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**MitiGate; an online meta-analysis database for quantification of mitigation strategies for enteric methane emissions**

Running head: MitiGate; a methane mitigation database

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## **Highlights**

- A meta-analysis database of research on options for mitigating enteric greenhouse gas emissions from livestock production
- An on-line interface allows data extraction, updating, and integration into modelling efforts or policy recommendations.
- Meta-analysis provides estimates of effect size, variance and heterogeneity of each mitigation strategy.
- Accuracy of mitigation potentials reduced by limited data for certain strategies, geographic regions or long term studies.

## ***Abstract***

The body of peer-reviewed papers on enteric methane mitigation strategies in ruminants is rapidly growing and allows for better estimation of the true effect of each strategy through the use of meta-analysis methods. Here we present the development of an online database of measured methane mitigation strategies called MitiGate, currently comprising 412 papers. The database is accessible through an online user-friendly interface that allows data extraction with various levels of aggregation on one hand and data-uploading for submission to the database allowing for future refinement and updates of mitigation estimates as well as providing easy access to relevant data for integration into modelling efforts or policy recommendations. To demonstrate and verify the usefulness of the MitiGate database those studies where methane emissions were expressed per unit of intake (293 papers resulting in 845 treatment comparisons) were used in a meta-analysis. The meta-analysis of the current database estimated the effect size of each of the mitigation strategies as well as the associated variance and measure of heterogeneity. Currently, under-representation of certain strategies, geographic regions and long term studies are the main limitations in providing an accurate quantitative estimation of the mitigation potential of each strategy under varying animal production systems. We have thus implemented the facility for researchers to upload meta-data of their peer reviewed research through a simple input form in the hope that MitiGate will grow into a fully inclusive resource for those wishing to model methane mitigation strategies in ruminants.

***Keywords: Methane, greenhouse gas mitigation, enteric fermentation, ruminants, meta-analysis.***

## **1 Introduction**

Animal production, and in particular ruminant production, carries with it a significant environmental cost both at the local and global level. Locally, this is mainly associated with nitrogenous compounds and phosphorous emissions from intensive operations. The global effect is predominantly due to the emissions of greenhouse gases (GHG), which occurs in both intensive and extensive systems. Agricultural production accounts for 10-12% of global annual GHG emissions (Smith et al., 2014) with livestock production being the most important contributing factor within this sector. The largest single contributor to agricultural GHG emissions is enteric fermentation which represents between 32 and 40% of the total GHG emitted from the sector (Smith et al., 2014). If the ruminant livestock sector is to remain a significant component of the agricultural industry, then strategies must be implemented which allows the sector to grow, while at the same time reducing **its** environmental impact.

A wide range of approaches aimed at decreasing enteric methane emissions have been described (Eckard et al., 2010) with the potential to reduce methane emissions by between 5-10% suggested as technically possible (Bellarby et al., 2013; Hristov et al., 2013; Oenema et al., 2001; Smith et al., 2007; Tubiello and Loudjani, 2010). However, such estimates are mostly based on qualitative reviews of current literature and are not always all inclusive. A more structured quantification of mitigation strategies will allow for better estimation of mitigation potentials at different levels (animal, farm and sector scale) to be used in modelling efforts and to inform policy recommendations. The body of research on enteric methane mitigation strategies in ruminants has grown exponentially in recent years. Fully utilizing this rapidly growing field through meta-analytical approaches will allow for better quantitative estimation of the effectiveness of

each strategy, as well as promoting a better understanding of the factors moderating the effect under different circumstances.

As with all other analyses, such extensive meta-analyses are frozen in time, representing the state of knowledge when they were completed but unable to accommodate new and emergent knowledge. As new studies are published, the whole procedure has to be repeated; a very time-consuming process from database searches, through meta-data collection to database development. Many examples can be found of repeated meta-analyses, either where the original meta-analysis was considered too narrow (Taub et al., 2008), because an updated analysis was considered necessary (Martin et al., 2010) or where a more detailed analysis was performed (Grainger and Beauchemin, 2011). In **most** cases the additional data, either through new publications or through analysing a broader range of data, lead to new conclusions and shows the need for continual updating and refinement of quantitative reviews.

Here we describe the development and implementation of an online database, MitiGate, which is accessible through a user friendly interface to facilitate meta-analysis of available data as well as continual updating of the meta-analysis as new data are made available. This flexible research platform allows for future refinement and updates of mitigation potential estimates for a range of animal production systems. It also provides easy access to relevant data for integration into modelling efforts or policy recommendations.

