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Background and objective

Supplementing a diet with nitrate is regarded as an effective and promising methane (CH₄) mitigation strategy by competing with methanogens for available hydrogen through its reduction of ammonia in the rumen. Studies have shown major reductions in CH₄ emissions with nitrate supplementation, but with large variation in response. The objective of this study was to collate data on nitrate supplementation for CH₄ mitigation and quantitatively evaluate the effects of dietary nitrate for enteric CH₄ production and yield in dairy and beef cattle. In the present meta-analysis, we hypothesize that, in addition to nitrate dose, dry matter intake (DMI), nutrient composition of the diet, birth weight (BW), measurement techniques [respiratory chamber, GreenFeed, and sulfur hexafluoride tracer (SF₆)] for CH₄ emissions, and type of cattle (dairy or beef) may explain a considerable proportion of the variability in CH₄ mitigation effect of nitrate. Therefore, this study quantitatively analyzed explanatory variables to account for the heterogeneity in CH₄ mitigation potential of nitrate using a meta-analytic approach.

Search strategy and selection criteria

A literature search was conducted using several sources including the Web of Science, Scopus, and Google Scholar online databases with all possible combinations of the key words "feed additives," "nitrate," "methane" (including variants of "CH₄" and "greenhouse gas"), "cattle" (including variants of "dairy," "beef," "steer," "cows," and "ruminants"). The period of the study covered from 1970 to 2020. 1) Have a control treatment group that did not receive supplementary nitrate; 2) trials conducted in vivo using cattle; 3) measured (i.e., not estimated) CH₄ emissions with standard deviation, standard error, or other relative data that can be used to calculate the standard error (e.g., least significant difference); 4) and other required variables described such as animal characteristics, DMI, dietary composition, and BW.

Data and analysis

To prepare for the meta-analysis, effect size estimates and corresponding sampling variances were obtained using the "metafor" (version 2.1-0) and "robumeta" (version 2.1) packages in R (version 3.6.1). To deal with the unknown correlations among these non-independent effect sizes, a robust variance estimation (RVE) method was used to conduct the meta-analysis. Studies selected in the meta-analysis were not identical in methods and sample characteristics, which may introduce variance of the true effect sizes. Therefore, RVE random-effects and RVE mixed-effects models were fitted to estimate between-study variability (heterogeneity) that was assumed to be purely random using the "robu" function in the "robumeta" package (version 2.1) in R (version 3.6.1).

Number of papers	Population	Intervention	Comparator	Outcome	Quality score
24	Dairy and beef cattle	Nitrates supplementation	No nitrate supplementation	Metric: 1) Methane (CH ₄) production (g/d); 2) CH ₄ yield (g/d); Effect size: Standardized difference of the considered metrics between intervention and control	87.5

Results

- Forest plots generated with SMD for CH₄ production and CH₄ yield indicate that nitrate mostly had an antimethanogenic effect, but that the size of effect varies across studies.
- Given the overall effect size that accounted for sampling variation within and between studies, at a mean nitrate dose of 16.7 g/kg of DM, the overall CH₄ production ($P < 0.001$) and CH₄ yield ($P < 0.001$) were reduced by $13.9 \pm 1.17\%$ and $11.4 \pm 1.36\%$, respectively, based on the random-effect RVE models.
- NULL
- NULL
- NULL

Factors influencing effect sizes

- Feed additive dose : The effectiveness of nitrate in reducing CH₄ production was positively associated with nitrate dose ($P < 0.001$). A 1 g/kg of DM increase in nitrate dose from its mean (16.7 g/kg of DM) enhanced the nitrate antimethanogenic effect on CH₄ production by $0.904 \pm 0.1461\%$.
- Cattle type : Nitrate has a stronger CH₄ yield mitigating effect for dairy cattle than for beef cattle, and that a higher nitrate dose is required for beef cattle to obtain the same CH₄ yield mitigation compared with dairy cattle. The efficacy of nitrate utilization appears to be greater and the potential of the nitrate inhibitory effect seems enhanced in dairy cattle, perhaps through a more complete nitrate reduction.
- Feed additive form : Nitrate provided in encapsulated form was given to only beef cattle in our database. Slow-release nitrate might have a lower capacity to decrease CH₄ emissions because it may have been washed out of the rumen before the nitrate is released to be reduced to ammonia, contributing to differences in type of cattle.

Conclusion

Nitrate supplementation reduced CH₄ emissions (production in g/d as well as yield in g/kg of DMI) in dairy and beef cattle in a dose-dependent manner. The mitigating effect of nitrate on CH₄ production and yield was greater in dairy than in beef cattle.