Mitigation Efficacy of Dietary Additives and Nutritional Strategies on Enteric Methane Emissions in Ruminant Livestock: A Protocol for Systematic Review and Meta-Analysis.

June 2026

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Citation: Saran, A, Young, S., Pandey, N., Shirodkar, S., Bhawalpuria, N., Mukherji, A., & Haddaway, N. (2026). *Mitigation Efficacy of Dietary Additives and Nutritional Strategies on Enteric Methane Emissions in Ruminant Livestock: A Protocol for Systematic Review and Meta-Analysis*. CGspace: {https://hdl.handle.net/10568/186249}.

Keywords: Livestock, low- and -middle income countries, nutrition, diet, breed

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Acknowledgments

We are grateful to Neal Haddaway and Aditi Mukherji for comments, and to various stakeholders who provided feedback on this protocol. This work was commissioned by ILRI as part of the CGIAR Climate Program. CGIAR research is supported by contributions to the CGIAR Trust Fund. CGIAR is a global research partnership for a food-secure future dedicated to transforming food, land, and water systems in a climate crisis.

We would like to thank all funders who supported this research through their contributions to the CGIAR Trust Fund (https://www.cgiar.org/funders/).

Collaborating Centers

Background

Description of the problem

Ruminant livestock production represents a major source of global anthropogenic greenhouse gas emissions, primarily through enteric fermentation which releases methane (CH4) Enteric methane is a short-lived climate pollutant with a high global warming potential, making its reduction an urgent target for short-term climate mitigation pathways. While agricultural emissions tracking requires comprehensive evaluation across all farming domains, large data vacuums persist globally in areas such as livestock genetics, animal health, and manure management. This skewed distribution makes it essential to systematically map and synthesize areas where evidence is dense enough to establish standardized, data-driven baselines for carbon accounting and agricultural policy. This challenge is profoundly acute in Low- and Middle-Income Countries (LMICs), where livestock systems face structural vulnerabilities that amplify both emission intensities and data uncertainties.

In LMICs, enteric methane represents a disproportionate ecological problem due to several compounding biophysical and socio-economic factors. First, ruminants in these regions are heavily reliant on low-quality native pastures, crop residues, and agro-industrial by-products (Gerber et al., 2013). These highly fibrous feedstuffs possess elevated concentrations of structural carbohydrates like lignocellulose, which prolong rumen retention time and shift microbial fermentation toward acetate production. This specific biochemical pathway generates a substantial excess of metabolic hydrogen, yielding high enteric CH4 per unit of voluntary dry matter intake (Hristov et al., 2013). Second, due to underlying challenges in animal health, heat stress, and suboptimal feed allocation, ruminants in LMICs exhibit significantly lower milk and meat productivity compared to high-income configurations. This creates a remarkably high emission intensity profile; while an individual animal in an LMIC may emit less absolute daily methane than a highly fed Western dairy cow, it emits vastly more methane per kilogram of milk or meat produced for human consumption (Herrero et al., 2013; FAO, 2018). Finally, driven by rapid population growth, expanding urbanization, and rising disposable incomes, the demand for animal-source proteins in LMICs is expanding exponentially in a phenomenon termed by the "livestock revolution" (Delgado, 2003). Because productivity gains per animal remain slow, LMICs meet this surging demand primarily by expanding absolute herd sizes rather than increasing efficiency. This structural growth threatens to accelerate global agricultural emissions unless scalable, locally viable nutritional interventions are successfully deployed (Steinfeld et al., 2006).

Description of the intervention

The focal interventions of this review comprise explicit technical changes to animal nutrition, broadly defined as Feed and Nutrition Strategies. To ensure rigorous data extraction and synthesis, these strategies are clearly classified into two primary operational categories and their respective technical sub-categories.

Category A consists of Dietary Additives, which involves the formulation and direct introduction of specialized compounds into the animal feed matrix to alter rumen chemistry or microbial activity. This category is sub-divided into synthetic chemical inhibitors, which are engineered chemical additives purposefully designed to disrupt metabolic pathways, such as 3-nitrooxypropanol (3-NOP); alternative hydrogen or electron sinks, which introduce competing biochemical pathways into the rumen environment, such as nitrates or sulfates that preferentially accept electrons and divert metabolic hydrogen away from methanogenesis; and natural bioactive compounds, including plant-derived extracts, secondary metabolites, or biomass additions like condensed tannins, saponins, essential oils, and specialized macroalgae or seaweed strains such as *Asparagopsis taxiformis*.