## ***2 Database development***

### *2.1 Classification of mitigation strategies*

When bringing together the full range of enteric methane mitigation strategies, the

question arises how to group or aggregate these strategies. No definitive rules exist on the most appropriate scale of aggregation for a meta-analysis (Laird and Mosteller, 1990; Lean et al., 2009). Choice of which level of aggregation is appropriate depends on the aim of the analysis and the level of inference intended (Laird and Mosteller, 1990; Sauvant et al., 2008). Studies combined need to refer to the same level of inference to avoid confusing or useless results (DerSimonian and Laird, 1986). Previous attempts to aggregate mitigation strategies have not been straightforward. Attempts have been made to classify strategies based on the targeted function (Hegarty, 1999; Monteny et al., 2006; Patra, 2012) though many strategies potentially have several targets (Patra, 2012) making classification of studies difficult. Other classifications are based more on the source of input (e.g. tannins, ionophores, lipids) such as in the review by Eckard *et al.* (2010). In practice, any classification has to make sense as distinct categories for all stakeholders interested in utilizing the data. In MitiGate we propose to use a classification structure based on those discussed in a number of recent reviews (Cottle et al., 2011; Eckard et al., 2010; Malik et al., 2012; Patra, 2012) which we think best summarises current practices and can be translated to several levels of aggregation depending on the output desired. By creating a database with multiple levels of classification, we hope to have provided a flexible platform for future meta-analyses or modelling efforts at many levels of aggregation. Studies can then in future be aggregated at the level most appropriate for specific modelling or policy recommendations.

In this database, we have focused our attention on mitigation strategies which reduce GHG emissions per animal per day (absolute emissions) or relative to dietary intake (either DMI or GE) as opposed to strategies which target emissions per unit product (emissions intensity). This approach excludes many potentially effective mitigation

strategies from our database, such as animal breeding or stock management. Selective breeding can achieve significant improvements in productivity and efficiency, which in turn mitigates GHG emissions through increasing productivity and hence decreasing emission intensity (Wall et al., 2010). The effectiveness of such strategies is best explored through heritability and selection models at the herd level. Stock management, such as faster turnover of stock or younger age structures, can also be cost-effective options to reduce emissions intensity (emissions per unit product) at the farm level through improved production efficiency (Mazzetto et al., 2015). However, as for breeding, herd management strategies are best modelled at the farm level rather than at the individual animal level which is the focus of this database.

## 2.2 Literature search strategy

Several searches were made of relevant databases (Web of Science ISI, CAB Abstracts, and Biosis), most recently in February 2016. A range of key words were used including specific animal terms (e.g. ruminant, cattle, dairy, and sheep), mitigation terms (general terms such as decrease and influence and more strategy specific such as ionophores or monensin, lipids or fatty acids, tannins or saponins) and methane. Further relevant papers were identified from cited references in relevant review or meta-analysis papers (including Eugène *et al.* 2004, 2008; Grainger & Beauchemin 2011; Jayanegara *et al.* 2011; Rabiee *et al.* 2012) as well as those cited in papers already discovered during the search process. Relevant papers identified were added to a database initially developed in Excel.

The database was limited to those studies which measured *in vivo* methane emissions from ruminants and where a control and mitigated measure could be identified. Studies where methane emissions were calculated from VFA concentrations (e.g. Chaturvedi *et*



*al.* 1973; Montoya *et al.* 2011) were not included. At the end of data collection, 412 papers had been added to the database. For each included paper a range of meta-data were extracted including reference information, study design, animals, diet and effect. Details regarding the database structure can be seen in **Table 1**. Mitigation strategies were classified according to the categories illustrated in **Table 2**. In addition treatments within strategies were identified to provide more detail and add further possible levels of classification. Multiple comparisons were included from an individual publication with multiple studies, where multiple mitigation strategies were tested or where multiple levels of a particular strategy were tested. Some long-term studies report methane emissions measured at several time-points during the study. For such studies where multiple emission measures are reported, each measurement and the respective treatment duration were included in the database.

Where multiple units of methane emissions were reported we chose to use emissions relative to intake (e.g. per g DMI, as % GE or similar). Feed intake level by itself is known to be a major determinant of total methane production and is negatively correlated with emissions per unit feed intake (Ellis *et al.*, 2010; Herd *et al.*, 2014; Muetzel *et al.*, 2009; Ramin and Huhtanen, 2013; Sauvant and Giger-Reverdin, 2010). Expressing methane emissions per unit of intake will partially account for potential differences in intake between studies and between production systems.

