

Research Article

Methane Emissions and Dry Matter Intake under Impact of *Saccharomyces cerevisiae* Supplementation on Dairy Cow

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Abstract | Methane emissions from livestock, particularly enteric methane, significantly contribute to global warming. As concerns about climate change intensify, the livestock sector, especially dairy and beef cattle, is under scrutiny for its substantial role in greenhouse gas emissions. The need for higher productivity has resulted in use of natural fermentation agents like *Saccharomyces cerevisiae* that can be methanogenic. The use of *Saccharomyces cerevisiae* has been under question for its possible methanogenic effects. This meta-analysis showed *Saccharomyces cerevisiae* supplementation did not significantly alter methane emissions (CH₄), dry matter intake (DMI), or rumen pH in dairy cows, this study provides valuable insights into future dose-dependent studies to address the methanogenic potential of yeast-based products recognizing the urgency of identifying effective mitigation strategies while maintaining productivity. As the cattle industry seeks sustainable solutions, further research should explore alternative strategies and optimize interventions for both productivity and environmental sustainability.

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Introduction

Methane (CH₄), a potent greenhouse gas, in terms of green-house-gases is one of three major contributors to global warming, along with nitrous oxide (N₂O) and carbon dioxide (CO₂) (Trenchev *et al.*, 2023). Dairy industry is a big source of Methane emissions, specially enteric CH₄, accounts for 50-60% of farm-level emissions (Martin *et al.*, 2010), livestock sector plays a crucial role in causing the concern (Moumen *et al.*, 2016). This is particularly

true for dairy and beef cattle (Ricci *et al.*, 2013). The urgency of identifying and mitigating greenhouse gas emissions caused by livestock is growing as the global community struggles to meet rising supply demands while coping with climate change strategies (Dangal *et al.*, 2017; Min *et al.*, 2022; Bačėninaitė *et al.*, 2022).

Methane emissions from livestock like sheep, goats, and cattle are on the rise in many areas (Gulich *et al.*, 2023; Ruden *et al.*, 2023); dairy cattle produce about 128 kg of methane per head per year (Meller

et al., 2019). In light of the damaging effects on the environment, scientists have started to look at practical solutions to reduce ruminant methane emissions (Króliczewska *et al.*, 2023). The metabolic activity of methanogenic archaea in ruminants, although providing fatty acids, are responsible for methane production (Patra and Puchala, 2023; O'Hara *et al.*, 2023; Singh *et al.*, 2023). Although methane is essential for effective decomposition of organic materials, its production is metabolically-expensive since it reduces energy levels and increases greenhouse gas emissions (Bošnjaković *et al.*, 2023).

Saccharomyces cerevisiae, a natural dietary supplement that can modify the rumen environment (Cattaneo *et al.*, 2023) and under certain conditions commonly enhance methane emissions, poses a new challenge to methane emission strategies (Benchaa *et al.*, 2024). Extensive research on yeast-derived products (YP) has shown varied degrees of success in enhancing the rumen environment for productivity parameters like milk yield, VFAs, beef quality, broiler egg production, broiler meat quality, rabbit carcass, etc. (Ahmed *et al.*, 2024; Ogbuwu and Mbajorgu, 2023; Benchaa *et al.*, 2024; Williams *et al.*, 2023; Sarwar *et al.*, 2023; Attia *et al.*, 2023; Adli *et al.*, 2023). All these productivity benefits come with an environmental damaging price as studies show it's boosting methanobacter development (Benchaa *et al.*, 2024) in host animals, especially cows. Improving productivity while decreasing methanogenic potential requires a thorough understanding of the relationship between the yeast strains, dosages, and its viability, along with the host animal's compatibility.

This meta-analysis delves into the complex connection between ruminant's daily methane output, dietary *Saccharomyces cerevisiae* supplementation, DMI, and pH and suggests optimal dosage for a stable and environment conscious farming. As the cattle

business explores sustainable solutions to lessen its environmental imprint, knowledge of the possibilities of yeast-based products in assisting methane emissions holds promise for possible second thoughts on shifting towards alternatives.

Materials and Methods

Eligibility criteria, search strategy, and data extraction

National Center for Biotechnology Information (NCBI) and Google Scholar were used to conduct a literature search. During the search, the keywords *Saccharomyces cerevisiae*, methane production, and cattle, were employed. The criteria for studies were as: (1) full-text publicly available original research publications published in English; (2) peer-reviewed journals; (3) direct comparison of control vs *Saccharomyces cerevisiae* diets; (4) CH₄ production reports with DMI and pH. The initial search provided a total of 30 results, (Figure 1). Following the screening, 4 were removed (not publicly available), 5 duplicates and 14 (reviews, *in vitro* studies, and irrelevant studies) were also removed. In the end, a total of 7 original research papers were utilized (Table 1) for data extraction and statistical analysis with adherence to the PRISMA-P guidelines throughout the entire process.

