RESEARCH PROTOCOL

Title
The effectiveness of feed efficiency and husbandry in mitigating the enteric methane emissions of dairy and beef cattle in temperate farming systems; a rapid synthesis and network meta-analysis

Registration
To be registered in EcoEvoRxiv. The Cochrane and PRISMA-EcoEvo protocol guidelines were used to inform the contents and structure of the protocol.

Authors

Jodie E. Vaughan (corresponding author)

Postgraduate Ecology and Biodiversity student, Newcastle University

Email: j.vaughan2@ncl.ac.uk

Dr. Gavin Stewart

Senior Lecturer in Evidence Synthesis, Newcastle University

Email: gavin.stewart@ncl.ac.uk

Contributions
JEV and GS led the project context conception, JEV leads the protocol development, data acquisition and extraction and writing, and GS leads statistical modelling and interpretation.

Amendments
Post-registration protocol amendments will be recorded and included in the updated copy.

Support

No sources of financial support are involved in this review. Authors are a postgraduate student and an academic staff member at Newcastle University.

Plain language summary

Beef and dairy cattle of temperate farming systems will be studied to investigate the relationships between their methane emissions as well as their efficiencies as milk and beef producers, in terms of how much food they require versus how much product they yield. The investigation will comprehensively utilise all research published on this topic to date via a formalised rapid review and analysing it together. The effects of organic versus conventional farming systems, the amount of time cattle spend grazing, the components of their diets and their breed as categorised by physical size will also be investigated for their effects on efficiencies and methane emissions. The bigger picture of subsequent implications on biodiversity, ecosystem services, welfare and cultural values will also be theoretically explored, as to address the often-speculated and assumed benefits of various practices such as the uses of native livestock breeds and organic farming.

INTRODUCTION

Rationale

Methane (CH_4) is the world's most abundant atmospheric hydrocarbon[1]. It has a global warming potential many times greater than carbon dioxide across both short- and long-term timescales[2,3] (Figure 1) and contributes to radiative forcing both directly and indirectly[5].

Greenhouse gas	Chemical formula	Lifetime (years)	Global warming potential by time horizon (years)		
			20	100	500
Carbon dioxide	CO_2	Variable	1	1	1
Methane	CH_4	12±3	56	21	6.5

Figure 1: Summary of the global warming potential of methane versus carbon dioxide over time[4].

Ice records have revealed substantial increases in CH_4 since the inception of the industrial revolution[6]. Although estimates of CH_4 sources by sector vary[5], within agriculture, ruminant livestock are understood to make the largest contributions[7], with approximately two thirds of this occurring in the form of enteric biogenic CH_4[8]. This is a result of their natural digestive systems, whereby microbes ferment plant components such as fibre, cellulose, and starch in the rumen to produce bioavailable energy for them, with the by-product of methane being released mostly via eructation and the small remainder through flatulence[9,10,11]. Larger ruminants such as cows are particularly significant contributors, in part due to their larger physical size inevitably requiring higher maintenance inputs[12]. Adding to rising emission rates are the increasing demands for both beef[13] and dairy products[14].

This has resulted in growing interests into potential means of mitigating the methane emissions of the beef and dairy industries, with the simultaneous intentions of maximising product yields while minimising human inputs. This can be measured in a variety of ways[15,16,17,18], but a conventional measurement is known as the feed efficiency ratio, generally defined as the ratio of product yields to inputs[19,20,21], with the latter referring to the weight of feed consumed. For beef cattle specifically, this is most commonly applied in terms of body weight gained[22,23] and, for dairy, the milk yield by weight[16]. A lower ratio indicates a greater efficiency[24], and a greater efficiency is commonly understood and expected to reduce enteric methane production[25]. Considering this, improvements in the cattle feed efficiency may have the potential to increase the profitability of these industries while simultaneously minimising its environmental impacts[26], and this measure has unsurprisingly become an important consideration regarding all facets of the farming process[27].

However, this is of course not the entire picture. In order to make well-informed attempts at reducing the emissions of these industries as much as possible, a sound understanding of any and all influencing factors is first required[28], of which there are many[29], and many of which are not fully understood. For instance, varied patterns have been observed between studies of enteric methane emissions in regards to the feed efficiency itself[30], as well as the breeds of cattle used[31,32], the application of organic versus conventional management schemes, the length of the grazing season and subsequent time spent in housing[33,34], and dietary components and proportions[35,36] amongst others. The common understanding amongst these researchers is that such factors influence enteric methane emissions also, however the extent to which is unclear and so a comprehensive review is essential to deepen our understanding[37].