This comprehensive database has now been made available on-line through a user friendly interface (<http://mitigate.ifers.aber.ac.uk>). The web-site provides a facility for open access to the database, as well as future updates of the database as more research is published on the topic. By registering for free as a user on the website, researchers can upload meta-data of their peer reviewed research through a simple input form. The

author will be asked provide the appropriate category of mitigation strategy along with relevant meta-data from their study according to the database structure seen in **Table 2**. To ensure compatibility for future analyses, data input has been restricted to allow only a small range of standardized units for reporting of emissions with preference indicated for emissions as g CH<sub>4</sub> / kg DMI. The database does not hold the raw data, only summary and meta-data as reported in a relevant scientific publication. Along with the reported meta-data, the author will be requested to include a reference to the publication which reports the results.

### *2.3 Estimation of mitigation potentials and heterogeneity*

Two statistical approaches are currently commonly in use for meta-analysis of agricultural research, based on estimation of the relative magnitude of the treatment effect (Hedges, 1992) or a prediction of the relationship between a predictor and a response variable (St-Pierre, 2001). In both approaches, study is treated as a random effect where the studies included in the analysis are considered a random sample of all possible studies and a mixed statistical model is used to evaluate study outcomes (St-Pierre, 2001; Viechtbauer, 2010). The MITIGATE database is made up of a very diverse range of mitigation strategies, animal production systems and study types which makes utilizing St-Pierre's (2001) method particularly challenging. Our aim for the analysis is to provide simple, broad scale parameter estimates for inclusion in future policy or modelling efforts. The calculation of an effect size (ES) allows the outcome of these diverse studies to be expressed on a common scale (Viechtbauer, 2010) and provides relevant comparisons of strategies. A wide range of potential effect sizes are available such as ratios, regression factors or relative risk (Hedges, 1992). For MITIGATE, the effect size (**ES**) was calculated as the natural logarithm of the response

ratio according to Equation 1

$$R_i = \ln(T_i / C_i) \quad (\text{Equation 1})$$

where  $R_i$  is the observed effect size,  $T_i$  is the reported mean methane emission for the treatment group and  $C_i$  is the reported methane emissions for the control group in the  $i^{\text{th}}$  study (Borenstein et al., 2009).

The variance of the ratio calculated based on the reported standard deviation or standard error of the mean for each comparison where possible according to Equation 2.

$$\text{var}R_i = s_i^2 * ((1/n_i^T * T_i^2) + (1/n_i^C * C_i^2)) \quad (\text{Equation 2})$$

where  $\text{var}R_i$  is the estimated variance of  $R_i$ ,  $s_i^2$  is the pooled standard deviation,  $n_i^T$  is the sample size of the treatment group and  $n_i^C$  is the sample size of the control group of the  $i^{\text{th}}$  study (Borenstein et al., 2009).

This provides a simple measure of the overall effectiveness of mitigation strategies for comparison and a useful starting point for future investigations of heterogeneity or inclusions in modelling efforts.

Although the database contains all available data and associated effect sizes, meta-analysis results reported in MITIGATE are restricted to those studies where methane emissions were expressed per unit of intake (either as related to feed energy intake (mainly % gross energy intake; GEI) or per weighed matter (mainly dry matter intake; DMI)) and where statistical variance was reported (either as standard deviation or standard error of the mean or as standard error of the mean difference). In total, 328 comparisons from 127 papers contained in the database have therefore been excluded from the reported meta-analysis results.

Mean mitigation potential and associated variance was computed by fitting a random-effects model with a DerSimonian-Laird estimator (DerSimonian and Laird, 1986) for assessing heterogeneity ( $\tau^2$ ) in the Metafor package of R (Viechtbauer, 2010). Models were fitted for each mitigation category separately and can be expressed as Equation 3.

$$\theta_i = \mu + u_i \quad (\text{Equation 3})$$

where  $\theta_i$  = true effect size in the  $i$ th study,  $\mu$  = overall true effect size and  $u_i$  = random deviation from the overall effect size [ $u_i \sim N(0, \tau^2)$ ], which was unknown but estimated from calculated ratio variances as described above (Appuhamy et al., 2012; Viechtbauer, 2010). Data heterogeneity was expressed as a percentage of total variability ( $\tau^2$  plus sample variance) indicated by the  $I^2$  statistics (Viechtbauer, 2010).