Category B consists of Nutritional Manipulations, which involves structural alterations to the physical formulation, component ratios, or agronomic quality of the basal ration itself. This category is sub-divided into lipid and fat supplementation, which introduces dietary lipids, oils, or oilseeds into the ration to suppress protozoal and archaeal populations and reduce organic matter fermentation; concentrate-to-

forage ratio adjustments, which systematically increase the proportion of concentrate feed relative to structural forage to shift volatile fatty acid production away from acetate and toward propionate; and high-quality or improved forage transitions, which shift animals from low-quality native pastures or crop residues onto highly digestible cultivated forages, improved pasture varieties, or legumes, which inherently lower the methane yield per unit of dry matter intake.

How the intervention might work

Dietary additives and nutritional manipulations lower enteric methane emissions through distinct biophysical and biochemical pathways. Synthetic additives like 3-NOP structurally bind to and inhibit the methyl-coenzyme M reductase enzyme, which catalyzes the final step of methanogenesis within the rumen archaea population (Duin et al., 2016). Additives such as nitrates act as preferred alternative electron acceptors in the rumen, outcompeting carbon dioxide for metabolic hydrogen and redirecting it away from methane formation (Latham et al., 2016). Bioactive compounds like condensed tannins or lipids exert toxic or inhibitory effects directly on methane-producing archaea or the ciliate protozoa associated with them, disrupting the symbiotic inter-species transfer of hydrogen (Goel & Makkar, 2012). Transitioning animals to higher-quality, highly digestible forages or increasing concentrate proportions decreases the production of acetate relative to propionate in the rumen (Janssen, 2010). Because propionate production acts as a net hydrogen sink rather than a source, this structural shift reduces the pool of metabolic hydrogen available for methanogenesis, decreasing methane yield per unit of dry matter intake.

Why it is important to do this review

From a comprehensive global evidence and gap map on agricultural climate mitigation, we identified a highly dense and specialized cluster of 42 active studies evaluating Feed and Nutrition Strategies for ruminants. Crucially, this dataset exhibits high methodological uniformity, with 39 of these studies tracking enteric CH4 emissions alone, representing 79.6% of the livestock records. The identified evidence base is tightly distributed across distinct ruminant sub-systems, including 17 studies on small ruminants, 12 studies on dairy cattle, and 9 studies on beef cattle. Because this sector has reached a high level of saturation and consistency, it satisfies all statistical prerequisites for rigorous evidence synthesis. Conducting a systematic review and meta-analysis in this domain is vital to pool this pre-identified data, resolve conflicting outcomes via statistical testing, and provide policymakers with definitive, species-specific reduction factors that a descriptive narrative synthesis alone cannot generate.

Objective of the Review

The primary objective of this review is to quantitatively synthesise experimental evidence to determine the mitigation efficacy of dietary additives and nutritional strategies on enteric methane emissions across different ruminant sub-systems, specifically small ruminants, dairy cattle, and beef cattle. The objective is guided by the research question below:

- What is the mitigation efficacy of feed and nutrition strategies on enteric methane CH4 emissions in managed ruminant livestock?
- How does the methane mitigation efficacy vary between Category A (Dietary Additives) and Category B (Nutritional Manipulations)?

Methods

Criteria for including studies in the review (PICOS)

To ensure explicit boundaries for this review, the eligibility criteria have been established using the formal PICOS framework. These criteria are systematically delineated in Table 1 below.

Table 1

PICOS Element	Inclusion Criteria	Exclusion Criteria
Population	• Managed ruminant livestock populations. • Classified into Small Ruminants	• Non-ruminant herbivores. • Monogastric livestock species (e.g.,

(P)	(sheep/goats), Dairy Cattle, Beef Cattle (grazing/feedlot), and Large Ruminants (buffaloes).	swine, poultry). • Wild, unmanaged ruminant populations.
Interventions (I)	• Category A (Dietary Additives): Synthetic chemical inhibitors, alternative electron sinks, and natural bioactive compounds. • Category B (Nutritional Manipulations): Lipid supplementation, concentrate-to-forage ratios, and high-quality forage transitions.	• Systems-level management lacking an isolated feed variable (e.g., general landscape grazing management). • Genetic or breeding improvement selections. • Veterinary health or vaccination treatments. • Downstream manure management interventions.
Comparators (C)	• Concurrent control cohorts of equivalent ruminant species. • Kept under identical baseline environmental conditions. • Subjected to baseline/conventional diets lacking the mitigation strategy.	Before-and-after studies
Outcomes (O)	Quantitative, isolated scientific measures of enteric methane CH4 output: • Methane yield • Methane intensity	• Total greenhouse gas parameters tracked as single metrics without isolated enteric CH4 • Purely metabolic or performance outcomes missing methane quantification data.
Study Designs (S)	Primary, controlled experimental designs containing concurrent control groups: • Randomized controlled trials (RCTs) • Randomized complete block designs • Latin square designs • Crossover configurations	• Non-controlled experimental configurations. • Observational or descriptive field surveys. • Macro-level simulation modeling or predictive exercises. • Review papers, conceptual articles, and perspective reports.

Search methods for identification of studies

Because the comprehensive evidence and gap map (Saran et al., 2026) has already been developed and finalized, the 42 primary studies mapped within that database constitute the complete and definitive sample for this systematic review. Consequently, traditional de novo bibliographic database searches, external screening phases, and new study identification processes are excluded from this scope, and the review will proceed directly to data extraction, coding, and quality analysis of these 39 pre-identified sources.

Data collection and analysis

Selection of studies

We used the comprehensive Evidence and Gap Map (EGM) to identify areas most relevant for targeted synthesis. Specifically, we filtered studies based on the cross-tabulation of intervention categories and target greenhouse gases (GHGs). This process identified 39 studies relevant to the selected intervention–GHG combinations. These studies had already been mapped during the EGM phase and therefore constituted the full, predetermined boundary for data extraction and synthesis. as the specific 42 studies mapped during the evidence and gap map phase serve as the full, predetermined boundary for extraction.

Data extraction and management

The 42 studies mapped from the evidence and gap map will be systematically extracted and coded using a structured coding tool designed for this review. A standardized extraction template will be utilized to capture data in duplicate (Table 2). The extracted information will include study characteristics such as author, year, publication journal, country, and experimental design; animal characteristics including ruminant sub-system classification, breed, age, sample size, and baseline body weight; diet and intervention details including basal feed composition, specific type of nutritional strategy or additive, exact

dosage, and total dry matter intake; and greenhouse gas outcomes including mean values, standard deviations, or standard errors for enteric methane emissions across both treatment and control cohorts.

Table 2: Data Extraction Form

A. Study Characteristics	
Study ID ***e.g. S01***	
Extractor initials ***e.g. NP/SS (first pass); second reviewer initials in notes***	
First author surname	
Year of publication	
Full journal title	
Country of study ***Where the trial was physically conducted***	
Income classification (World Bank FY2026 income classification)	□ Low-income country (LIC) □ Lower-middle-income country (LMIC) □ Upper-middle-income country (UMIC) □ High-income country (HIC) □ Not reported / unclear
Experimental design	□ Randomized controlled trial (RCT) □ Randomized complete block design (RCBD) □ Latin square design □ Crossover design □ Other controlled design — specify in notes
Trial duration (weeks)	
Number of treatment periods ***For crossover / Latin square designs***	
Washout / adaptation period ***Specify length and whether reported***	
Notes on study characteristics	
B. Animal Characteristics	

Ruminant sub-system	□ Small ruminants — sheep □ Small ruminants — goats □ Dairy cattle □ Beef cattle — grazing system □ Beef cattle — feedlot system □ Large ruminants — buffalo Other — specify
Breed / genotype	
Age / parity *e.g. 3rd parity; ~4 years old*	
Physiological status	□ Lactating □ Dry / non-lactating □ Growing / fattening □ Mature maintenance • Not reported
Total animals (n) *Across all treatment groups*	
Animals per treatment group (n)	
Number of treatment groups *Including control group*	
Baseline body weight — treatment (kg) *Mean ± SD or SE*	
Baseline body weight — control (kg) *Mean ± SD or SE*	
Baseline BW variability type	□ Standard deviation (SD) □ Standard error (SE) □ Range (min–max) • Not reported
Baseline milk yield (if dairy) ***Mean kg/d; state if not applicable***	
Notes on animal characteristics	
C. Diet & Intervention Details	
C1 Intervention classification	
Intervention category	□ Category A — Dietary Additive □ Category B — Nutritional Manipulation □ Both A + B (multi-component)
Sub-category (Category A)	□ Synthetic chemical inhibitor (e.g. 3-NOP) □ Alternative electron / hydrogen sink (e.g. nitrate, sulfate) □ Natural bioactive — condensed tannins □ Natural bioactive — saponins □ Natural bioactive — essential oils □ Natural bioactive — macroalgae / Asparagopsis taxiformis □ Natural bioactive — other plant extract (specify) □ Not applicable
Sub-category (Category B)	□ Lipid / fat / oilseed supplementation □ Concentrate-to-forage ratio adjustment □ High-quality / improved forage transition □ Other Cat. B — specify □ Not applicable
Specific additive or	

intervention name ***Full taxonomic/chemical name if available***	
C2. Dosage and formulation	
Dose / inclusion rate (treatment) ***e.g. 50 mg/kg DMI; 3% DM inclusion***	
Dose levels tested ***e.g. 0.5%, 1.0%, 1.5% DM — list all arms***	
Method of delivery ***e.g. mixed in TMR; bolus; top-dressed***	

C3. Basal diet composition		
Field	Treatment group	Control group
Basal feed description		
Total dry matter intake (kg/d)		
Concentrate: forage ratio (%)		
Crude protein content (% DM)		
Neutral detergent fibre, NDF (% DM)		
Ether extract / fat (% DM)		
Gross energy (MJ/kg DM)		
Notes on diet composition		

D. Greenhouse Gas Outcomes	
D1. Measurement details	
Methane measurement method	□ Open-circuit respiration chamber (gold standard) □ SF_6 tracer gas technique □ GreenFeed / automated head chamber □ Ventilated hood / headbox □ Sniffers / spot sampling □ Calculated from predictive equation (specify) □ Other / not specified
Outcome metric/unit reported	□ g CH_4/day (absolute daily emissions) □ g CH_4/kg DMI (methane yield) □ g CH_4/kg FCM or milk (methane intensity — milk) □ g CH_4/kg ADG (methane intensity — growth) □ L CH_4/day □ MJ CH_4/day □ % gross energy intake (Ym factor) □ CO_2-equivalent/day □ Other — specify

D2. Outcome data by treatment arm							
Treatment arm label	Baseline n (Treatment)	Baseline n (Control)	Analysis n (Treatment)	Analysis n (Control)	Mean — treatment	Mean — control	Variability (±)

D3. Statistical reporting	
Statistical test used ***e.g. ANOVA, linear mixed***	

model, t-test	
Gross energy intake —	
Additional performance metrics reported	□ Milk yield (kg/d) □ Average daily gain, ADG (kg/d) □ Body weight change □ Feed conversion ratio (FCR) □ Rumen pH □ VFA profile reported □ None reported
Notes on outcomes	

Critical Appraisal and Assessment of Risk of Bias

A comprehensive critical appraisal of the 39 included experimental trials will be systematically conducted in duplicate using a specialized critical appraisal framework optimized for animal agricultural research, where reviewers will independently rate each study across specific design vulnerabilities to determine internal validity and internal experimental quality. Critical appraisal will be conducted using the risk-of-bias tool described most recently in the review by Malhotra et al. (2025), which has been peer reviewed, piloted, and applied in previous Campbell Collaboration evidence syntheses. The tool assesses key domains of bias relevant to animal intervention studies and was adopted without substantive modification (Table 3).

Domain-level judgements will be combined into a single overall risk-of-bias rating per study following a "weakest link" principle, as indicated in the final row of Table 3. A study will be rated High overall only if it achieves a High rating on every domain (intervention description, evaluation questions, study design, outcome definition, sample size/power calculation, and attrition). A study will be rated Medium overall if it scores no lower than Medium on any domain. A study will be rated Low overall if it scores Low on at least one domain, irrespective of its ratings on the remaining domains. This approach ensures that a critical methodological weakness in a single domain — for example, absence of randomization or high differential attrition — is not offset by strong performance elsewhere, consistent with established Campbell Collaboration and Cochrane practice for risk-of-bias synthesis. Both the domain-level and overall ratings will be reported for each included study, so that readers can identify the specific source of any Low or Medium rating rather than relying on the summary judgement alone.

Risk-of-bias ratings will be used to qualify the synthesis of findings in the following ways. First, a risk-of-bias summary table and/or traffic-light plot will be presented to accompany the narrative and quantitative synthesis. Second, where meta-analysis is undertaken, a sensitivity analysis will be conducted excluding studies rated overall Low, to assess the robustness of pooled effect estimates to the inclusion of higher-risk studies.

Table 3: Critical Appraisal Tool

Item	Description	Key	Notes
Intervention	Is the intervention named and described, including all relevant components?	High: full and clear description, so that the main components and how they are delivered are clear Medium: Partial description Low: Little or no description	
Evaluation questions	Are the evaluation questions clearly stated?	High: full and clear description, so that the main components and how they are delivered are clear Medium: Partial description Low: Little or no description	

Study design	Use the study design coding	High: A randomised study Medium: Non-randomised study (quasi-experiment) Low: single time-point before versus after	
Outcomes	Are the outcomes clearly defined? Where appropriate do they use an existing, validated measurement tool?	High: full and clear definition using validated instruments where available (a researcher wishing to use these outcomes would have sufficient information to do so) Medium: Partial definition. May use validated instruments but without sufficient references to the source. Low: Little or no definition	
Sample size (power calculation)	Do the authors report a power calculation as the basis for sample size?	High: Power calculation report and sample size meet the necessary sample size Medium: Power calculation mentioned and sample size meets the necessary sample size Low: No mention of power calculation.	
Attrition	Reported for endline and longest follow-up. Calculate overall attrition and differential attrition (see example below). It is often necessary to calculate from the table of results. If sample size varies by outcome calculate for highest attrition.	High: total attrition <20% and differential attrition <10 percentage points Medium: total attrition >20% or differential attrition >10 percentage points Low: total attrition >20% and differential attrition >10 percentage points	
Overall (including questions for all studies)	The overall score uses the weakest link in the chain principle i.e., is the lowest score on any item	High: High on all items Medium: No lower than medium on any item Low: At least one low	

Data synthesis

Narrative Synthesis

A descriptive narrative synthesis will be conducted as a baseline to map the characteristics of the included literature, charting the geographical distribution of the trials, the varieties of dietary additives deployed, and the baseline nutritional compositions of the rations across the different ruminant systems. To characterize the evidence base and identify areas of evidence concentration, cross-tabulations will be conducted for **intervention category by target greenhouse gas**, **intervention category by animal species**, and **geographical region by publication period**, thereby enabling the assessment of thematic, biological, and temporal patterns in the distribution of studies.

Meta-Analysis

Quantitative synthesis is anticipated for groups of studies reporting sufficiently comparable interventions and methane-related outcomes. Random-effects meta-analysis will be conducted to estimate the overall effect of interventions on enteric methane emissions while accounting for between-study heterogeneity. Studies will be weighted by the inverse of their sampling variance. The weighted log response ratio (lnRR) will be the preferred effect measure for continuous outcomes reported on a common ratio scale, such as methane production (g/day), methane yield (g/kg dry matter intake), or methane intensity. The lnRR provides an intuitive estimate of proportional change and can readily be interpreted as percentage reduction relative to the control group. Hedges' g standardized mean difference will be calculated when outcomes are measured using different scales or when insufficient information is available to estimate response ratios. Between-study heterogeneity will be quantified using τ^2 and the I^2 statistic. Prespecified moderators will include **intervention category**, **animal species**, **methane measurement method**, **intervention duration**, and **risk-of-bias rating**, a Where sufficient studies are available, risk-of-bias rating will also be examined via sensitivity analysis, in which pooled effect estimates will be re-calculated after excluding studies rated overall Low, to assess whether the meta-analytic findings are robust to the exclusion of higher-risk studies. s these are expected to represent the principal sources of between-study heterogeneity.

Assessment of Publication Bias

Potential publication and selective reporting biases will be assessed using funnel plots to visually inspect asymmetry, which will be corroborated by Egger's linear regression test. If significant asymmetry is detected, the Duval and Tweedie trim-and-fill method will be applied to estimate the hypothetical effect size under a symmetric distribution.

Competing interests

The authors of the review state that they have no competing interests.

Funding information

This systematic evidence and gap map is funded by the International Livestock Research Institute (ILRI) and the Consortium of International Agricultural Research Centres (CGIAR).

Author's contributions

Ashrita Saran: Conceptualisation, Methodology, drafted and validated search strategy, software preparation for screening and coding and writing- original draft preparation and editing. **Nidhi Pandey**: Data curation, preparation, screening and data extraction. **Sujata Shirodkar**: Data curation, preparation, screening and data extraction. **Neal Haddway**: Reviewing, **Aditi Mukherji**: Reviewing

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