final report

Project code: B.CCH.1047

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Date published: February 2011

PUBLISHED BY
Meat & Livestock Australia
Limited Locked Bag 991
NORTH SYDNEY NSW 2059

Historic publications collection

Reducing Emissions from Livestock Research Program

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

SYNOPSIS

A publication database and collection was constructed from publically available literature reporting the effects of dietary mitigation on methane emissions. The data collection contained 782 sources from the international peer reviewed literature. The literature was subdivided into a number of individual collections – for example 'in vivo' and 'in vitro' literature, by species (sheep, cattle) and by technique. A meta-analysis was conducted on a proportion of the in vitro data collection identifying key factors affecting the overall usefulness of the analysis. For instance, there was a lack of consensus in the international literature concerning in vitro techniques, incubation parameters, reporting of fermentation and gas parameters. A recommendation for collaborative research to be undertaken to standardize methods and evaluation protocols (in a similar fashion to current work on meta-genomics) was made.

Monitoring and evaluation processes

Program Planning, Monitoring and Evaluation LEVELS	**Program Plan**	**Milestones and Performance Indicators**	**Evaluation Data Collection Methods**
1. Overall outcomes	The livestock industries are better positioned to optimally address the increased business risks and opportunities due to the contribution of methane to climate change and government policies related to the contribution of methane to climate change	• Future CPRS or alternative mitigation policy takes into account quantified RELRP R&D results • Industry has quantified options to reduce methane emissions from livestock (able to respond)	• Project milestones and deliverables met • Project report and consolidated program final report analysing R&D outputs and quantified national mitigation potential
2. Project objectives What is the project promising to deliver on?	Over the next 12 months The University of Melbourne will develop a publications collection that can allow the abstraction of data for meta-analysis of methane emissions from ruminant livestock to support the research, development and demonstration activities in the Reducing Emissions from Livestock Research Program.	**KPI 1:** By 1st July 2010, develop the decision tree and structural attributes of the publications collection. **KPI 2:** By 1st September 2010 Report on progress on populating and developing the database. Development of the interface of the meta-analysis tool. **KPI 3:** By 31st December 2010 report on the delivery of the final database with meta-analysis facility. Demonstrated single run output from database (e.g. effect of supplement X on methane production). Information session held for DAFF CCRP, RELRP researchers and MLA.	• Milestones achieved.

3. Uptake Strategies What methods will be used to help bring about change in capacity or practice change?	• Interaction with research leaders through RELRP technical meetings. • Fact sheets, print and web material that is produced from the program.	Level of collaboration between researchers within the RELRP.	• Milestone achieved.
4.Outputs What tangible extension, communication and/or research tools, information or resources will the project be developing?	Development of a database that allows meta-analysis of both historic data and current research.	Level of interest in the database by both producers and researchers.	• No. of projects within the RELRP that are able to utilize the database. • Participatio n in meetings/ technical workshops and other meetings as required.
5. Research & Development Activities to produce outputs	Engage producers and researchers to promote the use of the database as a resource.	• Objectives and deliverables produced as per project schedule. • Consistent and statistically significant data.	• Milestones met
6. Stakeholder engagement What committees, forums or other mechanisms will be used to engage with stakeholders during the project?	Information sessions held for DAFF CCRP, RELRP researchers and MLA.	• Acceptance of database by Government and Industry stakeholders.	• 6+No. of researchers and producers using the database.
7. Project structures and resources What is the program budget from what source? What staff time and other resources are allocated to the project? What management structure is in place?	DAFF $ 67,500.00	MLA approval of progress reports	• Budget reporting submitted on time. • Acceptance of progress reports.
8. Context and Issues The seasonal, environmental, policy, other programs and factors could affect the performance of the program?	• Methane mitigation research is relevant to government climate change policies e.g. CPRS and international negotiations in UNFCCC. • Methane reduction must not be at the expense if productivity for industry acceptance.	• Changes in policies and regulations, costs and trends and their implications for the program and industry. • Industry and producer needs for practical mitigation options.	• Regular reporting. • MLA board and peak council response. • Feedback from DAFF and DCC.