Observed heterogeneity can be further explored through the inclusion of relevant explanatory variables (moderators) in the model as illustrated in Equation 4:

$$\theta_i = \beta_0 + \beta_1 x_{1p} + \dots + \beta_p x_{ip} + u_i \quad (\text{Equation 4})$$

where  $\theta_i$  is the true effect size (ES) in the  $i$ th study,  $\beta_0$  the overall true effect size  $x_{ij}$  is the value of the  $j$ th explanatory variable ( $j = 1, 2, \dots, p$ ) for the  $i$ th study; and  $\beta_j$  is the change in the true effect size for unit increase in the  $j$ th explanatory variable. For this paper, moderators were chosen *a priori* from the meta-data and included animal species, animal physiological stage, and region of study.

Visitors to the MITIGATE site can view the estimated mean mitigation potentials, along with the estimated 95% confidence intervals and sample sizes, for each mitigation strategy. Options are available to filter the results based on classifications of animal type, mitigation type, geography, or production system. The website automatically re-

calculates and reports the updated mitigation potentials based on the filters selected. Either a filtered or complete database can be downloaded as a csv file including all reported meta-data for more detailed analyses.

### **3 Results and Discussion**

Several reviews of enteric methane mitigation strategies have recently been published, including comprehensive qualitative reviews (e.g. Hristov *et al.* 2013; Knapp *et al.* 2014) and quantitative meta-analyses focused on specific strategy options such as saponins (Jayanegara *et al.*, 2014), monensin (Appuhamy *et al.*, 2013), essential oils (Khiaosa-Ard and Zebeli, 2013), dietary lipids (Patra, 2013) and nitrates (Lee & Beauchemin, 2014). However, to the authors knowledge the current publication is the first comprehensive quantitative database of all tested mitigation strategies for enteric methane emissions from all ruminants. This allows for not only quantitative estimates of the technical mitigation potential in a wide range of production systems and mitigation strategies, but also for comparisons between strategies and an investigation of their heterogeneity. As illustrated by several recent meta-analyses with overlapping topics (e.g. effect of dietary fats in dairy production as seen in Eugène *et al.* 2008; Rabiee *et al.* 2012; Patra 2013), there is a need for continued updates and refinement of meta-analyses. Availability of a comprehensive database will simplify this process in future.

#### **4.1 Trends in mitigation research focus**

The potential for reducing methane emissions was first investigated due to the energetic loss to the animal, with the first publication appearing in the database from 1948 investigating the effect of dietary fat utilization and energy efficiency in sheep (Swift *et*

al., 1948). This theme continued through the 1960s and 1970s, with work only being published in the UK and USA. However, since the initial publications on the impact of ruminants on GHG emissions (e.g. Johnson & Johnson 1995) data available on this subject has grown exponentially (**Figure 1**) and is expected to continue to grow.

The MitiGate database currently includes 411 papers, resulting in 1173 treatment comparisons and representing a wide range of mitigation strategies as illustrated in **Table 1**. Overall, 78.9% of the publications originated from the year 2000 onwards, with 176 papers published in the last 5 years alone. This large increase in publications has largely been driven by research output from Europe, North America and to a smaller extent New Zealand and Australia (**Figure 1**). The last 5 years has seen an increasing contribution also from Asia and South America (**Figure 1**). This domination of research by Western Europe and North America has long been recognized in scientific publication more widely (Gálvez et al., 2000) but this domination may now be waning. Asia is now seeing the fastest rate of growth in research output particularly driven by China. This rapid growth in research output from China is seen in all areas of scientific output, not only in the field of methane mitigation, and is driven by a steady increase in research and development investment (Moiwo and Tao, 2013). Despite strong growth in research output, research is still dominated by only a small handful of countries, with USA, Canada, UK, Australia, and China producing the highest output in the past 5 years (**Figure 1**).

Although the underrepresentation of certain regions (such as Africa) partially reflects the higher priority given to adaptation strategies and sustainable intensification of livestock systems (Herrero et al., 2013; Ogle et al., 2014) many underrepresented regions are also lacking in research capabilities, both expertise and facilities (Gálvez et

al., 2000). This is likely to be partially alleviated through current funding and technology transfer initiatives (Bellarby et al., 2013; Clark, 2013). Each country or region will vary in their dominant production systems which could influence the effectiveness of some mitigation strategies (Smith et al., 2007). Comparisons representing a wide range of geographical regions and production systems will increase the certainty of effectiveness when approaches are implemented in these specific farming systems.

