900
Lipids	0.0408	0.0397	0.0324	0.0224
NDF	0.3943	0.5015	0.2176	0.3989

Table 2.5.2. Average feed evaluation values from AusBeef database for Lupins, Ryegrass Hay, and Canola seed. Data are expressed as proportions.

	Lupins	Ryegrass Hay	Canola seed
solubleAsh	0.0230	0.0537	0.0228
NDIN	NA	0.0145	0.0653
starch	0.0081	0.0179	0.0701
totalCHO	0.0713	0.1335	0.1548
ADF	0.2032	0.3140	0.2747
moisture	0.0905	0.1600	0.0665
ADIN	0.0034	0.0063	0.0171
SolubleCHO	0.0632	0.0861	0.0847
Ash	0.0302	0.0920	0.0394
Protein	0.3419	0.1600	0.3031
ADL	0.0099	0.0390	0.1518
Lipids	0.0775	0.0330	0.3406
NDF	0.2500	0.5500	0.3074

Table 2.5.3. Average feed evaluation values of amino acids (AA) from AusBeef database for Lupins and Canola seed. Data are expressed as proportions.

AA	Lupins	Ryegrass Hay	Canola seed
lys	0.0444	0.0485	0.0319
ile	0.0366	0.0396	0.0362
ser	0.0569	-	0.0507
his	0.0245	0.0194	0.0282
phe	0.0364	0.0478	0.0486
val	0.0355	0.0522	0.0509
cys	0.0114	0.0117	0.0222
tyr	0.0373	-	0.0355
pro	0.0388	-	0.0865
arg	0.1070	0.0410	0.0529
thr	0.0357	0.0410	0.0356
trp	0.0080	0.0209	0.0108
asx	0.1057	-	0.0671
glx	0.2734	-	0.2336
gly	0.0399	-	0.0422
leu	0.0696	0.0739	0.0916
met	0.0060	0.0164	0.0176
ala	0.0329	-	0.0578

Table 2.5.4. Average feed evaluation values of fatty acids from AusBeef database and literature for Lupins and Canola oil. Data are expressed as proportions.

Common name	Series	Lupins	Ryegrass Hay	Canola seed
Vaccenic	ω 7 18:1	0.0084	0.0000	0.0086
Eicosadienoic	ω 3 20:5	0.0033	0.0000	0.0000
Palmitoleic	ω 7 16:1	0.0029	0.0020	0.0009
Lignoceric	24:0	0.0040	0.0000	0.0005
Elaidic	ω 9 18:1,9	0.0000	0.0000	0.0003
Cis-11-eicosenoic	ω 9 20:1	0.0155	0.0000	0.0062
Linoleic	ω 6 18:2,9,12	0.3315	0.1080	0.5007
Sterols		0.0081	-	0.0057
Oleic	ω 9 18:1,9	0.2601	0.0310	0.2612
Pentacosandic	25:0	0.0000	0.0000	0.0004
Heptadecenoic	17:0	0.0110	0.0000	0.0000
Tricosanoic	23:0	0.0000	0.0000	0.0025
Docostetraenoic	ω 6 22:4	0.0018	0.0000	0.0010
Arachidic	20:0	0.0067	0.0050	0.0017
Stearic	18:0	0.1702	0.0260	0.0150
Linolenic	ω 6 gamma 18:3,6,9,12	0.0590	0.6010	0.0308
Erucic	ω 9 22:1,13	0.0224	0.0000	0.0000
Behenic	22:0	0.0201	0.0000	0.0021
Nonadecanoic	19:0	0.0000	0.0000	0.0190
Palmitic	16:0	0.1049	0.1960	0.1527

AusFarm

AusFarm is software that simulates both physical and biological systems to assist decision-making in agricultural enterprises ranging from paddock to whole landscapes. (AusFarm User Notes #1 Integrating APSIM Soil and Crop Models into AusFarm. Moore, May 2012). AusFarm has been successfully used to model mixed farming enterprises (Herrmann et al., 2015) based largely on the software design that enables users to integrate the GrazPlan animal model (Freer et al., 1997) and the APSIM cropping models (Hozworth et al., 2014). The NZ sheep rumen model developed by Vetharaniam et al. (2015) in MatLab was converted to C++ code and developed into a DLL so that AusFarm can call the NZ sheep rumen model. Modifications to the model to handle NO₃ and lipid supplementation are reported in 3.2. Tables A.1 to A.3, and A.5, A.6, and A.11 to A.15 of the appendix provide additional detail to run the simulation.

Calculation of VFA concentrations

Estimates of VFA concentrations from the sheep rumen were not used but empirical relationships of VFA concentrations *versus* DMI intake were developed using data from Vetharaniam et al. (2015) and unpublished data Oddy et al. (funded by Australian Government Department of Agriculture and Water Resources project 1194003-43 and MLA project B.CHH.2071) (Figures 2.5.1 to 2.5.3.).

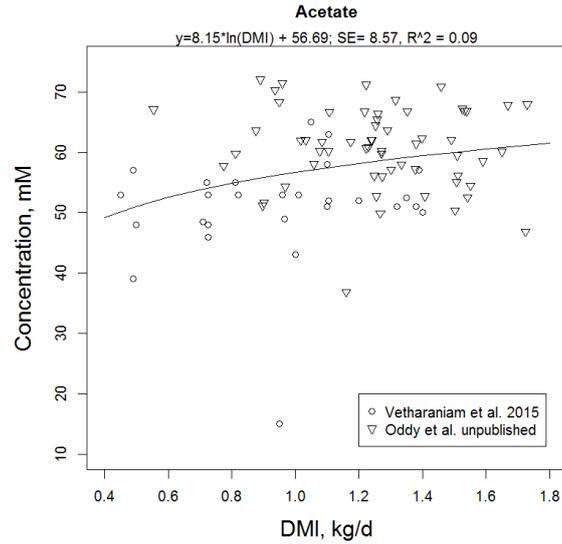


Figure 2.5.1. Relationship of the concentration of acetate (µM) to dry matter intake (DMI, kg/d) based on data from Vetharanim et al. (2015) and unpublished data Oddy et al. (funded by Australian Government Department of Agriculture and Water Resources project 1194003-43 and MLA project B.CHH.2071)

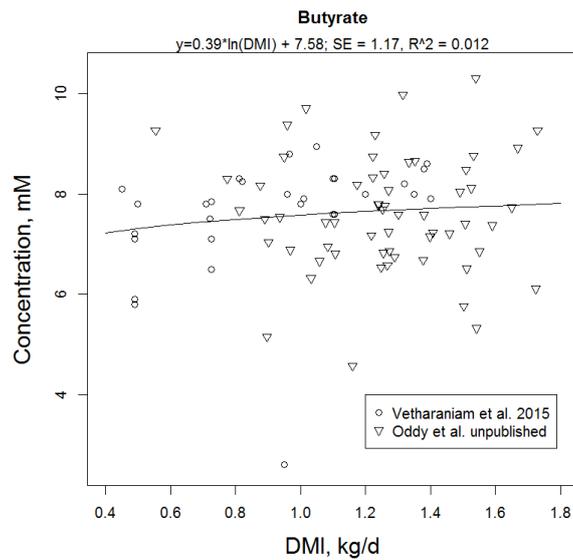


Figure 2.5.2. Relationship of the concentration of Butyrate (µM) to dry matter intake (DMI, kg/d) based on data from Vetharanim et al. (2015) and unpublished data Oddy et al. (funded by Australian Government Department of Agriculture and Water Resources project 1194003-43 and MLA project B.CHH.2071)

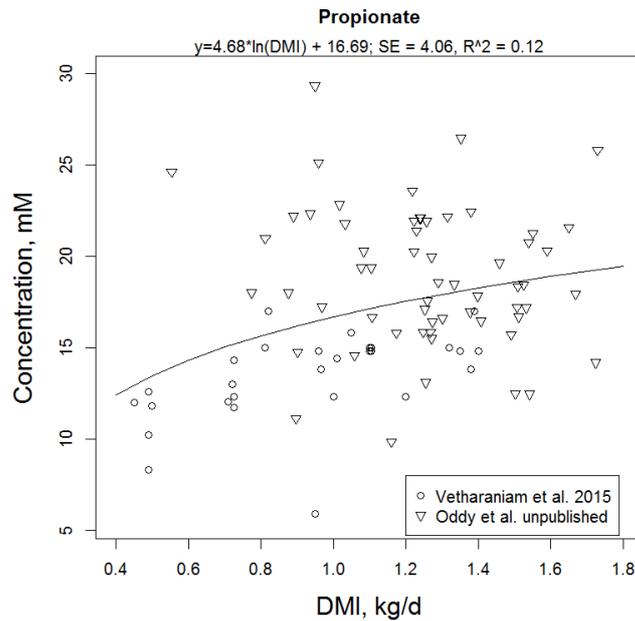


Figure 2.5.3. Relationship of the concentration of Propionate (μM) to dry matter intake (DMI, kg/d) based on data from Vetharanim et al. (2015) and unpublished data Oddy et al. (funded by Australian Government Department of Agriculture and Water Resources project 1194003-43 and MLA project B.CHH.2071)

Beef production

Data

Holbrook steers

In this scenario Angus steers weighing 230 kg at 9 months of age were purchased on May 1 each year and sold eight months later on 31 December. Results were modelled for a site in Holbrook (NSW, Australia) situated at Latitude $35^{\circ}43'S$ and Longitude $147^{\circ}19'E$. A single 1000 ha paddock was assessed comprising red duplex soil type with a pasture base of: Phalaris, Subterranean Clover (Seaton Park) and Annual Ryegrass (Table 2.5.5a and 2.2.5b). A stocking rate of 1.5 steers / ha was used. Tables A.1 to A.4 of the appendix provide additional detail to run the simulation.

University of California Sierra steers

In this scenario British x Charolais steers weighing 290 kg at 12 months of age were purchased on 10th December each year and sold the following year 14th May. Results were modelled for a site on the University of California Sierra Research Station situated at Latitude $39^{\circ}00'N$ and Longitude $123^{\circ}06'W$. A single 45 ha paddock was simulated with a pasture base of: Annual Grass, Sub Clover – Mt Barker, Annual Ryegrass and Perennial Ryegrass (Table 2.5.5a and 2.2.5b). A stocking rate of 1 steer / ha was used. Tables A.7 to A.10 of the appendix provide additional detail to run the simulation.

Table 2.5.5a. Feed evaluation of average (Avg) leaf and stem for phalaris, annual and perennial ryegrass, and subterranean clover (Seaton Park) of pasture availability. Data are expressed as proportions.

	Phalaris		Annual & Perennial Ryegrass		Sub Clover - Seaton Park	
	Avg. Leaf	Avg. stem	Avg. leaf	Avg. stem	Avg. leaf	Avg. stem
<i>Component</i>						
Soluble Ash	0.0589	0.0569	0.0376	0.0423	0.0825	0.0664
Starch	0.0354	0.0297	0.0277	0.0249	0.1059	0.0784
Total CHO	0.0988	0.0918	0.0642	0.0852	0.1685	0.1464
ADF	0.2803	0.3745	0.4073	0.4688	0.1969	0.3655
SolubleCHO	0.0634	0.0621	0.0365	0.0603	0.0626	0.0680
Ash	0.0600	0.1017	0.0900	0.0700	0.0992	0.0927
Protein	0.1769	0.0881	0.1556	0.0594	0.3613	0.1975
ADL	0.0681	0.0900	0.0684	0.0900	0.0614	0.1200
Lipids	0.0222	0.0084	0.0202	0.0118	0.0324	0.0224
NDF	0.4439	0.6300	0.5893	0.7174	0.2176	0.3989

Table 2.5.5b. Feed evaluation of average (Avg) leaf and stem for subterranean clover of pasture. Data are expressed as proportions.

	Sub Clover Mt Barker	
	Avg. Leaf	Avg. stem
<i>Component</i>		
Soluble Ash	0.0825	0.0664
Starch	0.1059	0.0784
Total CHO	0.1685	0.1464
ADF	0.1969	0.3655
SolubleCHO	0.0626	0.0680
Ash	0.0992	0.0927
Protein	0.3613	0.1975
ADL	0.0614	0.1889
Lipids	0.0324	0.0224
NDF	0.2176	0.3989

Because observed pasture quantity and quality values were not available, code was written in AusFarm to adjust the digestibility values to calibrate the simulated liveweight (LW) gain against the observed average LW gain (kg/day). The results of this calibration and an evaluation against subsequent growth rates are illustrated in Figure 2.5.1. Tables A.7 to A.10 of the appendix provide additional detail to run the simulation.

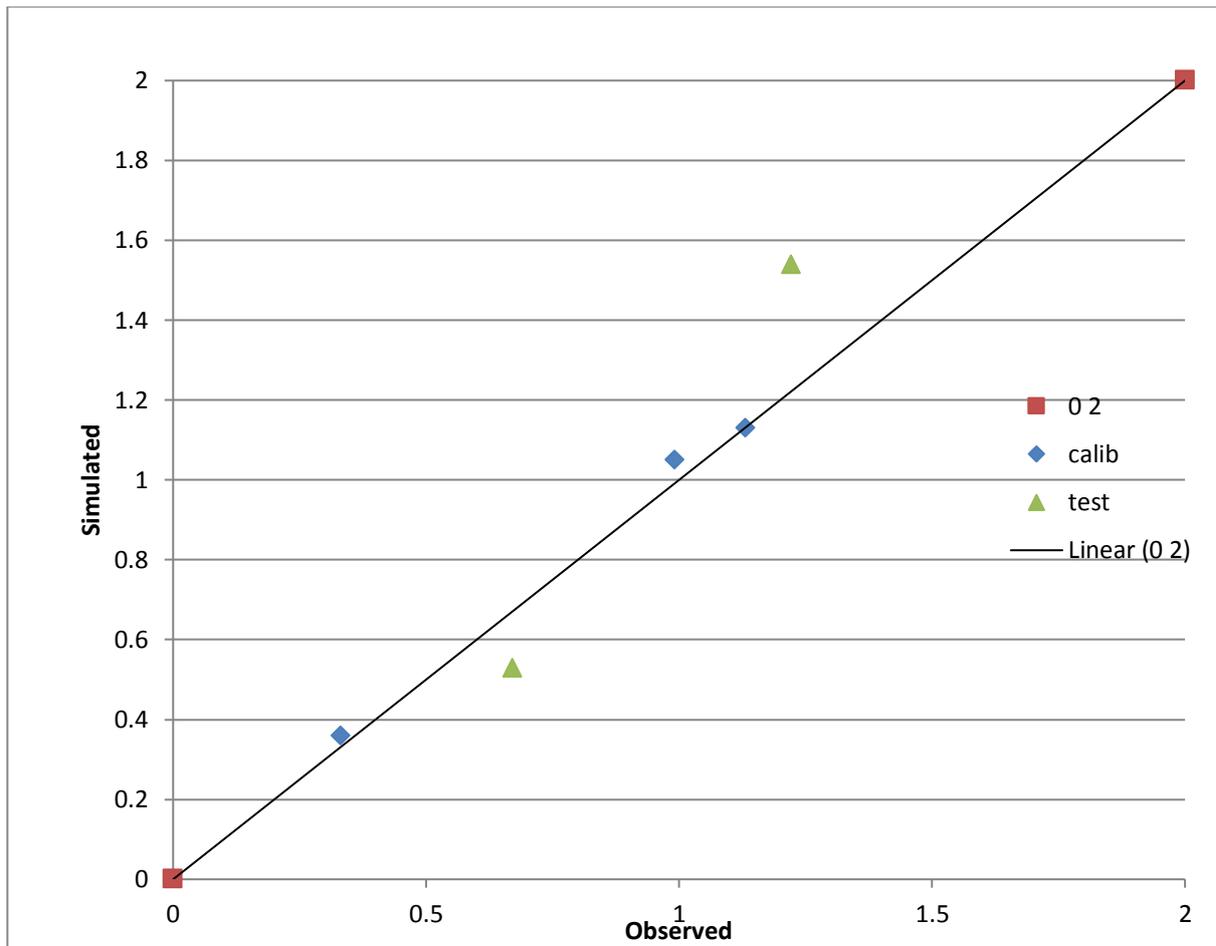


Figure 1. Relationship between observed and simulated live weight gain (kg/d) for a calibrated (calib) and test data set, solid line is a 1:1 relationship

Supplements

For the lipid treatment, a supplement of canola seed (Tables 2.5.6 to 2.5.8) was used to raise the lipid component to 5% on a dry-matter basis.

For the nitrate treatment, a supplement was used to provide 2% nitrate on a dry-matter basis. Note: 2% nitrate was coded into AusFarm so that 2% nitrate was distributed evenly over the grazing period.

Table 2.5.6. Average feed evaluation values from AusBeef database for Canola seed

Canola seed	
solubleAsh	0.0228
NDIN	0.0653
starch	0.0701
totalCHO	0.1548
ADF	0.2747
moisture	0.0665
ADIN	0.0171
solubleCHO	0.0847
Ash	0.0394
Protein	0.3031
ADL	0.1518
Lipids	0.3406
NDF	0.3074

Table 2.5.7. Average feed evaluation values of amino acids from AusBeef database for Canola seed

Canola seed	
lys	0.0319
ile	0.0362
ser	0.0507
his	0.0282
phe	0.0486
val	0.0509
cys	0.0222
tyr	0.0355
pro	0.0865
arg	0.0529
thr	0.0356
trp	0.0108
asx	0.0671
glx	0.2336
gly	0.0422
leu	0.0916
met	0.0176
ala	0.0578

Table 2.5.8. Average feed evaluation values of fatty acids (LCFA) from AusBeef database and literature for Canola seed

Common name	Series	Canola seed
Vaccenic	ω 7 18:1	0.0086
Eicosadienoic	ω 3 20:5	0.0000
Palmitoleic	ω 7 16:1	0.0009
Lignoceric	24:0	0.0005
Elaidic	ω 9 18:1,9	0.0003
Cis-11-eicosenoic	ω 9 20:1	0.0062
Linoleic	ω 6 18:2,9,12	0.5007
Sterols		0.0057
Oleic	ω 9 18:1,9	0.2612
Pentacosandic	25:0	0.0004
Heptadecenoic	17:0	0.0000
Tricosanoic	23:0	0.0025
Docostetraenoic	ω 6 22:4	0.0010
Arachidic	20:0	0.0017
Stearic	18:0	0.0150
Linolenic	ω 6 gamma 18:3,6,9,12	0.0308
Erucic	ω 9 22:1,13	0.0000
Behenic	22:0	0.0021
Nonadecanoic	19:0	0.0190
Palmitic	16:0	0.1527

AusFarm

As previously described, AusFarm is computer software that simulates both physical and biological systems to assist decision-making in agricultural enterprises ranging from paddock to whole landscapes. (AusFarm User Notes #1 Integrating APSIM Soil and Crop Models into AusFarm. Moore, May 2012). AusFarm has been successfully used to model mixed farming enterprises (Herrmann et al., 2015) based largely on the software design that enables users to integrate the GrazPlan animal model (Freer et al., 1997) and the APSIM cropping models (Hozworth et al., 2014). AusBeef (Nagorcka et al., 2000) originally written in VisSim has been converted to C++ code and the rumen model within AusBeef has been developed into a DLL module so that AusFarm, integrated with the DLL, models rumen function and provides the user to report on the mechanistic functions of the rumen e.g., VFAs and microbial protein output and the calculation of CH₄ using a mechanistic approach. The AusBeef rumen model has been enhanced to include the modelling of nitrate and lipid supplementation. Tables A.1 to A.4, and A.7 to A.10 of the appendix provide additional detail to run the simulation.

Statistical analysis of case studies

The R (R Development Core Team, 2013) statistical package was used to determine the least squares (LSM) means, and a 'pair-wise' comparison of *P*-values using the Tukey method.

Greenhouse gas calculation in case studies

The 'total greenhouse gas' values shown for the case studies are calculated as the sum of the enteric methane emissions and the nitrous oxide emissions, expressed as equivalent tonnes of CO₂ per hectare per year.

$$\text{GHG} = (\text{avg_animal_CH}_4\text{_g_per_day} \times 25 + \text{avg_animal_N}_2\text{O_g_per_day} \times 310) \times 10^{-6}$$

$$\times \text{stocking_rate}$$

$$\times \text{days_per_year_on_paddock}$$

Methane emissions are as generated by the AusBeef and NZ Molly rumen models while nitrous oxide emissions are calculated using equations from the Australian National Greenhouse Gas Inventory method as implemented in the GHG calculation tools of the Primary Industries Climate Challenges Centre.

Both the AusBeef and the NZ Molly models produce an estimate of urine N content and faecal N content. These values are used to calculate the nitrous oxide from excreta and from atmospheric deposition. In accordance with the PICCC spreadsheets, nitrous oxide from leaching and runoff is not included since the value of:

$$\text{annual_evapotranspiration_mm} / \text{annual_precipitation_mm}$$

is greater than 0.8 for all of the case studies.

3. RESULTS

Results are presented against each activity undertaken to deliver each of the required outcomes:

3.1 Activity 1. In depth assessment of current AusBeef rumen model

The main results from this activity were the development of flow charts, description of the rumen model and studies undertaken to assist in improving our understanding of microbial thermodynamics and of ruminal hydrogen flow in livestock on conventional diets and those subject to mitigation. The following sections provide the results obtained:

3.1.1 *Flow chart of existing AusBeef inputs, outputs; detailed description of models and parameter values.*

Two hundred and seventy-seven flow charts (e.g., Figure 3.1.1) of AusBeef as defined in the VisSim environment were developed. Thirty-nine interconnected modules with 230 sub-modules, each representing a different biological function were identified. The rumen is one of the 39 interconnected modules that include 147 sub-modules (e.g., the methane sub-module). A total of 5,310 variables were identified and a biological interpretation of each variable was conducted and collated into a table. For example a schematic representation of the AusBeef rumen is shown in Figure 3.1.2 and Table 3.1.1 provides a description of the state variables used in Figure 3.1.2.