Data extraction

The data extracted included the primary characteristic CH₄ emissions per day in control and treatment groups along standard deviation, breed, amount of *Saccharomyces cerevisiae* used in grams per day, number of control and treatment group animals, rumen pH, and DMI between control and treatment groups with Standard Deviations and the units for all extracted parameters were homogenized for further data analysis.

Table 1: *The studies used in the meta-analysis.*

Author	Year	Dosage (g)	Number of cows	CH ₄ measurement method	Feed type
A. N. Hristov	2009	56	8	Incubation Chmaber	TMR
J. Oh	2019	28	12	GreenFeed system	TMR
R. A. Meller	2019		6	GreenFeed system	TMR
Y.H. Chung	2010		10	Sulfur hexafluoride (SF ₆) tracer gas technique	TMR
Yan Li	2020	10, 20, 30	30	Sulfur hexafluoride (SF ₆) tracer gas technique	TMR
C. Muñoz	2016	0.5	20	Calorimetry chambers	TMR
Kampanat Phesatcha	2021	5, 10, 15	8	Equation of Moss using VFA proportions	TMR

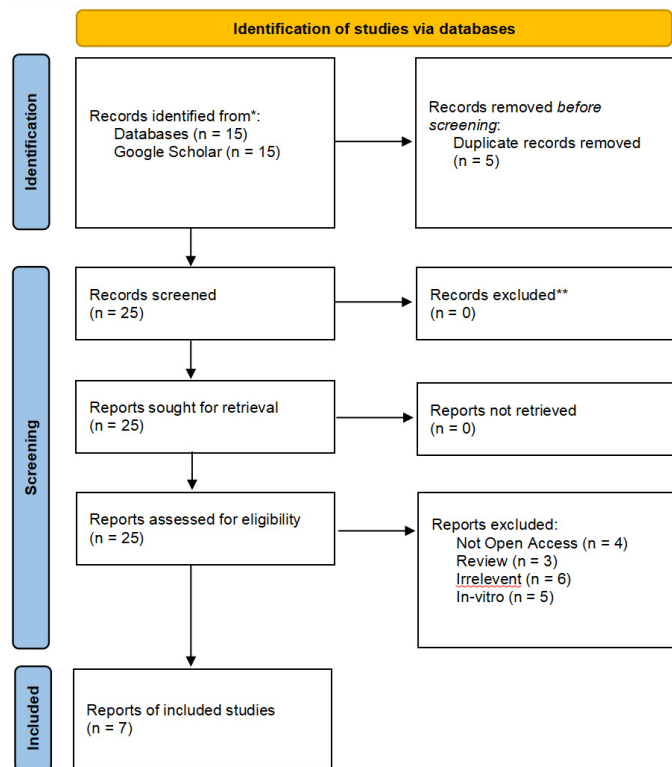


Figure 1: PRISM-P flowchart for study inclusion criteria.

Meta analysis using OpenMEE

The data analysis was conducted using the Hedges'd effect (Standardized Mean Difference/SMD) with a 95% confidence interval, employing the OpenMEE software to explore the effect sizes in the dataset (means of control and treatment groups of pH, CH₄ emissions per day, DMI and their standard deviations).

Hedges'd effect sizes, along with their corresponding 95% confidence intervals, were aggregated using a random-effects model. To assess heterogeneity across studies, the I^2 index was employed. A value of I^2 greater than 50% indicates sufficient heterogeneity among the studies. The entire meta-analysis was facilitated through OpenMEE software. The results were presented in forest plots, providing a visual representation for the comprehensive analysis of the effect sizes across the selected studies.

Results and Discussion

Methane emissions standard meta analysis

A standard meta-analysis, utilizing Hedges'd as the statistic, incorporating means and standard deviations across the studies. The continuous random-effects model using the DerSimonian-Laird approach was adopted, and weights allocated to each study reflected their impact on the total analysis. The model findings yielded a standardized mean difference estimate of

0.175, with a confidence range ranging from -0.571 to 0.920. The p-value of 0.646 revealed no statistically significant difference. Heterogeneity analysis indicated low τ^2 (1.150), a heterogeneity p-value of < 0.001, and an I^2 value of 78.188, suggesting small heterogeneity among the studies. The forest plot (Figure 2) displays the standardized mean differences for intestinal methane emission in dairy cows across studies, demonstrating a non-significant overall impact.

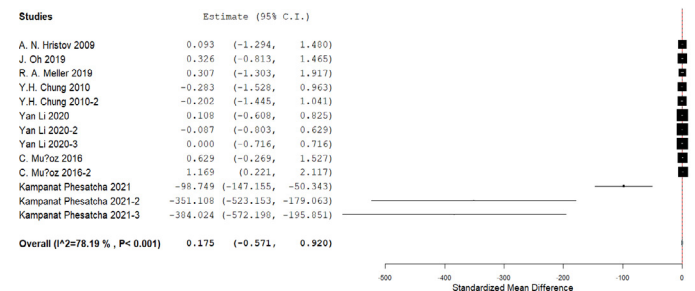


Figure 2: The forest plot for Methane Emissions illustrating standardized mean differences, demonstrating overall impact with a 95% confidence range from -0.361 to 0.295.

DMI standard meta analysis

The standard meta-analysis mainly concentrates on Hedges'd, assessing means and standard deviations linked to Dry Matter Intake (DMI) in dairy cows. The continuous random-effects model using the DerSimonian-Laird approach was applied. The summary of the continuous random-effects model suggested a standardized mean difference estimate of 0.459, with a confidence range from -0.364 to 1.281 and a non-significant p-value of 0.275. Heterogeneity analysis found low τ^2 (1.320), a heterogeneity p-value of <0.001, and an I^2 value of 78.984, indicating negligible heterogeneity among the studies. The forest plot (Figure 3) depicts the standardized mean differences and confidence intervals for Dry Matter Intake (DMI) in dairy cows across numerous studies, with all estimations crossing the null line, which indicates non-significant results.

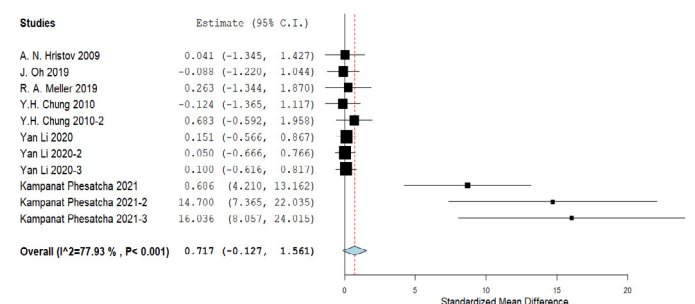


Figure 3: The forest plot for Dry Matter Intake illustrating standardized mean differences.

pH standard meta analysis

The continuous random-effects model using the DerSimonian-Laird approach was applied. The summary of the continuous random-effects model suggested a standardized mean difference estimate of 0.717, with a confidence range from -0.127 to 1.561 and a p-value of 0.096, demonstrating a non-significant trend towards pH in the experimental group. Heterogeneity analysis indicated a τ^2 of 1.243, a heterogeneity p-value of < 0.001 , and an I^2 value of 77.935, showing little heterogeneity among the studies.

The forest plot (Figure 4) illustrates standardized mean differences and confidence intervals for pH levels in dairy cows across several studies, indicating a non-significant trend towards lower pH in the experimental group as all estimations pass the null line.

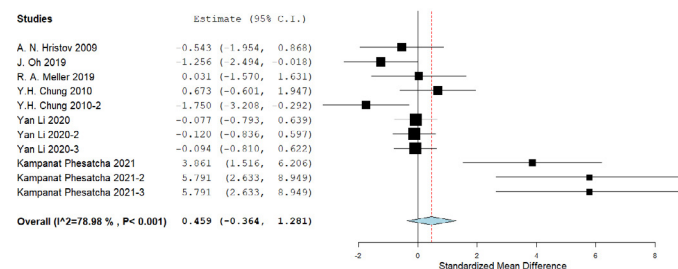


Figure 4: The forest plot for pH illustrating standardized mean differences.

The meta-analyses conducted in this study aimed to assess the impact of *Saccharomyces cerevisiae* supplementation on methane emissions, dry matter intake (DMI), and rumen pH in dairy cows. Methane emissions from livestock, particularly from ruminants like dairy cattle, contribute significantly to greenhouse gas emissions and are therefore of global concern (Moumen *et al.*, 2016). As such, finding effective strategies to mitigate methane production without compromising productivity is essential for sustainable livestock farming (Ricci *et al.*, 2013).

The analysis of methane emissions revealed that *Saccharomyces cerevisiae* supplementation did not have a significant overall effect. Despite variations among individual studies, the pooled estimate did not show a statistically significant difference between the control and supplemented groups. This suggests that while *Saccharomyces cerevisiae* may influence rumen fermentation, its impact on methane production in dairy cows may not be substantial. This finding

underscores the complexity of methane regulation in ruminants and highlights the need for further research to fully understand the mechanisms involved.

Similarly, the meta-analysis on dry matter intake (DMI) showed no significant difference between the control and supplemented groups. Previous studies have suggested potential effects of yeast-based supplements on feed intake, but our analysis did not find evidence to support this claim. The lack of significant variation in DMI between groups indicates that *Saccharomyces cerevisiae* supplementation may not have a pronounced effect on feed consumption in dairy cows. This finding has implications for understanding the nutritional management of dairy cattle and emphasizes the importance of considering multiple factors in dietary interventions (Dann *et al.*, 2000).

The meta-analysis of rumen pH revealed a non-significant trend towards lower pH in the experimental group receiving *Saccharomyces cerevisiae* supplementation. Although the difference was not statistically significant, the observed trend suggests a potential modulation of rumen fermentation by yeast-based supplements (Maamouri and Salem, 2022). Rumen pH plays a crucial role in microbial activity and nutrient utilization, and even subtle changes can impact animal health and performance. Further investigation is warranted to explore the mechanisms underlying the observed trend and its implications for rumen function in dairy cows.

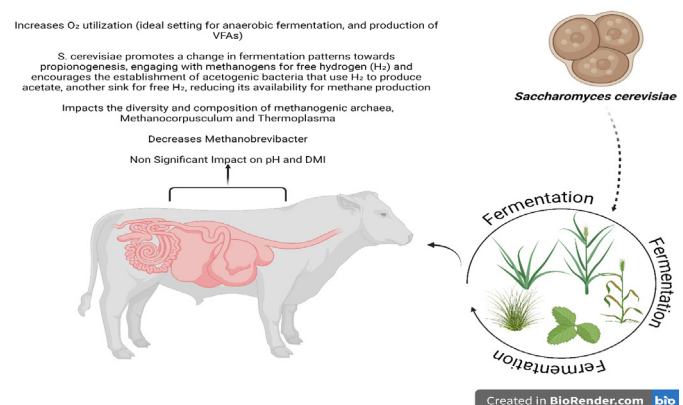


Figure 5: The supplementation of *Saccharomyces cerevisiae* in feed impacts Methane production in a number of ways as depicted in the illustration.

The addition of *Saccharomyces cerevisiae* impacts formation of methane via a number of interconnected pathways as illustrated in the Figure 5. Increased O_2 utilization creates an ideal setting for anaerobic

fermentation, leading to production of VFAs and lower pH. As a result, this environment inhibits the spread of methanogens and protozoa. Furthermore, *S. cerevisiae* promotes a change in fermentation patterns towards propionogenesis, engaging with methanogens for free hydrogen (H_2) and encouraging the establishment of acetogenic bacteria that use H_2 to produce acetate, another sink for free H_2 , reducing its availability for methane production (Gong *et al.*, 2018). Yeast supplementation enhances the diversity and composition of methanogenic archaea, notably the *Methanocorpusculum* and *Thermoplasma* species, while decreasing the presence of *Methanobrevibacter*. These alterations may provide light on the mechanisms through which yeast influences methane emissions and help to create methane mitigation measures (Jin *et al.*, 2017).

Overall, the findings of these meta-analyses contribute to our understanding of the effects of *Saccharomyces cerevisiae* supplementation in dairy cattle nutrition. While the results suggest limited effects on methane emissions and DMI, they provide valuable insights for future research directions. Further studies incorporating dose-dependent analyses and exploring interactions with other dietary factors are needed to fully elucidate the potential benefits of yeast-based supplements in dairy cow management. Additionally, the importance of considering individual variability and methodological differences in interpreting research outcomes in this field is emphasized.

Conclusions and Recommendations

In conclusion, this meta-analysis addressed an intricate link between ruminant methane emissions, dietary *Saccharomyces cerevisiae* supplementation (a natural growth stimulant), Dry Matter Intake (DMI), and rumen pH. The forest plots showed normalized mean differences and confidence intervals, which supports the conclusion that *Saccharomyces cerevisiae* supplementation in dairy cows had no significant effect on methane emissions, DMI, or rumen pH. However, future studies with dose-dependent data are required to answer disparity reported by some previous studies and to properly address the potential significance of *Saccharomyces cerevisiae* as a contributor to methane emissions.

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Novelty Statement

The meta-analysis highlights the environmental concern with the increasing use of yeasts as feed additives. The fermentation and breakdown of the feed components facilitates the production of methane which is of great concern and needs to be monitored. This meta analysis establishes the link between use of *Saccharomyces cerevisiae* on key parameters like dry matter intake, methane emissions, and ruminal pH.

Author's Contribution

Bilal Ahmed, Faheem Ahmed Khan and Nuruliarizki Shinta Pandupuspitasari: Conceptualized the meta-analysis.

Bilal Ahmed: Extracted, analyzed the data, and wrote the paper.

Asep Setiaji and Muhammad Rizwan Yousaf: Helped revise this manuscript.

All authors read and approved the final manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

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