Many strategies are being developed to address the environmental cost of methane emissions of livestock (Bellarby et al., 2013; Eckard et al., 2010; Hristov et al., 2013; Oenema et al., 2001; Smith et al., 2008; Tubiello and Loudjani, 2010), but there is a large variation in the coverage of different mitigation strategies included in the database (**Table 3**). Some strategies such as bacteriophages and reductive acetogenesis mentioned by (Eckard et al., 2010) and others (Boadi et al., 2004; Klieve and Hegarty, 1999; McAllister and Newbold, 2008) have not yet generated *in vivo* data suitable for inclusion in the database. The major research focus has also shifted over time. The main body of research has since the beginning focused on diet composition, as this is the main driver for energy use efficiency and production. Mitigation through the addition or substitution of concentrates (150 study comparisons) or the addition of dietary oils (179 study comparisons) has received the most attention and continues to be areas of rapid growth (**Figure 2**). Similarly, forage quality (104 study comparisons) or the addition of legumes (63 study comparisons) continued to receive considerable research interest over a long period of time (**Figure 2**). Only in the past 10 years have strategies specifically targeting methanogens and methane production such as the use of tannins or saponins or supplementation with hydrogen sinks such as nitrate received increasing attention (**Figure 2**). This mirrors the increasing attention on GHG emissions from

livestock production over the same period spurred on by a recognition of the very large contribution livestock production makes to overall GHG emissions (Steinfeld et al., 2006). Research into the use of ionophores peaked between 2005 and 2010 and has since decreased (**Figure 2**) showing very little further interest in this as a potential mitigation strategy.

With an increased focus on GHG emissions, the database also highlights technological developments in methane measurement techniques. Studies included in the database used either metabolic chambers (such as described by Pinares-Patiño *et al.* 2008) built to capture all the methane produced, the SF<sub>6</sub> tracer technique which uses the inert tracer gas sulphur hexafluoride (described by Johnson *et al.* 2007) or techniques which measure the composition of exhaled air such as masks (Wang et al., 2007) or hoods (Takahashi et al., 1999). The three latter strategies show higher variability compared to chamber measurements (Grainger et al., 2007; Pinares-Patiño et al., 2011). Chambers are less suitable for measuring emissions under grazing conditions and will therefore necessitate the refinement and use of a range of measurement techniques in the future. Among the latest developments in this field is the use of GreenFeed system introduced five years ago with results appearing in our database only in the last two years. The system allows the animal to move around freely, and records methane emissions in exhaled air within a hood with a feed supplement whenever the animal chooses to visit the GreenFeed (Hammond et al., 2015).

#### *4.2 Meta-analysis of mitigation effectiveness per unit of intake*

For the meta-analysis, studies were restricted to those studies where methane emissions were expressed per unit of intake (e.g. as % gross energy intake or per kg dry matter intake) and where statistical variance was reported (either as standard deviation or



standard error). The final restricted database held 845 study comparisons from 293 publications. Due to the large number of comparisons made,  $\alpha$  was restrictively set at 99% and only comparisons with a  $p < 0.01$  was considered significant.

Analysis of all mitigation strategies currently available identified clear differences in terms of their effectiveness in decreasing emissions (**Table 3**). For the mitigation strategies that were identified as effective, mitigation potentials varied between 6% and 25%. This is a much larger range than the 5-10% estimated in previous qualitative reviews (Bellarby et al., 2013; Hristov et al., 2013; Oenema et al., 2001; Smith et al., 2007; Tubiello and Loudjani, 2010) highlighting the need to better understand the heterogeneity in methane mitigation outcomes and applicability of strategies within specific production systems.

Our meta-analysis clearly identifies chemical inhibitors as the most effective mitigation strategy, reducing enteric methane emissions by 25% on average ( $p < 0.001$ ), closely followed by dietary supplements such as hydrogen sinks, tannins or lipids which all reduced methane emissions by more than 10% on average (**Table 3**). Vaccination appeared to have no impact on enteric methane emissions ( $p = 0.1$ ), whereas grazing intensity, probiotics and defaunation were not considered significantly effective in this meta-analysis ( $p = 0.02$ ).