Introduction

The original project brief of BCCH 1047 (Historic publications and meta-analysis tool) was part of the broader BCCH 1040 Information Integration and Delivery Program. BCCH 1047 is a minor component of the program however it has a role in assisting the development of scenario tools and calculators in providing a literature resource for end users. The BCCH 1040 program envisages that the data and information management system can be developed using a modular framework to facilitate rapid development of capacity required for the RELRP but with seamless capability that will allow researchers to access new and/or historic information concerning emissions from ruminants. The system will be stratified according to the thematic structure of RELRP *viz.* quantification and measurement technology; genetic approaches to reduce emissions; manipulation of rumen function; improvement of waste management; and farming systems research. BCCH 1047 will provide the historic publications collection that would allow the design of databases for interrogation and future possibilities to conduct meta-analysis, attributional life cycle analysis and create medium and long-term inventory statistics to understand the potential of mitigation and adaptation scenarios. The data and information management system developed by BCCH 1040 should also be able to inform the farm systems models and scenario evaluation tools required by the livestock industries. This approach will assist the on-going development of an on-farm scenario evaluation tool to incorporate evaluation of the implications of adopting the mitigation strategies.

The basis of BCCH 1040 projects is to develop a web based delivery system (initially via the basecamp system but in the future via farmGAS and other database systems) that is user friendly and can provide reports at various levels of detail. For instance, producers and industry practitioners should be able to access simplified tools to estimate impacts of various mitigation or adaptation scenarios whereas, researchers may be interested in detailed meta-analysis of animal response to a supplement and its impact on methane emissions per kg live weight gain. It is anticipated that partner organizations will develop the 'academic' inputs and outputs of the project, and specific contracts will be developed for the statistical approaches to data mining and the farm emissions calculator.

BCCH 1047 Historic publications collection

KP1 Publications and sources

Original searches for international literature were conducted using PubMed and SCIRUS. These search engines outputted 3080 literature sources linked to the search phrases 'rumen, methane' and 'emissions' and 'methane emissions, rumen'. Of the 3080 publication sources removal of web page materials, .html, patents and other electronic sources reduced the number to 1354 sources. Of these, 782 were found to be unique sources of which manuscripts were available within the international peer reviewed literature (excluding conference proceedings with local or national peer review). When consolidated into a .pdf readable source the publications collection was 401.6MB.

KP1 Decision tree and structural attributes of the collection

The decision tree was structured initially as 'in vivo', in vitro', 'unclassified' sources of literature concerned with methane emissions from ruminants. The important aspect of the subdivision is that it demonstrates the relatively paucity of literature based in 'in vivo' studies that focus specifically on the mitigation or abatement if methane (30.4%, n=238 compared to 47.7%, n=373 for in vitro studies) and also the potential for extrapolation of 'in vitro' data to 'in vivo' systems. The former is an important issue.

The majority of the literature concerning methane emissions or total yield of methane is derived from classical 'feed evaluation' experiments with the objective of measuring the energy content of the feed (expressed as ME, NE or DE). These experiments measure methane as part of the partition of digestible energy to ME at maintenance levels of feeding, and therefore, the methods deployed focus on single feed scenario, at a single level of feeding (normally maintenance) and do not reflect on-farm normal feeding practices. At best, the types of meta-analysis that could be conducted are collective summarisations of measurements that had a secondary role in the assessment of methane potential of forages or concentrates.

The 'unclassified' studies were publications that considered meta-analysis, policy development and systems modelling. Many of these publications are generic in their content building on limited extracts from the overall literature concerned with methane emissions from ruminant livestock. For instance, several of the modelling papers relied on IPCC methodologies developed from work in the 1960s and 1970s (e.g. Blaxter and Clapperton or Moe and Tyrell). These papers have their merits for public policy development and debate but do not provide further detailed information on mitigation or abatement.

In vivo based publications

Within the subset of literature identified as in vivo based studies, the publications were then subdivided by species (cattle, sheep, goats and other ruminants). The majority of studies (>70%) used sheep as their subject focusing on feed evaluation and nutritive value rather than methane mitigation. Furthermore, the studies that were focused on feed evaluation and nutritive value tended to offer various foods at maintenance to 1.3x maintenance. At this level of feeding, there would be concerns over extrapolation of the data on methane emissions to other feeding regimes or systems. Beef cattle were the second most studied animals (17%) with the majority of studies conducted using animals growing or managed under feedlot conditions. The number of studies recording emissions from grazing beef cattle were extremely limited (less than 5% of beef cattle studies) reflecting the complexities of measurement protocols required to obtain meaningful emissions data. There were a limited number of studies of methane emissions and mitigation technologies using dairy cattle. These studies were generally conducted using open path spectrometric techniques; however several studies also considered mitigation protocols by altering supplementation strategies.

