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EDITED BY

Virgilio Gavicho Uarota,
Universidad de O'Higgins, Chile

REVIEWED BY

Ravikanthreddy Poonooru,
University of Missouri, United States
Jorge Medina,
Universidad de O'Higgins, Chile

*CORRESPONDENCE

Yaniv Altshuler
✉ yanival@mit.edu

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AI-based predictive modeling for enteric methane mitigation: cross-farm validation using an allicin based essential oil

Yaniv Altshuler^{1,2*}, Tzruya Calvao Chebach², Shalom Cohen²
and Joao Gatica²

¹Massachusetts Institute of Technology, Cambridge, MA, United States, ²Alphabiome AI, Tel Aviv, Israel

Greenhouse gas emissions are a major global concern and reducing them is a key objective for governments and organizations worldwide. One of the largest agricultural contributors to greenhouse gas emissions is enteric methane, produced as a byproduct of microbial fermentation in the rumen during forage digestion. As previously reported, we developed an AI-driven model to address this challenge by predicting the efficacy of feed additives in mitigating methane emissions. Since the wide variety of feed additives available in the market, validating the model across a diverse range of additives is critical for its adoption in commercial farming practices. In this study, we extensively validate the model across ten commercial farms over a three-month period, involving 339 Holstein cows, and using an allicin-based essential oil (Allimax), an organosulfur compound obtained from garlic with potential to reduce enteric methane emissions. The validation process simulated two hypothetical scenarios: (i) a naive scenario in which the feed additive is applied uniformly across all participating farms, and (ii) an optimized scenario, based on a precision agriculture approach, in which the additive is supplied only to farms where the AI-driven model predicted a significant reduction in enteric methane emissions. Our results revealed two key findings: first, the AI-driven model demonstrated high accuracy in predicting the additive's effect on enteric methane emissions at the farm level. Second, the optimized scenario achieved greater overall methane reductions compared to the naive scenario, underscoring the value of a precision agriculture strategy that incorporates rumen microbiome genetics to guide additive applications. These results, which align with a previous validation using a different commercial feed additive, support the adoption of data-driven, customized additive strategies that enhance sustainability and productivity, promote precision agriculture practices, and facilitate compliance with evolving environmental regulations.

KEYWORDS

AI-driven model, predictive model, enteric methane emissions, feed additive, dairy, precision agriculture

1 Introduction

Greenhouse emissions from the agriculture sector account for approximately between 10 to 18% of global methane emissions and thereby contributing to climate change ([European Parliament, 2018](#); [Beauchemin et al., 2020](#); [EPA, 2025](#)). Moreover, enteric methane emissions, manure management and rice production for feed contribute to 54% of all livestock related emissions ([FAO, 2023](#)). Enteric methane emissions are produced in the rumen during microbial fermentation of feed, which generates hydrogen that is subsequently converted into

methane by methanogenic microorganisms, including bacteria, protozoa, methanogenic archaea, and fungi (Wang and McAllister, 2002).

Recent advancements in microbiome research have highlighted the pivotal role of the rumen microbial community in methane production (Mizrahi et al., 2021). Because mitigating these emissions is crucial for sustainable livestock production and environmental protection, different strategies have been developed or are at various steps of development; among these strategies, it is possible to find the use of microbial biomarkers to pinpoint high methane emitters for targeted interventions (Ramayo-Caldas et al., 2019), the analysis of microbial genes to distinguish between varying methane emission levels (Wallace et al., 2015), the use of vaccines against rumen methanogens (Roques et al., 2024), and the use of different feed additives (Honan et al., 2021). Feed additives reduce methane emissions by targeting different stages of rumen fermentation and microbial activity: some inhibit methanogenic archaea directly (e.g., 3-nitrooxypropanol), others redirect hydrogen toward alternative pathways such as propionate production (e.g., fumarate, nitrate), while plant secondary compounds (e.g., tannins, saponins, essential oils) modulate the rumen microbiome and decrease protozoa–methanogen interactions, thereby lowering methane formation without compromising feed digestion. Despite some promising results of feed additives reducing enteric methane emissions (Hart et al., 2019; Roque et al., 2019; Ma et al., 2024; Permata et al., 2024), also there are reports showing limited success (Hegarty et al., 2021; Arndt et al., 2022), which has negatively impacted their implementation on commercial farms (Roques et al., 2024).