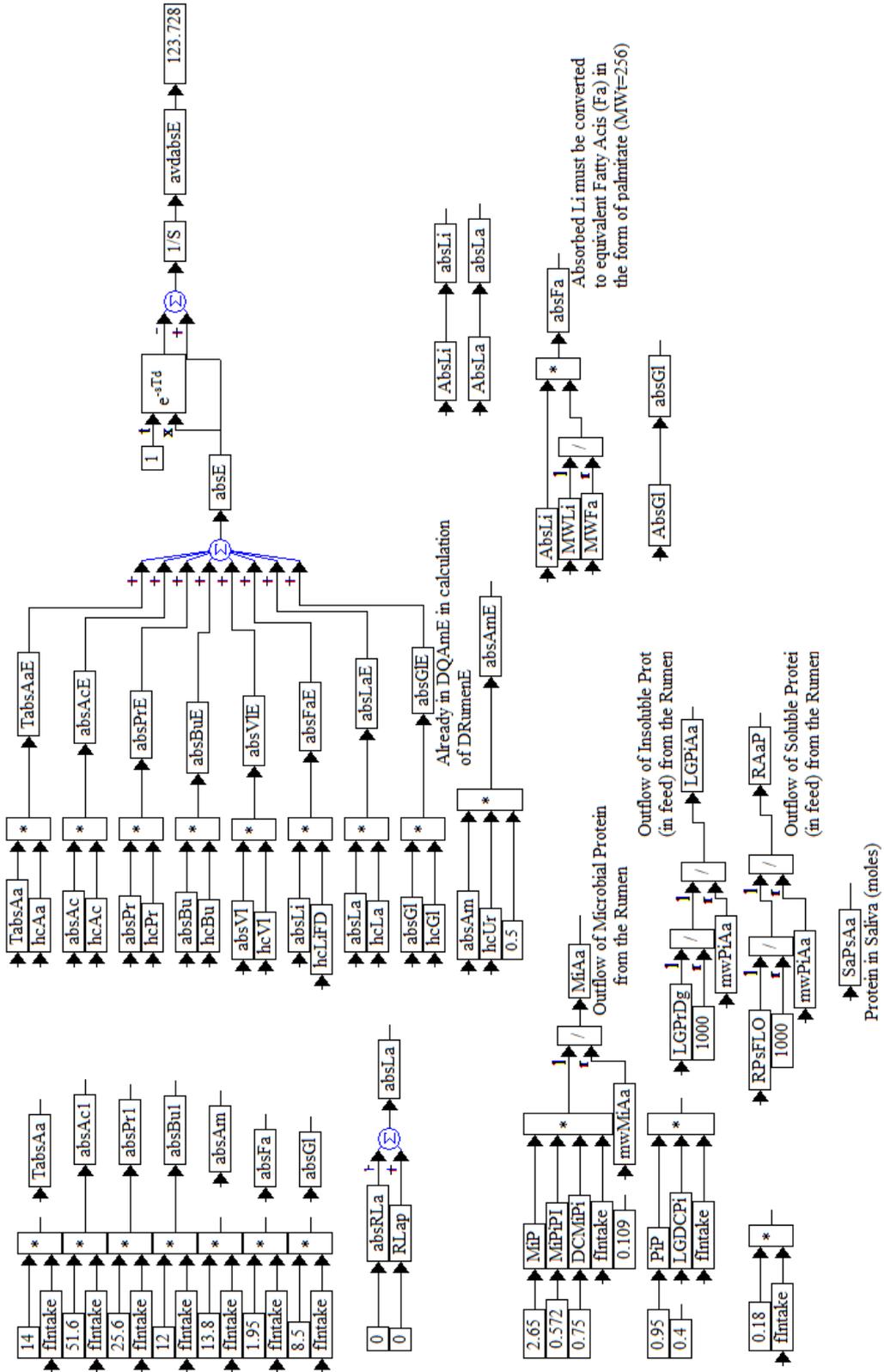


Figure 3.1.1. Absorbed nutrients of lower gut

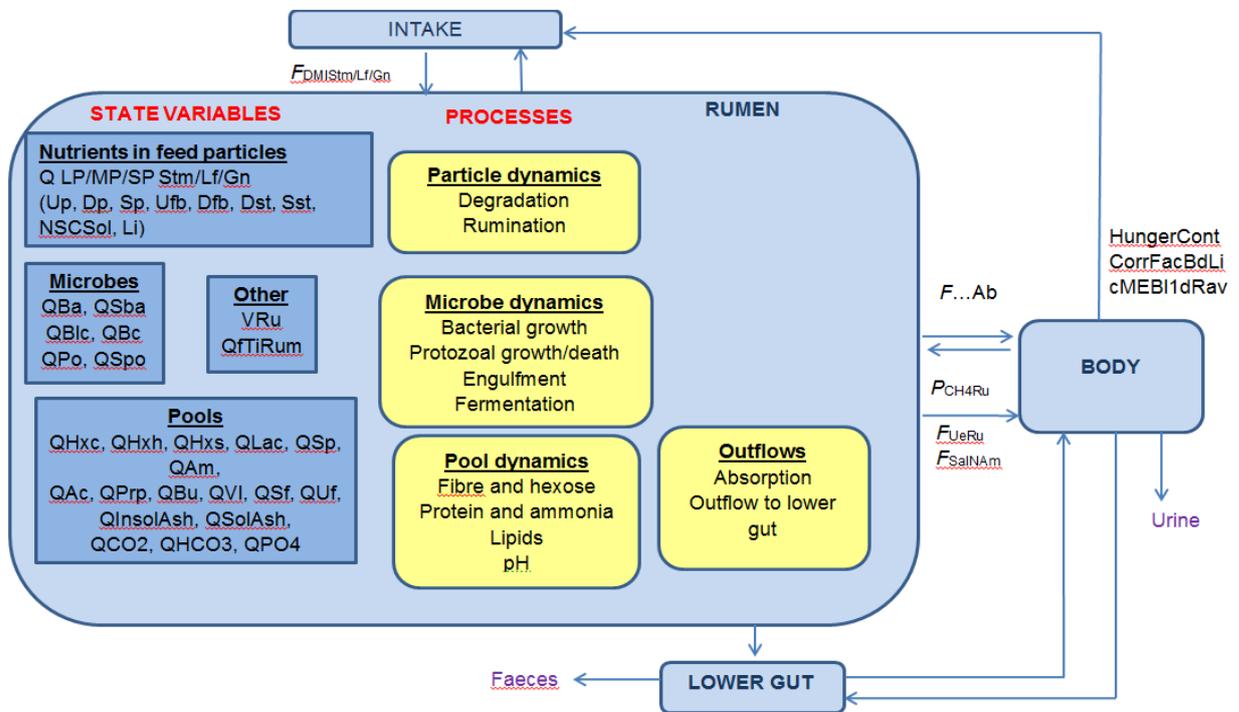


Figure 3.1.2. A schematic representation of the AusBeef rumen model. Further explanation is provided in Table 3.1.1.

Table 3.1.1. Explanation of terms in Figure 3.1.2.

Model Term	Description	Units
Q	Quantity	dimensionless
LP/MP/SP	Large/medium/small feed particles	grams/day
Stm/Lf/Gn	Stem/leaf/grain feed components	grams/day
Up/Dp/Sp	Undegradable/Degradable/Soluble protein	moles
Ufb/Dfb	Undegradable/Degradable fibre	moles
Dst/Sst	Degradable/Soluble starch	moles
NSCSol	Soluble non-starch carbohydrates	moles
Li	Lipids	moles
Ba/Sba	Amylolytic bacteria/storage polysaccharide	moles/grams
Blc	Lactolytic bacteria	moles
Po/Spo	Protozoa/storage polysaccharide	moles/grams
VRu	Rumen volume	liters
QfTiRu	Time spent ruminating	minutes
Hxc/Hxh/Hxs	Hexose from cellulose/hemicellulose/starch	moles
Lac	Lactate	moles
Am	Ammonia	moles
Prp	Propionate	moles
Ac	Acetate	moles
VI	Valerate	moles
Bu	Butyrate	moles
Sf/Uf	Saturated/Unsaturated lipid	moles
InsolAsh/SolAsh	Insoluble/Soluble Ash	grams
F...Ab	Absorption across the rumen wall	moles/day
F _{UeRu}	Flow of urea into the rumen	moles/day
F _{SalNA}	Rate of salivary nitrogen conversion to ammonia	moles/day
F _{DMIStm/Lf/Gn}	Dry matter intake of feed particles	grams/day
HungerCont/ CorrFacBdLi/ cMeBI1dRav	Energetic terms feeding into voluntary intake	dimensionless; dimensionless; MJ/kg live weight
P _{CH4Ru}	Production of CH ₄ in the rumen	moles/day

3.1.2 *Comprehensive understanding of microbial thermodynamics and of ruminal hydrogen flow in livestock on conventional diets and those subject to mitigation.*

Learnings from 3 studies are summarised here:

- (1) Effects of removing protozoa from the rumen as a means to reduce enteric methane production, while not implemented in AusBeef were studied in experiments with lambs, mature sheep and cattle (Nguyen et al., 2015, 2016a, 2016b). These effects on both rumen fermentation and animal production are summarised below and tabulated together with the findings of several reviews or meta-analyses.

Table 3.1.2.1 Rumen metabolite concentration and methane production in the rumen fluid of defaunated animals normalized relative to those in faunated animals (1.00)

	Total VFA	Acetate	Propionate	Butyrate	Acetate/Propionate	NH ₃	Methane production
Grazing sheep	1.04	1.08*	0.89*	0.76*	1.21	0.74*	0.93*
Hoggets pen fed	1.00	1.08	1.13	0.71*	0.96	0.63*	0.98
Lambs pen fed	0.84*	1.05*	0.85*	0.93	1.23*	0.66*	0.57*
Cattle in-vitro [‡]	0.79	1.02	1.04	0.84	0.98	0.76*	0.62*
Cattle	1.16	1.01	1.10	0.89	0.91	0.61*	0.90*
Thesis mean	0.97	1.05	1.00	0.83	1.06	0.68	0.80
<i>Published review</i>							
Jouany <i>et al.</i> (1988)	0.94	0.98	1.32	0.94	0.90	0.76	
Hegarty (1999)		0.86	1.23	1.08	0.70		0.87
Eugène <i>et al.</i> (2004a)	0.96	0.98	1.14	0.87	0.86	0.70	
Newbold <i>et al.</i> (2015)	0.95	1.03	1.00	0.78	1.03	0.74	0.89

(2) * Significant effect of defaunation ($P < 0.05$); † Experiment conducted under grazing environment; ‡ In vitro experiment.

The most consistent factor affecting hydrogen economy was the increased acetate proportion and increased acetate:propionate ratio but as found by Morgavi et al (2012), changes in propionate percentage were inconsistent. Regarding changes in nutrient fluxes, increased microbial protein flow was also consistent in response to defaunation (Table 3.1.2.2) while (surprisingly), wool production was not. This was unexpected as usually wool production reflects changes in protein supply to the intestine.

Table 3.1.2.2 Dry matter intake, digestibility, microbial protein outflow, and liveweight gain and wool growth of defaunated ruminants normalized relative to those in faunated ruminants (1.00)

	DM intake	DM digestibility	Microbial protein outflow	Liveweight gain	Wool production	Wool fibre diameter
Grazing sheep	0.98 [†]	0.97	1.17	1.04		
Hoggets pen fed				1.06	0.97	1.03
Lambs pen fed	0.94	0.94 [†]	1.03	1.11	0.95	1.01
Cattle in-vitro [‡]	0.98	1.03	1.29 [*]	1.56		
Thesis mean	0.97	0.98	1.16	1.19	0.94	1.02
Published review						
Jouany <i>et al.</i> 1988)	1.04	0.94	1.56	1.02		
Eugène <i>et al.</i> (2004a)	1.01	0.98	1.12	1.11	1.14	
Newbold <i>et al.</i> (2015)	0.98	0.96	1.30	1.09	1.01	

^{*} Significant effect of the defaunation ($P < 0.05$); [†] Experiment conducted under grazing environment

- (2) Dietary nitrate leads to a complex biology in the animal due to conversion of NO₂ to nitric oxide (NO) in the blood, and the known role of NO as a metabolic regulator. However, in the gut it has been shown to increase microbial crude protein outflow and the effect of increased acetate percentage was consistent in all studies (except the final 4x4 latin square), as was a reduction in daily methane production both in-vitro and in-vivo. Using both sheep and cattle digesta (de Raphelis *et al.*, 2016; Nguyen *et al.*, 2016a)
- (3) The effect of oil was evaluated in combination with NO₃ in both sheep and cattle studies and in both cases the reduction in emissions was greater than the sum of the mitigation caused by nitrate or oil alone.

3.2 Activity 2. Development of: (a) new parameter values; and (b) new model components

The main results from this activity were the development of enhanced rumen hydrogen and nutrient flow sub-models, development of a rumen fermentation model, rumen model code consistent with input/output code of AusFarm, and publications arising from this study. The following sections provide the results obtained:

3.2.1. *Development of enhanced rumen hydrogen and nutrient flow sub-models.*
and

3.2.2. *Develop a rumen fermentation model with new dual functionality for sheep and cattle. (Note: a variation to the contract was made to use the AgResearch sheep rumen model for all sheep simulations and interface this to AusFarm.)*

A combined report of both development of enhanced rumen hydrogen and nutrient flow sub-models (3.2.1) and the development of a rumen fermentation model (3.2.2) are reported for sheep and cattle rumen models in the following sections:

Sheep rumen model

A report by Kumar Vetharanim (Milestone report 5, 2.4, KPI 5.4) titled "Equations for nitrate and lipid supplementation and interfacing sheep rumen model with AusFarm" reports on the modification of a hydrogen sink equation developed by Vetharanim *et al.* (2015).

The following equations describe the modification to the hydrogen sink equation:

In the MollyRum14 version (Vetharaniem et al., 2015), a modified version of Molly (Baldwin et al., 1987a,b), Vetharaniem et al. (2015) introduced rumen hydrogen (H₂) as an explicit pool, (RH₂, in moles) with a governing differential equation (where DRH₂ is the time derivative of RH₂):

$$DRH_2 = DTHy (1 - k_1 * DMI/EBW^{0.75}) - KWAP * RH_2 - 4.0 * DTCH_4 \quad (1)$$

Where DTHy is the nett flux of hydrogen from several processes: fermentation of soluble carbohydrates and amino acids, hydrogen fixing during synthesis of microbial protein, bio-hydrogenation of unsaturated lipids, and production/consumption of hydrogen during the conversion of lactate to acetate and propionate. The factor $(1 - k_1 * DMI/EBW^{0.75})$ was introduced to account for effects such as hydrogen loss from belching, and changes in the rate of exchange with the head space and changes to actual fermentation stoichiometry with feeding level (MollyRum14, like Molly95 from which it was developed, does not have a dynamic approach to fermentation stoichiometries). The second term $(-KWAP * RH_2)$ represents the passage of hydrogen with water leaving the rumen, where KWAP is the rate of water passage (d⁻¹). The last term $(-4.0 * DTCH_4)$ represents hydrogen consumption due to production of methane, for which the following equation was developed (where DTCH₄ is the rate of methane production).

$$DTCH_4 = m_f * M_i * RH_2 / RLV \quad (2)$$

Here M_i is the microbial mass, RLV is rumen volume, and m_f is a rate constant which can be fitted to data.

The existence of the hydrogen pool as a dynamic variable facilitates modelling of nitrate supplementation in a dynamic way.

MollyRum15, the new version that includes nitrate and lipid models, introduced a rumen nitrate pool (RNO₃ in moles). Following Dr Hegarty's advice against including a nitrite pool, on the basis that it would be very difficult to measure in the rumen, it was assumed that nitrite will be reduced faster than nitrate, and hence will be a much smaller pool. Therefore we can lump the two together and model the two separate stages:

1. $NO_3^- + H_2 \rightarrow NO_2^- + H_2O$
2. $NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$

as the nett reaction:

3. $NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$

The following equation was used for the rate of this reaction:

$$NO3_NH4_rate = kNO3 * 1e6 * M_i * RNO3 * RH_2 / (RLV * RLV) \quad (3)$$

where kNO₃ is an adjustable parameter and 1e6 is simply a scaling factor. This equation assumes no pH affects and since Molly has an empirical estimation of pH, based on VFA concentrations, and does not account for H⁺ flux and buffering, the effect of this reaction on pH was ignored. A linear relationship between hydrogen concentration and the reaction rate was assumed although 4 moles of H₂ are consumed per mole of nitrate.

Equation (1) was then modified to account for this hydrogen sink, which will automatically reduce the hydrogen pool in the presence of nitrate and thus reduce the methane production predicted by Equation (2):

$$DRH_2 = DTHy (1 - k_1 * DMI/EBW^{0.75}) - 4.0 * DTCH_4 - KWAP * RH_2 - 4.0 * NO3_NH4_rate \quad (4)$$

MollyRum15 makes a distinction between supplementation with ammonium nitrate and with other forms of nitrate. Ammonium nitrate supplied is represented in the model by the input AmNitSup and other forms of nitrate by NitSup, both specified in terms of kg NO₃ supplied per kg of feed.

The AgResearch sheep rumen model, MollyRum14 has been modified to model the effect of nitrate and lipid supplementation on methane production. The rumen model was adapted so that it could be coupled to the animal growth model in GRAZPLAN. A report by Kumar Vetharaniem (Milestone 5: report 2.4, KPI

5.4) titled “Equations for nitrate and lipid supplementation and interfacing sheep rumen model with AusFarm” reports on the development of the nitrate and lipid models.

In brief:

After the development of the nitrate and lipid models (Milestone 5: report 2.4, KPI 5.4) simulations were performed for a 47.5kg sheep fed 1.4kg DM/day with one of the following supplement combinations.

- 1) 20g NO₃ from sodium nitrate per kg DM
- 2) 20g NO₃ from ammonium nitrate per kg DM
- 3) 30g fat per kg DM
- 4) Combination of (1) and (3)
- 5) Combination of (2) and (3)

Simulations were performed for 10 days from the start of supplementation. Results comparing the control (no supplement) are shown below (Figures 3.2.1 to 3.2.8).

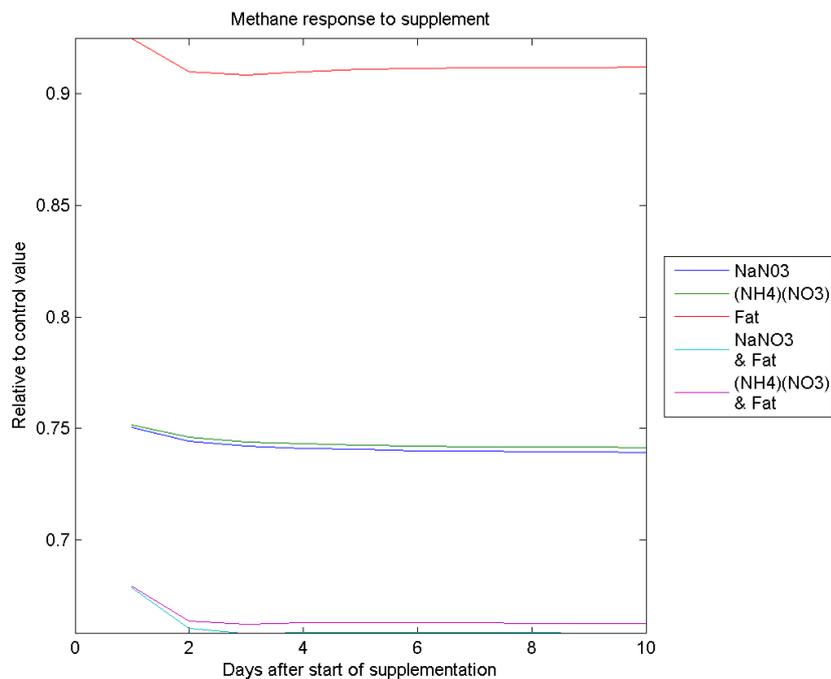


Figure 3.2.1. Methane response relative to control value, after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

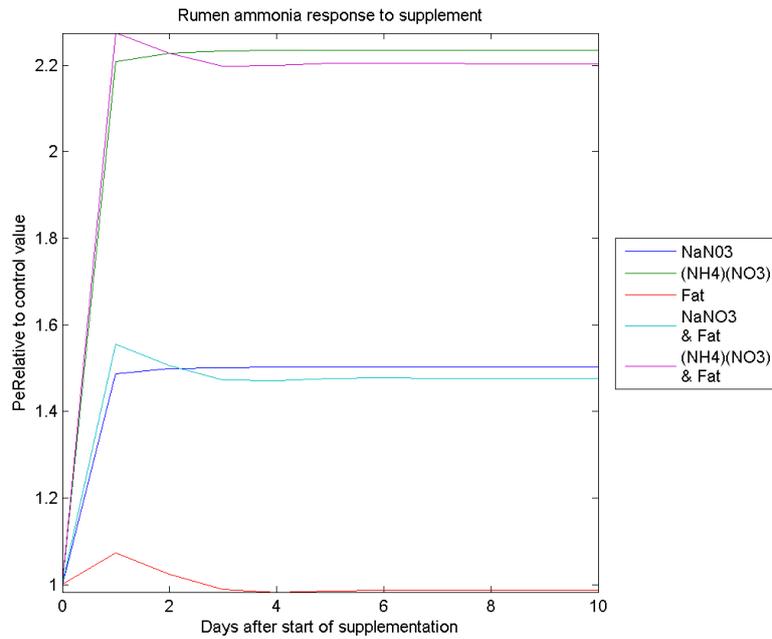


Figure 3.2.2. Rumen ammonia response relative to control value, after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