In vitro based publications

The most important subdivision of effort within in vitro studies was 'batch culture' vs. 'continuous culture' (RUSITEC) techniques. The former has been used, almost exclusively, as a 'proof of concept' screening tool (for example in studying plant bio-actives) and the latter have been deployed to understand longer-term microbial ecosystem changes (e.g. 3 to 5 day studies). There are major strengths and weaknesses in these data sets and their applicability to in vivo studies can be somewhat tenuous.

In vivo feed evaluation systems depend on a range of factors, including estimation of apparent digestibility, rate of digestion, fractional rumen outflow and efficiency of microbial production. Many of these processes are no mimicked in vitro and therefore in vitro evaluations estimate of dry matter (DM) and/or organic matter (OM) digestion and provide an indication of the end products of fermentation (e.g. gas, methane, H_2 and VFA). These systems are however popular as they have a high analytical capacity and low cost. One of the major problems that in vitro evaluation of methane production from feeds faces is the source and quality of rumen microbiota used. The majority of rumen fluid used in these studies (>90%) was collected from sheep as the donor animals maintained on intakes ranging from maintenance to 1.3x maintenance, and that there is little definition of the rations these donor animals were maintained on. The animals, without exception, were rumen cannulated.

KP2 Meta-analysis tools

One of the major considerations for stochastic modelling of cause-effect relationships is the fidelity of data. In general, the results from short-term experiments designed to answer specific research questions cannot be used as the basis of stochastic models. The designs of these experiments are 'narrow' and do not measure large numbers of factors that affect the principal variable (e.g. daily methane production). Also, it is rare that experiments are repeated by others to verify the repeatability of response and the applicability of the results and conclusions. In the past, the approach taken to evaluate multiple studies has been by qualitative literature review. This is limited in its application as it is subjective in its ability to choose which studies are important to include within the analysis. It is also limited by the non-inclusion of studies based on the knowledge and understanding of the authors. Meta-analytical techniques have been used recently in an attempt to quantify formally the evaluation of multiple studies. The four main aims of these analyses are:

1. Global hypothesis testing- for example the effect of feeding supplementary fat on daily methane production
2. Empirical modeling of biological responses – for example construction and comparisons of empirical models to determine the relationship between daily methane production and voluntary intake
3. Collective summarizations of measurements that only had a secondary or minor role in prior experiments – for example if a study is designed to evaluate the effect of starch supplementation on fibre digestion, information concerning rumen VFA concentrations or methane output may also be presented.

4. Parameter estimates and estimates of initial condition of state variables in mechanistic models may be derived from meta-analysis.

Types of Data and Factors in Meta-Analyses

One of the major problems facing meta-analysis is the quality of data available. Meta-analytical databases are characterised by the high frequency of missing data which reduces the possibility of using large multi-dimensional descriptive models, and therefore leads to the use of models which are based on a limited number of independent variable. These models are further affected by the original experimental design used for the study. The type of design of the experiment will affect the theoretical variance accounted for by independent variables (small effect) and studies (large effect) hence affects the overall precision of *post hoc* inclusion of studies in meta-analysis. This can lead to individual studies 'levering' the overall response or confounding or disconnect of independent variables. The overall processes of meta-analysis are in Figure 1 (St Pierre, 2001; J Dairy Science 84: 741-755).