Although several mitigation strategies are here identified as technically very effective, there are economic implications for implementation of these strategies which may mean that they are not cost effective. Our meta-analysis can be considered the first step in evaluating mitigation strategies, providing parameter estimates for further modelling and evaluation of cost-effectiveness. One such recent study used MitiGate estimates of technical potential to evaluate the cost-effectiveness of mitigation options for Chinese

agriculture (Wang et al., 2014). Utilizing dietary supplements such as lipids as a mitigation strategy, although technically very effective, was associated with large annual investment costs and hence not cost effective. Supplementation with probiotics or tea saponins on the other hand, had a much lower mitigation potential but at negative cost would provide a win-win strategy (Wang et al., 2014). Similar work comparing marginal abatement costs for EU dairy production, also based on inputs from the MitiGate database, has shown that there are considerable national differences in the cost effectiveness of strategies depending on national production scenarios (Koslowski, 2016).

#### *4.3 Heterogeneity of mitigation outcomes*

The broad meta-analysis reported here has shown significant heterogeneity for most of the mitigation strategies investigated. We investigated the potential for animal species or physiological stage, or geographic region to explain some of the observed heterogeneity in mitigation outcomes. All factors were found to explain a significant proportion of observed heterogeneity (animal species:  $F_{4,842}=78.62$ ,  $p<0.001$ ; physiological stage:  $F_{6,840}=54.89$ ,  $p<0.001$ ; region of publication:  $F_{7,839}=45.56$ ,  $p<0.001$ ). Differences in effect size between different ruminant species or physiological stage of the animal could be due to several factors. Lactating versus non-lactating animals have much higher energy requirements, will consume larger quantities and have higher rumen passage rates. This might have implications for dietary supplements in terms of daily dose and retention time of the supplement in the rumen. Furthermore, morphological differences in the anatomy of the rumen (Clauss et al., 2010) and differences in basal diet (Machmuller et al., 2001) could lead to changes in response for the different strategies between ruminants. Regional differences could be

explained both by animal genetic differences as well as differences in basal diet. It is important to note that in this database, many of the potential explanatory variables are likely to be correlated with each other. Mitigation strategies and animal production systems are not evenly represented in all regions. The very large heterogeneity in the dataset and clearly significant influence of broad explanatory variables illustrates the need for further exploration of heterogeneity in mitigation effectiveness.

#### 4.4 Suggested further database developments

For this meta-analysis, studies were restricted to those studies where methane emissions were expressed per unit of intake. Excluded comparisons were among those from older studies where methane was only reported per day, grazing studies where measuring feed intake is more challenging, but also studies where only rumen methane concentrations were reported (e.g. Perry & Weatherly 1976; Berchielli *et al.* 2003; Kongmun *et al.* 2010) or where methane was only measured for a short period after feeding (e.g. Chaturvedi *et al.* 1973; Agle *et al.* 2009). These latter techniques are either too indirect or not able to calculate daily methane production relative to daily intake making it difficult to compare the results with other studies.

Feed intake level by itself is known to be a major determinant of total methane production and is negatively correlated with emission per unit of feed intake (J. L. Ellis *et al.*, 2010; Gerber *et al.*, 2013; Herd *et al.*, 2014; Muetzel *et al.*, 2009; Ramin and Huhtanen, 2013; Sauvant and Giger-Reverdin, 2009). Expressing methane emissions per unit of intake will partially account for potential differences in intake when animals were fed *ad libitum*. We have therefore restricted the future input into the MitiGate database only to emissions reported per unit intake. Ideally, the effectiveness of different mitigation strategies should be compared as methane intensity, expressed on a

unit of output (being milk, live weight gain, wool or meat) produced. Together with other GHG produced, this results in the emission intensity (Ei) metric as an output unit. This metric is useful as it allows comparison within and between commodities and is closely related to the productivity per animal or herd, enabling identification of options that can both increase production while lowering Ei (Gerber et al., 2013). Data on these parameters is however very limited particularly in studies with small ruminants or non-dairy cattle. If future publications could publish methane per unit of output as well as per unit of feed intake both can be included in the database allowing for stronger and more accurate estimation of mitigation potentials.

An important limitation of current meta-analyses including this one, is the lack of long term studies. The persistency of strategies has been questioned for ionophores (Guan et al., 2006; Patra, 2012), chemical inhibitors (Knight et al., 2011) and fats (Grainger and Beauchemin, 2011; Grainger et al., 2010) as well as for strategies involving plant extracts (Patra, 2012). The typical short duration of animal trials is a severe limitation to our understanding of the persistency of mitigation effects (Hristov et al., 2013). The rumen ecosystem is known to be able to adapt to interventions, as can be seen under long term ionophore supplementation (Guan et al., 2006). Lack of persistency or a decrease in the effect results in an overestimation of the potential of a strategy. Clearly, more long-term studies are needed to better understand the persistency of mitigation strategies or any long-term consequences of rumen manipulation.