Ecological benefits of commonly-valued practices are oftentimes speculated and uncritically assumed. For instance, the often-assumed benefits of the use of native livestock breeds[38,39] include their superior adaption to poor-quality feed and pasture[40], and of organic farming systems, their improvements of biodiversity[41,42,43] and welfare[44]. In an attempt to shed light on matters such as these, the wider subsequent implications of methane mitigation approaches will also be explored through a meta-regression, in regards to biodiversity, ecosystem services, welfare and cultural values.

Existing meta-analyses of the kind proposed here are scarce, typically covering either a single covariate or a lesser range of the exampled covariates, and focusing on either tropical farming systems[45] or ruminant farming on a global scale, irrespective of the implications of varying climatic conditions and species[46]. This analysis is unique in both its geographical and contextual breadth in that it will assess cattle across the array of temperate farming systems currently in operation across the world, allowing a more comprehensive look at the issue that does not isolate the methane-mitigating effectiveness of improving feed efficiency from other contributing factors and the wider implications. Considering the potential importance of any existing relationships between such factors and subsequent enteric methane emissions and the wider implications that subsequent decisions may present, a comprehensive investigation into the effectiveness of improved feed efficiencies and other influencing factors of husbandry on the mitigation of enteric methane emissions produced by cattle is vital, and so is proposed here.

Objectives

The aim of the rapid review and meta-analysis is to synthesize all evidence available on the effectiveness of increased feed efficiencies and influencing farm management factors on the enteric methane emissions of beef and dairy cattle in the context of temperate farming systems. This will be achieved by meeting the following objectives:

1. Compile a comprehensive dataset of all existing research of feed efficiency ratios, husbandry and enteric methane emissions of beef and dairy cattle in a range of temperate farming systems which meet defined inclusion criteria.
2. Assess the effectiveness of an improved feed efficiency and the impacts of various farm management components on the mitigation of enteric methane emissions through a network meta-analysis.
3. Consider the factors of biodiversity, ecosystem services, welfare and cultural values that improved feed efficiencies, lowered methane emissions and the use of various farm management components interact with and discuss the potential subsequent trade-offs involved through a meta-regression.
4. Assess the quality of evidence through a critical appraisal.

The project will aim to assess a variety of dairy and beef cattle farming systems in all geographical areas possessing a temperate climate worldwide. Such areas will be identified using the Köppen climate classification[47,48]. Various farming conditions will be considered from the angles of organic versus conventional farming systems, the amount of time spent in pasture versus housing, the dietary components fed to cattle in terms of forage (herbage) to concentrate (supplement) ratios, the feed efficiency ratios and the cattle breed as categorised by its physical size. The extent to which the project will cover all of these areas and factors will entirely depend upon the availability of existing data at the time of the rapid review. The inclusion and exclusion criteria for the dairy and beef cattle populations to be studied, as well as the various farming systems, management practices and conditions they are subjected to, are identified in the methodology and justified in the methodology rationale.

Here, it is hypothesised that the methane emissions of both beef and dairy cattle are mitigated as a result of:

1. Higher feed efficiency ratios
2. Decreased forage to concentrate ratios
3. The use of conventional rather than organic farming systems
4. Decreased time spent in pasture
5. The use of physically smaller cattle

METHODOLOGY

A plain-language overview of the proposed methodology is provided here. For the rationale of the methodology, please refer to section 6. The following rapid synthesis design is guided by the Cochrane rapid review methods recommendations[49].