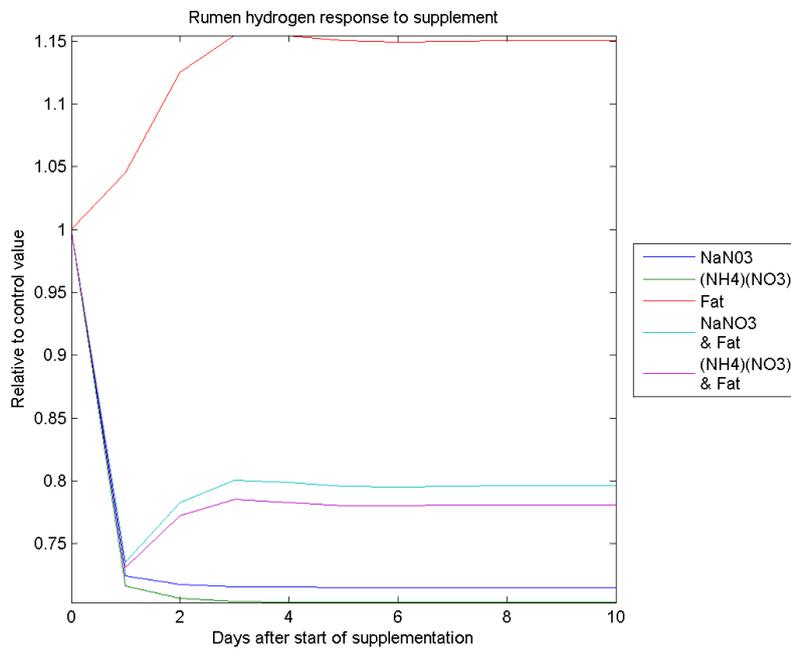


Figure 3.2.3. Rumen hydrogen response relative to control value, after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

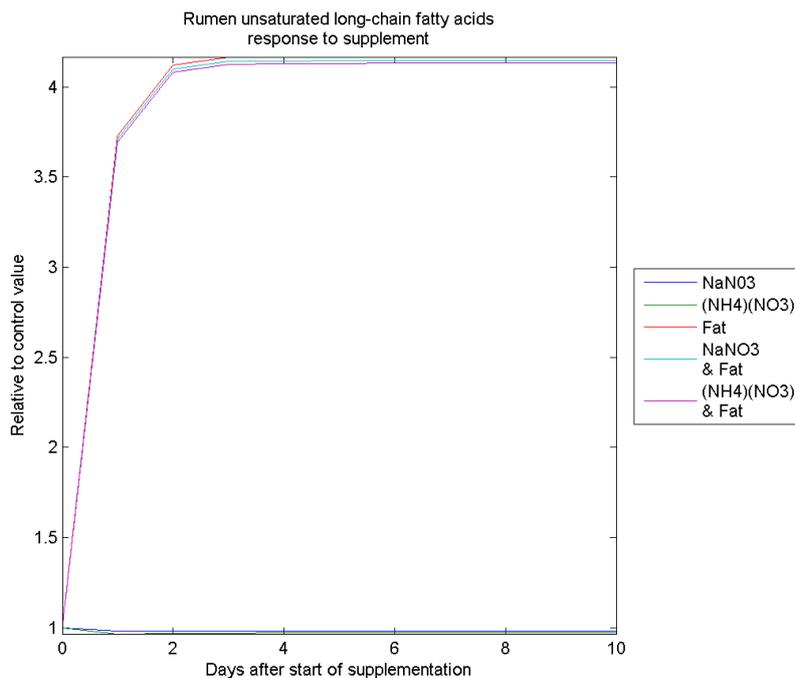


Figure 3.2.3. Rumen unsaturated long-chain fatty acids response relative to control value, after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

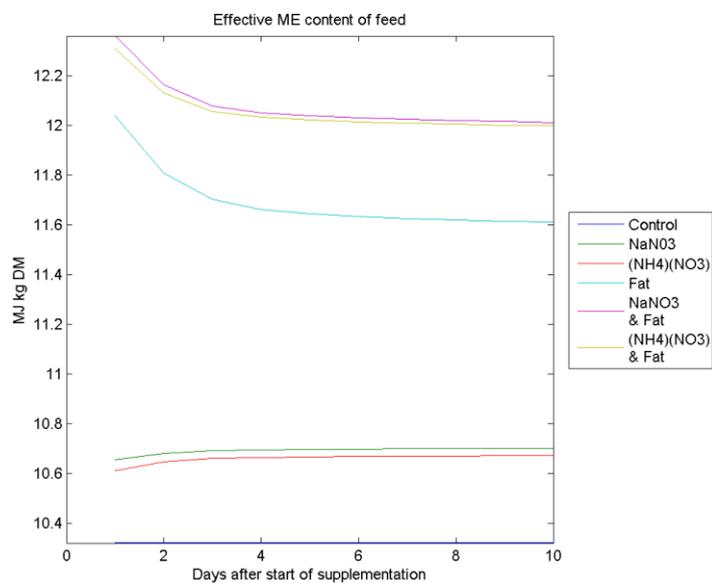


Figure 3.2.4. Effective metabolisable energy (ME) content of feed MJ kg/DM versus days after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

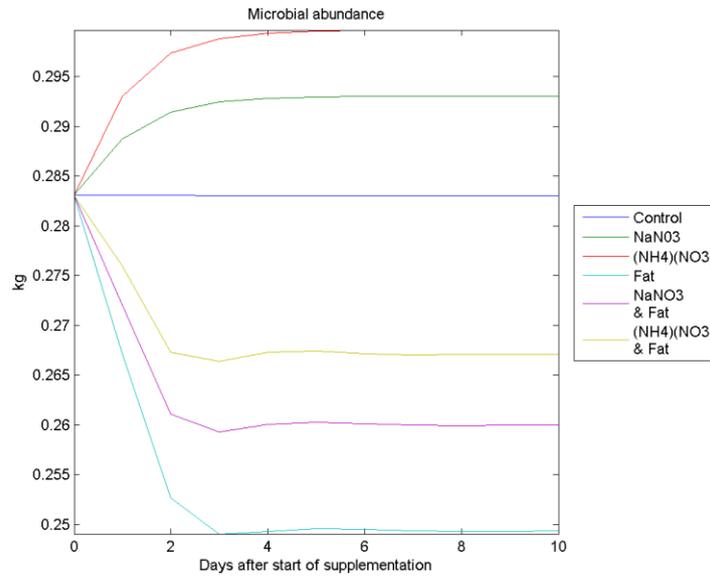


Figure 3.2.4. Microbial abundance (kg biomass) versus days after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

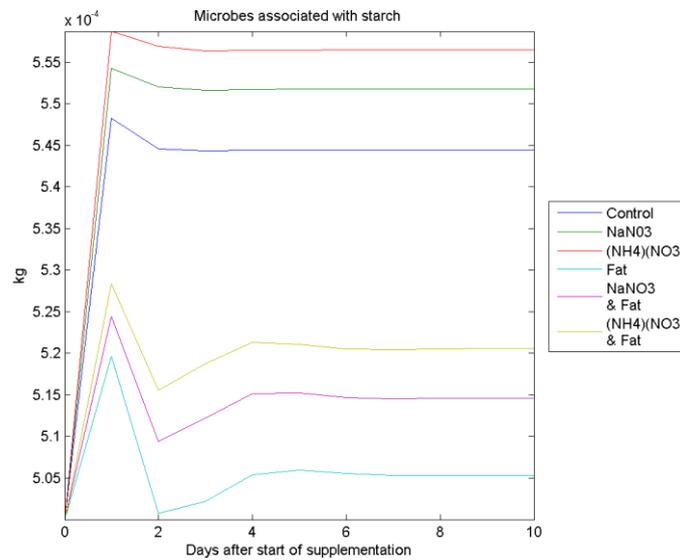


Figure 3.2.5. Microbes associated with starch (kg) versus days after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

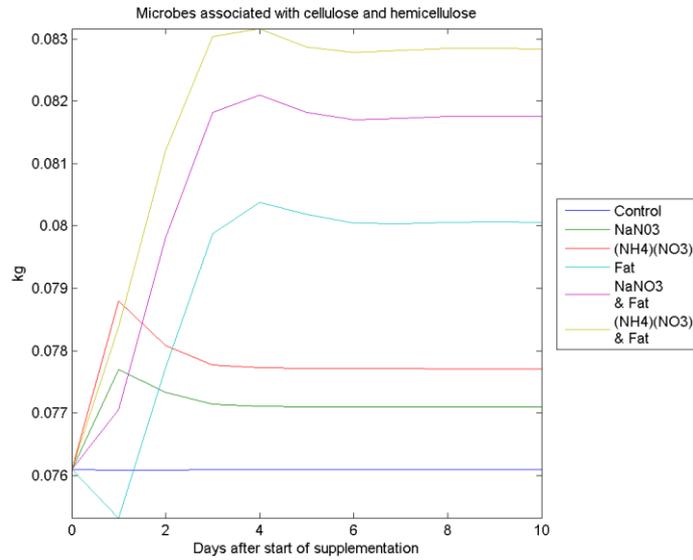


Figure 3.2.6. Microbes associated with cellulose and hemicellulose (kg) *versus* days after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

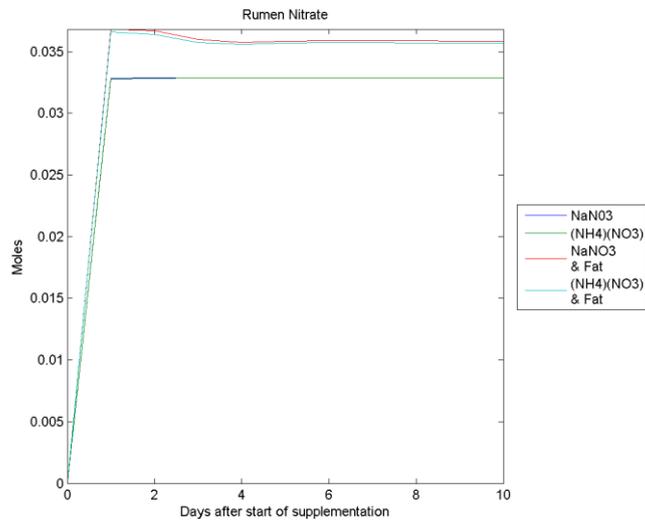


Figure 3.2.7. Rumen Nitrate (moles) *versus* days after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

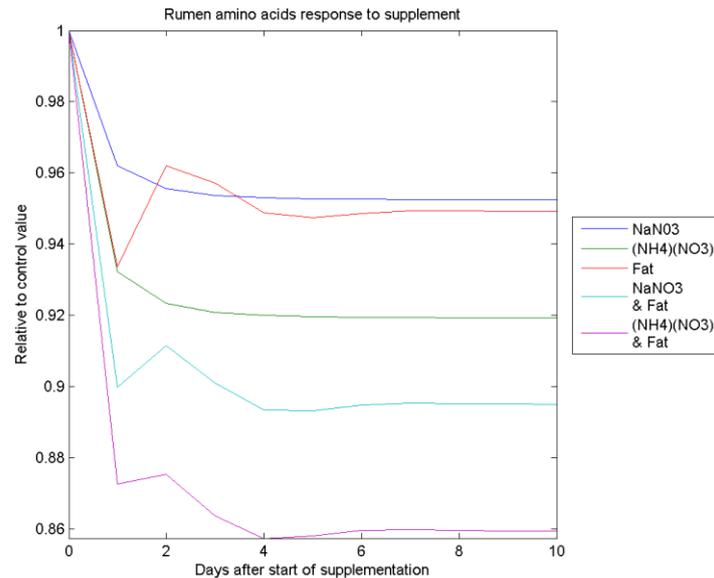


Figure 3.2.8. Rumen amino acid response to supplement relative to control value *versus* days after start of supplementation for (a) 20g NO₃ from sodium nitrate per kg DM (NaNO₃); (b) 20g NO₃ from ammonium nitrate per kg DM [(NH₄)(NO₃)]; (c) 30g fat per kg DM (Fat); (d) NaNO₃ & Fat; and (e) (NH₄)(NO₃) & Fat

Cattle rumen model

Nitrate sub-model

Assumptions:

The size of the NO₃ pool in the rumen is increased by nitrate intake in the diet and is decreased by:

- i) reduction to NO₂;
- ii) absorption through the rumen wall; and
- iii) outflow from the rumen with the rumen fluid

Nitrate salts in the diet are assumed to have a high enough solubility that the nitrate intake immediately adds to the pool. Absorption is assumed to occur at rates well below the maximum possible rate and is therefore treated as a linear function of pool size. The reduction rate is modelled by a simple Michaelis-Menten relationship with a 'Vmax' parameter representing the maximum possible reduction rate and a 'Km' parameter representing the pool concentration at which the reduction rate is half the maximum rate. Outflow of nitrate is based on the rumen fluid outflow rate which is calculated elsewhere in the model, as is the current rumen volume.

This nitrate model was expressed by the following equations:

$$dQNia/dt = FFdNia - (FNiaNii + FNiaAb + FNiaEx)$$

$$cNia = QNia / VRu$$

$$FNiaAb = fNiaAb \times QNia$$

$$FNiaEx = FFIEx \times QNia$$

$$FNiaNii = v_{max}NiaNii \times VRu / (1 + k_{m}NiaNii / cNia)$$

where the variables are as defined in Table 3.2.1 and the parameters as in Table 3.2.2.

Table 3.2.1: Variables used in the nitrate sub-model

Name	Meaning	Unit
QNia	Rumen nitrate pool size	mol
cNia	Rumen nitrate concentration	mol/L
FFdNia	Flow of nitrate into rumen from feed	mol/d
FNiaNii	Rate of nitrate to nitrite reduction in rumen	mol/d
FNiaAb	Absorption rate of nitrate through rumen wall	mol/d
FNiaEx	Outflow rate of nitrate in rumen fluid	mol/d
VRu	Rumen volume	L
FFIEx	Rumen fluid exit rate	fraction / d

Table 3.2.2: Parameters used in the nitrate sub-model

Name	Meaning	Unit	Value
fNiaAb	Fractional nitrate absorption rate	fraction/d	0.18
vmaxNiaNii	Maximum rate of nitrate to nitrite reduction	mol/L/d	0.144
kmNiaNii	Concentration of nitrate for half maximum rate of nitrate to nitrite reduction	mol/L	0.02

The values of $vmaxNiaNii$ and $kmNiaNii$ have been estimated from literature (Lewis, 1951; Wang et al., 1961; Ishigami and Inque, 1976).

The value of $fNiaAb$ has been estimated stoichiometrically as described in the CH₄ mitigation and determination of absorption rates section below.

Nitrite sub-model

The nitrite sub-model has the same basic structure as the nitrate sub-model. The size of the NO₂ pool in the rumen is increased by nitrate to nitrite reduction as given above and is decreased by:

- i) reduction to ammonia;
- ii) absorption through the rumen wall; and
- iii) outflow from the rumen with the rumen fluid

Absorption is again assumed to occur at rates well below a maximum possible rate and is therefore a linear function of pool size. The reduction rate is modelled by a simple Michaelis-Menten relationship with a parameter representing the maximum possible reduction rate and a parameter representing the pool concentration at which the reduction rate is half the maximum rate. Outflow of nitrite is again based on the rumen fluid outflow rate.

This nitrite model was expressed by the following equations:

$$dQNii/dt = FNiaNii - (FNiiAm + FNiiAb + FNiiEx)$$

$$cNii = QNii / VRu$$

$$FNiiAb = QNii \times fNiiAb$$

$$FNiiEx = QNii \times FFIEx$$

$$FNiiAm = vmaxNiiAm \times VRu / (1 + kmNiiAm / cNii)$$

where the additional variables are as defined in Table 3.2.3 and the parameters as in Table 3.2.4.

Table 3.2.3: Further variables for the nitrite sub-model

Name	Meaning	Unit
QNii	Rumen nitrite pool size	mol
cNii	Rumen nitrite concentration	mol/L
FNiiAm	Rate of nitrite to ammonia reduction in rumen	mol/d
FNiiAb	Absorption rate of nitrite through rumen wall	mol/d
FNiiEx	Outflow rate of nitrite in rumen fluid	mol/d

Table 3.2.4: Parameters used in the nitrite sub-model

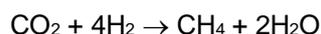
Name	Meaning	Unit	Value
fNiiAb	Fractional nitrite absorption rate	fraction/d	0.18
vmaxNiiAm	Maximum rate of nitrite to ammonia reduction	mol/L/d	0.122
kmNiiAm	Concentration of nitrite for half maximum rate of nitrite to ammonia reduction	mol/L	0.017

The values of *vmaxNiiAm* and *kmNiiAm* have been estimated from literature values as being slightly less than the corresponding nitrate reduction values.

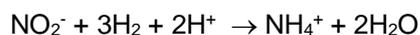
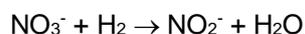
The value of *fNiiAb* has been estimated stoichiometrically as described in the next section.

CH₄ mitigation and determination of absorption rates

Methane production in the rumen results from the reduction of CO₂ by H₂:



Nitrate and nitrite reduction decrease methane production by consuming H₂ that would otherwise be used in the production of methane:



The amount of methane production prevented by nitrate supplement is therefore calculated as:

$$(FNiANii + FNiiAm \times 3) / 4 \quad (\text{mol/d})$$

Stoichiometry indicates that about 60% of nitrate supplementation is effective in decreasing methane, so, in a steady state:

$$(FNiANii + FNiiAm \times 3) / 4 = 0.6 \times FFdNia$$

It is further assumed that *fNiaAb* and *fNiiAb* are equal.

The model was optimised by varying the value of *fNiaAb* (and *fNiiAb*) in a near steady-state simulation scenario until the 60% effectiveness was obtained. The resulting values for the absorption parameters are as given in the tables above (Tables 3.2.2 and 3.2.4).

Model behaviour

Near steady-state simulation

The model was run with a supplement of 20g/kg calcium nitrate in the feed and with involuntary continuous feeding until flow rates stabilised. Table 3.2.5 shows the values obtained for the relevant variables.

Table 3.2.5: Steady-state values for model variables with 20g/kg Ca(NO₃)₂

Variable	Value	Unit
FFdNia	1.95	mol/d
cNia	0.00695	mol/L
FNiaNii	1.44	mol/d
FNiaAb	0.0487	mol/d
FNiaEx	0.458	mol/d
cNii	0.00500	mol/L
FNiiAm	1.08	mol/d
FNiiAb	0.0350	mol/d
FNiiEx	0.330	mol/d
CH4 decrease	1.17	mol/d

One-off addition of supplement

In this simulation run, feeding was twice per day at fixed times of the day. After a period of stabilisation, a one-off addition of 20g/kg calcium nitrate was added to the morning feed and then no afternoon feeding was done. Figure 3.2.9 shows the resulting response curves of nitrate and nitrite concentration.

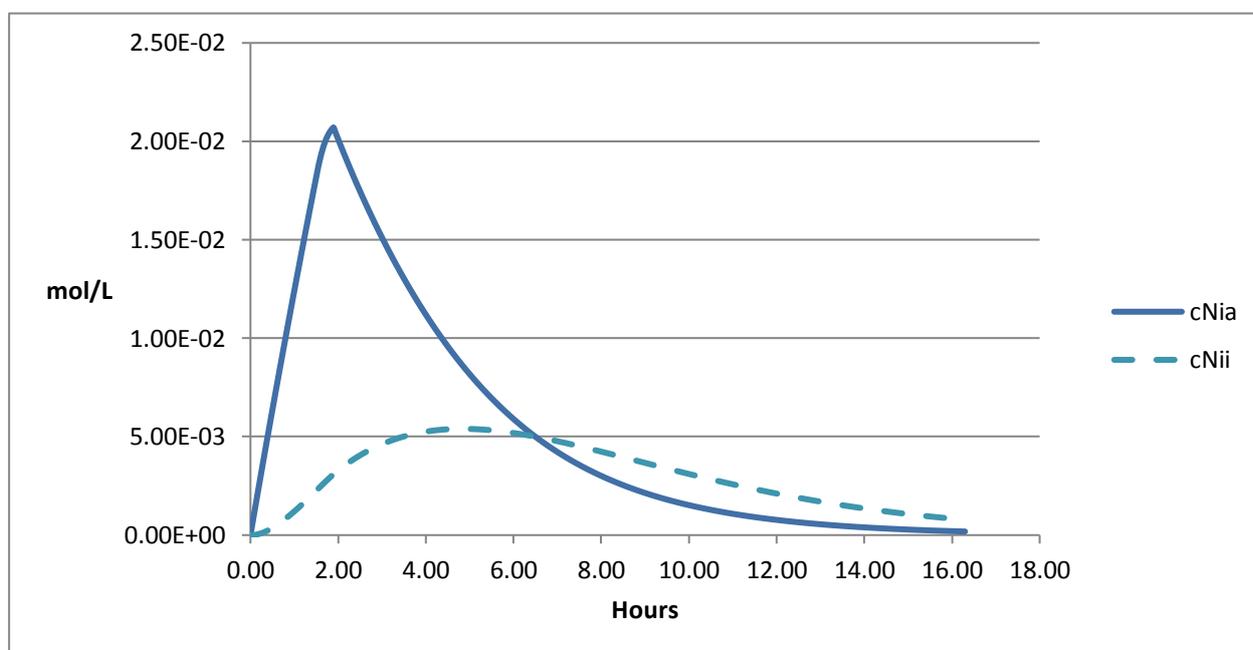


Figure 3.2.9: Response to one-off nitrate supplement

Twice-daily feeding with supplement

In this simulation run, feeding was twice per day at fixed times of the day with the addition of 20g/kg calcium nitrate supplement at each feeding. After a period of stabilisation, the concentration curves are as shown in Figure 3.2.10.

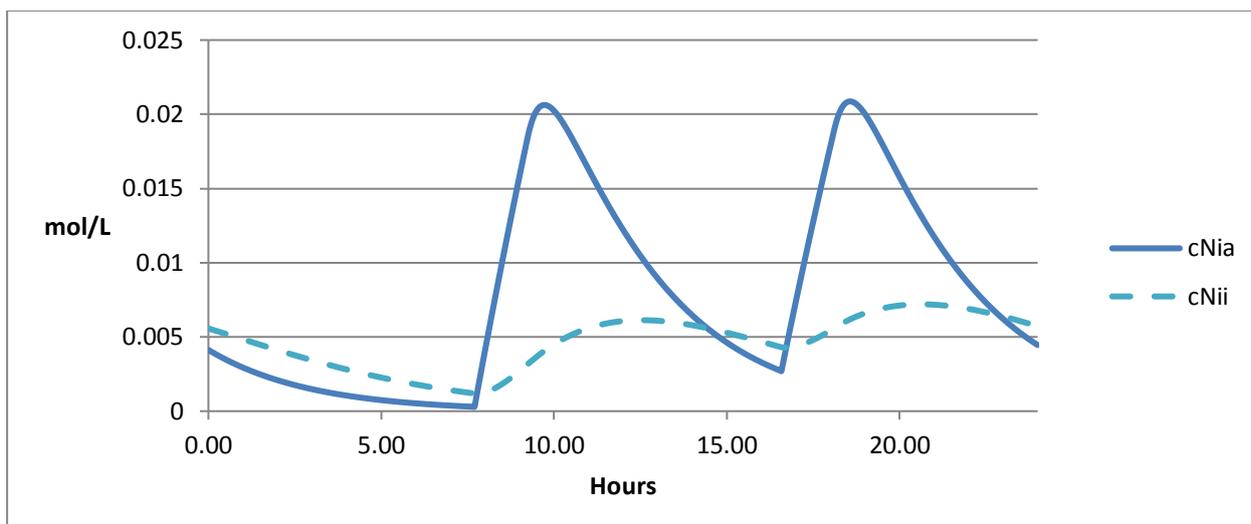


Figure 3.2.10: Response to twice-daily feeding with nitrate supplement

Lipid sub-model

Lipids in the diet already influence the model in at least four ways:

- i) the effect on plasma lipids / storage of triglycerides;
- ii) the effect on absorbed energy and hence voluntary intake;
- iii) the effect on protozoal attachment to feed particles; and
- iv) the effect of unsaturated fats on attachment of cellulolytic bacteria to feed particles.

