**A protocol for a systematic review and meta-analysis**

**Title:** Effects of Algae and Microalgae Supplementation on Beef Cattle Performance and Methane Emissions: A Systematic Review and Meta-Analysis

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**Author Contributions**

The review question and protocol described in this document were developed with the contribution and final approval of all co-authors. Gabriela Maria Leite and Vanessa Peripolli drafted the protocol, and all authors provided their input.

**Registration**

The protocol for this systematic review and meta-analysis was deposited on the public repository Open Science Framework (OSF; https://osf.io) and registered in Systematic Reviews for Animals and Food (SYREAF; www.syreaf.org) online. The article will be reported using the approved Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Page et al., 2021).

**Amendments**

This review is not an amendment of a previously completed or published protocol. Any amendments made after registration will be documented as Protocol Deviations in the systematic review.

**1. Introduction**

**1.1. Rationale**

As global demand for beef increases, sustainable and efficient production methods are imperative. Alternative feed sources, such as algae and microalgae, present an opportunity to improve cattle performance while reducing the environmental footprint (Halmemies-Beauchet-Filleau et al., 2018).

Algae and microalgae are rich in proteins, vitamins, minerals, and fatty acids. They offer advantages compared to cereal grains, including high photosynthetic efficiency, low water demand, and potential for carbon sequestration. Their potential to mitigate methane emissions from cattle digestion has been studied (Moorby and Fraser, 2021). However, the feasibility of using alternative feeds in ruminant nutrition depends on the nutritional value of the new feeds, the productive responses, and the costs of these feeds compared to conventional ones. Furthermore, the environmental footprint of animal feed production and the economic value of these emerging feeds in alternative uses are of great importance (Halmemies-Beauchet-Filleau et al., 2018).

Given these considerations, a systematic review (SR) and meta-analysis (MA) are essential to comprehensively assess and quantify the effects of algae and microalgae supplementation on beef cattle performance and methane emissions. Therefore, the objective of this study is to evaluate the impact of algae and microalgae supplementation on beef cattle productivity and methane emissions through a SR-MA.

**1.2. Objectives**

The objective of this protocol is to describe the methods to review and summarize the available information on the effects of supplementing algae and microalgae in the diet of beef cattle on performance and methane emissions using SR-MA methodology. The PICO elements are:

1. **P**opulation: Beef cattle

2. **I**nterest: Seaweed, microalgae, duckweed, aquatic feedstuffs

3. **C**omparison or control: Conventional feed-based diets or control groups without algae and microalgae supplementation

4. **O**utcomes: Performance (weight gain, feed intake, feed conversion) and methane emission

# 2. Methods

## 2.1 Study selection and screening criteria

The selected studies in the search will be screened by three different reviewers, who will evaluate all citations independently by reading the title and abstract of the article. The evaluation questions will be defined as: (1) is the evaluated abstract an original work?; (2) does this abstract evaluate the inclusion of algae and microalgae in the diet of beef cattle; (3) does this abstract evaluate feed intake, weight gain, feed conversion and/or methane emission? If there are negative responses from the reviewers to at least one of the above questions, the citation will be excluded from the evaluation.

The inclusion criteria will be original articles, which evaluates the use of supplementation with algae and microalgae in beef cattle. It will be considered observational or experimental studies. that evaluated at least one of the outcomes of interest: feed intake, weight gain, feed conversion and methane emission. Moreover, the studies must present the ingredients of the diet. We will consider articles with groups of cattle at any age, category breed and sex, castrated or not. We will consider only those articles conducted in a population of healthy cattle.

The exclusion criteria will be: unpublished articles; abstracts; articles with a population of cattle intended for breeding/rearing; articles with a population of cattle raised in an extensive system; articles with a population of cattle intended for dairy production; articles with a population of sick cattle; articles with the inclusion of medications in the treatment; articles with animals of other species; and studies that used algae and microalgae supplementation for less than 30 days.

## 2.2. Information sources

## Systematic reviews will be conducted using the internet server of the Instituto Federal Catarinense (IFC). The search will be conducted in the five electronic databases: PubMed, Scopus, Web of Science, Google Scholar and ProQuest Dissertations & Thesis Citation Index. All scientific publications, dissertations and theses will be considered, regardless of year or language of publication.

## 2.3. Search strategy

The search strategy adapted for each database search was described in Table 1 (search conducted on April 08, 2024).