## ***5 Conclusions***

We present here a comprehensive database of current research on the effectiveness of strategies for mitigation of enteric methane emissions from livestock production. It is hoped that this database will be instrumental in providing relevant data for on-going

modelling efforts or future policy recommendations. The database has been made available for further meta-analyses and continued updates as more research is published on the topic. As we have discussed above, there are significant caveats associated with general mitigation estimates based on the current body of literature. Data are currently limited from several regions and production systems, particularly in the developing world. Similarly, some animal types or mitigation strategies are under-represented in the database. There is also little data on the long-term effectiveness of any of the strategies described. In general, addition of more studies to the database will improve the power and estimation of the true effect. We hope that as the database becomes more widely publicised, more research will become available to fill these gaps.

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**Table 1 Database structure of the MitiGate database.**

Database structure	Category details
<i>Reference information</i>	
Author	First author only
Year	Year of publication
Country	Country where animal trial was performed
Region	Continent where animal trial was performed
Citation	Full bibliographic reference
<i>Study design</i>	
Mitigation strategy	Theme and category, categorized according to table 1
Mitigation measure	Chamber, hood, mask, SF6 tracer gas, other
Experimental design	Latin square, random trial, repeated measure, other
Treatment details	Details and assigned sub-category of specific treatment or additive
Dose	Dose of effective compound (g/kg DM increase to control)
Duration	Duration in weeks between treatment start and methane measurement
<i>Animals</i>	
Animal species	Cattle, Sheep, Goats, Other
Animal breed	Specific breed or crossbreed
Animal class	e.g. Lamb, Wether, Ewe, Ram
Physiological stage	Growth, Maintenance, Gestation, Lactation
Body weight	Average bodyweight (kg)
Management system	Housed, grazed or mixed systems
Diet type	Feedmix (roughage and concentrate or predominantly concentrate), Roughage (e.g. cut pasture, silage, hay), Grazed
DMI	Average dry matter intake, recorded for both treatment and control groups
<i>Effect</i>	
Unit	Unit of methane measurement recorded (i.e. % GE or g/kg DMI)
Emissions	Mean treatment and control emissions along with SD or SEM
Sample size	Number of animals/observations in control and treatment groups
Effect size	Ratio and log ratio of treatment to control along with estimated variance of the ratio

**Table 2 Classification of strategies to reduce enteric methane emissions in ruminants, based on the literature reviewed and corresponding number of publications and treatments comparisons within each strategy. Some publications have reported on more than one mitigation strategy.**

Strategy	Publications	Treatment comparisons
<i>Animal Management</i>		
Level of feed intake	28	71
Grazing intensity	6	27
<i>Diet Manipulation</i>		
Feed quality		
Forage quality	54	153
Concentrate quality	14	29
Increasing concentrate	69	167
Inclusion of legumes	30	81
Dietary supplements		
Dietary oils	63	236
Pre- and probiotics	13	34
Hydrogen sinks	34	72
Plant secondary compounds		
Tannins	27	62
Saponins	14	28
Essential oils and organosulphers	18	44
<i>Rumen manipulation</i>		
Antibiotics	34	76
Vaccination	3	7
Chemical inhibitors	22	62
Defaunation	12	25
<i>Total</i>	<i>412</i>	<i>1174</i>

**Table 3 Mean effect size and estimated heterogeneity parameters for mitigation strategies where results were reported on a per feed intake basis and included some measure of variance. Results were computed fitting a random-effects model with a DerSimonian-Laird estimator for assessing heterogeneity in the Metafor package of R.**