Each of these can and does affect methane production in the model but a more direct effect on methane, namely the process of bio-hydrogenation as an H₂ sink, has not previously been used in the model.

Bio-hydrogenation is assumed to occur at a rate which is a linear function of the pool size of unsaturated fat in the rumen. It adds to the rate of change of the saturated fat pool, subtracts from the rate of change of the unsaturated fat pool and consumes H₂ as in the following equations:

$$Biohydr = fbiohydr \times QFu$$

$$HyFa = Biohydr \times unsatbonds$$

where the variables are as defined in Table 3.2.6 and the parameters as in Table 3.2.7.

Table 3.2.6. Variables used in the calculation of biohydrogenation

Name	Meaning	Unit
QFu	Rumen unsaturated fat pool size	mol
QFs	Rumen saturated fat pool size	mol
Biohydr	Rate of biohydrogenation	mol/d
unsatbonds	Average double bonds per unsaturated LCFA	-
HyFa	H ₂ consumed by biohydrogenation in rumen	mol/d

Table 3.2.7: Parameters used in the calculation of biohydrogenation

Name	Meaning	Unit	Value
fbiohydr	Fractional rate of bio-hydrogenation of unsaturated fats	fraction/d	4.8

The value chosen for the parameter *fbiohydr* has been determined by running a near steady-state simulation and aiming for an overall value of 85% of the unsaturated fat becoming saturated. It corresponds to a rate of 20% per hour.

Table 3.2.8 summarises the effect of lipid supplementation with and without taking bio-hydrogenation into account. The supplement is 8% of feed, with 50% of this saturated and 50% unsaturated (linoleic).

Table 3.2.8. Methane production rate with no lipid supplementation (1), with lipid supplementation but without allowing for biohydrogenation (2) and with lipid supplementation including biohydrogenation (3).

Treatment	CH ₄ production rate (mol/d)
1	11.08
2	10.21
3	10.10

3.2.3 *AusBeef* rumen model coding consistent with input/output code of *AusFarm*.

Major areas of work:

- Analysis/documentation of *AusBeef* ruminant model
- Ruminant model integration into *AusFarm*
- Extensions to *AusBeef* ruminant model
- Maintenance/development of *AusBeef* program and management of model code

Several routines from CSIRO, with assistance from CSIRO, were made available to code *AusBeef* to be consistent with *AusFarm*. A sheep rumen model, developed by AgResearch, was also interfaced with *AusFarm*. The outputs attached to this report are 2 DLL's, one for sheep and one for cattle that can be used by *AusFarm* programmers.

3.2.4. *A conference and refereed journal paper on the structure and fit of original and revised models, including sensitivity analysis identifying critical inputs submitted.*

Several conference abstracts have been presented and are reported in the references. The following extracts from publications in preparation for journal articles on the structure and fit of original and revised models compared with MOLLY and COWPOLL and a sensitivity analysis paper are reported below.

Paper titled. Advances in rumen-scale enteric methane modelling: structure of *AusBeef* and qualitative comparison with MOLLY, and COWPOLL.

This paper is about the structure of *AusBeef* and a qualitative comparison with MOLLY and COWPOLL therefore the results provided from this paper provide detail of the models that forms the basis of discussion.

General description of models

AusBeef

AusBeef was written in VisSim (PTV 2004) and the user interface was constructed in Delphi with C++ code generated from VisSim. The *AusBeef* model is integrated on a time step of 0.001 day (1.44 minutes) allowing for precise calculation of changes and trends in physiological processes. The model is an integration of four sub-models: rumen, body growth, lower gut, and voluntary feed intake, each of which contains submodules that mechanistically represent ongoing physiological processes. In this study, discussion is limited to the rumen sub-model, specifically as it pertains to CH₄ emissions, as body growth and lower gut models depend on output from the rumen sub-model. The *AusBeef* interface allows users to input the age, sex, breed, and initial weight of the average animal in their herd, and then specify feed

composition and feed management strategies. Feed characteristics are drawn from an internal database or specified by the user, which, combined with user-supplied costs and market price data, are used to simulate the growth and economics of beef cattle production under a variety of conditions.

MOLLY

MOLLY (Baldwin, 1995) is a dynamic mechanistic model of nutrient utilization in cattle originally developed at the University of California, Davis. MOLLY has undergone several updates by Hanigan *et al.* (2006, 2007, 2013) and Gregorini *et al.* (2015). Methane production is predicted as described by Baldwin (1995). Briefly, ruminal CH₄ production was predicted based on hydrogen net balance. Excess hydrogen produced during fermentation of carbohydrates and protein to lipogenic volatile fatty acids (VFA) (i.e., acetate and butyrate) is partitioned between use for microbial growth, biohydrogenation of unsaturated fatty acids and production of glucogenic VFA (i.e., propionate and valerate). The assumption is made that the remaining hydrogen is used solely and completely for methanogenesis. The VFA stoichiometry in MOLLY is based on equations developed by Murphy *et al.* (1982).

COWPOLL

The rumen model of Dijkstra *et al.* (1992; 1994a) is the basis for the mechanistic model used in the present study. Methane production in the rumen and hindgut was introduced by Mills *et al.* (2001) following the principles of Baldwin (1995). Later, Kebreab *et al.* (2004) incorporated the rumen model to a whole animal model that included N and P utilization. Bannink *et al.* (2006) developed a new stoichiometry for fermentation within the rumen based entirely on experimental observations with lactating dairy cows, therefore, COWPOLL was modified to accommodate these stoichiometric coefficients. Microbial groups that are involved in fermentation and transformation of substrate to VFA are represented by three main groups in COWPOLL. Recently, Mills *et al.* (2014) also described a fourth group, which will be discussed later.

Comparison of AusBeef with MOLLY and COWPOLL

All three models are dynamic, mechanistic, and deterministic, and detail representations of biological processes. Even though the three models: AusBeef, MOLLY, and COWPOLL share similarities in overall structure and aims, there are distinct differences. Both COWPOLL and MOLLY were developed for the dairy industry whereas AusBeef was developed for beef production and uses parameters derived for beef cattle. However, following evaluation of COWPOLL by Kebreab *et al.* (2008), Ellis *et al.* (2014) modified rumen coefficients in COWPOLL for estimating CH₄ emissions in beef cattle.

Microbial groups

The microbial groups in MOLLY are represented by one state variable while COWPOLL has three distinct microbial pools, including two bacterial pools (amylolytic, Ba and cellulolytic, Bc) and one protozoal pool. AusBeef represents four different microbial pools including three bacterial pools (Ba, Bc and lactolytic bacteria, Blc), as well as amylolytic storage polysaccharides and protozoal storage polysaccharides. In both COWPOLL and AusBeef, each microbial group has distinct coefficients for representing digestion and fermentation, and all three models use Michaelis-Menten dynamics to represent microbial growth.

An important distinction is the inclusion of lactate fermentation in the AusBeef rumen model. Lactate is produced in the rumen, and is then utilized and fermented to VFA. The balance between production and utilization usually keeps the concentration of lactate in the rumen low (Owens *et al.* 1988). However, in animals fed large quantities of rapidly degradable starch or soluble sugars, as in feedlots, or animals fed high-concentrate diets multiple times a day, lactate can accumulate, decreasing rumen pH and resulting in lactic acidosis (Nagaraja and Titgemeyer 2007). Mills *et al.* (2014) also developed a model for lactic acid metabolism in the rumen, which can be used to evaluate diets for their propensity to induce lactic acidosis. AusBeef and the model of Mills *et al.* (2014) allow for simulation of peak rumen lactic acid concentration in the rumen and assessment of the potential of the feed to cause acute or sub-acute rumen acidosis. This will in turn affect rumen pH and VFA stoichiometry, affecting CH₄ production.

Source and particle size of feed

Feed composition is represented as a singular item in both MOLLY and COWPOLL. In AusBeef, feed is split between grain, leaf and stem based on feed composition and user inputs, and coefficients for degradation vary depending on the source of the feed. Unlike COWPOLL, both MOLLY and AusBeef account for differences in particle size in the rumen. The difference is that in the former three size classes of feed particles i.e., large, medium, and small particles are represented and have different degradation rates. AusBeef represents four particle size classes: i.e., large, medium, small, and fine or dissolved. Large

particles are defined as > 2mm for grain and >1 mm for leaf and stem; medium as 1-2 mm for grain, 0.5-1 mm for leaf and stem; and small as <1 mm for grain; < 0.5 mm for leaf and stem, and fine particles are classified as the dissolved individual nutrients within the rumen.

In AusBeef, feed source and particle size classes are divided into pools of specific nutrients contained in each particle and size class combination. Microbial and physical degradation rates are then calculated for each combination of size, source, substrate, and microbial group, which can be customized to any ration. The model uses a series of degradation coefficients to calculate a distribution of the quantity of particles in each particle size class, as well as the proportion chewed and regurgitated during each rumination cycle. Microbial degradation is also represented by vectors of attachment and degradation rates, which are dependent on particle size and source, ruminal pH, and feed particle nutrient availability. As particles are degraded, the nutrients contained in these particles become available to microbes, which in turn feedback in to microbial attachment rates and the distribution of particles in each size and source class.

To represent rumination, the model of Kennedy (1985) was modified to fit the particle and feed source classes represented in AusBeef. The distribution of particles in each size and source class is multiplied by a vector representing the percentage of each class that is regurgitated and chewed during each time step, with the probability of a given particle being chewed decreasing proportionally with size. The specific fragility of a given feed source also influences how long it takes to pass between size classes, as more fragile feeds break down faster when chewed. Time spent ruminating is represented as a function of the amount of particles in the rumen, current rumen volume relative to the total empty rumen volume, and the size distribution of particles being chewed in the cud. This allows for a flexible representation of the rumination process and for representation of the influences of specific feed types on particle breakdown during rumination. This comprehensive mechanistic representation of both physical and chemical degradation allows the model to represent degradation processes based on particle size instead of having specific sets of coefficients for forage, concentrate, and mixed diets. AusBeef's expanded representation of biological processes offers a unique feature compared to the other two models.

Substrates for VFA production

All three models represent similar sets of substrates for the production of volatile fatty acids (VFAs) via microbial degradation and fermentation: protein, starch, cellulose, hemicellulose, and soluble non-starch carbohydrates. However, in addition to these, AusBeef also represents microbial lipids and lactate as substrates for VFA production. In all models, protein in the rumen primarily involves the soluble protein pool and the ammonia pool. Protein that does not enter the soluble protein pool may remain bound to feed particles or exit as rumen undegradable protein (RUP). Both "true" RUP and protein that exits the rumen before being fully degraded enter the lower gut for utilization. Ammonia is formed as a byproduct of protein fermentation, from the conversion of urea to ammonia in the rumen, ammonia from salivary nitrogen, ammonia contained in feed, plasma urea N, and ammonia from engulfed bacteria. Ammonia is either absorbed directly via the rumen wall, used as a N source for microbial growth, or exits to the lower gut.

Fibre in feed belongs to two classes: degradable and undegradable fibre. Undegradable fibre exits the rumen in the solid phase, whereas degradable fibre undergoes microbial attachment and degradation to hexose from cellulose and hexose from hemicellulose in all models. In AusBeef, the rate of microbial attachment and degradation depends on the type of microbes, specifically protozoa (Po) or cellulolytic bacteria (Bc), rumen pH, and particle passage rates out of the rumen. While some cellulose and hemicellulose will exit the rumen as dissolved nutrients, the majority is broken down into hexoses by cellulolytic bacteria, which are then used by cellulolytic bacteria for growth using ammonia or soluble protein as N sources, or exit the rumen as dissolved hexose.

Starch use in the rumen primarily involves the pool representing hexose from starch, non-hexose sugars, and other soluble non-starch carbohydrates. Starch in feed culminates in either degradable starch or soluble starch. Degradable starch, soluble starch and soluble non-starch carbohydrates can flow out of the rumen as dissolved nutrients or be fermented or hydrolyzed in the rumen. Soluble starch is hydrolyzed to hexose from starch (Hxs) and then fermented by amylolytic bacteria (Ba) to produce VFA. Degradable starch is used by bacteria for growth and maintenance, fermented by Ba to VFA, hydrolyzed to Hxs, or incorporated into the protozoal and amylolytic storage polysaccharide pools, which can then be hydrolyzed and fermented to VFAs. Soluble non-starch carbohydrates are hydrolyzed and then fermented by Ba and Po to produce VFAs and hexose, which can then be used by Ba and Po for growth with ammonia and protein as N sources, yielding VFA as a by-product.

In all three models, lipids in the rumen involve the state variables representing saturated and unsaturated lipids. Lipids are degraded and can flow out of the rumen as dissolved lipids, microbial lipids, or lipids bound to feed particles. Microbes can hydrolyze lipids to free fatty acids and glycerol or galactose, which can then be fermented to VFA and hexose, or used for microbial growth and maintenance along with free fatty acids. AusBeef, unlike COWPOLL or Molly, also includes microbial lipids in the pool of potential substrates for the production of VFAs. After microbial lysis or protozoal engulfment of microbes, these lipids can be hydrolyzed and the resulting glycerol or galactose fermented to produce VFAs, a process not represented by other models but which expands the representation of biological processes in the rumen to be closer to *in vivo* mechanisms. Unsaturated lipids in the rumen undergo bio-hydrogenation and enter the saturated lipid pool for microbial use or exit rumen for absorption in the lower gut. In AusBeef, the rate of microbial bio-hydrogenation is represented as proportional to the amount and concentration of unsaturated lipids in the rumen, and is an input to the saturated fat pool. As shown by Beauchemin *et al* (2008), bio-hydrogenation of lipids in the rumen acts as a hydrogen sink, further reducing the hydrogen available for methanogenesis.

As discussed earlier, AusBeef includes lactolytic bacteria that are capable of fermenting lactate produced by rumen microbes such as *Streptococcus bovis* and producing propionate and acetate as potential end products. Lactate can also be used as a substrate for microbial growth, further reducing the supply of lactic acid in the rumen. This expanded representation allows for more detailed representative of the mechanisms behind rumen pH, as well as the production of VFAs and microbial growth due to usage by lactolytic bacteria.

Volatile fatty acid stoichiometry

The VFA stoichiometries used in all three models differ from each other. The VFA stoichiometry in MOLLY is based on Argyle and Baldwin (1988) who modified the model of Murphy *et al.* (1982) that used data sources from beef cattle and sheep by relating the fermentation of soluble carbohydrates and starch to rumen fluid pH. The digestive parameters in MOLLY that affect VFA stoichiometry were further modified by Hanigan *et al.* (2013). The VFA stoichiometry used in COWPOLL is based on Bannink *et al.* (2006) who used a similar approach to Murphy *et al.* (1982), but used data from lactating Holstein cows to fit the stoichiometric parameters to obtain values more representative of the dairy system. Both MOLLY and COWPOLL use different sets of VFA production and substrate digestion coefficients based on the type of diet fed. These could be either mostly concentrate, mostly forages or intermediate (mixed) diets (40 to 60% forage) with the latter using coefficient means of the first two categories (Dijkstra *et al.* 1992; Baldwin *et al.* 1987). The VFA molar proportions are then related to observed amounts of digested protein, cellulose, hemicellulose, starch and soluble carbohydrates.

Morvay *et al.* (2011) and Alemu *et al.* (2011) compared several VFA stoichiometries including those used by extant models. The authors concluded that rumen VFA stoichiometric approaches explain a large part of the variation in VFA molar proportions among diets, especially for acetate but predictive power for propionate and butyrate differ considerably among approaches. For example, the Bannink *et al.* (2006) model performed best in predicting propionate while the Argyle and Baldwin (1988) model had the best performance in predicting acetate. This could further explain differences in CH₄ predictions between models, as VFA molar proportions are a major driving force of ruminal hydrogen fluxes and therefore CH₄ production rates.

AusBeef does not include specific coefficients based on diet but instead determines degradation (and hence amounts of digested substrates) based on size and source of feed. AusBeef calculates digestion and VFA production from cellulose, hemicellulose, soluble non-starch carbohydrates, starch, protein, lactate, and microbial lipids. AusBeef calculates VFA production using all four microbial groups represented in the model via a series of consensus stoichiometries, as first described in Nagorcka *et al.* (2000). These were created by deriving fermentation stoichiometries for individual microbial genera and then creating a weighted average consensus stoichiometry based on the proportion of each genera in the overall pool to which that genera belongs. This weighted average for each substrate became the stoichiometry behind AusBeef. Each group has its own different patterns for the production of VFA and other by-products from each set of substrates: hexoses for Ba, Bc, and Po, lactate for Blc, and protein for all groups, all of which are affected by rumen pH. This allows for a flexible and biological representation of ruminal fermentation that can adjust to changing physiological conditions and feed inputs. By representing these differences in fermentation patterns across microbial groups the VFA fermentation stoichiometry in AusBeef is an

advance on those used in the other two models, both in terms of mechanistic representation of biological processes and prediction accuracy (Nagorcka *et al.* 2000). These stoichiometries were shown to improve VFA prediction compared to Murphy *et al.* (1982). The squared correlation coefficient (observed vs estimated) for acetate, butyrate, propionate and total VFA production in AusBeef was 0.62, 0.74, 0.37 and 0.84, respectively compared to 0.26, 0.38, 0.13 and 0.80 from Murphy *et al.* (1982) as used by Dijkstra (1993), respectively.

Methane calculation

All three models compute enteric CH₄ production by calculating hydrogen balance in the rumen and assuming that any excess hydrogen is converted to CH₄. However, each model uses different terms to calculate the overall fluxes of rumen hydrogen and CH₄. Methane in AusBeef is treated as a 'zero pool' derived from hydrogen balance. Inputs to the hydrogen pool come from the fermentation of protein and hexoses to acetate and butyrate, as well as hydrogen from the growth of Ba, Bc, and Po with amino acids as a N source. Hydrogen is used in the fermentation of hexoses and protein to produce propionate and valerate, the growth of Ba and Bc using ammonia as a N source, and the bio-hydrogenation of unsaturated rumen lipids (Eqns 1-2). The remaining hydrogen is assumed to be used by methanogens as an energy source with CH₄ as a byproduct, which is then eructated during rumination.

$$P_{CH4Ru} = P_{HyRu}/4 \quad [1]$$

$$P_{HyRu} = (2 P_{AcHx} + 2 P_{BuHx} + 2 P_{AcPrt} + 2 P_{BuPrt} + (0.00042 P_{MiGrPrt}) - (P_{PrpHx} + P_{VIHx} + P_{PrpPrt} + P_{VIPrt} + (0.00271 P_{BacGrAm}) + U_{HyLi})) \quad [2]$$

Where P_{CH4Ru} is the production rate (moles/day) of CH₄ in the rumen and P_{HyRu} is the production rate of hydrogen in the rumen. P_{AcHx} , P_{BuHx} , P_{AcPrt} , P_{BuPrt} , P_{PrpHx} , P_{VIHx} , P_{PrpPrt} , and P_{VIPrt} are the rates of production of VFAs by rumen microbes using either hexose or protein as the substrate for fermentation. $P_{MiGrPrt}$ is the growth rate of rumen microbes using protein as a nitrogen source, which releases hydrogen, and $P_{BacGrAm}$ is the growth rate of microbes with ammonia as a nitrogen source, which requires hydrogen. U_{HyLi} represents the use of rumen hydrogen for microbial biohydrogenation of unsaturated lipids.