Eligibility criteria

Judgement of eligibility based upon the proceeding criteria will first be made based upon titles and abstracts. A second judgement will then be made based upon the study's level of relevance, consisting of two levels:

1. Adheres to all inclusion criteria and non-essential additional criteria
2. Only adheres to all inclusion criteria

Problem

Eligible studies must adhere to all of the following criteria:

1. Study assesses at least one of the following;
    a. Dairy cows
    b. Beef cows
2. Study was conducted in temperate climate
3. All measurements taken from individual cows
4. Replicates for each treatment group

And must also report:

1. Location at which study was conducted

Any of the following further details that are published in eligible studies will be extracted, however are not necessary for inclusion in the review:

1. Coordinates of location at which study was conducted
2. Details of climatic conditions at study location

Intervention

To obtain the values necessary to calculate the feed efficiency ratio, eligible studies must include the following data, all of which must have been measured directly from individual cows with the sampling designs, methods and timeframes specified and either standard deviations or standard errors of means reported:

1. **Dry matter intake**
    a. Provides for the amounts of forage and supplement on a dry matter basis in at least one of the following forms:
        i. Masses
        ii. Ratio
    b. Diet digestibility %

2. **Product yields and components**

   c. Dairy cattle: Milk yield
      i. Fat yield
      ii. Protein yield
   d. Beef cattle: Average daily weight gain

Comparison

Details of the following factors must be provided:

1. **Cattle characteristics**
   a. Breed
   b. Sex
   c. Physiological stage
      i. Dairy cattle: must be in lactation
      ii. Beef cattle: must be immature
2. **Conventional or organic farming management**
   a. Organic: must have organic certification
3. **Housed, grazed or mixed farming system**
   a. Housed: housing season period and length must be specified.
   b. Mixed: grazing season period and length must be specified.
4. **Sampling details**
   a. Sampling design
   b. Sampling year, period, length and dates
   c. Measurement methods (e.g. equipment, calculations)

Any of the following further details that are published in eligible studies will be extracted, however are not necessary for inclusion in the review:

1. **Further cattle characteristics**
   a. Age/life stage (e.g. yearling, substage of lactation)
   b. Behavioural (e.g. trained to be accustomed to environments, apparatus and conditions used for measurements)
2. **Feed component compositions**
   a. Forage (herbage) component: herbage type(s) and nutritional profile, timing of harvest
   b. Concentrate (supplement) component: supplement contents and nutritional profile
3. **Medical procedures** undertaken on cows (e.g. vaccination, antibiotic treatments)

Outcome
Primary outcomes

Eligible studies must include the following primary outcomes, all of which must have been measured directly from individual cows with the sampling designs, methods and timeframes specified:

1. **Enteric CH_4 emissions**
    a. Must be measured via either indirect open-circuit calorimetry respiration chambers or sulphur hexafluoride tracers
        i. Sulphur hexafluoride tracers: must specify whether rectal emissions were measured

In addition, eligible studies must provide at least one of the following for all primary outcomes so that standard deviations can be obtained:

1. Standard deviations
2. Standard errors
3. P-values

Secondary outcomes

Any of the following secondary outcomes that are published in eligible studies will be extracted, however are not necessary for inclusion:

1. **Non-enteric CH_4 and CO_2 emissions**

Search strategy and screening

A systematic search strategy will be applied through the use of a series of databases; CABI, Google Scholar, Newcastle University Library Search, SCOPUS and Web of Science. Prior to conducting the formal screening process, a pilot screening will be conducted to calibrate and refine search strings as required.

Sample search strategies:
Dairy cattle

1. Google Scholar (search for dairy cattle with methane measured via the sulphur hexafluoride technique, using the American spelling *sulfur*):

All of the words:
Dry matter, DMI, milk yield, fat, protein, methane, sulfur hexafluoride

At least one of the words:
grazing grazed housing housed

2. Newcastle University Library Search

Any field contains:

"dry matter" AND ((forage OR graz OR herbage) AND (concentrate OR supplement OR grain) AND ratio) AND ("milk yield*") AND fat AND protein AND lactat* AND (graz* OR hous*) AND methane AND ("Sulfur hexafluoride" OR "sulphur hexafluoride" OR "respiration chamber*") AND (temperate OR Argentina OR Austria OR Belgium OR Canada OR Chile OR China OR Denmark OR Egypt OR Finland OR France OR Germany OR Greece OR Hungary OR Iran OR Italy OR Japan OR Jordon OR Korea OR Luxembourg OR Mexico OR Morocco OR Netherlands OR "New Zealand" OR Norway OR Poland OR Portugal OR Spain OR Sweden OR Switzerland OR Turkey OR "United Kingdom" OR UK OR England OR Ireland OR Wales OR Scotland OR "United States of America" OR "United States" OR USA OR America)*