In recent years, the rise of artificial intelligence (AI) has enabled its application across various fields of agriculture (Mana et al., 2024), opening new opportunities to reduce methane emissions in livestock (Nejad et al., 2024). Moreover, the integration of AI-driven tools with deep sequencing techniques offers a promising approach to explore, and even predicts, the efficacy of feed additives in reducing enteric methane emissions according to the herd specific characteristics. Thus, we developed an AI-driven tool that using rumen microbiome samples, can accurately predict how feed additives could impact on enteric methane emissions, considerably improving the farmer's capacity to make decisions based on herd genetics (Altshuler et al., 2024, 2025). The performance of this tool relies, among other parameters, on DNA sequences obtained from rumen samples, but

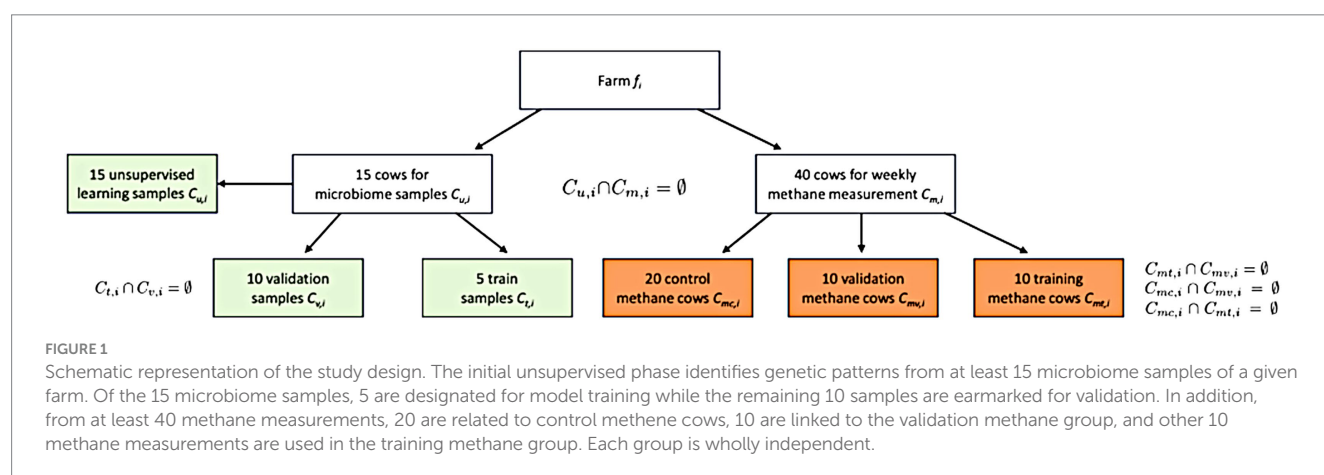
does not require from taxonomical assignments or the detection of specific metabolic pathways. Instead, the model uses the most basic information of these DNA sequences in a two steps process to generate biomarkers correlating with methane production, which are then used to predict the effect of feed additives on enteric methane emissions.

The aim of this study was to validate, across several commercial farms, the accuracy of the model's predictions using a widely used commercial feed additive (Allimax, Vaasen, Netherlands), which is based on garlic essential oil (allicin). The feed additive was administered across several commercial dairy herds, and *in-situ* methane emission measurements were compared with the model's predictions. The additive was chosen for its global availability, high bioavailability, and scientific evidence supporting its methane-reducing effects (Busquet et al., 2005; Roque et al., 2019; Ding et al., 2023).

2 Materials and methods

2.1 Model's framework and validation strategy

The AI-driven model predicts the efficacy of feed additives in reducing enteric methane emissions by leveraging two primary inputs: rumen microbiome data and methane emission measurements. The model was developed through two distinct phases. In the first phase (training), a subset of cows was used to learn associations between microbiome-derived biomarkers and methane emission measurements. Importantly, none of these animals were in the validation phase. In the second phase (validation), predictions were tested on a completely independent cohort of cows, with methane measurements collected under identical protocols. This design constitutes a true out-of-scope validation, as the model was evaluated on animals that had never contributed to model training, ensuring results were not influenced by overlap or resampling. Additionally, each farm included control groups (untreated animals) alongside treated animals, allowing efficacy to be normalized within farm conditions and minimizing biases from management or environmental variation (Figure 1). This two-stage process results in a predictive model that links microbiome profiles to the efficacy of a given feed additive (or any other biological trait), generating a “predictive efficacy score” for each specific farm (Altshuler et al., 2024, 2025).



For the model's validation, *in-situ* enteric methane emissions were measured biweekly over a three-month period at consistent hours following additive administration. The methane emissions obtained post-additive application were then compared to the predictive scores previously generated by the AI-driven model. To control for seasonal effects, validation was carried out across 10 commercial farms, with one of them participating in two separate rounds, located in diverse geographical regions of Israel, including the areas of Golan heights, west Galilee, lower Galilee, and central plains. Despite the geographical spread, all farms fall within the hot-summer Mediterranean climate region (Figure 2), characterized by a mean annual rainfall of 401–650 mm, concentrated in winter with sporadic events in transitional seasons. Temperatures are generally warm to hot but can be cooler and drier at night. Additionally, transitional seasons may include episodes of extreme heat and drought (Kutiel, 2024).