**Table 1 -** Database search strategy.

|  |  |
| --- | --- |
| **Database** | **Search (April 08, 2024)** |
| **PubMed**  292 | ("cattle"[MeSH Terms] OR "cattle"[All Fields] OR "bovine"[All Fields] OR "beef"[All Fields] OR "bull"[All Fields] OR "cow"[All Fields] OR "steer"[All Fields] OR "heifer"[All Fields]) AND ("microalgae"[MeSH Terms] OR "microalgae"[All Fields] OR "algae"[All Fields] OR "seaweed"[MeSH Terms] OR "seaweed"[All Fields] OR "duckweed"[All Fields] AND ("efficiency"[MeSH Terms] OR "efficiency"[All Fields] OR "productivity"[All Fields] OR "performance"[All Fields] OR "weight"[All Fields] OR "dry matter intake"[All Fields] OR "DMI"[All Fields] OR "ADG"[All Fields] OR "feed:gain"[All Fields] OR "feed conversion"[All Fields] OR "gain:feed"[All Fields] OR "feed intake"[All Fields] OR "feed efficiency"[All Fields] OR "daily gain"[All Fields] OR "methane"[MeSH Terms] OR "methane"[All Fields] OR "CH4"[All Fields]) |
| **Scopus**  561 | ( TITLE-ABS-KEY ( ( "cattle"  OR  "bovine"  OR  "beef"  OR  "bull"  OR  "cow"  OR  "steer"  OR  "heifer" ) )  AND  TITLE-ABS-KEY ( ( "microalgae"  OR  "algae"  OR  "seaweed"  OR  "duckweed" ))  AND  TITLE-ABS-KEY ( ( "efficiency"  OR  "productivity"  OR  "performance"  OR  "weight"  OR  "dry matter intake"  OR  "DMI"  OR  "ADG"  OR  "feed:gain"  OR  "feed conversion"  OR  "gain:feed"  OR  "feed intake"  OR  "feed efficiency"  OR  "daily gain"  OR  "methane"  OR  "CH4" ) ) ) |
| **Web of Science Core Collection**  802 | (“cattle” OR “bovine” OR “beef” OR “bull” OR “cow” OR “steer” OR “heifer”) (All Fields) AND ("microalgae" OR “algae” OR "seaweed" OR "duckweed") (All Fields) AND (“efficiency” OR “productivity” OR “performance” OR “weight” OR “dry matter intake” OR “DMI” OR “ADG” OR “feed:gain” OR “feed conversion” OR “gain:feed” OR “feed intake” OR “feed efficiency” OR “daily gain” OR "methane" OR "CH4") (All Fields) |
| **Proquest**  41 | (“cattle” OR “bovine” OR “beef” OR “bull” OR “cow” OR “steer” OR “heifer”) (Topic) AND ("microalgae" OR “algae” OR "seaweed" OR "duckweed") (Topic) AND (“efficiency” OR “productivity” OR “performance” OR “weight” OR “dry matter intake” OR “DMI” OR “ADG” OR “feed:gain” OR “feed conversion” OR “gain:feed” OR “feed intake” OR “feed efficiency” OR “daily gain” OR "methane" OR "CH4") (Topic) |
| **Google Scholar**  120 de 974.000 | (“cattle” OR “bovine” OR “beef” OR “bull” OR “cow” OR “steer” OR “heifer”) AND ("microalgae" OR “algae” OR "seaweed" OR "duckweed") AND (“efficiency” OR “productivity” OR “performance” OR “weight” OR “dry matter intake” OR “DMI” OR “ADG” OR “feed:gain” OR “feed conversion” OR “gain:feed” OR “feed intake” OR “feed efficiency” OR “daily gain” OR "methane" OR "CH4") |

## 2.4. Study Records

***Data management***

All citations will be exported to EndNote Web® bibliographic management software for manual removal of duplicate references. The file obtained after deduplication will be uploaded in Rayyan Web® program for the screening process.

***Data extraction***

Data will be extracted from relevant publications in a predefined protocol. Each publication will be previously assessed for the language of interest (English, Spanish or Portuguese); whether it has an appropriate control group and provides sufficient data for an MA.

The information collected from each publication will include characteristics of the population, intervention and outcomes of interest, as well as publication information (journal name, author(s), year of publication and language). For each result, the mean, standard deviation (SD) or any available measure of dispersion, the unit of measurement, p-value and the number of animals in the control and treated groups will be extracted.

An Excel spreadsheet will be constructed with the extracted data, as well as a dataset containing results from controlled trials. A pooled standard deviation (Sp) will be calculated using the general standard error of the mean (SEMp) presented in the control and treated groups (Mederos et al., 2012; Canozzi et al., 2017). For studies that present only the p-value, an estimate of the pooled standard deviation will be calculated using the t-statistic, assuming a normal distribution of the data (Mederos et al., 2012).

The risk of bias will be assessed by the first author, individually for each included study using the Cochrane Collaboration Risk of Bias Tool (Higgins et al., 2011).

**2.6. Data synthesis**

All pooled analyses will be based on random effects models with the means of the control and treated groups. Forest plot will be used for the presentation of the pooled result. With the assumption of the presence of heterogeneity among the studies, randomized MA and meta-regression analyses will be performed (Der Simonian and Laird, 1986). This heterogeneity may be due to unknown or unrecorded methodological characteristics (Thompson, 2001). The Cochrane’s Q test and I2 will be used to evaluate the heterogeneity among studies, and the sensibility test to evaluate the possible sources of heterogeneity. Publication bias will be assessed by using both funnel plots and Egger’s weighted regression tests.