Beef cattle

1. SCOPUS

   "dry matter" AND ((forage OR graz OR herbage) AND (concentrate OR supplement OR grain) AND ratio) AND yield* AND (beef OR meat) AND (graz* or hous*) AND methane AND ("Sulfur hexafluoride" OR "sulphur hexafluoride" OR chamber OR "respiration chamber*") AND (temperate OR Argentina OR Austria OR Belgium OR Canada OR Chile OR China OR Denmark OR Egypt OR Finland OR France OR Germany OR Greece OR Hungary OR Iran OR Italy OR Japan OR Jordon OR Korea OR Luxembourg OR Mexico OR Morocco OR Netherlands OR "New Zealand" OR Norway OR Poland OR Portugal OR Spain OR Sweden OR Switzerland OR Turkey OR "United Kingdom" OR UK OR England OR Ireland OR Wales OR Scotland OR "United States of America" OR "United States" OR USA OR America)*

2. Web of Science

   Abstract:
   "dry matter" AND ((forage OR graz* OR herbage) AND (concentrate OR supplement OR grain) AND ratio) AND yield* AND (beef OR meat) AND (graz* or hous*) AND methane AND ("Sulfur hexafluoride" OR "sulphur hexafluoride" OR chamber OR "respiration chamber*")

Data extraction and management

Extraction

All data extraction will be conducted by a single individual. All search results, including the count of results for each search, and the abstracts of referenced material will be compiled into EndNote. Data will be extracted manually from eligible studies and compiled into Microsoft Excel (Appendix 1). For all eligible studies included in the analysis, available data beyond that to be assessed in the meta-analysis as mentioned will be extracted and compiled into data extraction forms (Appendix 2). Due to the high number of eligible studies anticipated to be used, to ensure data sources are not confused or lost, an additional record of all studies used will be compiled into excel consisting of first author, publication year, publication title, DOI and a unique identifying number, allocated in numerical order by their order of extraction.

Management
All data will be backed up following every work session, both within the device used to conduct the study and on an external drive. Data will be processed to standardise their forms and units as follows:

The various variations of means reported by studies will be standardised into standard deviations.

$$SD = SEM\ \sqrt{N}$$

Where SD is standard deviation, SEM is standard error of the mean and N is the sample size.

The following will be standardised into kilograms per day (kg/day/cow):

1. **Dry matter intake**
2. **Product yield**
   a. Dairy cattle: milk, milk fat and milk protein yields
   b. Beef cattle: average daily weight gain

Study treatments will be grouped by their following qualities into a concise spreadsheet in preparation for analysis (Appendix 3) as follows:

1. **Forage to supplement ratio**
   a. Very high: >80:20
   b. High: 70-79:30-21
   c. Moderate: 60-69:40-31
   d. Low: 50-59:50-41
   e. Very low: <50:50
2. **Treatment type**
   a. Dietary (e.g. active component added to diet)
   b. Medical (e.g. antibiotic added to diet, inoculation)
2. **Potential treatment effects**
   a. Methane mitigator
   b. Product yield enhancer
   c. Methane mitigator AND product yield enhancer

For dairy cattle, energy-corrected milk will be calculated as follows[50]:

$$ECM = (0.25\ MY) + (12.2\ MFY) + (7.7\ MPY)$$

Where ECM is energy-corrected milk yield (kg/day/cow), MY is milk yield (kg/day/cow), MFY is milk fat yield (kg/day/cow) and MPY is milk protein yield (kg/day/cow).

Breeds will be categorised by their registered average physical size (small, medium, large). Feed efficiency ratios, forage to concentrate ratios and enteric methane yields and intensities will be calculated as follows:

1. **Feed efficiency ratio**
    a. Dairy cattle

$$FE = \frac{ECM}{DMI}$$

Where FE is feed efficiency, ECM is energy-corrected milk yield (kg/day/cow) and DMI is dry matter intake (kg/day/cow).

    b. Beef cattle

$$FE = \frac{ADG}{DMI}$$

Where FE is feed efficiency, ADG is average daily weight gain (kg/day/cow) and DMI is dry matter intake (kg/day/cow).

2. **Forage to concentrate ratio**

$$F : C$$

Where F is the foraged (i.e. grazed, herbage) component of the DMI (kg/day/cow), and C is the concentrate (i.e. supplement) component of the DMI (kg/day/cow).