Two analytical scenarios were considered. In the first (“naive scenario”), the feed additive was assumed to be applied uniformly across all participating farms. In the second (“optimized scenario”), the additive was assumed to be administered only to those farms identified by the model as having a high likelihood of achieving methane reduction.

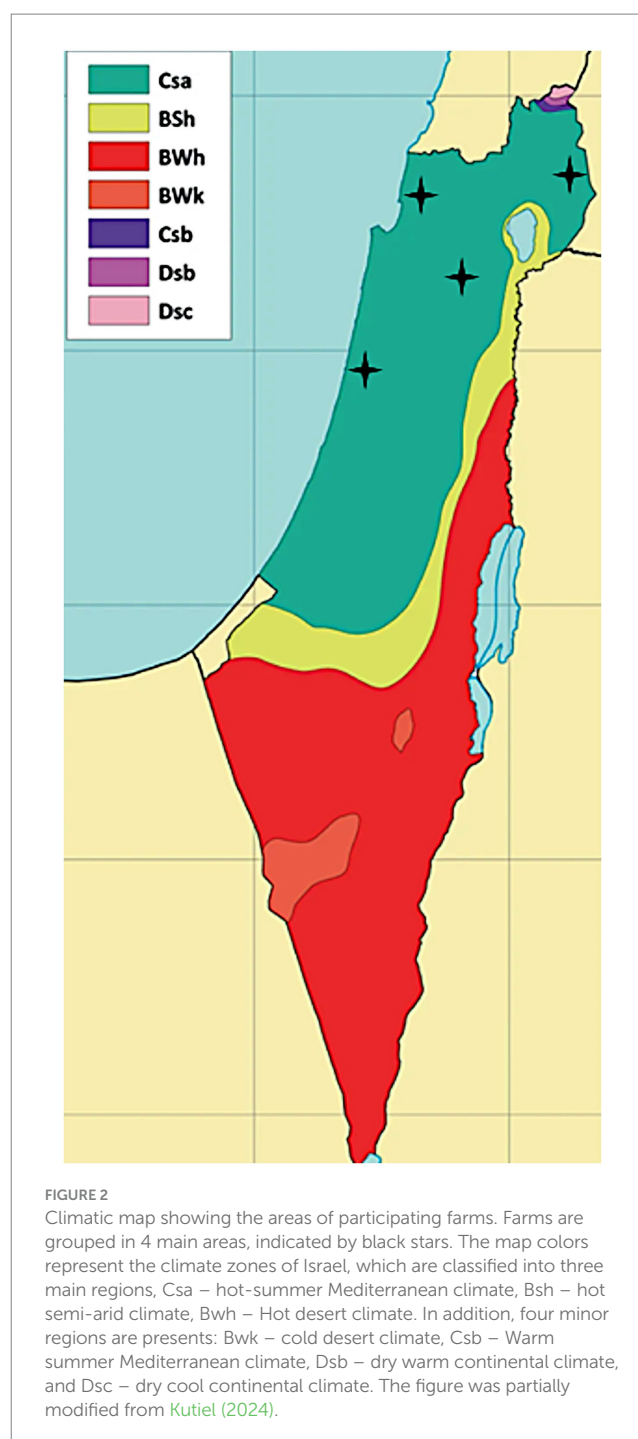
2.2 Animals and tested feed additive

The cows included in this study were randomly selected from commercial dairy farms in Israel. The diet followed a total mixed ration diet consisting of 32% concentrates and 68% of wheat, corn and/or sorghum silage on a dry matter basis (diet components on Supplementary material 1). Cows were blocked based on parity and days in milk (DIM) which was at the start of these trials 120 \pm 60 (mean \pm SEM). The blocked cows were randomly allocated into treatment and control groups. All animals were managed under standard commercial farming practices, and due to the small size of each herd (less than 900), cows were housed together in the same barn. It is important to indicate that all procedures were explained and detailed to each farm staff, and they were responsible for its correct implementation; for example, the selected feed additive was supplied to the randomly selected animals (166 cows in total) by each local farm staff according to the manufacturer's instructions (one bolus per animal), while the control animals (173 in total) consumed the standard ration without additive. Animals from both groups were identified by ear tags for future methane measurements.

As previously indicated, the feed additive selected for this validation was a widely used commercial product (Allimax), commonly applied in both dairy and beef cattle. Its primary active component is allicine, which has been shown to function as an antimicrobial, antiparasitic, and immune system modifier (Ankri and Mirelman, 1999). Moreover, allicin-based products reduce populations of protozoa and methanogenic archaea in the rumen (Roque et al., 2019), thereby altering the proportion of key short-chain volatile fatty acids, such as propionate and acetate, which correlate negatively and positively, respectively, with methane production (Ding et al., 2023).