For all statistical analyses, Stata software (version 16, StataCorp., CollegeStation, TX, USA) will be used. The interpretation of the results will be assessed using the GRADE approach (Grading of Recommendations Assessment, Development and Evaluation, Santesso et al., 2020) based on the imprecision of the results (p-value and 95% CI) and inconsistency (heterogeneity).

# 3. Conclusions

This SR-MA will provide a comprehensive assessment of the effects of algae and microalgae supplementation on beef cattle performance and methane emissions. By synthesizing existing research, this study aims to generate evidence-based insights that can guide livestock producers, researchers, and policymakers in adopting sustainable feeding strategies. The findings will contribute to the development of more efficient and environmentally responsible beef production systems, supporting global efforts to reduce agricultural methane emissions while maintaining animal productivity.

**Review stage at the time of this submission**

|  |  |  |
| --- | --- | --- |
| Stage | Started | Completed |
| Search | Yes | Yes |
| Search results screened according to eligibility criteria | Yes | Yes |
| Data extraction | Yes | No |
| Data analysis | No | No |

# References

CANOZZI, M.E.A.; MEDEROS, A.; MANTECA, X.; TURNER, S.; MCMANUS, C.; ZAGO, D.; BARCELLOS, J.O.J. A meta-analysis of cortisol concentration, vocalization, and average daily gain associated with castration in beef cattle. Research in Veterinary Science, 114, 430-443, 2017.

DER SIMONIAN, R.; LAIRD, N. Meta-analysis in clinical trials. Controlled Clinical Trials, 7, 177-188, 1986.

GEADA, P.; MOREIRA, C.; SILVA, M.; NUNES, R.; MADUREIRA, L.; ROCHA, C.M.R.; PEREIRA, R.N.; VICENTE, A.A.; TEIXEIRA, J.A. Algal proteins: Production strategies and nutritional and functional properties. Bioresource Technology, 332, 125125, 2021.

HALMEMIES-BEAUCHET-FILLEAU, A.; RINNE M.; LAMMINEN, M.; MAPATO, C.; AMPAPON, T.; WANAPAT, M.; VANHATALO, A. Review: Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. Animal, 12, 295-309. 2018.

HIGGINS, J.P.T.; ALTMAN, D.G.; GOTZSCHE, P.C.; JUNI, P.; MOHER, D.; OXMAN, A.D. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. British Medical Journal, 343, 9-20, 2011.

MEDEROS, A.; WADDELL, L.; SÁNCHEZ, J.; KELTON, D.; PEREGRINE, A.S.; MENZIES, P.; VANLEEUWEN, J.; RAJIC, A. A systematic review-meta-analysis of primary research investigating the effect of selected alternative treatments on gastrointestinal nematodes in sheep under field conditions. Preventive Veterinary Medicine, 104, 1-14, 2012.

MOORBY, J. M; FRASER, M.D. Review: New feeds and new feeding systems in intensive and semiintensive forage-fed ruminant livestock systems. Animal, 15, 100297, 2021.

PAGE, M.J.; MCKENZIE, J.E.; BOSSUYT, P.M.; BOUTRON, I.; HOFFMANN, T.C.; MULROW, C.D.; SHAMSEER, L.; TETZLAFF, J.M.; AKL, E.A.; BRENNAN, S.E.; CHOU, R.; GLANVILLE, J.; GRIMSHAW, J.M.; HR´OBJARTSSON, A.; LALU, M.M.; LI, T.; LODER, E.W.; MAYO-WILSON, E.; MCDONALD, E.; MCGUINNESS, L.A.; STEWART, L.A.; THOMAS, J.; TRICCO, A.C.; WELCH, V.A.; WHITING, P.; MOHER, D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. The BMJ, 71, 372, 2021.

SANTESSO, N.; GLENTON, C.; DAHM, P.; GARNER, P.; AKL, E.A.; ALPER, B.; BRIGNARDELLO-PETERSEN, R.; CARRASCO-LABRA, A.; DE BEER, H.; HULTCRANTZ, M.; KUIJPERS, T.; MEERPOHL, J.; MORGAN, R.; MUSTAFA, R.; SKOETZ, N.; SULTAN, S.; WIYSONGE, C.; GUYATT, G.; SCHÜNEMANN, H.J. GRADE guidelines 26: informative statements to communicate the findings of systematic reviews of interventions. Journal of Clinical Epidemiology, 119, 126-135, 2020.

THOMPSON, S.G. Why and how sources of heterogeneity should be investigated. In: Systematic Reviews in Health Care: Meta-Analysis in Context. Egger, M.; Smith, G.D.; Altman, D.G. (Eds.). British Medical Journal Books, London, UK, pp. 157-175, 2001.