COWPOLL represents CH₄ similarly to AusBeef, as shown in the following equations (Mills *et al.*, 2001, Eqns. 3-4). As in AusBeef, hydrogen is produced from the fermentation of carbohydrates and protein to Ac and Bu, and from microbial growth with protein. Hydrogen is consumed by the production of propionate and valerate, microbial growth with ammonia, and biohydrogenation of lipids.

$$P_{CH4} = Hy/4 \quad [3]$$

$$Hy = P_{Hyferm} + P_{HyMg} - U_{Hyferm} - U_{HyMg} - U_{HyLi} \quad [4]$$

MOLLY, as originally created by Baldwin (1995), represents hydrogen in the rumen from fermentation (DThy) as the weighted sum of hydrogen produced from the fermentation of carbohydrates and protein to VFA (DCsHy and DRAaHy) and hydrogen produced from the conversion of lactate to acetate (RLaAc), minus hydrogen consumed by microbial protein synthesis (DHyMi), biohydrogenation (DHyFIF), and the conversion of lactate to propionate (RLaPr) (Eqn.5). Unlike AusBeef and COWPOLL, the 1995 MOLLY model does not explicitly represent microbial growth with amino acids for N as a source of hydrogen.

$$DThy = DCsHy + DRAaHy - DhyMi - DhyFIF + 2 \times RLaAc - RLaPr \quad [5]$$

A recent update to MOLLY by Vetharaniem *et al.* (2015) modified MOLLY to work for sheep production systems, and modified the CH₄ prediction equations as follows:

$$DTCH4 = m_f \times Mi \times RH2/RLV \quad [6]$$

$$DRH2 = DThy_{R14} \times (1 - k_f \times DMI/EBW^{0.75}) - 4 \times DTCH4 - KWAP \times RH2, \quad [7]$$

Where DTCH4 is the rate of CH₄ formation, m_f is a coefficient governing CH₄ formation, Mi is microbial biomass, RH2 is the amount of hydrogen in the rumen, and RLV is rumen volume. DRH2 is the derivative of the hydrogen pool, and DThy_{R14} is the new term for hydrogen from fermentation, which is similar to the 1995 version but also incorporates hydrogen produced and consumed by microbial growth. The term (1-

$k_1 \times \text{DMI/EBW}^{0.75}$) adjusts hydrogen from fermentation for unaccounted H_2 losses such as losses due to eructation and changes in fermentation stoichiometries, and KWAP is the fluid passage rate out of the rumen, which, multiplied by RH2, represents dissolved CH_4 formation and loss due to fluid exiting the rumen. These changes bring MOLLY more in line with how AusBeef and COWPOLL in representing CH_4 production in the rumen, but at a different level of mechanistic detail and with parameter values fitted to a different species. While the initial CH_4 calculation structures seem similar across the models, the fermentation stoichiometries in each model are all different, leading to the potential for considerably different results between the three models when used to predict enteric CH_4 emissions.

pH effects

All three models represent the effects of rumen pH on rumen processes. An update by Hanigan *et al.* (2013) in MOLLY included the effect of rumen pH on microbial activity and fibre degradation. MOLLY also includes terms representing the effects of VFA production on rumen pH. Conversely, COWPOLL represents the effects of rumen pH on VFA production via effects on microbial activity. AusBeef incorporates the effects of rumen pH on VFA production and absorption, on microbial population size and activity, and on microbial attachment and degradation of feed particles. Rumen pH also has the potential to stimulate or suppress appetite in the animal. By dynamically calculating ruminal pH and its effects on biological processes, trends in pH can be calculated and the diets designed to prevent ruminal acidosis can then be incorporated into livestock management strategies.

Feed intake

All three models treat feed intake differently. COWPOLL assumes continuous feeding, whereas MOLLY allows for two feeding systems: continuous eating or multiple set meals per day. In addition to these, AusBeef incorporates a novel voluntary feed intake submodel, which is controlled mechanistically via several feedback loops that will be described in a future paper on overall model structure, and enables AusBeef to predict the changes in rumen pH and VFA production and absorption due to variations in feed intake. AusBeef also offers the option for users to assume continuous feeding, or for users to set specific meal size and feeding times throughout each day.

Passage rates

All three models have different methods of calculating passage rates. COWPOLL uses constants that depend on the particular substrate exiting the rumen, with each VFA having its own constant exit rate to the lower gut, and with substrate exit rates calculated as a function of a constant solid or fluid passage rate. Amylolytic bacteria and protozoa exit at the fluid passage rate, while cellulolytic bacteria exit at the solid passage rate. MOLLY, as updated by Gregorini *et al.* (2015), calculates nutrient passage rates as a function of the liquid passage rate and the total solid particle passage rate. The fluid passage rate is calculated as a function of fluid in the rumen and a fluid exit rate constant, and the solid passage rate is calculated as a function of total particles in the rumen and a solid exit rate constant. AusBeef calculates passage rates dynamically, with microbes exiting at a rate based on the fluid exit rate, the amount of feed particles in the rumen, and the amount of each microbial group in the rumen. The rumen fluid exit rate is calculated as a percentage of rumen contents per day, and is calculated from the time it takes for rumen contents to completely turn over as well as the ratio of current rumen volume relative to a normal rumen volume for a given age and weight. Solids in the rumen exit at a rate calculated based on the amount of particulate matter exiting the rumen, derived from the sum of exit rates across all particle sources and size classes, and the total amount of particulate matter in the rumen. Exit rates help determine CH_4 production rates, as degradation and fermentation increase with time until maximum degradation is reached. The more time microbes have to ferment a substrate, the more potential hydrogen is freed up for use in methanogenesis, therefore, accurate modelling of passage rates is necessary for accurate prediction of CH_4 production rates.

The following paper, as mentioned above, has also been written on a local and global sensitivity analysis of the AusBeef model.

Paper titled. Local and global sensitivity analysis of methane production in the AusBeef rumen model for cattle production.

Local sensitivity analysis

The local sensitivity analysis for the four outputs chosen is as follows: For the acetate:propionate ratio, there were 224, 221 and 233 parameters with normalized $|S_i| > 0$ for AUS, US and NZ diets, respectively. There were 157, 111, and 140 parameters with normalized $|S_i| > 0.01$ for AUS, US and NZ diets, respectively. Only nonzero parameter sensitivities were considered for this graph and analysis. Using the US diet resulted in the smallest variance of sensitivities, while the NZ diet had the lowest minimum and highest maximum values. The AUS diet showed the greatest variance (Figure 3.2.1).

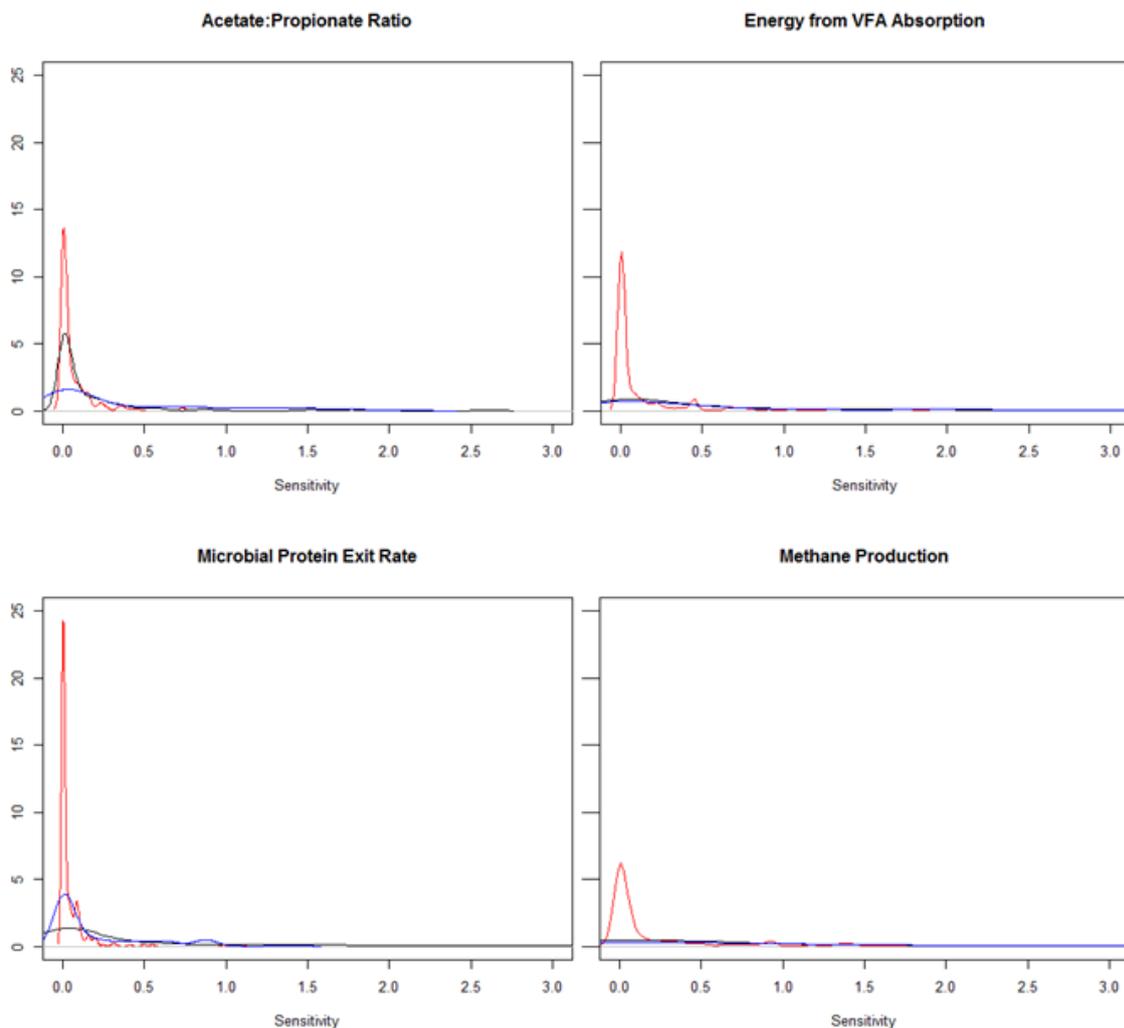


Figure 3.2.1 Distribution of Local Sensitivities (Blue = AUS, Red = US, Black = NZ) for Acetate:Propionate ratio, energy from VFA absorption, microbial protein exit rate, and methane production.

There were 254, 253 and 234 parameters with normalized $|S_i| > 0$ for AUS, US and NZ diets, respectively, related to energy absorbed from VFA. The number of parameters with normalized $|S_i| > 0.01$ were 157, 111 and 155 for AUS, US and NZ diets, respectively. The US data showed the lowest variance of sensitivity values and the smallest range of sensitivities. The NZ data showed both the highest maximum and the highest variance. Parameters with normalized $|S_i| > 0$ related to MiPrt were 259, 264, 249 for AUS, US and NZ diets, respectively. There were 147, 105, 158 parameters with normalized $|S_i| > 0.01$ for AUS, US and NZ diets, respectively. The majority of the parameter sensitivities were between 0 and 0.2, with the US data having the highest density near 0 and the lowest overall variance. The NZ diet sensitivities had the highest

variance and range, and the AUS data was intermediate to the other two datasets but closer to the US data in distribution. There were 255, 252 and 233 parameters with normalized $|S_i| > 0$ for AUS, US and NZ diets, respectively, related to CH₄ production. The number of parameters with normalized $|S_i| > 0.01$ were 174, 127 and 162 for AUS, US and NZ diets, respectively. The NZ dataset had the widest range and greatest variance overall, with the US dataset showing the smallest range and variance, being primarily clustered between 0 and 2.

There were 199 parameters with sensitivities > 0 across all three diets for the acetate to propionate ratio; 195 parameters for energy from absorbed VFA, 249 parameters for MiPrt and 227 parameters for CH₄ production. Overall, 195 parameters were sensitive across all diets and measures, indicating a high conservation of sensitivity across measures. There were 55 parameters with sensitivities > 0.01 for all three diets and measures. Individually, there were 81, 83, 75 and 101 parameters with sensitivities > 0.01 for acetate to propionate ratio, energy from absorbed VFA, MiPrt, and CH₄ production, respectively, across all diets. For the acetate to propionate ratio, there were no parameters with sensitivities > 1 across all diets. For energy from absorbed VFAs, only three parameters had $S_i > 1$ across all diets, and for microbial protein exit rates, there was only one parameter with $S_i > 1$ across all diets. Methane production had the most parameters with sensitivities > 1 across all diets, with 10.

There were 79, 82, and 70 non-sensitive parameters for AUS, US and NZ diets, respectively, with 49 parameters overall for acetate:propionate ratio. For energy from VFA, there were 49, 50, and 69 non-sensitive parameters for AUS, US and NZ diets, respectively, with 47 parameters overall. There were 44, 39, and 54 non-sensitive parameters for AUS, US and NZ diets, respectively, with 38 parameters overall for MiPrt. For CH₄, there were 48, 51, and 70 non-sensitive parameters for AUS, US and NZ diets, respectively, with 44 parameters overall. The model was non-sensitive to 36 parameters across all diets/measures.

Global sensitivity analysis

Twenty parameters from the local sensitivity analysis were chosen as the parameters of interest for the global sensitivity analysis. Parameter choice was based on several factors, including biological meaning, magnitude of S_i across diets and measures, and how many times each parameter appeared in the top 20 parameters for each diet/output combination. The twenty parameters of interest are given in Table 3.2.1.

Table 3.2.1: Parameters Used in Global Sensitivity Analysis

Term	Meaning
CorrFacAmBa	Adjustment factor for ammonia uptake by amylolytic bacteria
CorrFacSpBa	Adjustment factor for soluble protein uptake by amylolytic bacteria
FracOutP7	Fraction of small grain particles exiting in rumen fluid
JpHAcAb	Inhibition of acetate absorption due to pH effects
KdegSPgrain	Microbial degradation rate for small grain particles
MMPoPo	Michaelis-Menten constant for engulfment of protozoa by other protozoa
pHCalcP1	Rumen pH calculation offset factor (intercept)
EngEffPo	Engulfment efficiency of protozoa
ReqHxcAmGrBc	Requirement of hexose from cellulose for growth of cellulolytic bacteria using ammonia as a nitrogen source
SpecFragP2	Specific fragility of leaf particles in relation to chewing
SpecFragP4	Factor relating large particle fragilities to cud chews required
TotRuminP1	Factor relating amount of large particles in cud to required rumination time
VmaxAcAbRu	Maximum velocity of acetate absorption in the rumen

VmaxBaPo	Maximum velocity of amylolytic engulfment by protozoa
VmaxPrtAm	Maximum velocity of ammonia production from fermentation of protein
YAcCeBc	Yield of acetate from fermentation of cellulose by cellulolytic bacteria
YSpAm	Yield of ammonia from fermentation of soluble protein
YBaSp	Yield of amylolytic bacteria from growth with soluble protein as a nitrogen source
YLiFdLi	Yield of lipids into the rumen from lipids contained in feed
YPoCho	Yield of protozoa from growth with carbohydrates

The results of the global sensitivity to the outputs: acetate:propionate ratio, energy from absorbed VFA (EVFA), microbial protein passage rate, and methane production follow:

Acetate:propionate ratio

For the AUS diet, protozoal engulfment efficiency (EngEffPo) had the highest main and total effect (Figure 3.2.2). The yield of amylolytic bacteria from growth with soluble protein as a nitrogen source (YBaSp) had the highest interaction. When interactions were expressed as a percentage of total effects, the factor relating the amount of large particles in cud to the required rumination time (TotRuminP1) was the highest (93%).

For the US diet, the yield of protozoa from growth with carbohydrates (YPoCho) (Figure 3.2.2) had the highest main effect and the highest total effect. In terms of raw interactions, the inhibition of acetate absorption due to pH effects (JpHAcAb) had the highest effect. When interactions were expressed as a percentage of total effects, again the factor TotRuminP1 was the highest (93%).

For the forage-based NZ diet, the yield of acetate from fermentation of cellulose by cellulolytic bacteria (YAcCeBc) (Figure 3.2.2) had the highest main and total effect. The highest interaction was from YBaSp, The term representing the fraction of small grain particles exiting in rumen fluid (FracOutP7) had the highest percentage of interactions (98%).

In general, there was little interaction present for most terms, indicating that the majority of sensitivity of these measures was due to independent main effects. The yield of protozoal growth from carbohydrates (YPoCho) was relatively important across all three diets. While not the highest term, the maximum velocity of protozoal engulfment of amylolytic bacteria by protozoa (VmaxBaPo) was also important across all diets. Particle degradation and rumination terms were also important, especially in terms of interaction.

Energy from absorbed VFA

For the AUS diet, the highest main effect came from YBaSp (Figure 3.2.3). The highest total effect and highest interaction effect was JpHAcAb, which is about equally split between main effects and interactions. The Michaelis-Menten constant for protozoal engulfment by other protozoa (MMPoPo) had the highest (96%) interaction effect as a percentage of the total effect.



Figure 3.2.2 Global sensitivity analysis for main effects and interactions of acetate:propionate ratio (Top to bottom: AUS, US, NZ)

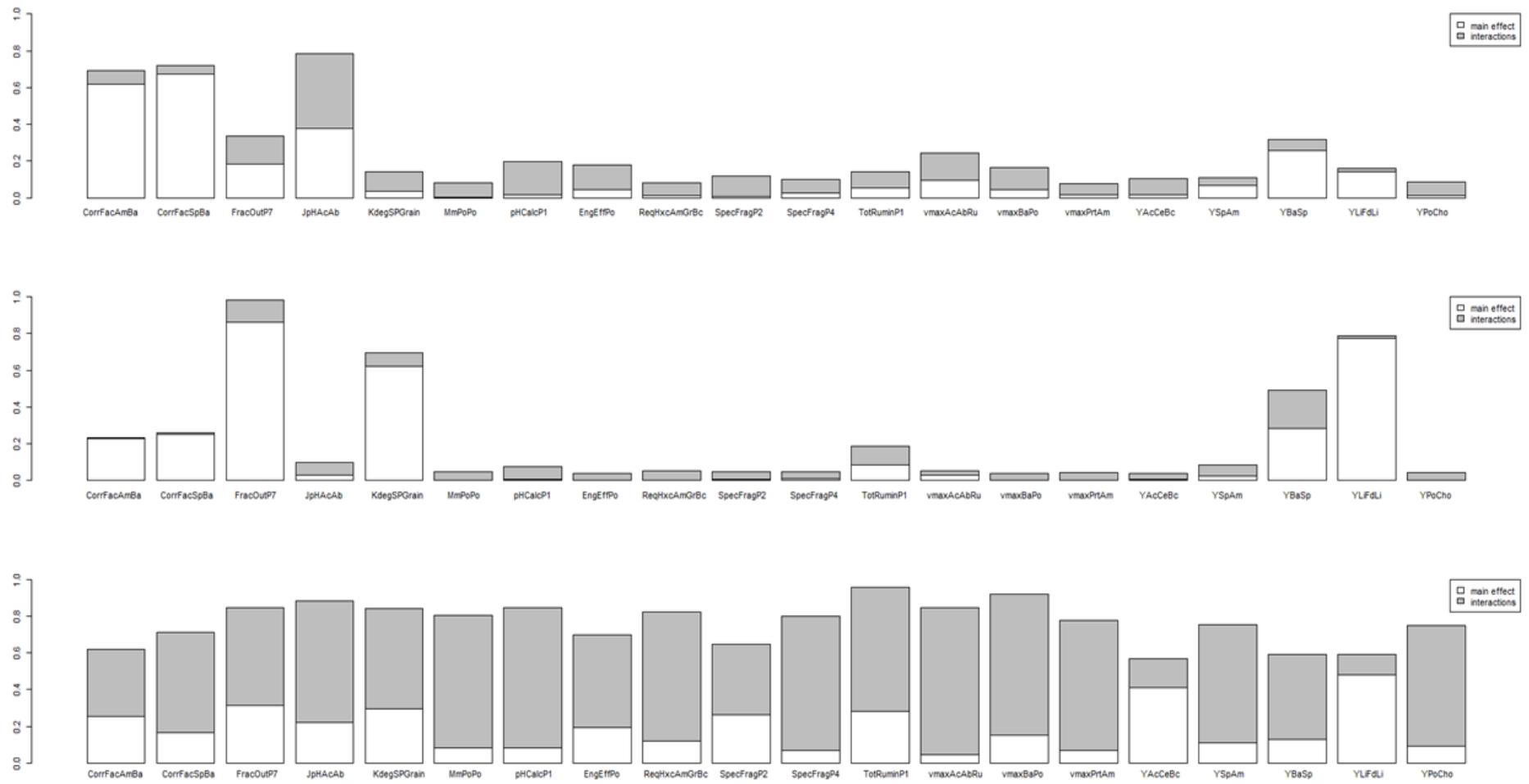


Figure 3.2.3 Global Sensitivity analysis for main effects and interactions of Energy from absorbed VFA (Top to bottom: AUS, US, NZ)

For the US diet, FracOutP7 (Figure 3.2.3) had the highest main and total effects; microbial degradation rate for small grain particles (KdegSPgrain) and the yield of lipids from lipids in feed (YLiFdLi) also ranked highly. YBaSp had the highest degree of interaction

For the forage-based NZ diet (Figure 3.2.3), most of the parameters display a high degree of interaction, with only two parameters, YAcCeBc and YLiFdLi, having less than 50% interaction. The maximum velocity of acetate absorption in the rumen (VmaxAcAbRu) had both the highest interaction and the highest percentage of interaction of total effects (95%).

Across all three diets, EVFA displays significantly more interaction between terms, particularly on the NZ diet, where most of the terms have significant interaction. There were also more highly sensitive terms than seen when looking at the acetate:propionate ratio. For the barley-based US diet, the adjustment factor for ammonia uptake by amylolytic bacteria (CorrFacAmBa) and the adjustment factor for soluble protein uptake by amylolytic bacteria (CorrFacSpBa) were more sensitive (main effect >0.6) than on the other two diets, whereas the US diet had the highest main effect on terms dealing with grain. The NZ diet had the highest total sensitivities, but the majority of these were due to interactions, perhaps indicating different mechanisms in play on forage diets as compared to concentrate-based feedlot diets.

Microbial Protein passage rate

For the AUS diet, the highest main and total effects come from YBaSp (Figure 3.2.4). The highest amount of interaction comes from JpHAcAb. The VmaxPrtAm parameter had the highest interaction effect as a percentage of the total effect.

Overall, the US diet had relatively little interaction between terms. The highest main and total effects came from YBaSp (Figure 3.2.4). The highest amount of interaction came from JpHAcAb. The VmaxPrtAm had the highest interaction effect as a percentage of the total effect.

The highest main and total effects of the NZ diet come from YBaSp (Figure 3.2.4). The YBaSp accounts for the highest main and total effects, and JpHAcAb had the highest interaction. The VmaxAcAbRu parameter had the highest interaction effect as a percentage of the total effect.

Similar to the acetate:propionate ratio, the majority of terms for microbial protein exit quantities have more main effect than interaction, with few parameters having large main effects. Across all three diets, YBaSp has both the highest main effect and highest total effect. The JpHAcAb parameter had the highest interaction across all diets.

Methane production

For the AUS diet, CorrFacSpBa (Figure 3.2.5), which is an offset factor for soluble protein uptake by amylolytic bacteria had the highest amount of main and total effects. The highest interaction came from JpHAcAb. The highest percentage of interaction came from pHCalcP1, the offset factor for rumen pH calculation.