2.3 Ruminal fluid sampling and sequencing

The sample size was set as 15 rumen samples per farm to account for potential sequencing failures. Preliminary analysis revealed no



significant correlation between farm size and the prediction engine's accuracy. Based on this finding, we postulate that a sample size representing at least a 0.5% of the herd population is sufficient for the model described herein (Altshuler et al., 2024, 2025). Rumen samples were collected by a qualified veterinarian using a stomach tubing (ST) approach (Supplementary material 2), with periodic washings after each use to avoid cross-contamination. Rumen samples were treated with a DNA-preservation buffer (Zymo Research, Cat. No. R1100-250) and immediately transported for sequencing. DNA was extracted using a commercial kit (ZymoBIOMICS 96 MagBead DNA Kit, Zymo Research, Cat. no. D4308) and libraries were prepared using Illumina

DNA Prep (Illumina, Cat. no. 20060059). Libraries were paired end sequenced (2×150) in an Illumina NovaSeq 6,000 instrument (300 cycles). Cleaning and trimming were performed with BBDOUK software, discarding sequences smaller than 100 bp or with a Phred Score under 35. Filtered sequences were used as input for the AI-driven model, without performing any taxonomic or functional classification.

2.4 Methane emissions

Enteric methane emissions were obtained using an ATEX Gas Analyzer (Geotech SEM 5000), a specialized methane measurement instrument. The selection of the instrument was based on its performance compared to other well-known instruments (Supplementary material 3) and based on other important advantages such as ease of use, non-intrusive operation, no need for animal training, and onsite performance (Altshuler et al., 2024, 2025). Additionally, the laser sensor of the instrument has been previously recommended for use in cattle (Rey et al., 2019; Ribeiro et al., 2020).

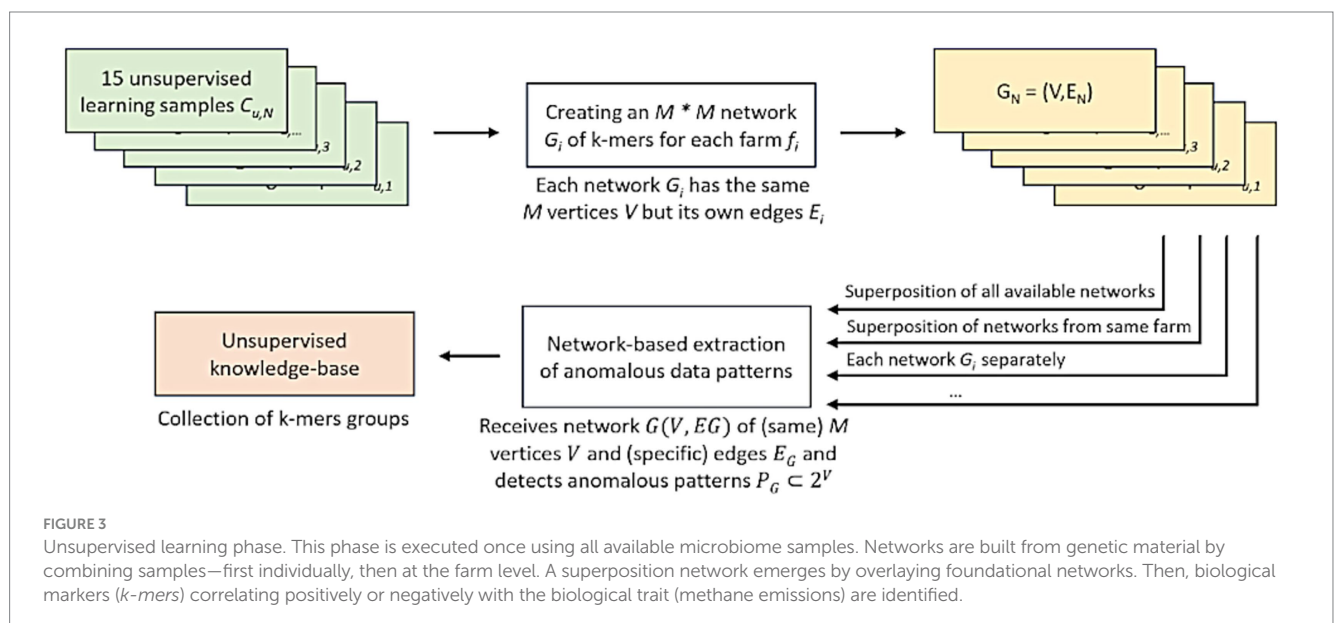
In practice, the instrument recorded methane-measurements continuously, generating hundreds of methane readings per cow in each procedure. Reading under 5 ppm were discarded with the aim to improve consistency; this threshold was set based on *in-situ* observations, suggesting that values under 5 ppm also can be obtained in open spaces far from the animals, corresponding to a background reading.

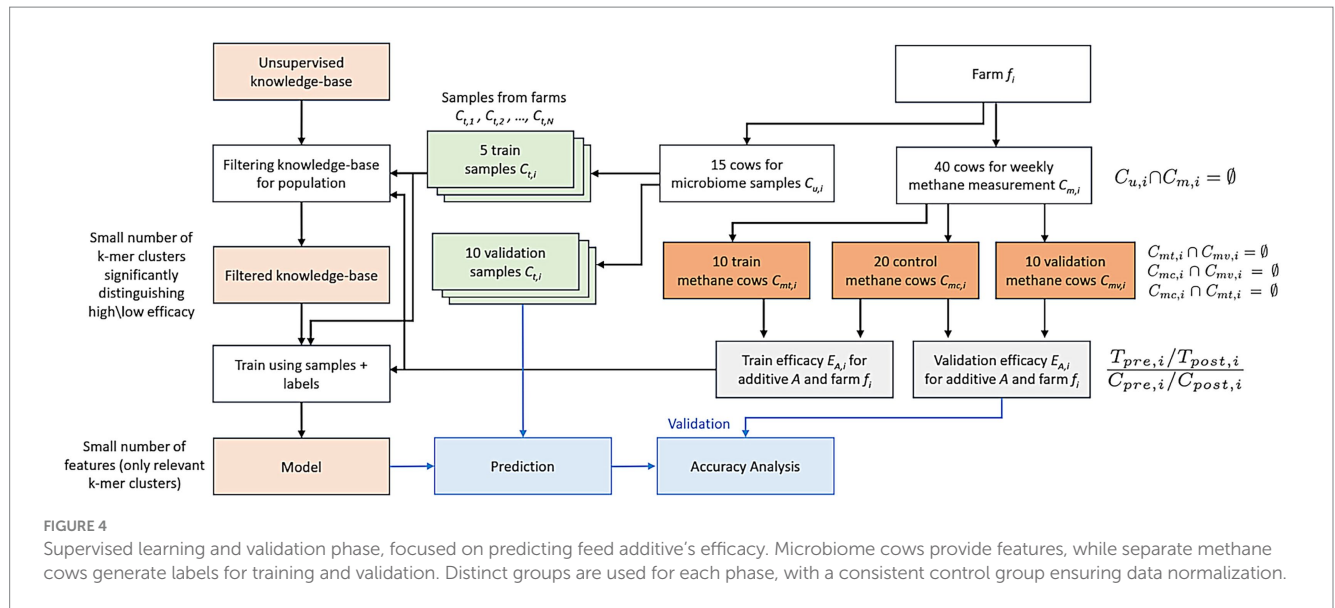
2.5 Model performance

The model processed the sequenced reads from each rumen sample, approximately 14–16 million paired-end reads per sample, each with a length of 150 nucleotides, which were decomposed into subsequences of 30 nucleotides (*k-mer* 30). This step generated millions of unique *k-mers* per sample, representing an extremely high-dimensional space. To reduce noise and focus on

biologically informative signals, *k-mers* occurring below a minimum frequency threshold across samples were excluded. Each remaining *k-mer* was then incorporated into a co-occurrence network, where nodes represented *k-mers* and edges denoted joint appearance within the same read. Groups of *k-mers* exhibiting consistent patterns of co-occurrence across animals were flagged as candidate biomarkers. Each group was then engineered into a single feature by aggregating its normalized frequency within a sample, yielding a biologically expressive representation that captures coordinated microbial signals rather than isolated fragments.

Given the enormous number of possible *k-mers* and their combinations, a dedicated dimensionality reduction step was implemented. Candidate biomarkers were evaluated for anomalous internal connectivity within the co-occurrence networks, defined as deviations from the expected power-law distribution of *k-mer* associations. Only groups showing statistically significant deviation were retained, dramatically reducing the feature space from billions of potential variables to a tractable number of validated biomarker clusters. Later, networks from each rumen microbiome sample are combined to generate a superposition network, which provides a more exact representation at farm level (Figure 3; Supplementary material 4). The superposition network reveals frequent *k-mers*, correlating with enteric methane emissions, although this target could be changed by any other biological target (milk production, susceptibility to diseases, etc.), producing specific biomarkers. Each biomarker is then analyzed and validated to ensure it is unlikely to occur randomly. Next, the identified biomarkers are grouped into two sets of DNA subsequences; with biomarkers positively associated with the reduction of enteric methane emissions being placed in the “top list,” while the biomarkers negatively associated with methane emissions are placed in the “bottom list.” Both lists are used to analyze the farm’s overall response to the tested feed additive, generating a predictive score ranging from 0 to 1 (Figure 4). Scores close to one indicate a high expected efficacy, while scores close to zero indicate low expected additive efficacy (Supplementary material 5). This step requires a model training step in which methane measurements of 40





R2024b software (Matlab, 2024), with significance levels set at $\alpha = 0.05$, ensuring that the findings are statistically reliable.

3 Results

3.1 Simulated scenarios

Figure 5 presents the efficacy analysis for the tested feed additive based on the obtained biomarkers (Supplementary material 7). The efficacy is initially represented as a normal distribution under naive deployment conditions, without farm selection ("Naive Deployment," in blue). The results of this scenario are contrasted with the optimized strategy ("Optimized Deployment," in green) where the additive is applied only to farms with high likelihood to respond positively to the feed additive. Thus, the optimized deployment exhibited a higher methane reduction (33%) than the naive deployment (22%); in other words, the optimized scenario produced an 11% more reduction of enteric methane emissions, than the observed in the naive scenario.

Although both scenarios achieved substantial methane reductions, the results suggest that the two approaches lead to clearly distinct outcomes (Cohen's $d = 0.92$, Hedges' $g = 0.87$). This means that the differences between both scenarios are substantial and unlikely to be due to random variation. Therefore, the overall additive efficacy to reduce enteric methane emissions, was dependent on the scenario analyzed.

At farm level, methane emissions revealed a significant reduction in the treatment groups following the administration of the additive compared to the control groups; thus, the normalized efficacy column in [Table 1](#) reflects the normalized mean change in methane emissions, accounting for variations in control and treatment groups. Only one farm (MP3) did not reduce methane emissions; however, it was aligned with the model prediction (prediction score <0.2). In general,

except for the MP3 site, the recorded methane reductions fluctuated between 0.8 to 41.1%, with eight sites exhibiting reductions higher than 20% in methane emissions.

The proficiency of our predictive model in accurately identifying the farms that are most likely to benefit from the use of the feed additive is shown in Figure 6. This figure illustrates the anticipated efficacy of the additive by a predicted efficacy score (axis x) ranging from 0 (no expected efficacy) to 1 (high efficacy) versus the real methane measurements (axis y). Thus, the model predicted a higher additive efficacy for sites VL3, FG2, and LV1 (predictive score equal or higher than 0.8) and this was confirmed by reductions in methane emissions higher than 30%. But the model not only successfully predicted the positive effects of the additive, but also the cases in which the additive efficacy is expected to be low, for example in sites

MP3 and SI2, where the predictive score was lower than 0.2 and the methane measurements did not exhibit reduction respect to the control groups. In overall, the model accurately predicted the additive efficacy in nine sites, while two sites (YE1 and KS3) exhibited higher reductions than expected.

Statistical analysis corroborates the accuracy of the model. Kendall's Tau and the Spearman's Rho metrics indicate a strong negative monotonic relationship between the model's predictions and the actual methane emissions (-0.67 and -0.85 respectively). This means the larger the score, the more methane reductions are expected, but when the predictive score is low, then the additive efficacy is low.

4 Discussion

This study successfully validated the AI-driven model, which uses rumen microbiome data of a given herd to accurately predict the efficacy of feed additives in reducing methane emissions. The model was able to successfully predict in most cases the effect of the selected feed additive, even in cases where the additive did not reduce methane emissions (sites MP3, SI2). The validation process demonstrated robustness by accounting for a wide range of parameters, including the diverse geographical and climatological conditions across the sites, variations in dietary regimes, differences in farm size and management practices, and genetic diversity among the participating herds. Despite this degree of variability among the commercial farms evaluated, the model consistently demonstrated a high predictive accuracy. The grade of accuracy observed in this study aligns well with previous findings (Altshuler et al., 2025), indicating a strong integration of herd-level genomic data into the model's performance.

The reductions observed in enteric methane emissions at the farm level highlight that the efficacy of the feed additive is influenced by herd genetics and by factors shaping the rumen microbiome. In this study, the additive's effectiveness varied widely, from no measurable impact to reductions of up to 40.1% in enteric methane emissions. Comparable variability has been reported across *in vivo* studies assessing allicin-based products, with reductions ranging from 10.7 to

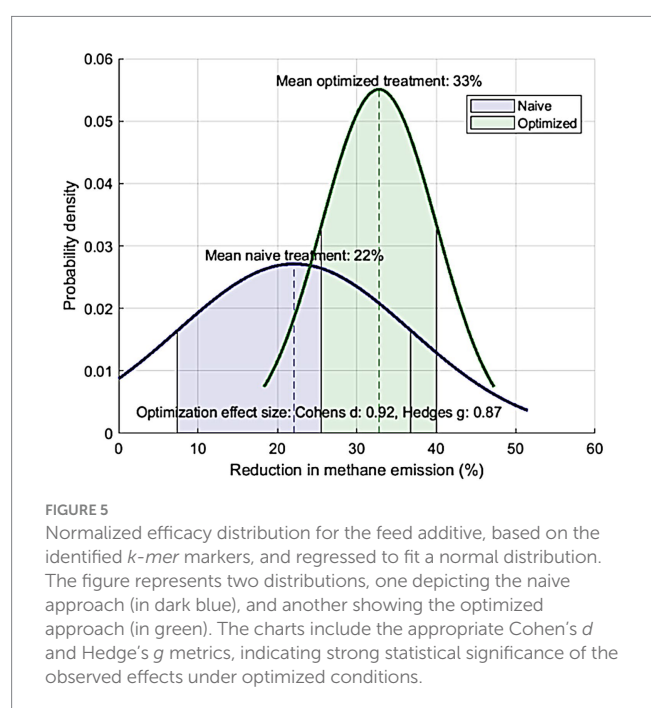
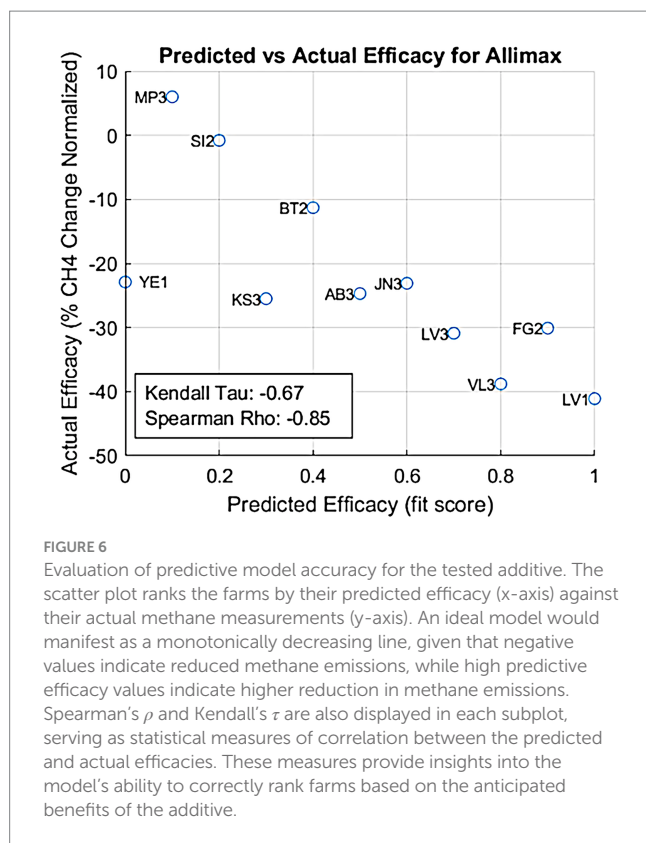


TABLE 1 Summary of methane emission changes across farms.

Farm	CH ₄ change (treatment)	CH ₄ change (control)	Normalized efficacy $\eta A, f_i$	Effect size	Cohen's D
MP3	-61.0%	-63.2%	6.0%	2.4	0.11
SI2	161.0%	163.0%	-0.8%	-0.7	-0.01
BT2	-24.4%	-14.7%	-11.3%	-12.2	-0.16
YE1	-41.6%	-24.3%	-22.9%	-21.2	-0.28
KS3	0.3%	34.6%	-25.5%	-67.3	-0.26
AB3	-52.1%	-36.3%	-24.7%	-24.9	-0.32
JN3	-10.4%	16.5%	-23.1%	-27.5	-0.75
LV1	-27.9%	22.3%	-41.1%	-224.4	-0.76
LV3	2.2%	47.8%	-30.9%	-60.9	-0.52
FG2	78.7%	155.6%	-30.1%	-84.4	-0.33
VL3	-26.8%	19.6%	-38.8%	-71.0	-0.55

Effect size indicates the raw impact of the additive; Cohen's D indicates the standardized treatment-control differences.



23.2% (Roque et al., 2019; Khurana et al., 2023). Even greater variability is observed *in vitro*, as demonstrated by a recent meta-analysis (Sari et al., 2022). This discrepancy arises because *in vitro* studies assays operate under simplified and controlled conditions, while *in vivo* systems represent a far more complex environment, where dilution, metabolism, microbial adaptation, and host responses attenuate the additive's effect. Among *in vivo* studies, several non-standardized factors contribute to variability in methane reduction, including compound stability, formulation and dosage, diet composition, microbial community structure, experimental design, and even the measurement device employed. A particularly illustrative case of this variability emerged at the LV site, which participated in two separate rounds of the study (labeled LV1 and LV3). In the first round, methane emissions decreased by 41.1% compared to the control group, while in the second round, the reduction was 30.9%. This 10.2% difference in efficacy was also predicted by the model, which assigned a predictive score of 1.0 for the first round and 0.8 for the second. These findings demonstrated that the model could detect subtle variations in the rumen microbiome that drive differences in additive efficacy, thereby capturing the biological mechanisms underlying methane responses. Such mechanisms include herd genetics, diet, management practices, climate conditions, and even soil and pasture characteristics; we are aware, however, that the patterns of variability captured by the model could differ if the study were extended to farms in more distant climatic and geographical regions. Notably, the model achieves this without requiring taxonomic classification of rumen populations or prior knowledge of methane-related metabolic pathways, as this information is inherently encoded in the DNA sequences extracted from rumen samples. Finally, as with

any data-driven tool, the model requires periodic updates with new datasets to reflect the evolving state of the rumen microbiome and to ensure reliable support for the implementation of emerging feed additive strategies.

From an overall perspective, the study revealed significant differences between a traditional strategy (naive approach), where the additive is applied without consideration of herd genetics or farm management, and a precision agriculture-oriented strategy, where the additive is used only when a higher impact is expected. Thus, both approaches showed an 11% difference in methane emission reduction, with a reduction of 22% reduction under the naive approach compared to a 33% reduction under the optimized approach. These differences may help explain some contradictory results observed in studies evaluating feed additive efficacy, which ultimately affect the adoption of feed additives as an effective strategy to reduce enteric methane emissions in the dairy sector.

Although the results are promising, the model could not provide accurate predictions in all cases. Specifically, the model predicted a low expected additive efficacy for sites YE1 and KS3; however, reductions in methane emissions exceeded 20% in both cases. This discrepancy likely reflects normal biological variability and limitations in data generalization rather than model overfitting, as overall predictive accuracy across all sites remained high. These observations highlight certain limitations of the model, including the selection of representative cows for rumen sampling and the implementation of the study protocol on commercial farms. Cow selection was based on parameters such as age, productivity levels, and visual inspection; however, it cannot completely prevent the inclusion of cows in the early stages of disease, which may influence rumen microbiome composition and, consequently, model performance. In addition, while the study protocol was executed by local farm staff under close supervision and no deviations were observed, minor inconsistencies cannot be entirely ruled out.

Given the wide variety of feed additives available on the market, the current validation process is not sufficient to ensure the same level of precision across all commercial products. Therefore, based on the promising results obtained in this study, we plan to continue validating the model with other commonly used commercial feed additives under real farm conditions. Future studies should include farms from diverse climate regions, where greater variability in factors such as temperature and precipitations is expected, and should also incorporate microbiome community analyses to provide a stronger basis for understanding how a given feed additive affects microbial populations and, consequently, the metabolic pathways involved in methane production. Given the model's flexibility in addressing diverse biological conditions, future research should also explore its applicability in key areas such as animal health, productivity, and product quality. Expanding the scope to include beef cattle and other livestock species could further enhance the model's utility and generalizability.

Based on the reductions in enteric methane emissions observed at global and farm levels, and on the demonstrated accuracy of the AI-driven model in predicting feed additive efficacy, we consider the model to be a valuable tool for deployment in dairy farms as part of a precision agriculture strategy. This approach can help reduce the environmental impact of farming activities, improve farm profitability, and support alignment with emerging global policies affecting the sector.

5 Conclusion

The AI-driven model successfully predicted the effect of the tested feed additive on most of the farms included in the study, identifying those where a high impact was expected and others where the effect was negligible. The reduction in enteric methane emissions peaked at over 40%, with most farms showing reductions greater than 20%. When evaluated overall, the optimized approaches achieved a 33% reduction, compared to a 22% reduction under the naive approach, where the feed additive was applied without targeting. These results underscore the potential of the AI-driven model as a precision agriculture tool, enabling dairy farmers to implement data-driven strategies in line with emerging methane reduction policies.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: microbiome data used in this research was collected from privately owned dairy farms. To respect the confidentiality agreements established with these farms and to protect the privacy of the farm owners, access to the raw data is restricted and will be made available upon request. Requests to access these datasets should be directed to YA, yanival@mit.edu.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because all animal procedures were conducted following the Agricultural Animals in Research and Teaching (Ag Guide, FASS, 2010) and supervised by a qualified veterinarian. The staff responsible for methane measurements were carefully trained to use the methane measuring instrument before performing in-situ measurements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

YA: Methodology, Conceptualization, Project administration, Validation, Software, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Data curation. TC: Project administration, Writing – review & editing. SC: Writing – review & editing, Investigation, Supervision. JG: Writing – review & editing, Investigation, Writing – original draft.

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Conflict of interest

All authors were employed by company Alphabiome AI.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1637380/full#supplementary-material>

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