Mitigation strategy	Mean effect size		<i>n</i>	<i>P</i>	Heterogeneity	
	(95% CI)				<i>I</i> <sup>2</sup>	<i>P</i>
<i>Animal management</i>						
Level of feed intake	0.90 (0.85-0.94)	44	<0.001	95.2	<0.001	
Grazing intensity	0.95 (0.91-0.99)	15	0.022	26.9	0.160	
<i>Diet manipulation</i>						
Feed quality						
Forage quality	0.95 (0.93-0.98)	105	<0.001	91.2	<0.001	
Concentrate quality	0.88 (0.81-0.96)	26	0.003	94.0	<0.001	
Increasing concentrate	0.93 (0.90-0.95)	124	<0.001	77.9	<0.001	
Inclusion of legumes	0.94 (0.90-0.98)	63	0.004	79.8	<0.001	
Dietary supplements						
Dietary oils	0.86 (0.83-0.89)	179	<0.001	93.5	<0.001	
Pre- and probiotics	0.96 (0.94-0.99)	22	0.020	0	0.801	
H sinks	0.85 (0.81-0.90)	54	<0.001	87.1	<0.001	
Plant secondary compounds						
Tannins	0.82 (0.78-0.87)	40	<0.001	88.8	<0.001	
Saponins	0.94 (0.91-0.97)	24	<0.001	35.7	0.043	
Essential oils	0.92 (0.89-0.94)	40	<0.001	0	0.769	
<i>Rumen manipulation</i>						
Antibiotics	0.91 (0.88-0.95)	40	<0.001	98.7	<0.001	
Vaccination	1.12 (0.98-1.29)	7	0.100	0	0.993	
Chemical inhibitors	0.75 (0.70-0.79)	52	<0.001	52.2	<0.001	
Defaunation	0.83 (0.71-0.96)	11	0.015	60.6	0.005	

### ***Figure captions***

**Figure 1:** Number of publications in the database for each year. Data is separated by geographical region, based on the location where the research was conducted as reported in each publication. Very little research is available from Africa or South America, although South America is showing considerable growth in research output in the last 5 years.

**Figure 2:** Number of publications per year reporting on each mitigation strategy. Strategies focusing on dietary quality, such as concentrate inclusion, forage composition or lipid supplementation, have received the greatest research interest. Very little research is available on manipulation of rumen microbiota through defaunation or probiotic supplementation.

Figure 1

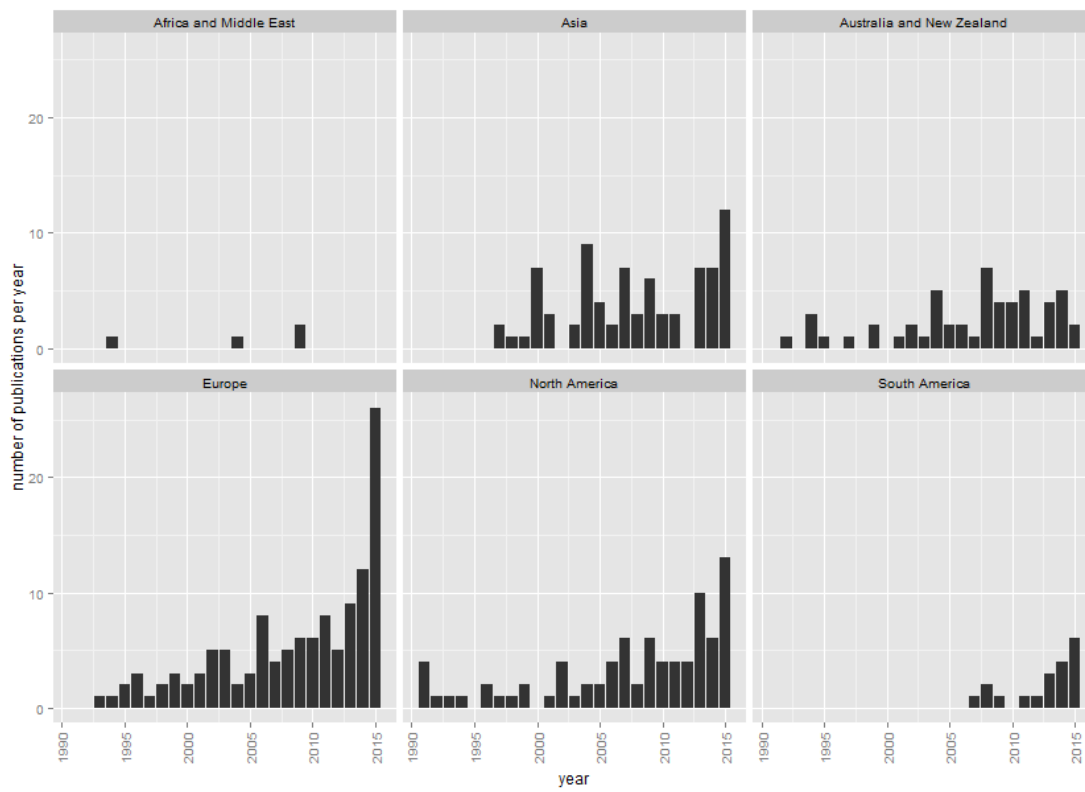


Figure 2:

