



Re-Livestock
RESILIENT FARMING SYSTEMS

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Feed additives for methane mitigation: How to account for the mitigating potential of antimethanogenic feed additives - Approaches and recommendations 9

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Recommendations for identification and selection of bioactive compounds to develop antimethanogenic feed additives

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Abstract

This work provides technical recommendations and guidelines for research to identify and screen different compounds to reduce enteric methane (CH₄) emissions before they can be further assessed in vivo. For the initial identification of anti-methanogenic feed additives (AMFA) candidates, 2 approaches can be used: mechanistic (combining computational tools—in silico—and docking studies, using a modeling technique to predict how target enzymes interact with candidate molecules, and then synthesizing the candidate compounds), and empirical (finding published evidence of specific bioactive effects, by searching different repositories, followed by obtaining bioactive material from the identified source). Although both approaches offer potential to identify a large number of compounds, sophisticated equipment and specific expertise are required for computational/modeling work and extracting bioactive compounds from a biological matrix, respectively, in mechanistic and empirical studies. Once AMFA candidates have been identified, the next step is to evaluate experimentally the anti-methanogenic activity and there is an array of in vitro methodologies that can be used for this purpose (enzymatic assays, pure cultures of methanogens batch cultures and continuous or semi-continuous cultures). We will present and discuss the main aspects to consider when using different approaches, including identifying the target enzymes to study, developing the enzyme material for running inhibition kinetics, culturing conditions and selection of archaeal species to study and the processing of the rumen inoculum used for experiments conducted using batch cultures and fermenters. In addition, critical overarching methodological considerations for all in vitro approaches are (1) appropriate experimental design and statistical tests, (2) selection of doses depending on the nature and mode of action of the AMFA, (3) careful preparation of solid or liquid formats for appropriate delivery of the additives, and (4) when and how to measure CH₄ and units to express its production.

Key Words: in silico, docking, in vitro



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Recommendations for testing enteric methane-mitigating feed additives in ruminant studies

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Abstract

There is a need for rigorous and scientifically based testing standards for existing and new enteric methane mitigation technologies, including antimethanogenic feed additives (AMFA). This review provides guidelines for conducting and analyzing data from experiments with ruminants intended to test the antimethanogenic and animal production and physiological effects of AMFA. Recommendations include study design and statistical analysis of the data, interactions with diet composition, associative effect of AMFA with other mitigation strategies, appropriate methods for measuring methane emissions, production, and physiological responses to AMFA, and effects on animal health and product quality. Animal experiments should be planned based on clear hypotheses and experimental designs must be chosen to best answer scientific questions, with pre-experimental power analysis and robust postexperimental statistical analyses being important requisites. Experimental conditions should be representative of the production system of interest, to ensure that results and conclusions are applicable and practical. To explore additivity and synergism, as well as trade-



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offs, including relevant manure emissions, AMFA may be combined with other methane mitigation strategies and studied in appropriately designed experiments. Methane emissions can be successfully measured, and efficacy of AMFA determined, using respiration chambers, the sulfur hexafluoride method, and the GreenFeed system. For proper assessment of an AMFA, it is critically important that representative animal production data are collected and reported. In addition, evaluating the effects of AMFA on nutrient digestibility, animal physiology, animal health and reproduction, product quality, and how AMFA interact with nutrient composition of the diet is necessary and should be conducted at various stages of the evaluation process. The authors emphasize that enteric methane mitigation claims should not be made until efficacy of AMFA is confirmed in animal studies designed and conducted considering the guidelines provided in the review.

Key Words: feed additive, enteric methane mitigation, guideline



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Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants - Approaches and recommendations

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Abstract

Over the past decade, there has been significant focus on using antimethanogenic feed additives (AMFA) to mitigate enteric methane (CH₄) emissions from ruminants. Administered in small quantities, these additives can considerably reduce methanogenesis. Mathematical models are essential for understanding and predicting the quantitative impact of AMFA on enteric CH₄ emissions across diverse diets and production systems. This study provides a comprehensive overview of methodologies for modeling the impact of AMFA on enteric CH₄ emissions in ruminants, concluding with recommendations for modeling approaches to quantify AMFA's impact on CH₄ emissions. Key considerations encompass the type of models employed (i.e., empirical models including meta-analyses, machine learning models, and mechanistic models), alignment of modeling objectives, data availability, modeling synergies and trade-offs associated with using AMFA, and model applications for enhanced understanding, prediction, and integration into higher levels of aggregation. Based on an evaluation of these critical aspects, recommendations are provided for modeling approaches to quantify AMFA's impact on CH₄ emissions and in support of farm-level, national, regional, and global inventories for accounting greenhouse gas emissions in ruminant production systems. These recommendations emphasize that data quality is critical in modeling approaches, with a strong preference for peer-reviewed sources. Careful evaluation of additive dosage, delivery methods, and transient effects is essential in quantitative analyses. The chosen model and modeling approach should be clearly defined and aligned with specific objectives, while exploring the synergy of diverse modeling methodologies, including machine learning, to improve understanding and predictive accuracy of AMFA's impact on CH₄ emissions in ruminants. An integral, quantitative assessment is advised to evaluate AMFA's CH₄ mitigation effects, considering potential synergies or trade-offs with other greenhouse gas sources.