3. **Methane yield**

$$CH_4\ yield = \frac{CH4}{DMI}$$

Where CH_4 yield is enteric methane yield (g CH_4/kg DMI), CH_4 is enteric methane (g/day/cow) and DMI is dry matter intake (kg/day/cow).

4. **Methane intensity**
    a. Dairy cattle

$$CH_4\ intensity = \frac{CH4}{ECM}$$

Where CH_4 intensity is enteric methane intensity, CH_4 is enteric methane (g/day/cow) and ECM is energy-corrected milk yield (kg/day/cow).

    b. Beef cattle

$$CH_4\ intensity = \frac{CH4}{ADG}$$

Where CH_4 intensity is enteric methane intensity, CH_4 is enteric methane (g/day/cow) and ADG is average daily weight gain (kg/day/cow).

Narrative synthesis

In order to reveal any existing heterogeneities between eligible studies for data beyond that to be statistically assessed, a narrative synthesis will be performed. This will be conducted using the completed data extraction forms (Appendices 1-2).

Analyses

Meta-analysis

A Bayesian random effects meta-analysis[51] will be conducted using the R package 'multinma' in the RStudio[52] software environment. Enteric methane emissions and intensities will be assessed against feed efficiency ratios, as well as farming systems, grazing season lengths, dietary forage-concentrate ratios and breeds by size as described. The network connectivity will be displayed via network plots in order to visualise which studies compare which treatments, aiding the estimations of treatment effects. The convergence of the algorithm will first be evaluated via trace plots for each comparison across the iterations, followed by density plots of effect size estimates[53], and finally Gelman-Rubin plots to compare variations within versus between chains[54]. A nodesplit analysis will then be conducted to evaluate the network model's consistency, and related to the Bayesian p-values as well as the study characteristics forms[55].

Meta-regression

The biodiversity, ecosystem services, welfare and cultural values involved in improving feed efficiencies, mitigating methane emissions, and incorporating the various farming management factors assessed will be considered and ranked by factor values through a meta-regression. Trade-offs between these factors will then be evaluated and implications on future practices will be theoretically, qualitatively explored. The 'metareg' function of the 'meta' package will be used to conduct the meta-regression, and the results of which will be presented in a bubble plot to visualise the estimated regression along with the relative effect sizes of each study[52]. The meta-regression will be conducted this way at both the within- and between-cow levels to maximise the understanding of existing heterogeneity[55].

Critical appraisal

The strength of evidence used will be assessed using the GRADE approach, based on the following considerations:

- Imprecision
- Inconsistency
- Indirectness
- Publication bias

Each outcome will be assessed individually. The evidence will then be rated based on the evidence quality. Recommendations will then be made based on the ratings.

Presenting results

A world map identifying the locations at which all utilised studies were conducted will be generated with ArcMap. RStudio will be used to visualise results into appropriate figures. For example, publication biases will be presented and visualised through any existing asymmetry in a funnel plot.

Methodology rationale

A rapid synthesis will be conducted as opposed to a full systematic review due to the limited timeframe in which the project must be completed and the project being undertaken primarily by one individual. For the same reasons, it appears unlikely that every eligible study will be able to be used in the project, therefore every detail of the data sourcing and inclusion strategy will be defined explicitly and transparently. The assessment will constrict eligible studies to those conducted in temperate climates in an attempt to account for the factor of climatic variation between the various farming environments and the subsequent influence this may have on the various variables to be assessed[26].

Enteric methane emissions can be measured using an array of techniques, with varying reliability between them, and each possessing its own limitations. The indirect open-circuit calorimetry respiration chamber is generally considered the gold standard for such measurements due to its unmatched control, accuracy and precision[56,57,58] (Figure 2). Its physical setup allows for the control of various environmental conditions[60]. This creates a double-edged sword in that extraneous variables can be accounted for more so than with any other technique, however it cannot be directly applied to grazed cows and can only attempt to mimic grazing conditions through the dietary composition. It is therefore largely unknown to what extent its results can be reliably extrapolated to grazed cattle[61].

Figure 2: An example of the apparatus used in the indirect open-circuit calorimetry respiration chamber technique for measuring enteric methane emission[59].

