Peer Community In Animal Science

Modeling the Shifting Impacts of *Asparagopsis taxiformis* on Rumen Fermentation and Methane Production

Arda Yildirim *based on peer reviews by 2 anonymous reviewers*

Paul Blondiaux, Tristan Senga Kiessé, Maguy Eugène, Rafael Muñoz Tamayo (2025) Dynamic sensitivity analysis of a mathematical model describing the effect of the macroalgae *Asparagopsis taxiformis* on rumen fermentation and methane production under *in vitro* continuous conditions. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Animal Science. https://doi.org/10.1101/2024.06.19.599712

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Published: 10 June 2025

Reducing enteric methane emissions remains one of the key environmental and economic challenges in ruminant livestock systems. The red macroalgae *Asparagopsis taxiformis* has emerged as a promising natural feed additive for methane mitigation due to its bioactive compounds such as bromoform, which can inhibit methanogenesis in the rumen (Machado et al., 2014). While experimental studies have demonstrated its effect on reducing methane output, there is still a need to better understand the mechanistic underpinnings of this mitigation and how they interact dynamically within the rumen microbial ecosystem. In particular, modeling its impact under continuous fermentation conditions and assessing the sensitivity of key model parameters over time have not been extensively explored.

The present study by Blondiaux et al. (2024) addresses this gap by performing a dynamic sensitivity analysis using the Shapley effects method on a previously developed mathematical model describing the effect of *A. taxiformis* on rumen fermentation and methane production under *in vitro* continuous conditions. This approach provides a robust global measure of parameter influence over time. The model outputs assessed include methane production, volatile fatty acid (VFA) profiles, and hydrogen concentration.

One of the most notable contributions of this study is the demonstration that sensitivity is not static but evolves dynamically over the course of the simulation. The analysis reveals that different parameters dominate influence at different time points and for different output variables. For example, the rate of bromoform release and its interaction with methanogenic inhibition are shown to exert varying levels of influence over

time on methane concentration. These findings underscore the complex temporal interactions in the rumen microbial ecosystem and the value of using Shapley values to unravel them.

The manuscript is clearly written, logically structured, and demonstrates strong methodological rigor. The authors made commendable efforts to respond to reviewer feedback and further improved the clarity of their figures, terminology, and explanation of model assumptions. Their discussion of the rationale for using the Shapley procedure and their reflection on the implications of parameter influence over time are particularly insightful.

Nonetheless, the authors acknowledge that the model's applicability to *in vivo* conditions still requires empirical validation, particularly for its assumptions around hydrogen dynamics and methanogen inhibition. Despite this, the study provides meaningful advances in the use of mathematical modeling for guiding experimental design and optimizing mitigation strategies in ruminant systems.

In conclusion, this study stands out for its scientific clarity, methodological depth, and relevance to ongoing efforts in precision livestock feeding and environmental sustainability. It contributes meaningful insights into the use of computational tools to complement empirical research and optimize methane mitigation strategies. I strongly recommend the publication of this preprint in PCI Animal Science.

References:

Blondiaux P, Senga Kiessé T, Eugène M, Muñoz-Tamayo R. 2024. Dynamic sensitivity analysis of a mathematical model describing the effect of the macroalgae Asparagopsis taxiformis on rumen fermentation and methane production under in vitro continuous conditions. bioRxiv 2024.06.19.599712 ver. 2 peer-reviewed and recommended by PCI Animal Science.
https://doi.org/10.1101/2024.06.19.599712

Machado L, Magnusson M, Paul NA, de Nys R, Tomkins N. 2014. Effects of marine and freshwater macroalgae on in vitro total gas and methane production. PLoS One 9,932 e85289.
https://doi.org/10.1371/journal.pone.0085289

Reviews

Evaluation round #2

Reviewed by anonymous reviewer 2, 27 May 2025

Dear authors,
Thank you for this new version of the manuscript. The changes made at the end of the first proofreading phase have greatly improved understanding of the methodology used and the highlighting of the major results and their implications. I have nothing more to add to this version.
Yours sincerely