Figure 3.2.4 Global Sensitivity analysis for main effects and interactions of microbial protein (Top to bottom: AUS, US, NZ)

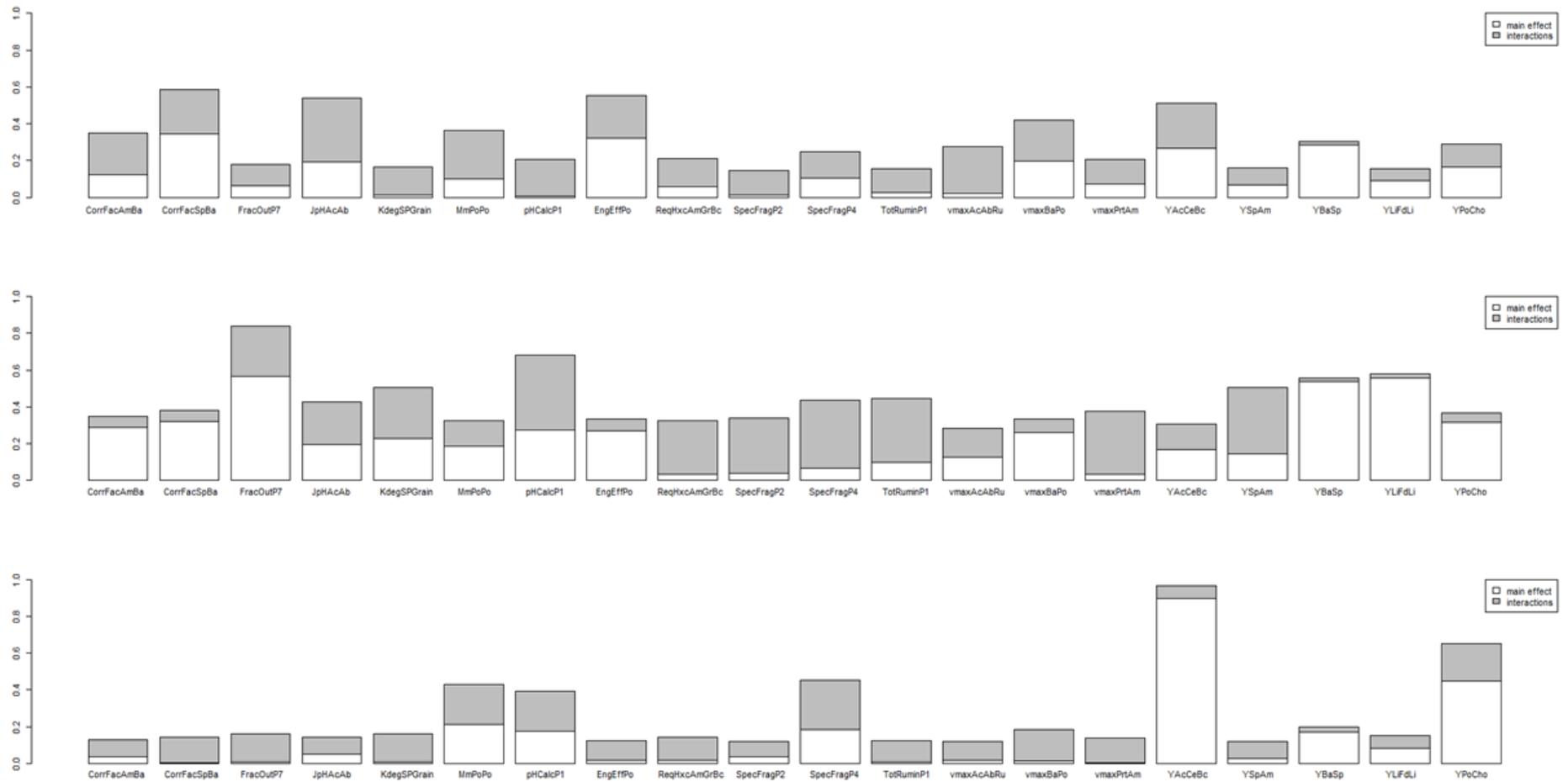


Figure 3.2.5 Global Sensitivity analysis for main effects and interactions of methane production (Top to bottom: AUS, US, NZ)

For the US diet, FracOutP7 (Figure 3.2.5), the fraction of small grain particles exiting rumen fluid, had the highest main and total effects. The highest quantity of interaction came from the pH calculation offset term. The VmaxPrtAm parameter had the highest percentage of interactions.

The forage-based NZ diet had the highest main and total effects from YAcCeBc (Figure 3.2.5). The highest interaction amount comes from the factor relating large feed particles to the number of cud chews required (SpecFragP4). The CorrFacSpBa parameter had the highest percentage of interaction.

As in EVFA, CH₄ production had many terms with a large amount of interaction, as well as many terms with intermediate sensitivities across all diets. The majority of important terms are those related to processes that affect ruminal hydrogen balance, such as soluble protein utilization or VFA production.

3.2.5. *Report on the effects of methane inhibition on fermentation array and nutrient supply completed (in-vitro and in-vivo) and prepared as draft conference and refereed journal papers.*

While the H₂ recovery is typically >95% in the unperturbed rumen, introduction of methane inhibitors can dramatically reduce this efficacy, indirectly H₂ is either not produced by conventional pathways or is used by other reductive processes (H₂ sinks). Thermodynamics was not tested in these studies but there was some evidence of potential reductive acetogenesis across all studies, with distinct increases in acetate concentration while also reducing methane production (Table 3.1.2.1).

It was also apparent that the direct effect of mitigation do not support 'hydrogen starvation' of methanogenesis as being the direct cause of mitigation, and this is consistent with addition of the additional H₂ acceptor leading to increased H₂ accumulation (Janssen 2012). Certainly this was evident in a large number of the in-vitro studies undertaken with addition of nitrate and nitrite, where a sustained period of H₂ gas accumulation followed NO₃ and NO₂ addition.

Nitrate and canola oil are synergistic in reducing methanogenesis in cattle

There was no difference in daily DMI between treatments ($P = 0.51$; Table 3.2.2). In combination however, NO₃ and oil caused a larger reduction in MY (6.82 g/kg DMI) than the sum of their individual contributions without compromising DMI or total VFA concentration.

Table 3.2.2: Dry matter intake (DMI), methane yield, total VFA concentration and VFA molar percentages in rumen fluid of cannulated steers fed: 1) a control diet (CON: 7.5 kg air dry; 40% of lucerne chaff and 60% barley grain; 2) nitrate diet (NO3: CON + 5% calcium nitrate; 3) oil diet (OIL: CON+5% canola oil) and 4) nitrate plus oil diet (NO3+OIL: CON with 5% canola oil plus 5% calcium nitrate)

Item					Treatment	
	CON	NO3	OIL	NO3-OIL	SEM	P-value
DMI, (kg)	6.75	6.25	6.75	6.34	0.14	0.51
Methane Yield (g/kg DMI)	23.6 ^a	20.1 ^b	22.4 ^{ab}	16.8 ^c	0.72	< 0.01
pH	6.24 ^a	6.27 ^a	5.97 ^b	6.40 ^a	0.03	< 0.01
Total VFA (mmol/l)	104	99.5	106.5	100.1	1.61	0.3
VFA (molar %)						
Acetate	62.5	67.6	58.7	64.1	1.09	0.45
Propionate	16.6 ^a	15.2 ^{ac}	19.6 ^b	15.8 ^{acd}	0.46	< 0.01
Butyrate	17.1 ^a	13.4 ^b	17.0	16.3 ^{ac}	0.39	< 0.01
Acetate:propionate ratio	3.77 ^a	4.43 ^b	3.00 ^c	4.04 ^a	0.09	< 0.01

^{a-d} Within a row, means without a common superscript letter differ, $P < 0.05$

3.3 Activity 3. Impacts of CFI methodologies on whole-farm systems

The main results from this activity were the *Whole-farm model capable of allowing for the impacts of mitigation on animal enterprise productivity available to science, policy and producer community*, and *Case studies showing long-term productivity and emission responses to methane mitigation and nitrous oxide emissions at an enterprise level*. The following sections provide the results obtained:

3.3.1. *Whole-farm model capable of allowing for the impacts of mitigation on animal enterprise productivity available to science, policy and producer community.*

and

3.3.2. *Case studies showing long-term productivity and emission responses to methane mitigation and nitrous oxide emissions at an enterprise level.*

A combined report on both the impacts of mitigation on animal enterprise productivity available to science, policy and producer community (3.3.1) and case studies showing long-term productivity and emission responses to methane mitigation and nitrous oxide emissions at an enterprise level (3.3.2) are reported in the following section.

Sheep production

These studies report 25 year simulations of wether and 30 year simulations of ewe flocks at Holbrook (NSW) and ewes in New Zealand, with statistical analyses of treatment effects based on replication over years.

Holbrook wethers

Long-term productivity effects

There was no significant difference ($P > 0.05$) between baseline and NO_3 for DMI, however there was a significant difference ($P < 0.05$) between baseline and lipid and lipidx NO_3 for DMI. There was no significant difference ($P > 0.05$) for sale of LW (kg) or LW gain (g/d). There was a significant difference ($P < 0.01$) between baseline and lipidx NO_3 for wool (kg) but no significant effects ($P > 0.05$) between baseline and NO_3 and lipid. There was a significant difference ($P < 0.01$) between baseline and all mitigation strategies for CH_4 (g/d) and CH_4 yield (g/kg DMI). There was no significant ($P > 0.05$) difference between NO_3 and lipid for LWG CH_4 production (g/kg LWG) and wool CH_4 production (g/kg wool), however significant differences ($P < 0.01$) were found between baseline, NO_3 , lipid, and lipidx NO_3 for LWG CH_4 production (g/kg LWG) and wool CH_4 production (g/kg wool). No significant difference ($P > 0.05$) between baseline and NO_3 mitigation for pH, however a significant difference ($P < 0.01$) was found between baseline, lipid, and lipidx NO_3 for pH. There was no significant difference ($P > 0.05$) between baseline and NO_3 for Ac, Pr, Bu (%) and Ac:Pr ratio, however there was a significant effect ($P > 0.01$) between baseline, lipid and lipidx NO_3 for Ac, Pr, Bu (%), and Ac:Pr ratio. No significant difference ($P > 0.05$) between baseline and all mitigation strategies for microbial protein output (g/d), however there was a significant difference ($P < 0.05$) between NO_3 and lipid for microbial protein output (g/d). There was no significant difference ($P > 0.05$) between baseline and NO_3 for VFA energy (MJ/d), however there was a significant difference ($P < 0.01$) between baseline and lipid and lipidx NO_3 for VFA energy (MJ/d) (Table 3.3.1). Table 3.3.4 provides concentration values of Ac, Pr, and Bu.

Emissions responses to methane mitigation

No significant differences ($P > 0.05$) for atmospheric leaching of N_2O (t CO_2) and N_2O from stock (t CO_2) for mitigation strategies. There was a significant difference ($P < 0.01$) between baseline and NO_3 and lipidx NO_3 for CH_4 (t CO_2 /ha) emissions and no significant difference ($P > 0.05$) between NO_3 and lipid. No significant differences ($P > 0.05$) for N_2O (t CO_2 /ha) were found. There were significant differences ($P < 0.01$) for total GHG (t CO_2 /ha) emissions between baseline and NO_3 and lipidx NO_3 but no significant difference ($P > 0.05$) between NO_3 and lipid (Table 3.3.1).

Table 3.3.1. Mean values of AusFarm simulations across 21 years[§] of Holbrook wethers purchased on 18th April and sold after a 2 year period on 17th April for baseline and mitigation strategies [Nitrate (NO₃), Canola seed (Lipid), LipidxNO₃]

Item	Baseline [%]	Mitigation strategies			SEM	Mitigation P-value
		NO ₃ [%]	Lipid ^{%%}	LipidxNO ₃ ^{%%}		
DMI^{&} (kg/d)	1.33 ^a	1.33 ^a	1.25 ^b	1.25 ^b	0.029	< 0.01
Sale LW⁺ (kg)	65.59	67.29	67.96	69.49	2.38	0.43
LW gain[*] (g/d)	57.62	59.09	59.57	61.28	50.21	0.91
Wool (kg)	6.37 ^A	6.59	6.75	6.97 ^B	0.18	< 0.01
CH₄ (g/d)	28.18 ^A	22.31 ^B	23.70 ^B	17.77 ^C	0.60	< 0.01
CH₄ yield (g/kg DMI)	21.06 ^A	16.65 ^B	17.25 ^C	12.92 ^D	0.13	< 0.01
CH₄ prod (g/kg LWG)	525.24 ^A	399.64 ^B	417.96 ^B	302.36 ^C	28.57	< 0.01
CH₄ prod⁺⁺ (kg/kg wool)	1.63 ^A	1.25 ^B	1.29 ^B	0.94 ^C	0.050	< 0.01
pH	6.64 ^A	6.64 ^A	6.69 ^B	6.69 ^B	0.0067	< 0.01
Ac (%)	61.75 ^A	61.76 ^A	59.17 ^B	59.19 ^B	0.44	< 0.01
Pr (%)	24.76 ^A	24.74 ^A	27.11 ^B	27.09 ^B	0.38	< 0.01
Bu (%)	13.49 ^A	13.50 ^A	13.71 ^B	13.71 ^B	0.061	< 0.01
Ac:Pr	2.50 ^A	2.50 ^A	2.19 ^B	2.19 ^B	0.045	< 0.01
Micr. Prt. Out^{##} (g/d)	13.55	13.84 ^a	12.69 ^b	12.92	0.39	< 0.05
VFA energy (MJ/d)	5.71 ^A	5.76 ^A	5.21 ^B	5.25 ^B	0.108	< 0.01
Atmos^{**} N₂O (t CO₂)	66.56	66.87	69.98	70.21	1.59	< 0.05
Stock^{***} N₂O (t CO₂)	147.40	148.06	154.06	154.55	3.44	0.07
CH₄ (t CO₂/ha)	2.11 ^A	1.67 ^B	1.77 ^B	1.33 ^c	0.045	< 0.01
N₂O (t CO₂/ha)	0.21	0.21	0.22	0.22	0.005	0.06
GHG (t CO₂/ha)	2.32 ^A	1.88 ^B	2.00 ^B	1.55 ^c	0.050	< 0.01

^{a-c} Within a row, means without a common superscript letter differ, P < 0.05

^{A-D} Within a row, means without a common superscript letter differ, P < 0.01

[&]The DMI is pasture only, supplementation was as follows 0.01, 0.01, 0.12, 0.12 (kg/d) for baseline, NO₃, lipid and lipidxNO₃ mitigation strategies, respectively

[§]Mean value of the yearly mean (years 1995, 1998, 2003, 2004, 2006, 2007, 2009, 2010, and 2013 were removed because of low LW gains)

[%]Lupins supplement averaged over the year but only provided 40 g/head/day between 1st March and 15th May

^{%%}Canola seed supplement supplied all year round

⁺Average of stock being sold

^{*}Average of all stock

[#]Volatile fatty acid (VFA): acetate (Ac), propionate (Pr), and butyrate (Bu)

^{##}Microbial protein output

^{**}Atmospheric leaching of nitrous oxide (N₂O) without run off

^{***}Faeces and urine

⁺⁺Methane production over 365 days

Holbrook ewes

Long-term productivity effects

There was no significant difference ($P > 0.05$) between strategies for DMI. There was a significant difference ($P < 0.01$) between baseline and lipidxNO₃ for sale of LW (kg) or LW gain (g/d) but no significant difference ($P < 0.05$) between NO₃ and lipid. There was no significant difference ($P > 0.05$) between strategies for lambing rate. There was a significant difference ($P < 0.05$) between baseline and lipidxNO₃ for wool (kg) but no significant effects ($P > 0.05$) between baseline and NO₃ and lipid. There was a significant difference ($P < 0.01$) between baseline and NO₃ and lipidxNO₃ for CH₄ (g/d) and a significant effect ($P < 0.05$) between NO₃ and lipid. There was a significant difference ($P < 0.01$) between baseline and all mitigation strategies for CH₄ yield (g/kg DMI). There was a significant difference ($P < 0.01$) between baseline and NO₃ and lipidxNO₃ for LWG CH₄ production (g/kg LWG) and no significant difference between NO₃ and lipid for LWG CH₄ production (g/kg LWG). There was a significant difference ($P < 0.01$) between baseline and all mitigation strategies for wool CH₄ production (g/kg wool). No significant difference ($P > 0.05$) between baseline and NO₃ mitigation for pH, however a significant difference ($P < 0.01$) was found between baseline, lipid, and lipidxNO₃ for pH. There was no significant difference ($P > 0.05$) between baseline and NO₃ for Ac, and Pr(%), however there was a significant effect ($P > 0.01$) between baseline, lipid and lipidxNO₃ for Ac, and Pr(%). There was no significant difference ($P > 0.05$) between baseline and all mitigation strategies for Bu (%), however there was a significant difference ($P < 0.05$) between NO₃ and lipidxNO₃ for Bu (%). There was no significant difference ($P > 0.05$) between baseline and NO₃ for Ac:Pr ratio, however there was a significant effect ($P > 0.01$) between baseline, lipid and lipidxNO₃ for Ac:Pr ratio. There were no significant differences ($P > 0.05$) between baseline and all mitigation strategies for microbial protein output (g/d) and VFA energy (MJ/d) (Table 3.3.2). Table 3.3.4 provides concentration values of Ac, Pr, and Bu.

Emissions responses to methane mitigation

No significant differences ($P > 0.05$) for atmospheric leaching of N₂O (t CO₂) and N₂O from stock (t CO₂) for mitigation strategies. There was a significant difference ($P < 0.01$) between baseline and NO₃, lipid, and lipidxNO₃ for CH₄ (t CO₂/ha) emissions and a significant difference ($P < 0.05$) between NO₃ and lipid. No significant differences ($P > 0.05$) for N₂O (t CO₂/ha) were found. There were significant differences ($P < 0.01$) for total GHG (t CO₂/ha) emissions between baseline and NO₃ and lipidxNO₃ but no significant difference ($P > 0.05$) between NO₃ and lipid (Table 3.3.2).

Table 3.3.2. Mean values of AusFarm simulations across 30 years[§] of Holbrook self-replacing ewes on the 1st January and stock aged 5 to 6 years sold on 31st December for baseline and mitigation strategies [Nitrate (NO₃), Canola seed (Lipid), LipidxNO₃]

Item	Baseline [%]	Mitigation strategies			SEM	Mitigation
		NO ₃ [%]	Lipid ^{%%}	LipidxNO ₃ ^{%%}		P-value
DMI^{&} (kg/d)	1.11	1.12	1.06	1.06	0.035	0.14
Sale LW⁺ (kg)	63.03 ^A	64.29	65.21	66.70 ^B	1.15	< 0.05
LW gain[*] (g/d)	114.99 ^A	117.57	119.44	122.50 ^B	2.35	< 0.05
Lambing rate	1.24	1.27	1.28	1.26	0.066	0.92
Wool (kg)	7.31 ^a	7.57	7.73	8.00 ^b	0.25	< 0.05
CH₄ (g/d)	24.22 ^A	19.01 ^{B,a}	20.84 ^{B,b}	15.35 ^C	0.70	< 0.01
CH₄ yield (g/kg DMI)	21.42 ^A	16.78 ^B	17.95 ^C	13.23 ^D	0.16	< 0.01
CH₄ prod (g/kg LWG)	177.09 ^{A,a}	133.66 ^{B,b}	145.30 ^b	103.73 ^{B,c}	10.89	< 0.01
CH₄ prod (kg/kg wool)	1.21 ^A	0.92 ^B	0.99 ^C	0.70 ^D	0.018	< 0.01
pH	6.59 ^A	6.59 ^A	6.65 ^B	6.64 ^B	0.009	< 0.01
Ac (%)	58.70 ^A	58.96 ^A	56.69 ^B	56.56 ^B	0.53	< 0.01
Pr (%)	27.41 ^A	27.19 ^A	29.27 ^B	29.37 ^B	0.45	< 0.01
Bu (%)	13.89	13.86 ^a	14.04	14.07 ^b	0.073	< 0.01
Ac:Pr	2.16 ^A	2.18 ^A	1.94 ^B	1.94 ^B	0.052	< 0.01
Micr. Prt. Out^{##} (g/d)	10.92	11.08	10.36	10.46	0.243	0.27
VFA energy (MJ/d)	4.96	5.00	4.57	4.62	0.17	< 0.05
Atmos^{**} N₂O (t CO₂)	62.33	62.40	64.78	66.03	2.87	0.49
Stock^{***} N₂O (t CO₂)	135.92	136.25	140.84	143.36	6.00	0.54
CH₄ (t CO₂/ha)	1.83 ^A	1.44 ^{B,a}	1.58 ^{C,b}	1.16 ^D	0.53	< 0.01
N₂O (t CO₂/ha)	0.20	0.20	0.21	0.21	0.009	0.52
GHG (t CO₂/ha)	2.03 ^A	1.64 ^B	1.78 ^B	1.37 ^C	0.056	< 0.01

^{a-c} Within a row, means without a common superscript letter differ, P < 0.05

^{A-D} Within a row, means without a common superscript letter differ, P < 0.01

[&]The DMI is pasture only, supplementation was as follows 0.02, 0.02, 0.10, 0.10 (kg/d) for baseline, NO₃, lipid and lipidxNO₃ mitigation strategies, respectively

[§]Mean value of the yearly mean;

[%]Lupins supplement averaged over the year but only provided 40 g/head/day between 1st March and 15th May

^{%%}Canola seed supplement supplied all year round

⁺Average of stock being sold

^{*}Average of all stock

[#]Volatile fatty acid (VFA): acetate (Ac), propionate (Pr), and butyrate (Bu)

^{##}Microbial protein output

^{**}Atmospheric leaching of nitrous oxide (N₂O) without run off

^{***}Faeces and urine

New Zealand ewes

Long-term productivity effects

There was no significant difference ($P > 0.05$) between baseline and NO_3 for DMI, however there was a significant difference ($P < 0.05$) between baseline and lipid and lipidx NO_3 for DMI. There was no significant difference ($P > 0.05$) between baseline and lipid, however there were significant differences ($P < 0.01$) between baseline and lipidx NO_3 for sale of LW (kg) and LW gain (g/d) but no significant difference ($P > 0.05$) between NO_3 and lipid, however a significant ($P < 0.01$) effect between NO_3 and lipidx NO_3 . There was no significant difference ($P > 0.05$) between strategies for lambing rate. There was a significant difference ($P < 0.05$) between baseline and lipidx NO_3 for wool (kg) but no significant difference ($P > 0.05$) between NO_3 and lipid, however a significant ($P < 0.01$) effect between NO_3 and lipidx NO_3 . There was a significant difference ($P < 0.01$) between baseline and all mitigation strategies for CH_4 (g/d) and CH_4 yield (g/kg DMI). There was a significant difference ($P < 0.01$) between baseline and NO_3 and lipidx NO_3 for LWG CH_4 production (g/kg LWG) and no significant difference between NO_3 and lipid for LWG CH_4 production (g/kg LWG). There was a significant difference ($P < 0.01$) between baseline and all mitigation strategies for wool CH_4 production (g/kg wool). There was no significant difference ($P > 0.05$) between strategies for pH, Ac, Pr, Bu (%), Ac:Pr ratio, microbial protein output (g/d) and VFA energy (MJ/d) (Table 3.3.3). Table 3.3.4 provides concentration values of Ac, Pr, and Bu.

Emissions responses to methane mitigation

No significant differences ($P > 0.05$) for atmospheric leaching of N_2O (t CO_2) and N_2O from stock (t CO_2) for mitigation strategies. There was a significant difference ($P < 0.01$) between all mitigation strategies for CH_4 (t CO_2/ha) emissions. No significant differences ($P > 0.05$) for N_2O (t CO_2/ha) were found. There was a significant difference ($P < 0.01$) between all mitigation strategies for total GHG (t CO_2/ha) (Table 3.3.3).

Table 3.3.3. Mean values of AusFarm simulations across 30 years[§] of New Zealand ewes purchased on the 1st January at 7 months of age and sold at 6 to 7 years on 15th January for baseline and mitigation strategies [Nitrate (NO_3), Canola seed (Lipid), Lipidx NO_3]

Item	Baseline%	Mitigation strategies			SEM	Mitigation P-value
		$\text{NO}_3\%$	Lipid%%	Lipidx $\text{NO}_3\%$		
DMI^{&} (kg/d)	1.48 ^A	1.48 ^A	1.39 ^B	1.39 ^B	0.022	< 0.01
Sale LW⁺ (kg)	64.29 ^A	66.86 ^{A,B}	68.99 ^{B,C}	70.66 ^C	1.052	< 0.01
LW gain[*] (g/d)	321.20 ^A	335.51 ^{A,B}	347.45 ^{B,C}	356.78 ^C	5.88	< 0.01
Lambing rate	1.48	1.48	1.62	1.55	0.069	0.14
Wool (kg)	4.76 ^A	4.91 ^{A,B}	5.17 ^{B,C}	5.32 ^C	0.108	< 0.01
CH₄ (g/d)	35.36 ^A	27.10 ^B	33.27 ^C	24.72 ^D	0.46	< 0.01
CH₄ yield (g/kg DMI)	23.74 ^A	18.18 ^B	21.77 ^C	16.16 ^D	0.11	< 0.01
CH₄ prod (g/kg LWG)	77.41 ^A	56.59 ^B	61.00 ^B	46.11 ^C	3.20	< 0.01
CH₄ prod (kg/kg wool)	2.72 ^A	2.02 ^B	2.36 ^C	1.70 ^D	0.032	< 0.01
pH	6.27	6.27	6.28	6.29	0.025	0.75
Ac (%)	51.56	51.58	50.38	50.41	0.62	0.07
Pr (%)	33.60	33.59	34.66	34.64	0.47	< 0.05
Bu (%)	14.84	14.84	14.96	14.95	0.15	0.76
Ac:Pr	1.54	1.55	1.46	1.46	0.04	< 0.05
Micr. Prt. Out^{##} (g/d)	19.31	19.31	19.74	19.80	0.47	0.60
VFA energy (MJ/d)	7.93	7.95	7.77	7.76	0.21	0.71
Atmos^{**} N₂O (t CO_2)	113.61	107.46	106.09	103.81	8.30	0.68
Stock^{***} N₂O (t CO_2)	233.73	222.95	220.15	215.60	16.62	0.73
CH₄ (t CO_2/ha)	2.68 ^A	2.05 ^B	2.52 ^C	1.87 ^D	0.035	< 0.01
N₂O (t CO_2/ha)	0.35	0.33	0.33	0.32	0.025	0.72
GHG (t CO_2/ha)	3.03 ^A	2.38 ^B	2.85 ^C	2.19 ^D	0.052	< 0.01

^{A-D} Within a row, means without a common superscript letter differ, $P < 0.01$

[&]The DMI is pasture only, supplementation was as follows 0.01, 0.02, 0.14, 0.14 (kg/d) for baseline, NO_3 , lipid and lipidx NO_3 mitigation strategies, respectively

§Mean value of the yearly mean;

%Rye grass hay supplement averaged over the year but only provided 73 g/head/day between 1st May and 31st July

%%Canola seed supplement supplied all year round

+Average of stock being sold

*Average of all stock

#Volatile fatty acid (VFA): acetate (Ac), propionate (Pr), and butyrate (Bu)

##Microbial protein output

**Atmospheric leaching of nitrous oxide (N₂O) without run off

***Faeces and urine

Table 3.3.4. Concentrations of VFA calculated from empirical relationships for Holbrook wethers, Holbrook ewes and New Zealand ewes.

Item	Baseline%	Treatments		
		NO ₃ %	Lipid%%	LipidxNO ₃ %%
Holbrook wethers				
Ac (mmol/l)	58.26	58.27	58.54	58.55
Pr (mmol/l)	17.59	17.60	17.75	17.76
Bu (mmol/l)	7.65	7.66	7.67	7.67
Total VFA# (mmol/l)	83.50	83.52	83.96	83.98
Holbrook ewes				
Ac (mmol/l)	57.35	57.38	57.59	57.59
Pr (mmol/l)	17.07	17.08	17.21	17.21
Bu (mmol/l)	7.61	7.61	7.62	7.62
Total VFA# (mmol/l)	82.03	82.07	82.42	82.42
New Zealand ewes				
Ac (mmol/l)	59.49	59.49	59.66	59.67
Pr (mmol/l)	18.30	18.30	18.39	18.40
Bu (mmol/l)	7.71	7.71	7.72	7.72
Total VFA# (mmol/l)	85.50	85.50	85.78	85.79

#Volatile fatty acid (VFA): acetate (Ac), propionate (Pr), and butyrate (Bu)

Beef production

Simulation of emission and performance of beef cattle in Australia (Hollbrook NSW) and the USA (California) were conducted over 30 years variable climate and comparison of treatment effects within each site based on replication over years.

Holbrook steers

Long-term productivity effects

There was no significant difference ($P > 0.05$) between all mitigation strategies for DMI. There was no significant difference ($P > 0.05$) between baseline and NO₃ and a significant difference ($P < 0.01$) between baseline and lipid and lipidxNO₃ for final LW (kg) and LW gain (kg/d). There was no significant difference ($P > 0.05$) between baseline NO₃ and lipid, however there was a significant difference ($P < 0.01$) between baseline and lipidxNO₃ and a significant difference ($P < 0.05$) between lipid and lipidxNO₃ for CH₄ (g/d). There was a significant difference ($P < 0.01$) between baseline and NO₃, and lipidxNO₃ and no significant difference ($P > 0.05$) between NO₃, and lipid for CH₄ yield (g/kg DMI). There was a significant ($P < 0.05$) difference between baseline and NO₃ and there was a significant difference ($P < 0.01$) between baseline and lipid and lipidxNO₃ for LWG CH₄ production (g/kg LWG). No significant difference ($P > 0.05$) between baseline and all mitigation strategies for pH. There was no significant difference ($P > 0.05$) between baseline and NO₃, however there was a significant difference ($P < 0.01$) between baseline, lipid and lipidxNO₃ for the concentration of Ac (mmol/l). There was no significant difference ($P > 0.05$) between all mitigation strategies for Pr (mmol/l). There was no significant difference ($P > 0.05$) between baseline and NO₃, however there was a significant difference ($P < 0.01$) between baseline, lipid and lipidxNO₃ for the concentration of Bu (mmol/l) and total VFA (mmol/l). There was no significant difference ($P < 0.05$) between baseline and NO₃ and lipid, however there was a significant difference ($P < 0.05$) between baseline and lipidxNO₃ for Ac (%). There was no significant difference ($P > 0.05$) between all mitigation strategies for Pr (%), Bu (%), Ac:Pr ratio, microbial protein output (g/d) and VFA energy (MJ/d) (Table 3.3.5).

Emissions responses to methane mitigation

There was a significant difference ($P < 0.05$) between baseline and NO₃ and a significant difference ($P < 0.01$) between baseline and lipid and lipidxNO₃ and N₂O, there was also a significant difference ($P < 0.05$) between lipid and lipidxNO₃ for atmospheric leaching of N₂O (t CO₂). There was no significant difference ($P > 0.05$) between baseline and NO₃ and lipid, however there was a significant difference ($P < 0.01$)

between baseline and lipidxNO₃ and a significant difference ($P < 0.05$) between lipid and lipidxNO₃ for stock N₂O (t CO₂/ha). There was no significant difference ($P > 0.05$) between baseline and NO₃ and lipid, however there was a significant difference ($P < 0.01$) between baseline and lipidxNO₃ and a significant difference ($P < 0.05$) between lipid and lipidxNO₃ for CH₄ (t CO₂/ha) emissions. There was no significant difference ($P > 0.05$) between baseline and NO₃ and lipid, however there was a significant difference ($P < 0.01$) between baseline and lipidxNO₃ and a significant difference ($P < 0.05$) between NO₃ and lipidxNO₃ for N₂O (t CO₂/ha). There was no significant difference ($P < 0.05$) between baseline and NO₃ and lipid, however there was a significant difference ($P < 0.05$) between baseline and lipidxNO₃ for total GHG (t CO₂/ha) emissions (Table 3.3.5).

Table 3.3.5. Mean values across 30 years[§] from 1st May to 31st December of AusFarm simulations for baseline and mitigation strategies [Nitrate (NO₃), Canola seed (Lipid), LipidxNO₃) of Holbrook steers

Item	Baseline	Mitigation strategies			SEM	Mitigation P-value
		NO ₃	Lipid	LipidxNO ₃		
DMI^{&} (kg/d)	7.65	7.66	7.50	7.51	0.28	0.96
Final LW (kg)	449.55 ^A	453.77 ^A	516.93 ^B	519.11 ^B	15.35	< 0.01
LW gain (kg/d)	0.90 ^A	0.92 ^A	1.18 ^B	1.19 ^B	0.063	< 0.01
CH₄ (g/d)	194.37 ^A	172.67	183.29 ^a	160.11 ^{B,b}	8.39	< 0.01
CH₄ yield (g/kg DMI)	25.51 ^A	22.68 ^B	22.18 ^B	19.38 ^C	0.28	< 0.01
CH₄ prod (g/kg LWG)	227.94 ^{A,a}	198.82 ^{A,b}	157.86 ^B	136.96 ^B	10.53	< 0.01
pH	6.31	6.33	6.34	6.35	0.028	0.44
Ac⁺ (mmol/l)	74.74 ^A	74.40 ^A	64.52 ^B	64.35 ^B	2.80	< 0.01
Pr⁺ (mmol/l)	15.73	15.81	13.81	13.85	0.87	0.023
Bu⁺ (mmol/l)	20.61 ^A	20.49 ^A	17.84 ^B	17.80 ^B	0.47	< 0.01
Total VFA⁺ (mmol/l)	111.09 ^A	110.70 ^A	96.18 ^B	96.00 ^B	4.04	< 0.01
Ac⁺ (%)	67.23 ^a	67.16	67.04	66.98 ^b	0.094	< 0.05
Pr⁺ (%)	14.02	14.15	14.19	14.27	0.37	0.93
Bu⁺ (%)	18.75	18.69	18.78	18.75	0.43	1.00
Ac:Pr⁺	4.84	4.79	4.78	4.75	0.13	0.91
Micr. Prt. Out⁺⁺ (g/d)	465.81	492.48	467.28	488.00	31.50	0.76
VFA⁺ energy (MJ/d)	44.57	44.44	42.41	42.26	2.22	0.59
Atmos[*] N₂O (t CO₂)	46.45 ^{A,a}	51.64 ^{a,b}	54.60 ^{b,c}	60.39 ^{B,c}	2.94	< 0.01
Stock^{**} N₂O (t CO₂)	105.43 ^A	116.13 ^a	121.84	133.65 ^{B,b}	4.66	< 0.01
CH₄ (t CO₂/ha)	1.76 ^A	1.57	1.66 ^a	1.45 ^{B,b}	0.076	< 0.01
N₂O (t CO₂/ha)	0.15 ^A	0.17 ^a	0.18	0.19 ^{B,b}	0.0095	< 0.01
GHG (t CO₂/ha)	1.92 ^a	1.73	1.84	1.65 ^b	0.085	< 0.05

^{a-c} Within a row, means without a common superscript letter differ, $P < 0.05$

^{A-D} Within a row, means without a common superscript letter differ, $P < 0.01$

[&]The DMI is pasture only, supplementation was as follows 0, 0, 0.79, 0.79 (kg/d) for baseline, NO₃, lipid and lipidxNO₃ mitigation strategies, respectively

[§]Mean value of the yearly mean

⁺Volatile fatty acid (VFA): acetate (Ac), propionate (Pr), and butyrate (Bu)

⁺⁺Microbial protein output

^{*}Atmospheric leaching of nitrous oxide (N₂O) without run off

^{**}Faeces and urine

California steers

Long-term productivity effects

There was no significant difference ($P > 0.05$) between all mitigation strategies for DMI. There was no significant difference ($P > 0.05$) between baseline and NO_3 and a significant difference ($P < 0.01$) between baseline and lipid and lipidx NO_3 for final LW (kg) and LW gain (kg/d). There was a significant difference ($P < 0.01$) between baseline, NO_3 and lipidx NO_3 , however there was no significant difference ($P > 0.05$) between baseline and lipid and there was no significant difference ($P > 0.05$) between NO_3 and lipidx NO_3 but a significant difference ($P < 0.05$) between lipid and lipidx NO_3 for CH_4 (g/d). There was a significant difference ($P < 0.01$) between baseline and NO_3 , and lipidx NO_3 and a significant difference ($P < 0.05$) between NO_3 , and lipid for CH_4 yield (g/kg DMI). There was a significant ($P < 0.01$) difference between baseline, NO_3 and lipidx NO_3 and there was a significant difference ($P < 0.05$) between NO_3 and lipid for LWG CH_4 production (g/kg LWG). There was a significant difference ($P < 0.01$) between baseline, NO_3 and lipid and a significant difference ($P < 0.01$) between NO_3 and lipid for pH. There was no significant difference ($P > 0.05$) between baseline and NO_3 , however there was a significant difference ($P < 0.05$) between baseline and lipid and a significant difference ($P < 0.01$) between baseline and lipidx NO_3 for the concentration of Ac (mmol/l). There was no significant difference ($P > 0.05$) between baseline and NO_3 however there was a significant difference ($P < 0.05$) between baseline, lipid and lipidx NO_3 for Pr (mmol/l). There was no significant difference ($P > 0.05$) between baseline, NO_3 and lipid however there was a significant difference ($P < 0.05$) between baseline and lipidx NO_3 for the concentration of Bu (mmol/l). There was no significant difference ($P > 0.05$) between baseline and NO_3 however there was a significant difference ($P < 0.05$) between baseline and lipid and a significant difference ($P < 0.01$) between baseline and lipidx NO_3 for total VFA (mmol/l). There was no significant difference ($P > 0.05$) between baseline and NO_3 and lipid, however there was a significant difference ($P < 0.01$) between baseline and lipidx NO_3 for Ac (%). There was no significant difference ($P > 0.05$) between all mitigation strategies for Pr (%). There was no significant difference ($P > 0.05$) between baseline and all management strategies however there was a significant difference ($P < 0.01$) between NO_3 and lipid and lipidx NO_3 for Bu (%). There was no significant difference ($P > 0.05$) between all mitigation strategies for Ac:Pr ratio, microbial protein output (g/d) and VFA energy (MJ/d) (Table 3.3.6).

Emissions responses to methane mitigation

There was no significant difference ($P > 0.05$) between baseline, NO_3 , and lipid, however there was a significant difference ($P < 0.01$) between baseline and lipidx NO_3 for atmospheric leaching of N_2O (t CO_2). There was no significant difference ($P > 0.05$) between baseline, NO_3 , and lipid, however there was a significant difference ($P < 0.05$) between baseline and lipidx NO_3 for stock N_2O (t CO_2 /ha). There was a significant difference ($P > 0.01$) between baseline and NO_3 and lipidx NO_3 , and there was a significant difference ($P < 0.05$) between lipid and lipidx NO_3 for CH_4 (t CO_2 /ha) emissions. There was no significant difference ($P > 0.05$) between baseline, NO_3 , and lipid, however there was a significant difference ($P < 0.05$) between baseline and lipidx NO_3 for N_2O (t CO_2 /ha). There was a significant difference ($P < 0.05$) between baseline and NO_3 , no significant difference ($P < 0.05$) between baseline and lipid, however there was a significant difference ($P < 0.01$) between baseline and lipidx NO_3 for total GHG (t CO_2 /ha) emissions (Table 3.3.6).

Table 3.3.6. Mean values across 30 years[§] from 1st May to 31st December of AusFarm simulations for baseline and mitigation strategies [Nitrate (NO₃), Canola seed (Lipid), LipidxNO₃) of California steers

Item	Mitigation strategies				Mitigation	
	Baseline	NO ₃	Lipid	LipidxNO ₃	SEM	P-value
DMI^{&} (kg/d)	7.33	6.99	6.83	6.85	0.37	0.47
Final LW (kg)	414.97 ^A	413.53 ^A	440.44 ^B	445.22 ^B	7.47	< 0.01
LW gain (kg/d)	0.99 ^A	0.98 ^A	1.19 ^B	1.23 ^B	0.059	< 0.01
CH₄ (g/d)	167.32 ^A	134.93 ^B	153.36 ^{A,B,a}	129.59 ^{B,b}	8.75	< 0.01
CH₄ yield (g/kg DMI)	22.72 ^A	19.17 ^B	20.76 ^C	17.43 ^D	0.18	< 0.01
CH₄ prod (g/kg LWG)	170.20 ^A	138.23 ^{B,a}	128.18 ^{B,b}	104.60 ^C	3.77	< 0.01
pH	6.58 ^{A,C}	6.65 ^B	6.59 ^C	6.62	0.017	< 0.01
Ac⁺ (mmol/l)	75.28 ^{A,a}	71.08	67.21 ^b	65.91 ^B	3.00	< 0.01
Pr⁺ (mmol/l)	22.53 ^{A,a}	21.73 ^{a,c}	20.37 ^{b,c}	20.15 ^{B,b}	0.73	< 0.01
Bu⁺ (mmol/l)	13.66 ^a	12.76	12.35	12.09 ^b	0.58	< 0.05
Total VFA⁺ (mmol/l)	111.46 ^{A,a}	105.57	99.93 ^b	98.15 ^B	4.30	< 0.01
Ac⁺ (%)	67.47 ^A	67.26	67.20	67.08 ^B	0.12	< 0.01
Pr⁺ (%)	20.31	20.68	20.48	20.64	0.18	0.17
Bu⁺ (%)	12.22	12.06 ^A	12.33 ^B	12.28 ^B	0.072	< 0.01
Ac:Pr⁺	3.33	3.26	3.29	3.26	0.033	0.12
Micr. Prt. Out⁺⁺ (g/d)	570.76	594.68	560.98	591.86	30.10	0.66
VFA⁺ energy (MJ/d)	42.38	39.77	39.47	38.90	2.23	0.36
Atmos[*] N₂O (t CO₂)	0.94 ^A	1.00	1.00	1.10 ^B	0.056	< 0.01
Stock^{**} N₂O (t CO₂)	2.13 ^a	2.26	2.24	2.45 ^b	0.13	< 0.05
CH₄ (t CO₂/ha)	0.53 ^A	0.43 ^B	0.48 ^a	0.41 ^{B,b}	0.028	< 0.01
N₂O (t CO₂/ha)	0.07 ^a	0.07	0.07	0.08 ^b	0.004	< 0.05
GHG (t CO₂/ha)	0.60 ^{A,a}	0.50 ^b	0.56	0.49 ^B	0.032	< 0.01

^{a-c} Within a row, means without a common superscript letter differ, P < 0.05

^{A-D} Within a row, means without a common superscript letter differ, P < 0.01

[&]The DMI is pasture only, supplementation was as follows 0, 0, 0.53, 0.53 (kg/d) for baseline, NO₃, lipid and lipidxNO₃ mitigation strategies, respectively

[§]Mean value of the yearly mean

^{*}Volatile fatty acid (VFA): acetate (Ac), propionate (Pr), and butyrate (Bu)

⁺⁺Microbial protein output

^{*}Atmospheric leaching of nitrous oxide (N₂O) without run off

^{**}Faeces and urine

4. DISCUSSION

The key findings of this project and the implication and limitations for agriculture are discussed. This is quite different than the discussion which would be provided in a scientific paper. The discussion covers: (1) New AusFarm modules; (2) Log-term productivity effects; and (3) Long-term emission and productivity responses to methane mitigation.

4.1 New AusFarm modules

The development of a new AusFarm modules for both sheep and beef production will provide the sheep and beef industries with a tool to estimate CH₄ (g/d) and CH₄ yield (g/kg DMI) with a mechanistic model that also provides additional capacity to report the utilisation of energy (e.g., reporting of VFA and microbial protein outflow). These new modules also provide the opportunity for the industry to evaluate NO₃, lipid, and lipidxNO₃ supplementation on long-term production effects and emission responses to CH₄ mitigation that have the potential to not only reduce GHG emissions but improve productivity. The development of the new modules is a significant contribution to achieving the aim and expected outcome of this project.

Sheep production

The NZ sheep rumen model was integrated into a C++ module so that AusFarm sheep production simulations could be evaluated using a sheep rumen model. Modifications were made to the hydrogen sink equations to reduce the hydrogen pool in the presence of nitrate (i.e., 4 mols of H₂ are consumed per mole of nitrate). The development of this module involved an international engagement with NZ researchers that facilitated this development.

Beef production

The integration of the beef rumen model into AusFarm has involved the reengineering of an existing piece of software developed for the feedlot industry (i.e., AusBeef) into a package that can now be used by the grazing industry. The details of the model have been described in Section 3.2.4. The new AusFarm modules have the potential to be used by researchers to increase their understanding of the underlying mechanism in CH₄ production and explore new and novel ways to reduce CH₄ emissions and increase productivity.