In response and in attempt to fill this gap in enteric methane measurement techniques, the sulphur hexafluoride (SF_6) tracer technique was developed (Figure 3), which operates by sampling the CH_4 contents of air from eructation released from the nose and mouth. Several investigations into the accuracy and precision of the SF_6 tracer technique have been conducted. There are general agreements that the results of the technique are comparable to that of the respiration chamber technique, but also that a small amount of enteric methane is produced beyond the rumen and so is released by flatulence rather than eructation[62], and that it is associated with significant between-cow variability in emission measures from cows fed a standardised diet[63]. It is unclear to what extent this latter characteristic may imply potential flaws in the technique, as a variety of other factors such as environmental, behavioural, and genetic variations may be to blame. Nonetheless, the mobility and financial accessibility of the technique make it a very popular one[64] and is generally considered to be the most similar and reliable alternative technique to the respiration chamber[65,66,67,68] while possessing the great advantage of making enteric methane measurements in outdoor grazing conditions possible.

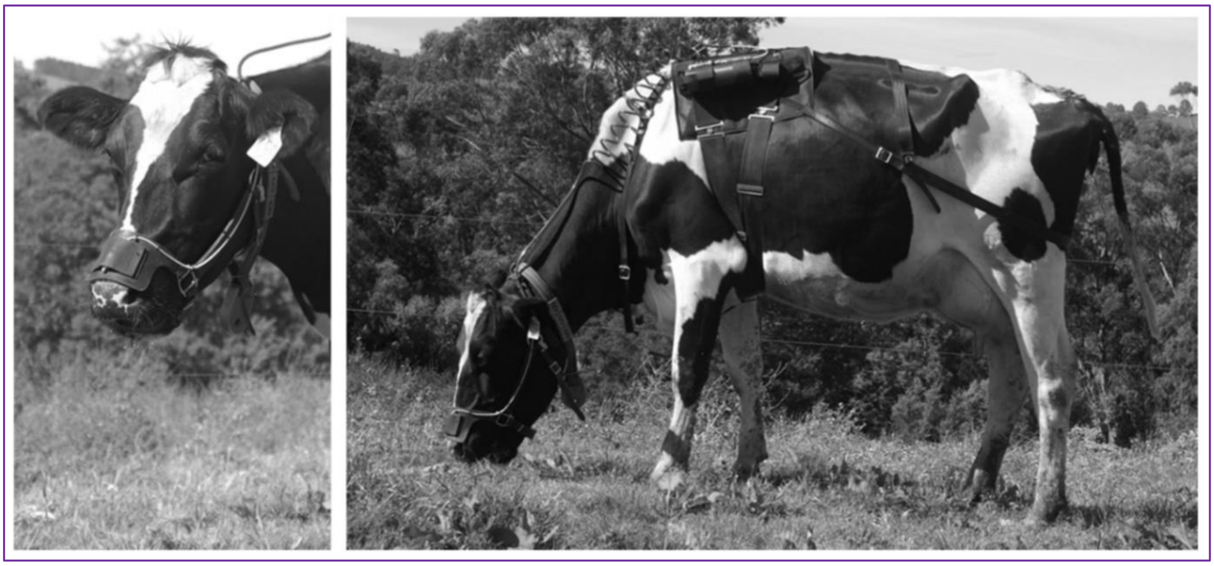

Figure 3: An example of the halter and saddle vehicles used for the gas collection cannisters and apparatus involved in the sulphur hexafluoride (SH6) tracer technique for measuring enteric CH4 emissions[56].

The components of biodiversity, ecosystem services, welfare and cultural values are critical factors to consider when considering the implications of the results in the wider context[69,70], yet is not generally discussed within studies of this kind. For instance, organic versus conventional cattle farming methods as well as extents of grazing are commonly understood to impact biodiversity in a variety of ways[71], and native cattle breeds often hold high cultural value on both local and national scales[72]. In the interests of considering the wider implications of the meta-analysis results, a meta-regression of such factors will be performed.

The factors of farming systems, time spent in pasture, dietary compositions and cattle breed and size are all important to consider as they are commonly understood to influence enteric methane emissions. For instance, organic cattle farming systems have in many cases been found to produce greater methane yields and intensities compared to conventional systems[73,74], and this is thought to be at least partially explained by the increased proportions[75,76] of herbage within the diet, which in turn relates to the extent of time spent in pasture[77]. Interest into genetic variations between cattle breeds is also growing[78], with a common conclusion being that the greatest contributing factor to between-breed differences being related to physical size due to their input requirements typically being lower[79,80], although breed differences in methane emissions have also been attributed to physical rumen size and retention times[81]. For reasons such as these, it is essential to include methane-mitigating factors other than the feed efficiency ratio in order to gain a more thorough, comprehensive understanding and perspective on the full picture of enteric methane emissions in the dairy and beef farming industries.