Key Words: feed additive, methane mitigation, modeling



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A guideline to uncover the mode of action of antimethanogenic feed additives for ruminants.

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Abstract

We discuss a set of guidelines of research and knowledge required for understanding the mode of action of antimethanogenic feed additives (AMFA). According to their mode of action, AMFA can be classified into 4 categories: (1) lowering H₂ production, (2) inhibiting methanogens, (3) promoting alternative H₂-incorporating pathways, and (4) oxidizing methane. Identifying the exact mode of action of AMFA is complex and costly. Thus, we recommend that in-depth research should only be pursued after the effectiveness and safety of an AMFA has been proven. Key research questions that guide the investigation should cover 5 perspectives: (1) microbiology, identifying the targeted microbes and the potential side effects on nontargeted microbes; (2) cell and molecular biochemistry; identifying the active compounds, the subcellular mechanisms of action on the targeted molecules, and the mechanisms of resistance or adaptation to AMFA; (3) microbial ecology, analyzing the effects of AMFA on the metabolic pathways at the microbial community level, including accumulation of fermentation products and AMFA degradation; (4) animal metabolism, studying

effects on feed intake, digestibility, absorption, metabolism, excretion, and accumulation of active compounds or their metabolites to assess safety for animals, consumers, and the environment; and (5) cross cutting, describing the modulatory effects of the diet, type of animal, management, and other factors on the mode of action and effectiveness of AMFA. The research proposed and discussed herein implies multidisciplinary and complementary approaches to fully understand the mode of action of AMFA at different depths. It also addresses existing critical knowledge gaps about the consequences of inhibiting rumen methanogenesis on the microbial ecosystem and host animal, which must be understood for the successful adoption of AMFA to mitigate methane emissions from ruminants.

Key Words: methanogens, mitigation, rumen methanogenesis



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Regulations and evidence requirements for the authorization of enteric methane-mitigating feed additives

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Abstract

A review of the regulatory and evidence requirements for authorizing feed additives aimed at reducing enteric methane emissions from ruminants was conducted for 7 illustrative jurisdictions. The primary objective was to provide a broad overview and synthesis to help applicants and scientists comply with regulations and understand evidence requirements needed for authorization of antimethanogenic feed additives (AMFA) in Australia, Canada, the European Union, New Zealand, South Korea, the United Kingdom, and the United States. The review also identified differences and similarities in regulations and evidence requirements, which may aid global regulatory harmonization, and provides recommendations for applicants and scientists. Regulations protect animal health, promote food safety, and prevent unsafe practices and misleading claims. These mandatory regulations cover ingredient safety, manufacturing practices, product labeling, and permissible limits for substances. The intended use, or declared purpose for which the additive is authorized, influences its legal classification or regulatory status, and determines conforming evaluation and approval processes. This includes practical aspects of administration like active ingredients, dosage, mixing, and feeding frequency. Each jurisdiction has unique criteria for legally classifying AMFA, making it challenging to meet all legal classifications with a single set of scientific evidence. However, there is consistency in the need for robust evidence for efficacy, safety, and product quality, despite differences in study requirements. The burden of proof is on the applicant to provide all necessary scientific evidence. Applicants and scientists must consult with authorities before designing research trials. Scientists conduct efficacy and risk assessments that inform regulatory decisions and must follow good scientific practices. Collaboration with regulatory agencies can refine legal classifications and improve harmonization. Educating stakeholders about AMFA's advantages and proper use can encourage correct usage and enhance understanding and transparency.

Key Words: intended use, regulatory status, conditions of use



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Feed additives for methane mitigation: How to account for the mitigating potential of antimethanogenic feed additives - Approaches and recommendations

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Abstract

Recent advances in our understanding of methanogenesis have led to the development of antimethanogenic feed additives (AMFA) that can reduce enteric methane (CH₄) to varying extents. Here we examine current and emerging approaches used for accounting (or quantifying) of enteric CH₄ abatement through the use of AMFA in livestock systems across scales, from the individual animal to the global level. The accounting process for on-farm enteric CH₄ emissions with the use of AMFA starts with the ruminant animal, with estimates obtained from simple empirical emission factors or equations, to complex processbased models. However, the enteric CH₄ abatement from the use of AMFA per se remains to be fully quantified in most accounting systems and scales. The accounting also needs to consider the AMFA delivery method and the accounting of synergies and trade-offs in greenhouse gas emissions at levels before (e.g., emissions from AMFA production and distribution) and after (e.g., effects on feed efficiency) being offered to the animal to fully assess the impact of AMFA use. The choice of methodology and level of complexity to account for AMFA abatement in livestock systems must be tailored to the targeted scale of analysis, the availability of input data to represent contextualized conditions, and the accounting objectives. In the accounting of AMFA abatement, it is critical to consider the implications of efficacy (results from controlled interventions) versus effectiveness (results from real-world conditions). Assumptions on the additivity of AMFA abatement should follow insights from experimental work, and in the future, from models as these continue to improve the simulation of combined AMFA efficacy and effectiveness. Collectively, the accounting of enteric CH₄ abatement by feed additives remains to be fully assessed beyond experimental results to address pragmatism, potential for adoption and societal acceptance.

Key Words: life cycle assessment, emissions trading schemes, emission factors



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