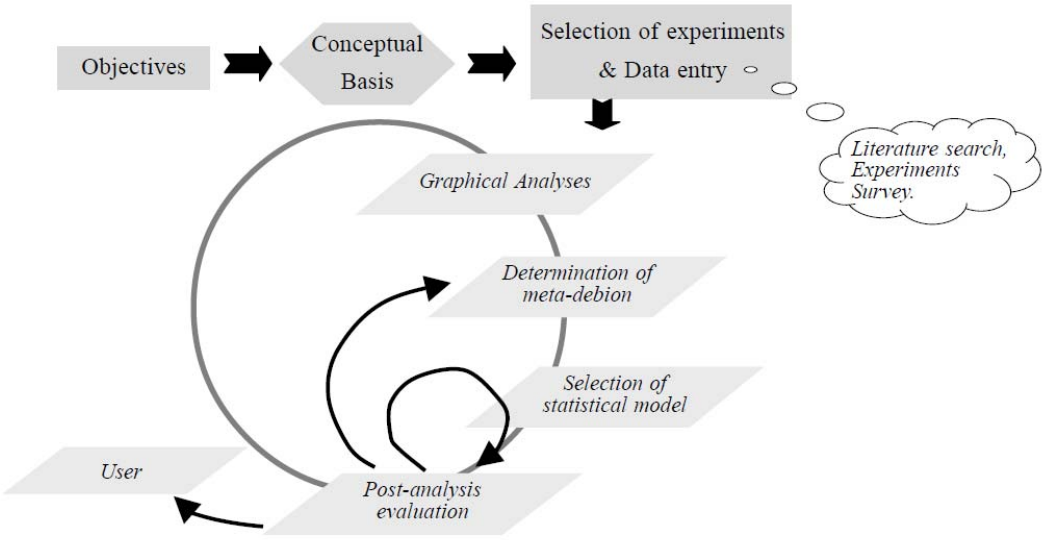


Figure 1 Theoretical and conceptual considerations for meta-analysis.

The approaches used for meta-analysis of the relationship between VFA and methane yield are those reported by St Pierre (2001). The approach is to use regression tools to identify linear and quadratic models (as well as possible interactions between dependent variables) that predict the relationship between variables (for example, fat in diet and methane yield). The effects of experiment are included in the models, both as a main random effect and, initially, as their random interaction with independent variables. These terms correct the regression equations for random experiment effects and their possible interactions with the independent variables. The regressions were calculated using REML in Genstat.

KP3 Case study (in vitro data)

Introduction

The majority of *post hoc* analyses conducted on data sets related to methane emissions from ruminants have been focussed on in vivo based work. These studies have reported the effects of nutritional strategies on the mitigation of methane emissions. For instance, Moate et al. (2010) recently identified the relationships between incremental increases in fat content of a ration and reduction in methane yield (3.4% reduction per 1% increase in fat in total ration). The observations of Moate et al. need further examination to understand the possibilities and problems associated with meta-analysis of response experiments. Moate's work was based on 17 experiments from which data on 76 dietary treatments were obtained. The response was constructed from 4 experiments based on beef cattle and 13 experiments on dairy cows with methane measurement being made using two methods (SF_6 technique in 6 experiments and respiration chambers in 11 experiments). The average fat content of the ration was 4.97% (range 1.2 – 11.4%) with beef cattle diets containing 4.67% ± 3.02, and dairy rations containing 5.00% ± 2.93. Regression analysis demonstrated:

$$Y\ (CH_4/kg\ DM\ intake) = 24.51\ (\pm 1.48) - 0.788(\pm 0.157)\ \%Fat$$

The back-transformed fitted line (weighted mean) from the random linear coefficients model on the log-transformed data, shows slight curvature corresponding to a constant percentage decrease in CH4 /kg DMI per percentage unit of dietary fat equal to 3.4% with 95% confidence interval: [2.28%, 4.59%]. No significant difference in means of CH_4 emissions associated with methodology of measurement: chambers 20.4 ± 4.01 vs. SF_6 technique 23.9 ± 9.21 g/kg DM. The estimate of variance between experiment means for experiments using SF_6 technology (40.9) was considerably larger than between chamber experiments (14.2).

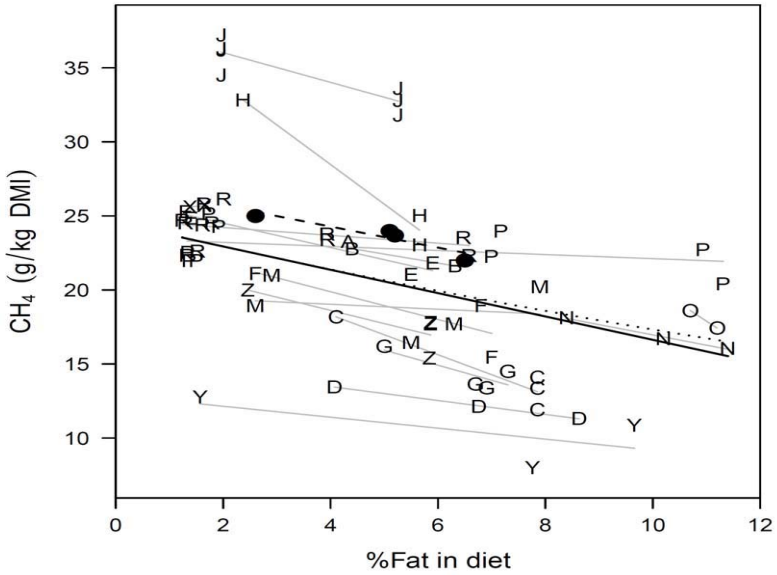


Figure 2 Effect of fat inclusion in ration on methane (g/kg DMI) production.

Nine of the 17 experiments were designed to evaluate the effects of fat supplementation on lactation performance. The primary aim of these experiments was not to determine the mitigation effects of fat on methane production but to further understand the role of fat as a supplement to energy metabolism. It is therefore suggested that the meta-analysis is categorised as a collective summarisation of measurement and a secondary objective of the work was to determine methane production. The second key issue is the measurement protocols used to determine methane emissions from the animals. Approximately one-third of the information in the study has been derived from SF_6 proxies of methane production. There is also a substantial difference between the variance accounted for by measurement with the variance of SF_6 measures being substantially greater than that observed for respiration chambers. Recently, Clark (2010) demonstrated similar observations where the variability of emissions measured using calorimeters was about half that of SF_6. The inherent differences in variability due to measurement protocol would lead to an increase in 95% confidence interval associated with the regression slope (mean = 3.4 95%CI = 2.28 to 4.59).

In a second example, Ungerfield et al. (2007) reported the relationship between methane production and fumarate addition in vitro.

CH_4, µmol = 642 (±110, *P* <0.001) – [2.05 (±0.91, *P* = 0.029) x initial fumarate, m*M*].

This study is a typical example of a 'global hyopthesis' analysis as it considers experiments focused on the effects of fumaric acid on methane production. Increased supplementation of the animal with fumarate provides an opportunity for the rumen ecosystem to reduce its production of H_2 and hence reduced methane production via the mixotrophic pathway. The authors identified the numbers of studies and some of the problems faced in conducting the meta-analysis viz. "This relationship was based on 74 treatment means for CH_4 production and VFA production or concentration from 7 experiments in 6

published studies (Callaway and Martin, 1996; Asanuma et al., 1999; López et al., 1999; Carro and Ranilla, 2003; García-Martínez et al., 2005; Newbold et al., 2005) and the 2 unpublished experiments. However, VFA production could not be calculated because only final VFA concentrations were reported. Changes in VFA production caused by fumarate addition with respect to controls were calculated from the liquid volumes and final VFA concentrations of the incubations because initial VFA concentrations were assumed to be equal for all treatments".

Incubation techniques

There is a range of incubation techniques used in in vitro studies related to methanogenesis. These were identified in the collection as temperature, rumen fluid preparation effects or instrumentation effects (syringe vs. bottle). Under normal conditions the preparation of incubation fluid requires filtration through course muslin cloth, and dilution into a rumen in vitro buffer. In the majority of in vitro studies using batch culture techniques, 30% addition of rumen fluid (s.d. 6.77) was used. The incubation was normally conducted at 39^0C (38.7^0C, sd 0.48; n=108) for 24 hours (Figure 3).