Project assessments

Resources and budget

As an entirely desk-based project, the following resources and outlets will be required:

- Computer
- Digital storage
- Internet access
- Workspace
- Database access
- Software access

A computer, multiple digital storage outlets and an internet connection are already personally owned with unlimited, reliable access. A personal, quiet, at-home workspace with unlimited access is available. All databases required to conduct the analysis is available through the university, and all software required to conduct the analysis is already owned and licensed on the personal device thanks to the Student Software services of the university. Overall, there is no foreseeable financial budget for the project.

Skill requirements

As mentioned, only non-specialist equipment will be required for this entirely desk-based assessment. The technical nature of R and the RStudio software to be used will be met in this project by skills developed and strengthened through the within-degree module 'NES8010 – Quantitative Ecological Research Methods' provided by Newcastle University, with skills in presenting the results through such software being further strengthened by the additional within-degree module 'BIO8069 – Geographical Information Systems and Remote Sensing.' The proposed network meta-analysis is highly complex and its application and technicalities will be supported by the supervisor's expertise, as well as wider research and personal practice with its use.

Project risk assessment

Due to the purely desk-based nature of the review and analysis, an ethics risk assessment was not conducted. The existing risks and subsequent mitigation measures to be taken in the project are summarised below (Figure 4). Several risks are interlinked, emphasising the importance of adhering to all mitigation measures throughout the project.