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2024.06.19.599712
Version of the preprint: 1

Authors' reply, 07 April 2025

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Decision by Arda Yildirim, posted 09 November 2024, validated 20 November 2024

Dear Authors,

Thank you for submitting your manuscript titled "Dynamic sensitivity analysis of a mathematical model describing the effect of the macroalgae Asparagopsis taxiformis on rumen fermentation and methane production under in vitro continuous conditions." The reviewers have provided insightful feedback that will strengthen the clarity and impact of your study. To enhance readability, please focus the results section more directly on the equations and the effects of the three rations tested, possibly consolidating results and discussion. Moreover incorporating main results into Figure 3 may help to succinctly convey the key mechanisms affected, relevant parameters, and the magnitude of impact.

To increase the clarity of your study's methodological approach, we suggest revisiting the introduction to explain the rationale for using two sensitivity analysis methods and to better distinguish your model from previous studies. In the Materials and Methods, it would be helpful to justify the variation ranges of the input parameters and emphasize the added value of combining these methods in this context. Providing a more general overview at the beginning of the methodology section, along with definitions of key symbols in the figures, will make it easier for readers to navigate through the technical aspects.

We encourage you to highlight the broader scientific insights gained from applying these methods, beyond the mathematical framework, especially regarding the implications for methane inhibition in animal feed management. Strengthening the discussion with a narrative on the scenarios chosen, the scientific meaning of applying sensitivity analysis in this context, and potential implications for animal diet management based on your findings would add depth to the paper. We look forward to receiving your revised manuscript with these points addressed for a clearer and more concise presentation of your work. Sincerely,

Reviewed by anonymous reviewer 1, 24 September 2024

**** OVERALL ASSESSMENT ****

Title and abstract

Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

Introduction

Are the research questions/hypotheses/predictions clearly presented? [ ] Yes, [X] No (please explain), [ ] I don't know

Explanations are below in the main review

Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know

I think yes, both in the science addressed (although its presentation can be improved) and in the methods used

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don't know

Methods are explained in detail, what is missing to me is an overview of the whole methodological architecture, but see details below

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [ ] I don't know

Not applicable

Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don't know

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

**** REVIEW ****

The paper presents a thorough and well-executed study, applying two sensitivity analyses to a simulation model of the fermentation process in the rumen. One of the study's key innovations is the dynamic nature of the sensitivity analyses, where sensitivity indices were computed over time. Surely the study is well conducted and the methodological aspects are well done with rigour.

Herewith a summary of the main comments about the manuscript in general

While the mathematical and computational aspects are clearly central to this work, I encourage the authors to emphasize the broader scientific insights gained from the study, beyond the application of the methods themselves.The authors selected specific scenarios and addressed a particular issue—the use of a microalgae for methane inhibition. It would be beneficial to provide more narrative around the rationale for these choices (why certain scenarios are chosen and not others?) and to elaborate on the insights gained from applying sensitivity analysis to these particular cases. Strengthening the introduction and discussion sections with this additional context would enhance the overall impact of the paper.

Concerning the methodology, I suggest to provide an overall description of its rationale at the beginning of the section. For example, you can say in advance all the operations done (model description, application of two SA methods, definition of the scenarios and how the elements of the methods are articulated one another...and how it links to the scientific questions posed) in this way the reader better knows how to navigate the method section.

It would be great to make a clear representation of which are the elements involved in the sensitivity analysis. My suggestion is to make this in Table 2.

It would be great to go a little bit beyond mathematics. What is the scientific meaning of the scenarios considered? The scientific meaning of the sensitivity analysis applied to these scenarios, on that model, and what is the meaning of the results observed?

The added value of the methods chosen for sensitivity analysis can be made clear since the introduction. Why these specific methods? Why two methods and not one? What do we gain by applying these sensitivity analyses to this specific problem related to methane inhibition?

Detailed comments

ABSTRACT

First line: Ruminant play (no s)

INTRODUCTION

The introduction is well written and gives a good overview of the importance of the sensitivity analysis and makes a good job in placing the study in the relevant literature. My suggestion for improving is the following. It seems that the aim of the study is to perform a sensitivity analysis in order to better understand the impact of IP on the outputs for rumen fermentation models. However you also point out the the SA is applied to the specific case of simulations of the micro algae AT. So, what does it mean to apply SA in this specific context?

Which are the scientific insights that SA can give about the role of AT in the fermentation process? Answering to these questions would give a better understanding of the scientific relevance beyond the mathematical approach applied.

Line 4: FAO (not Fao)

Line 5: Not sure if it is needed to add a specific sentence about France, to me it seems that the paper is not dedicated to France specifically, so I would suggest to keep it of general interest

Line 72: Maybe you can explain a little bit more what is the meaning of "modelling rumen fermentation under in vitro condition". The meaning of "in vitro" in the context of modelling might not be clear to non specialists. The same can be said in the following lines for "in vivo" and "in silico".

METHODS

I suggest to state clearly in the very beginning if the model is spatially implicit or spatially explicit. Also it would great to know from the beginning if the time is continuous or discrete.

Line 89: In which sense is the flow transport a biochemical phenomenon?

As a general question: what do you mean by feed? Grain? Grass? A specific dietary composition? Or is it general? I guess that the process changes according to the chemical composition of the specific intake.

Figure 1: In this version, this figure is unfortunately not fully understandable. Some symbols (e.g., z_i) are not defined. I suggest to either define the symbols in the caption or to make the figure bigger and indicate the different elements in words in rectangles/rounds. I know that many of the symbols are duly defined in the main text, however, it is important that a figure is fully informative independently from the text.

2.1.1 the model description goes directly into the parameterization. However, I suggest to clearly describe the model conceptualization before in abstract way: e.g., the rumen is represented as a reactor with a liquid phase of volume V_l and a gas phase of volume V_g… in this way the reader understand the conceptual representation in advance.

Line 99: To my understanding DMI is not the total dry matter intake (which is DM_total, right?), it is a function of time, a kind of instantaneous intake. Please make sure that the use of symbols and definitions are coherent in these lines.

Line 101: the concept of "feed distribution" is not immediately clear. I understand that it corresponds to the act of the feed being distributed by the farmer to the animal?

Line 102: Maybe I am wrong, but something is not convincing to me with this equation, should there be a sum over j? j is not appearing on the other side of the =. Is j lambda_n_j an input occurring at a specific time? Please check.

Figure 3: how does this figure relate to Figure 1? There are symbols in the figures which are not defined… Also, where is feed intake in Figure 3? Please but the "2" in subscript in the black rectangle ("H2 utilizers").

Line 119 and following: I understand that it is good to present the assumptions. However it would be useful to use the text to gently help the reader to understand figure 3, which is not totally readable for non specialists. The text could be used to describe the process. No need to go in the detail, but at least give an overall description of the process.

Lines 139 and following: you can define the symbols close to the elements they represent (eg, you can put Z_ndf after "neutral detergent fiber")

Line 150: should not be a time continuous equation as equation 1?

Table 2: in the majority of the cells the columns "Reference" is empty. Therefore I suggest that you explain what is the criteria used if the reference is absent. I understand that a reference for everything is impossible

Table 2: You can also add a column in which you say, by means of a acronym, if the elements are parameters, state variables, input, outputs variables and if they are involved or not in the sensitivity analysis.

Line 304: I suggest replace "use a strong theoretical framework" with "are grounded on a strong theoretical framework".

Line 305: dynamicS

Line 306:Shapley effects and Sobol indices are not necessarily known by non specialists. I suggest to give a description of their meaning.

Line 310: I am not sure to understand this statement, do you mean that the combination of the two methods constitute a new method?

Beginning of 2.2.2: it is not clear which is the difference between intake and dietary scenarios. Are they playing on different parameters and then combined together? The arrangement of Figure 5 is a bit unclear

Line 387: what is "cost" exactly?

RESULTS

Lines 486: What is the reason for having negative results?

Figure 12: when lines get too many, readability becomes quite challenging...

DISCUSSION

Line 679: no need to make a new paragraph here

The discussion goes very much into technical details about modelling, however I miss some high level considerations about the lessons learnt from the scenarios. Which are the implication in management for having the obtained results? What is the insight obtained for considering sensitivity analysis over time? These lessons learnt could be grouped together. The originalities highlighted in 4.4.1 all regard the method application, which is good. But it would be great to have some considerations about the lessons learnt from the mathematical efforts done for future modelling, data collection, and management.

Reviewed by anonymous reviewer 2, 08 November 2024

Title and abstract

Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

Introduction

Are the research questions/hypotheses/predictions clearly presented? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the introduction build on relevant research in the field? [X] Yes, [ ] No (please explain), [ ] I don't know

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [ ] I don't know

Are the results described and interpreted correctly? [X] Yes, [ ] No (please explain), [ ] I don't know

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [X] Yes, [ ] No (please explain), [ ] I don't know

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

General comments:

This article looks at an original aspect of animal feed management to reduce methane emissions by adding different levels of a microalga Asparagopsis taxiformis (AT) to the diet of animals. It focuses on two dynamic sensitivity analysis methods (Shapley effects and Sobol indices). The first method measures the contribution of the variation in input parameters to the variability of model outputs using a single index, but does not allow the effects of interactions between a given parameter and the others to be distinguished. The second method

provides information about the nature of the contributions (even if the exercise here is only academic because the parameters are not correlated). Dynamic sensitivity analysis is an interesting tool for pinpointing the key 'moments' when parameters make a strong contribution to outputs, and is therefore an interesting tool in modelling work presenting temporal or serial outputs, as it can lead to the identification of key moments for implementing a specific action.

The article is well written, but it is long and a bit tedious to read. Perhaps focusing the results and description on the equations and the effects of the three rations tested would make it possible to reduce and clarify certain aspects. Similarly, dividing up the article into results and discussion in the same section would make it possible to focus on the important points raised by the use of dynamic sensitivity analyses. Perhaps also give the results by output and not by method.

One suggestion would be to take Figure 3 and incorporate the main results obtained from the sensitivity analyses. This would make it possible to present the major results in a more concise way (which mechanisms are most affected, which parameters are concerned, what is the magnitude of the impact, etc.).

Specific comments :

Introduction.

The interest in combining the two AS methods should be justified in the objectives in the introduction. Similarly, it would be interesting to include references to analyses carried out in other animal science disciplines (such as epidemiology).

Materials and method.

The differences between the Munoz-Tamayo et al. 2021 model and the model presented here are not obvious.

In the Materials and Methods section, the justification for using the two methods (even though there is no dependency between the input parameters) just to introduce these methods in the context of animal science does not really seem relevant to me. It seems to me that Sobol indices have already been used in epidemiological studies in particular.

Emphasising the value of combining these methods in terms of the relevant information they provide would be more justified than simply exploring the possibility of using two AI methods.

Why use different ranges of variation for the input parameters used by the SA (as for p1 to p6). The results obtained are based on different ranges of variation of the input parameters, and this should be discussed.

Presenting section 2.2.2. at the beginning of the Materials and Methods section could clarify the general approach used in the paper.

Results.

As mentioned above, the article is very long and some elements seem redundant with the Discussion section. This section in particular should be reworked to make it more concise.

It might have been interesting to present the distribution of the simulations for all the simulations carried out (at least with the confidence intervals), in order to visualise the ranges of variation of the outputs (range of variation of the model responses).

The AS results are given for just a few parameters, whereas a great deal of detail has been given upstream to describe the models properly. Would it be possible to append some of the model equations?

Discussion.

In the discussion, in 4.1.1, perhaps the title could be changed to CH4 production to be consistent with the following titles. When hypotheses are formulated, it would sometimes be interesting to go further in proposing ways of improving the accuracy of the model (for example, the interactions to be specified between feed characteristics and fermentation (l.691).

Although the aim of the paper is to present sensitivity analysis methods carried out on animal science models, discussions going as far as recommendations concerning diets including AT could be envisaged.