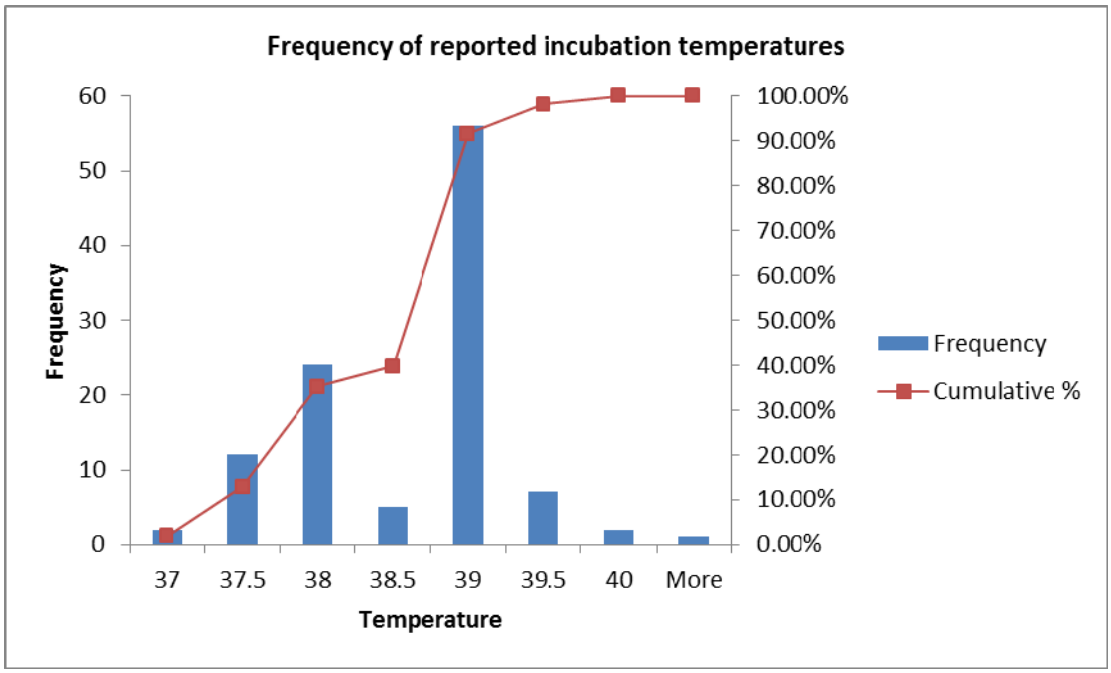


Figure 3 Distribution of published incubation temperatures

The incubation volume (nominally 100 ml) was 93.3 (sd 19.0) with 305.7 mg (sd 277.7) substrate. The ratio of substrate to incubation fluid was 3.27 mg/ml (%CV 90.8). This lack of standardisation of incubation parameters is an important area of future work, especially when attempting to compare studies that demonstrate mitigation potential of a range of plant bio-actives.

Substrate characterisation

In many studies, there is limited information concerning the voluntary intake or nutritive value of diets of donor animal. Furthermore, there is limited information on the microbiology of the donor samples. This lack of nutritional and microbiological information poses two significant questions – do the samples of rumen fluid used in the experiment reflect 'normal' rumen conditions? And does the preparation of the samples for incubation alter the structural attributes of the microbial community selected and hence its functionality?

The majority (>80%) of studies report DM, CP and OM contents of the basal rations and the incubation diets only. Less than 50% of studies report NDF content or DM digestibility. This situation leads to a lack of information on the C:N ratio of the incubation and it is therefore not known if the incubation is limited in N or in surplus.

Fermentation parameters

The majority of studies report clearly differences in fermentation parameters between control and various treatments. Normally these parameters are measured at the end of the incubation period. The range of measurements are pH, VFA (acetate, propionate, butyrate – both isomeric forms, branch chain VFA), ammonium and occasionally lactate. There are a range of units of measurement, for instance mol/mol, total mmol, µmol, molar%, mg/l, mg/dl. Inter-conversion of units of measurement, and calculation of total or molar proportions can be conducted, however there is always a loss of precision using this approach. Further, the reporting of data in each paper varies with a number of papers reporting VFA concentrations to 1 or 2 dp. Some papers (<40%) report the acetate:propionate ratio (A:P) of the fermentation. This measure is a useful indicator of the fermentation processes however there is a lack of uniformity in reporting (again 1 or 2 dp. is commonplace). Various relationships can be developed between molar proportions of VFA and head space concentration of methane:

Methane (umol) = -16.69acetate (mol%) + 1507.22 r^2= 0.23*

Methane (umol) = -9.22 propionate (mol%) + 716.64 $r^2 = 0.04^{ns}$

Methane (umol) = 35.19 butyrate (mol%) + 117.86 r^2 = 0.43**

Similar observations for changes in propionate concentration and mol% on methane emissions have been reported (for instance by Ungerfield et al. 2007) for range of mitigants.