The sensitivity analysis (3.2.4) has provided detail on the most sensitivity parameters in the AusBeef rumen model. For example, the most sensitive parameters were related to processes that affect ruminal hydrogen balance (i.e., soluble protein utilization or VFA production). The outcomes from the sensitivity analysis provides, for both modellers and researchers, information on the key drivers for not only CH₄ production but also Ac:Pr ratio, energy from absorbed VFA, and microbial protein passage rate.

The development of the nitrate models (3.2) has utilized michaelis-menton relationships for the nitrate modes that have explored the relationship of substrate utilization (i.e., reduction of nitrate to nitrite and nitrite to ammonia), the absorption through the rumen wall and the outflow from the rumen with rumen fluid. The development of the nitrate model has provided good interaction between modellers and researchers that have challenged our current understanding (personal communications. Emeritus Professor of UNE John Nolan).

The development of the lipid model included the process of bio-hydrogenation as an H₂ sink that had not previously been developed in the AusBeef model. It was assumed that bio-hydrogenation occurs at a rate which is a linear function of the pool size of unsaturated fat in the rumen. The rate of change of the saturated fat pool subtracts from the rate of change of the unsaturated fat pool and consumes H₂. This lipid model has been developed based on our current understanding.

4.2 Long-term productivity effects

The long-term productivity effects reported provide the Australian and overseas agricultural communities with information on the application of 3 mitigation strategies (NO₃, lipid, and lipidxNO₃) to reduce greenhouse gas emissions. The simulation case studies are a significant contribution to achieving the aim and expected outcome of this project. The following sections provide detail on sheep and beef production case studies.

Sheep production

Hoolbrook, NSW wether production

The long-term productivity effects for wool production over 21 years of variable weather data for wethers at Hoolbrook, New South Wales demonstrate that mitigation strategies for CH₄ production in relationship to wool production (kg/kg wool) over 365 days reduce CH₄ production (kg/kg wool) by 23, 21, and 42% of the baseline (i.e., control) value for NO₃, lipid, and lipidxNO₃, respectively. The highest reduction of 42% was from the lipidxNO₃ mitigation strategy a synergistic effect from both NO₃ and lipid supplementation, as was found in the final 4x4 cattle study and the lamb growth study (Nguyen et al., 2016a). The CH₄ production (kg/kg wool) reduction of 42% is a substantial reduction that would benefit the sheep industry if such a mitigation strategy was implemented. Sale of LW for the lipidxNO₃ mitigation strategy also increased by 6% and would increase profitability by \$4 per animal; wool production increased by 0.6kg and at \$12.91/kg clean (25th May 2016) this equates to \$7.75 per animal. On 1000 head a producer could potentially reduce CH₄ by 42% and increase productivity profitability by \$11,750.

Hoolbrook, NSW ewe self-replacing production

The long-term productivity effects for wool production over 30 years of variable weather data for a self-replacing ewe flock at Hoolbrook, New South Wales demonstrate: (1) that mitigation strategies for CH₄ production in relationship to LWG production (kg/kg LWG) over 365 days reduce CH₄ production (kg/kg LWG) by 25, 18, and 41%, of baseline levels for NO₃, lipid, and lipidxNO₃, respectively; and (2) that

mitigation strategies for CH₄ production in relationship to wool production (kg/kg wool) over 365 days reduce CH₄ production (kg/kg wool) by 24, 18, and 42%, for NO₃, lipid, and lipidxNO₃, respectively. The highest reduction of 41 and 42% for LWG and wool production, respectively was from the lipidxNO₃ mitigation strategy an additive effect from the NO₃ and lipid supplementation. Substantial reduction in CH₄ and an increase in productivity would potentially benefit the sheep industry if such a mitigation strategy was implemented. Sale of LW for the lipidxNO₃ mitigation strategy increased by 6% and would increase profitability by \$4.70 per animal; wool production increased by 0.7kg and at \$12.91/kg clean (25th May 2016) this equates to \$9.00 per animal. Live weight gain of lambs, at 8 months of age, of 1.8 kg and at \$5.80/kg (25th May 2016) equates to \$10.40 per animal. On 1000 head a producer could potentially reduce CH₄ to 41% of its baseline value and increase profitability by \$24,100. Lambing rates were not affected by the mitigation strategies & it is important to recognise the financial gain is moving from saleable physical product not a cash value on the methane mitigated.

New Zealand ewes (non-replacing flock)

The long-term productivity effects for wool production over 30 years of variable weather data for a self-replacing ewe flock at Whykikapaki New Zealand demonstrate: (1) that mitigation strategies for CH₄ production in relationship to LWG production (kg/kg LWG) over 365 days reduce CH₄ production (kg/kg LWG) by 27, 21, and 40%, of baseline levels for NO₃, lipid, and lipidxNO₃, respectively; and (2) that mitigation strategies for CH₄ production in relationship to wool production (kg/kg wool) over 365 days reduce CH₄ production (kg/kg wool) by 26, 13, and 38%, for NO₃, lipid, and lipidxNO₃, respectively. The highest reduction of 40 and 38% for LWG and wool production respectively was from the lipidxNO₃ mitigation strategy an additive effect from the NO₃ and lipid supplementation. Substantial reduction in CH₄ and an increase in productivity would potentially benefit the NZ sheep industry if such a mitigation strategy was implemented. Sale of LW for the lipidxNO₃ mitigation strategy increased by 10% and would increase profitability by \$8.15 per animal; wool production increased by 0.6kg and at \$12.91/kg clean (25th May 2016) this equates to \$7.75 per animal. Live weight gain of lambs at 8 months of age of 8.6 kg and at \$5.80/kg (25th May 2016) equates to \$49.90 per animal. On 1000 head a producer could potentially reduce CH₄ to 40% of its baseline value and increase profitability by \$65,800. Values based on \$Aus. Lambing rates were not affected by the mitigation strategies & it is important to recognise the financial gain is moving from saleable physical product not a cash value on the methane mitigated.

Beef production

Hoolbrook steers

The long-term productivity effects for beef production of steers over 30 years of variable weather data at Hoolbrook, New South Wales demonstrate that mitigation strategies for CH₄ production in relationship to LWG production (kg/kg LWG) over 245 days reduce CH₄ production (kg/kg LWG) by 13, 31, and 40%, of baseline levels for NO₃, lipid, and lipidxNO₃, respectively. The highest reduction of 40% for LWG was from the lipidxNO₃ mitigation strategy an additive effect from the NO₃ and lipid supplementation. Substantial reduction in CH₄ and an increase in productivity would potentially benefit the beef industry if such a mitigation strategy was implemented. The final LW for the lipidxNO₃ mitigation strategy increased by 15.5% and would increase profitability by \$350 per steer (25th May 2016). On 1000 head a producer could potentially reduce CH₄ to 40% of its baseline value and increase profitability by \$350,000. It is important to recognise the financial gain is moving from saleable physical product not a cash value on the methane mitigated.

California steers

The long-term productivity effects for beef production of steers over 30 years of variable weather data at Hoolbrook, New South Wales demonstrate that mitigation strategies for CH₄ production in relationship to LWG production (kg/kg LWG) over 245 days reduce CH₄ production (kg/kg LWG) by 19, 25, and 39%, of baseline levels for NO₃, lipid, and lipidxNO₃, respectively. The highest reduction of 39% for LWG was from the lipidxNO₃ mitigation strategy an additive effect from the NO₃ and lipid supplementation. Substantial reduction in CH₄ and an increase in productivity would potentially benefit the beef industry if such a mitigation strategy was implemented. The final LW for the lipidxNO₃ mitigation strategy increased by 7% and would increase profitability by \$90 per steer (25th May 2016). On 1000 head a producer could potentially reduce CH₄ to 39% of its baseline value and increase profitability by \$90,000. It is important to recognise the financial gain is moving from saleable physical product not a cash value on the methane mitigated.

4.3 Emission responses to methane mitigation

Sheep production

Holbrook, NSW wether production

In terms of total GHG (t CO₂/ha) emissions the lipidxNO₃ mitigation strategy had the greatest overall reduction to 33% of baseline. Atmospheric leaching of N₂O (t CO₂) increased by 5% and N₂O (t CO₂/ha) emissions remained the same across all mitigation strategies.

Holbrook, NSW ewe self-replacing production

In terms of total GHG (t CO₂/ha) emissions the lipidxNO₃ mitigation strategy had the greatest overall reduction to 33% of baseline. Atmospheric leaching of N₂O (t CO₂) increased by 6% and N₂O (t CO₂/ha) emissions remained the same across all mitigation strategies.

New Zealand ewes (non-replacing flock)

In terms of total GHG (t CO₂/ha) emissions the lipidxNO₃ mitigation strategy had the greatest overall reduction of 28%. Atmospheric leaching of N₂O (t CO₂) was reduced by 9% and N₂O (t CO₂/ha) emissions remained the same across all mitigation strategies.

Beef production

Holbrook steers

In terms of total GHG (t CO₂/ha) emissions the lipidxNO₃ mitigation strategy had the greatest overall reduction to 14% of baseline. Atmospheric leaching of N₂O (t CO₂) increased by 30% and N₂O (t CO₂/ha) emissions increased by 27% for the lipidxNO₃ strategy.

California steers

In terms of total GHG (t CO₂/ha) emissions the lipidxNO₃ mitigation strategy had the greatest overall reduction to 18% of baseline. Atmospheric leaching of N₂O (t CO₂) increased by 20% and N₂O (t CO₂/ha) emissions increased by 14% for the lipidxNO₃ strategy.

5. FUTURE RESEARCH NEEDS

Further development of the nitrate and lipid models is required as new data becomes available to assist with this modelling process, in particular some new research recently conducted by (de Raphélis-Soissan et al. 2016) would greatly enhance this process. A modification to the sheep rumen model for predicting rumen volume will be required to calculate VFA concentrations. Maternal productivity parameter values also need to be developed for the Beef module to enable it to be used with a breeding cow herd, not just a growing steer enterprise.. A workshop showcasing the sheep and rumen models to scientists working on developing methods to reduce CH₄ may facilitate would provide them with an increased understanding of rumen function from a whole systems approach & would lead to further enhancements of the model. Further studies to: (1) determine practical methods to feed nitrate to sheep and cattle; and (2) determine upper and lower limits of lipid and nitrate supplementation are warranted.

PUBLICATIONS

de Raphélis-Soissan V., Nolan J.V, Godwin I.R, Newbold J.R, and Hegarty R.S, (2016) Can adaptation to nitrate and provision of fermentable energy reduce methane production and nitrite accumulation in rumen contents in vitro? *Animal Production Science* 56, 605-612.

Villar L., R.S. Hegarty, D. Ebert, R. Woodgate, I.R. Godwin, J.V. Nolan and M.J. McPhee (2016). Nitrate and canola oil are synergistic in reducing methanogenesis in cattle. *Accepted abstract for July 2016 conference of the Australian Society of Animal Production*

Nguyen S. H., L. Li, R. S. Hegarty (2015) Effects of Rumen Protozoa of Brahman Heifers and Nitrate on Fermentation and *In vitro* Methane Production. *Asian Australas. J. Anim. Sci.* 2016;29(6):807-813. Published online 23 December 2015

Nguyen S, Barnett M, Hegarty R.S (2016a) Use of dietary nitrate to increase productivity and reduce methane production of defaunated and faunated lambs consuming protein deficient chaff. *Animal Production Science* (In press Dec 2015)

- Nguyen, S.H. and R.S. Hegarty. (2016b). Effects of defaunation and dietary coconut oil distillate on fermentation, digesta kinetics and methane production of Brahman heifers. *Journal of Animal Physiology and Nutrition* (Accepted, *in press*)
- Dougherty H.C., E. Kebreab, M. Evered, B.A. Little, A.B. Ingham, R.S. Hegarty, D. Pacheco, and M.J. McPhee. (2016) Overview of the Methane Prediction Module in the AusBeef Rumen Model. California Animal Nutrition Conference
- Dougherty H.C., E. Kebreab, M. Evered, B.A. Little, A.B. Ingham, R.S. Hegarty, D. Pacheco, and M.J. McPhee. (Draft prepared) Advances in rumen-scale enteric methane modelling: a qualitative comparison of AusBeef, MOLLY, and COWPOLL and model evaluation of AusBeef. To be submitted to *Journal of Agricultural Science* in July 2016.
- Dougherty H.C., E. Kebreab, M. Evered, B.A. Little, A.B. Ingham, R.S. Hegarty, D. Pacheco, and M.J. McPhee. (Draft prepared) Local and global sensitivity analysis of methane production relevant parameters in the AusBeef rumen model of beef production To be submitted to *Journal of Agricultural Science* in July 2016.
- Dougherty H.C., E. Kebreab, M. Evered, B.A. Little, A.B. Ingham, R.S. Hegarty, D. Pacheco, and M.J. McPhee. (Draft prepared) Description of the AusBeef rumen model - Ration optimization and assessment of environmental impact for the feedlot industry
- Dougherty H.C., E. Kebreab, B.A. Little, A.B. Ingham, R.S. Hegarty, D. Pacheco, B.N. Nagorcka, and M.J. McPhee. (2014) Overview of the Methane Prediction Module in the AusBEEF Rumen Model. In K.J. Harper, D.M. McNeill and A.W. Bell (eds) 8th International Workshop on Modelling Nutrient Digestion & Utilization in Farm Animals p.33
- Evered M. Report on sensitivity analysis. (2015) Milestone 5 (KPI 5.2). Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).
- Evered M. (2014) Report on flow chart of inputs, outputs; detailed description of models and parameter values. Milestone 3 (KPI 1.2 and 2.1). Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).
- Hegarty R.S. and M.J. McPhee. (2015) Report on the quantification of hydrogen stoichiometry in rumen of sheep and cattle on conventional diets based on existing measurements. Milestone 4 (KPI 3.2) Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).
- Hegarty R.S. and M.J. McPhee. (2015) Report on in-vitro studies of H₂ recovery completed. Milestone 4 (KPI 3.3) Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).
- Kebreab E. and Dougherty H.C. (2014) Report on the outline of strengths and weaknesses of AusBeef. Milestone 3 (KPI 2.3) Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).
- Little B.A., A.B. Ingham, R.S. Hegarty, E. Kebreab, D. Pacheco, B.N. Nagorcka and M.J. McPhee. (2014) AusBEEF: revision of the model to simulate animal and enterprise impacts of methane mitigation implemented on farm. *Proceedings of the 30th Biennial Conference of the Australian Society of Animal Production a joint ISNH/ISRP International conference*. 30:311
- Pacheco D., M. Evered, B.A. Little, A.B. Ingham, E. Kebreab, H.C. Dougherty, R.S. Hegarty, & M.J. McPhee (2016). Evaluation of AusBeef Methane Predictions from Beef Cattle. Presentation at GGAA conference.
- Pacheco D. (2014) Report on the evaluation of existing AusBeef model using two independent data sets Milestone 3 (KPI 3.1) Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).
- Vetharanim K. (2015) Report on sheep parameters and the updated beef parameters. Milestone 5 (KPI 5.4). Federal Department of Agriculture filling the research gap program "Impacts of CFI methodologies on whole farm systems". (Department of Agriculture No: 01200.047).

APPENDICES

Table A.1. Weather data for Holbrook wethers, ewes and steers

Item	Value/File
Latitude	35°43'S
Longitude	147°19'E
Data period	1 Jan 1969 to 31 Dec 2015
SIL0 file	Holbrook.zip
Wind speed	2.0 m/s

Table A.2. Paddock and soil detail for Holbrook wethers, ewes and steers

Item	Value	
<i>Paddock</i>		
Area	1000 ha	
Steepness	Undulating	
Fertility	0.80	
Reduce wind to	100%	
<i>Soil</i>		
Description	Red Duplex	
Soil albedo	0.17	
Soil evaporation	3.3 mm/d ^{1/2}	
SCS runoff curve no.	Default	
<i>Soil</i>		
	Topsoil	Subsoil
Cumulative depth (mm)	300	900
Field capacity (m ³ /m ³)	0.31	0.41
Wilting point (m ³ /m ³)	0.16	0.29
Bulk density (Mg/m ³)	1.40	1.40
Saturated conductivity (mm/hr)	100.0	30.00
Initial water (m ³ /m ³)	0.15	0.23

Table A.3. Pasture base for Holbrook wethers, ewes and steers

	Phalaris	Sub clover – Seaton Park	Annual Ryegrass
Phenology	S. Dormant (15)	Senescent	Senescent
Live DM (kg/ha)	0	0	0
Standing dead DM (kg/ha)	2000	1000	1000
Litter DM (kg/ha)	500	200	200
Below ground DM (kg/ha)	200	0	0
Max. rooting depth (mm)	900	300	580
Seed DM (kg/ha)	-	400	400

Table A.4. Livestock detail on Holbrook steers

Item	Detail
Breed	Angus
Standard reference weight	550 kg
Death rate: adults	2.0%/year
Death rate: weaners	2.0%/year

Table A.5. Livestock detail on Holbrook ewes

Item	Detail
Breed	Medium Merino
Standard reference weight	55 kg
Greasy fleece weight	5.50 kg
Fibre diameter	19.5 microns
Fleece yield	70%
Ram breed	Medium Merino (Mature ram: 77 kg)
Death rate: adults	4.0%/year

Death rate: weaners 5.0%/year

Table A.6. Reproduction detail on Holbrook ewes

Item	Detail
First join at	1 years
Mating date	20 th Feb
Conception at CS 3 [#]	(1) 45%
	(2) 50%
	(3) 0%
Birth date	18 th July
Castration	Yes
Weaning date	25 th Oct
One ram per	100 ewes
Keep rams for	4 years
Sell young ewes	Sell 1 year old animals on 20 th Nov
Sell young wethers	Sell 1 year old animals on 15 th Nov

[#]Probability of 1, 2, or 3 offspring per ewe.

Table A.7. Weather data for California steers

Item	Value/File
Latitude	39°00'N
Longitude	123°06'W
Data period	19 Oct 1982 to 20 Feb 2016
SIL0 file	US weather data 1982-2016.txt
Wind speed	2.0 m/s

Table A.8. Paddock and soil detail for California steers

Item	Value	
<i>Paddock</i>		
Area	45 ha	
Steepness	Gentle	
Fertility	0.80	
Reduce wind to	100%	
<i>Soil</i>		
Description	Sierra Research Station	
Soil albedo	0.23	
Soil evaporation	3.3 mm/d ^{1/2}	
SCS runoff curve no.	Default	
<i>Soil</i>		
	Topsoil	Subsoil
Cumulative depth (mm)	180	800
Field capacity (m ³ /m ³)	0.22	0.35
Wilting point (m ³ /m ³)	0.11	0.21
Bulk density (Mg/m ³)	1.61	1.88
Saturated conductivity (mm/hr)	32.40	32.40
Initial water (m ³ /m ³)	0.24	0.38

Table A.9. Pasture base for California steers

	Annual Grass	Sub clover – Mt Baker	Annual Ryegrass	Perennial Ryegrass	Red Clover
Phenology	Reproductive (1200)	Reproductive (1200)	Reproductive (1200)	Reproductive (1200)	
Live DM (kg/ha)	2000	600	1000	1000	
Standing dead DM (kg/ha)	1000	500	500	500	
Litter DM (kg/ha)	1000	200	500	5000	
Below ground DM (kg/ha)	3000	1000	1500	2000	
Max. rooting depth (mm)	700	300	500	500	
Seed DM (kg/ha)	25	10	25	-	

Table A.10. Livestock detail on California steers

Item	Detail
Breed	British x Charolais
Standard reference weight	500 kg
Death rate: adults	2.0%/year
Death rate: weaners	2.0%/year

Table A.11. Weather data for New Zealand ewes in the Hawkes Bay region

Item	Value/File
Latitude	39°50'S
Longitude	176°38'W
Data period	1st Jan 1982 to 31 Dec 2015
SIL0 file	NZ weather dat.txt
Wind speed	2.0 m/s

Table A.12. Paddock and soil detail for New Zealand ewes in the Hawkes Bay region

Item	Value	
<i>Paddock</i>		
Area	570 ha	
Steepness	Moderate	
Fertility	0.80	
Reduce wind to	100%	
<i>Soil</i>		
Description	Calcareous Orthic Melanic	
Soil albedo	0.17	
Soil evaporation	3.0 mm/d ^{1/2}	
SCS runoff curve no.	Default	
<i>Soil</i>		
	Topsoil	Subsoil
Cumulative depth (mm)	300	1000
Field capacity (m ³ /m ³)	0.35	0.35
Wilting point (m ³ /m ³)	0.17	0.21
Bulk density (Mg/m ³)	1.20	1.40
Saturated conductivity (mm/hr)	50.00	30.00
Initial water (m ³ /m ³)	0.15	0.23

Table A.13. Pasture base for New Zealand simulations of ewes in the Hawkes Bay region

	Perennial Ryegrass	Annual Ryegrass	White clover
Phenology	Vegetative (0)	Vegetative (0)	Vegetative (0)
Live DM (kg/ha)	700	500	300
Standing dead DM (kg/ha)	500	200	50
Litter DM (kg/ha)	500	100	50
Below ground DM (kg/ha)	2000	1000	500
Max. rooting depth (mm)	350	300	200
Seed DM (kg/ha)	-	500	500

Table A.14. Livestock detail on New Zealand ewes in the Hawkes Bay region

Item	Detail
Breed	Suffolk
Standard reference weight	55 kg
Greasy fleece weight	3.47 kg
Fibre diameter	26 microns
Fleece yield	70%
Ram breed	Dorset (Mature ram: 77 kg)
Death rate: adults	2.0%/year
Death rate: weaners	2.0%/year

Table A.15. Reproduction detail on New Zealand ewes in the Hawkes Bay region

Item	Detail
First join at	1 years
Mating date	8 th Mar
Conception at CS 3 [#]	(1) 90%
	(2) 10%
	(3) 0%
Birth date	4 th Aug
Castration	Yes
Weaning date	15 th Dec
One ram per	50 ewes
Keep rams for	5 years
Sell young ewes	Sell 7 month old animals on 30 th Jan
Sell young wethers	Sell 7 month old animals on 30 th Jan

[#]Probability of 1, 2, or 3 offspring per ewe.