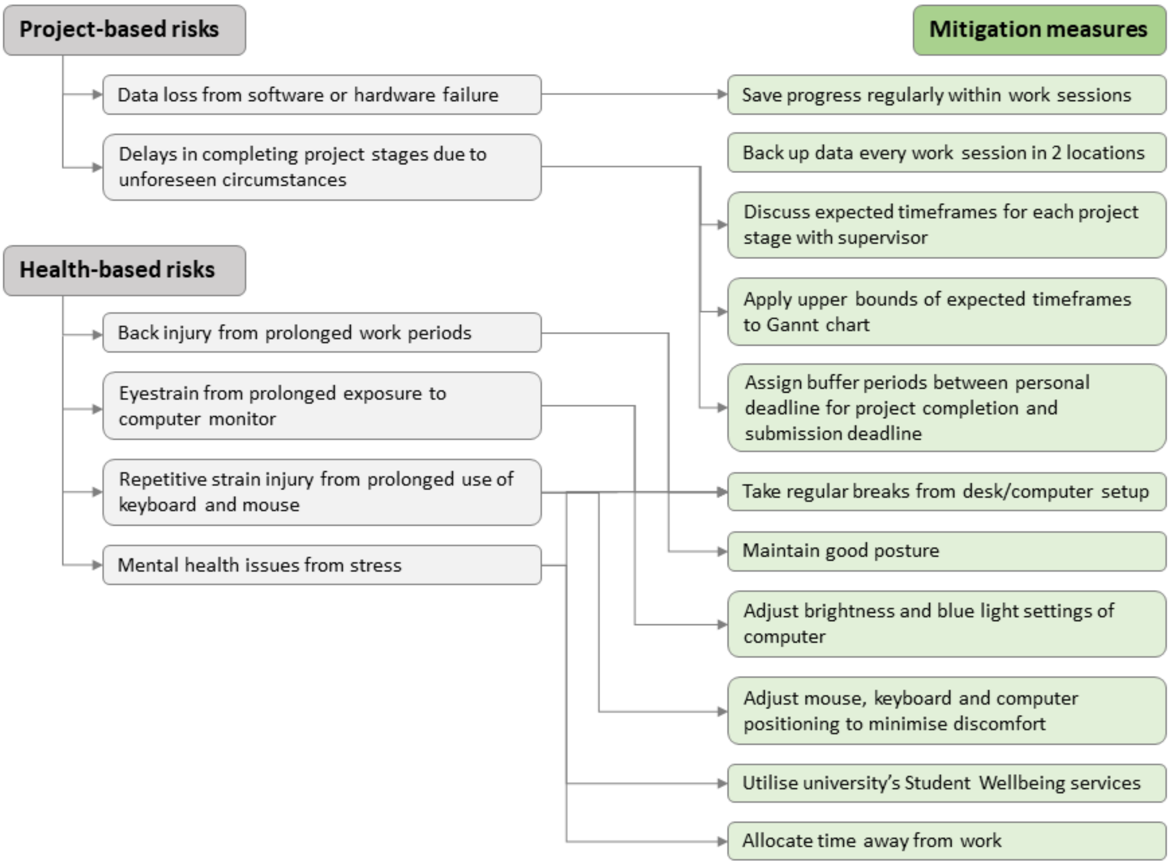

Figure 4: Flow chart of all risks associated with the project and the relevant mitigation measured to be taken in response.

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APPENDICES

Appendix 1

A sample of the initial data extraction spreadsheet framework columns that will be used to compile data from eligible studies. Please note that these columns are only displayed by row here for the practical purposes of portrait display. In this display, the second column contains all possible levels used for each component of the data, each separated by a comma. The column 'Level' refers to the level of relevance rankings of eligible studies (as described in section 4.1). 'M' refers to the mean, 'SD' refers to standard deviation, 'SEM' refers to standard error of the mean, 'DMI' refers to dry matter intake.

Unique publication number	
Publication author	
Publication year	
Publication title	
DOI	
Level	1, 2
Study country	
Study location	
Farming system	conventional, organic
Farming conditions	housed, mixed, grazed
Treatment overview	
Breed	
Breed size	small, medium large
Control or treatment by row	control, treatment (1, 2, 3…)
Treatment description by row	
Treatment type	dietary, medical
Potential treatment effects	methane mitigator, product yield enhancer, methane mitigator AND product yield enhancer
M DMI	
SD DMI	
SEM DMI	
M herbage DMI	
SD herbage DMI	
SEM herbage DMI	
M concentrate DMI	
SD concentrate DMI	
SEM herbage DMI	
Forage supplement ratio	
M average daily gain	
SD average daily gain	
SEM average daily gain	
M milk yield	
SD milk yield	
SEM milk yield	

M milk fat yield	
SD milk fat yield	
SEM milk fat yield	
M milk protein yield	
SD milk protein yield	
SEM milk protein yield	
M energy-corrected milk yield	
SD energy-corrected milk yield	
M CH4	
SD CH4	
SEM CH4	
M CH4 yield	
SD CH4 yield	
M CH4 intensity	
SD CH4 intensity	

Appendix 2

A sample of the study characteristics forms that will be completed for each eligible study used in the meta-analysis. In this display, the second column gives non-exhaustive examples of the qualities that will be recorded for each section.

Reference no. – author, year	
DOI	
Location	Specific location, coordinates, region, country
Participants	Sample size, sex, breed, age, parity, physiological stage
Intervention(s)	
Methodology	Length of grazing season, medical/management procedures (vaccination, antibiotics, etc) Treatment details, source/batch/location of treatments, number of trials, study's predicted/understood effects of treatments Sampling year, period, count by days, number of observations per cow per trial, sampling designs and measurement methods for DMI, yield and enteric methane emissions
Potential biases	
Outcomes	
Additional notes	Feeding and milking frequencies, specific contents of grazing and concentrate constituents, grazed grass heights, adaption period to conditions/treatments, days in milk, body condition scores, grazing intensity, purebred/crossbreed

Appendix 3

A sample of the prepared data spreadsheet that will be used to compile data once it has been corrected into standardised formats and thus has been made ready for the analyses. 'no' refers to the unique number allocated to each study, 'trt' refers to the categorised treatment applied to each row (for categorisation, see methodology), 'n' refers to the sample size for that treatment, 'fe' refers to the feed efficiency ratios, 'mey_m' refers to the mean enteric methane yields, 'mey_sd' refers to the standard deviations of enteric methane yields, 'mei_m' refers to the mean enteric methane intensities, 'sys' refers to the farming system used, 'con' refers to the farming conditions cattle were subjected to, 'f_c' refers to the forage to concentrate ratio of the diet and 'sz' refers to the cattle breed as categorised by physical size.

no	trt	n	fe	mey_m	mey_sd	mei_sd	sys	con	f_c	sz