The question of acetate:propionate ratio is an important one to be examined to a greater depth (Figure 4). In the literature, there are many references that suggest that enhanced propionogenesis leads to a reduction in methane production reflecting the reduction in metabolic H_2 production in situ. Under these conditions, there is generally a reduction in the production of acetate as demonstrated by Bannink et al. (2006: J Theoretical Biol 238: 36-51).

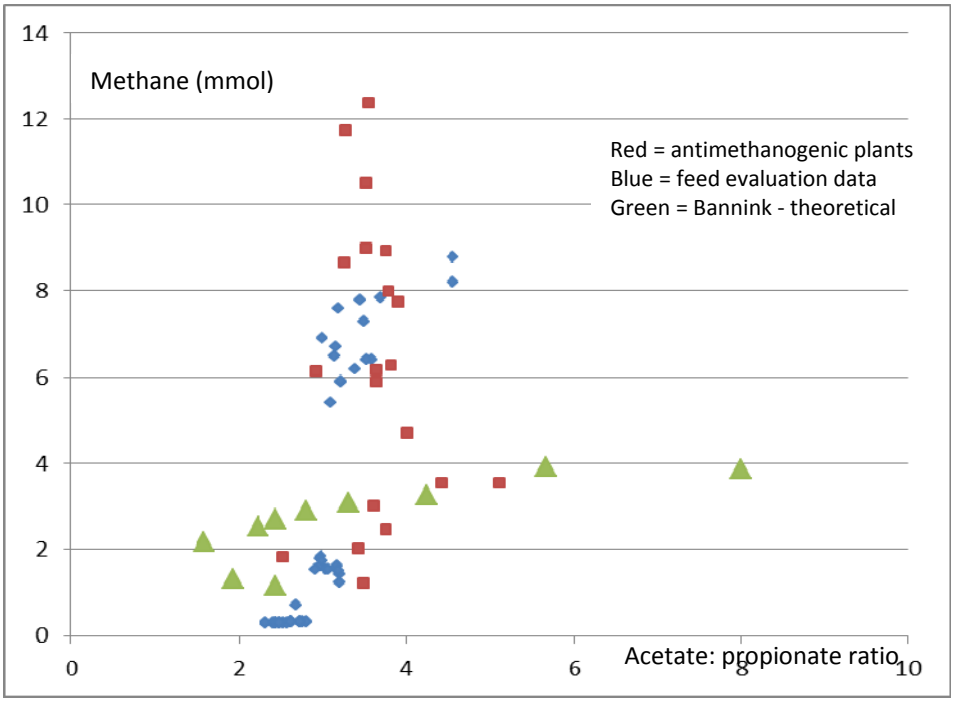
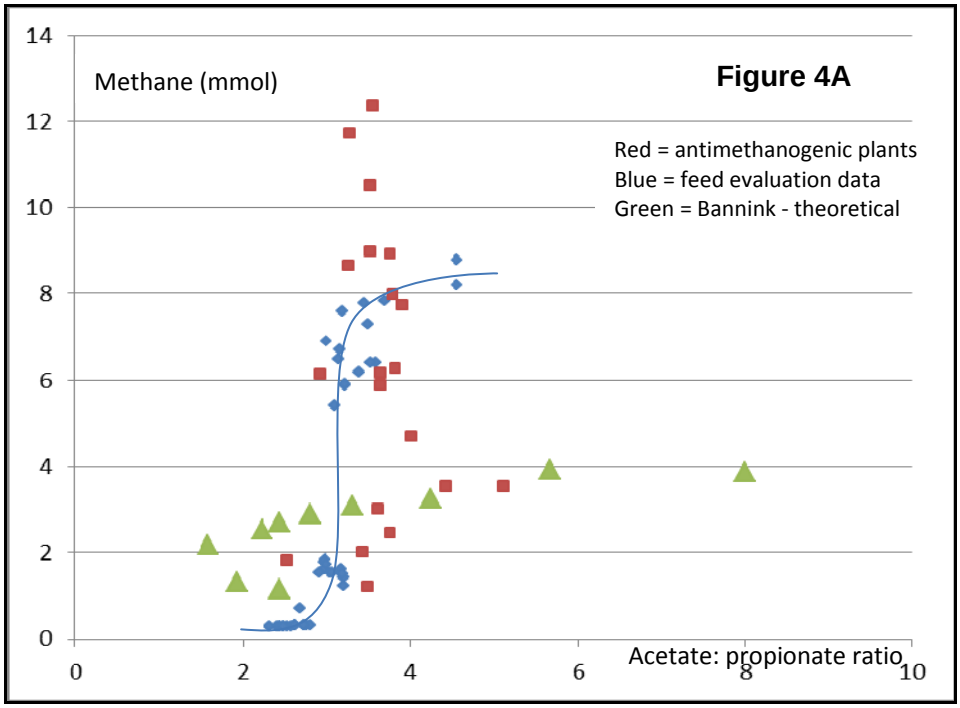


Figure 4 Meta-analysis of effect of change in acetate:priopionate ratio on methane yield. Blue symbols are the response of 8 studies, red symbols one study with different experimental objective and green are Bannink et al. 2006 theoretical model data.

In Figure 4A, the response of methane yield to change in acetate:propionate ratio (AP) is presented. This generalised response demonstrates two clear groupings of data, those related to AP greater than 3.2 yielding greater than 5 mmol methane and those less than 3.2 yielding < 2 mmol methane. The relationship would suggest the stoichemimetry of the relationship between acetate:propionate:butyrate (mol/mol) would be 0.64:0.20:0.16. These data reflect rations assessed for nutritive value using feed evaluation studies where the diets were incubated at fixed ratio of rumen fluid to feed.



The antimethanogenic screening data represents a group of studies focussed on the mitigation of methane using novel plants or bioactive extracts. The range in methane production is substantially greater. These studies generally rank plants in terms of their methane potential (presumably in a similar fashion to the studies conducted in RELRP at UWA). Finally, the work of Bannink et al. (20006) using a complex mechanistic model to determine rumen function demonstrates a reduction in methane production as a result of increased propionate, however, the slope of the regression does not represent what actually occurs in vitro.

Methane and hydrogen concentrations

The measurement of methane and hydrogen in vitro can pose considerable technical difficulties. In general methane concentration is reported as umol or mmol per day, per unit of DM digestible feed or per g degradable NDF or OM. These units can pose considerable difficulties in meta-analysis. First, the reporting of umol or mmol/d does not back calculate to per mg of DM or OM incubated leading to considerable problems for between study comparisons. The main issue facing between study comparisons is identified by the high coefficient of variation of the ratio of feed incubated to incubation volume (>90%) and a lack of standardization in the methodologies. The second issue facing the meta-analysis of methane data is the lack of information concerning the basis of the degradable DM, OM or NDF. It is far from clear how these parameters were calculated and if they reflect comparable studies using the same feeds in vivo.

Similar comments to those made for methane emission can be made for hydrogen. There are a number of reports of mmol H_2/d or umol/d. However the majority of literature reporting H_2 identifies derived parameters such as H_2 produced, H_2 utilised or % recovered. These derived parameters are essential to understand the stoichemimetry of the fermentation, but they do not readily allow the data sets to be evaluated or compared between studies.

Microbial enumeration

In general, microbial enumeration is reported as CFU data or as cells per ml. Occasionally there is microbial structural data reported, however this is generally very limited in its reporting and segments the microbial community into fibre utilizing bacteria, methanogens, fungal, protozoa etc. Further, in studies that specifically relate protozoa, there is subdivision of species reported.

Conclusion and recommendations

1. Integration of publications collection to the AFI FarmGAS calculator help wizard. This has been requested by AFI to support the new version of the calculator. The impact of this will be to disseminate international peer reviewed literature to many users of the calculator, thus communicating the underpinning science to the calculations derived from NCAT, NGGI and other sources.

2. A recommendation for future work to underpin the standardization of reporting of data from in vivo and in vitro studies, standardization of techniques used and provision for future meta-analysis on standardised data.

3. Further work is required to mine the historic collection to draw out important relationships between methane production and attributes of rumen biochemistry.