The authors aimed to model the effect of *A. taxiformis* supplementation on the dynamics of rumen microbial fermentation under in vitro conditions. A previously developed model (Munoz-Tamayo et al 2016) was extended by accounting for rumen methane inhibitor *A. taxiformis*. The overall framework and model simulations seem relevant and worth publishing in the light of sustainable production from ruminants and metabolic implications of macroalgae supplementation to ruminant diets. However, various aspects of the present study need further clarification or reconsideration.

Overall modeling framework

First of all, *A. taxiformis* does not appear in the flow chart (Fig. 1), which I recommend the authors to add. Second, glucose utilizers, amino acid utilizers and hydrogen utilizers appear to contribute to input of non-fiber carbohydrates and proteins, for which no physiological description is given. Fiber carbohydrates appear without any influx. I would like the authors to provide more details. Third, it is shown that glucose utilization, and acetate, butyrate and propionate are controlled by *A. taxiformis* as is the production of methane. However, based on the equations, glucose utilization and the production of the three volatile fatty acids appear controlled by hydrogen rather than *A. taxiformis*. Certainly, I understand the indirect control on VFA production, but still methane production is controlled directly and indicated by the same symbol. Please correct. Fourth, three metabolic pathways for volatile fatty acid production are shown in Table 1 (R_1, R_2 and R_3), of which R_2 shows a zero hydrogen balance, and R_1 and R_3 result in net hydrogen production. Then, there is a λ_1 and a λ_2 equation that seem to correspond to R_1 and R_2. As expected, λ_1 is inversely related to hydrogen, whereas λ_2 appears positively related to hydrogen, despite R_2 showing a zero hydrogen balance. As such, the latter choice may be confusing to the reader of the present paper. Therefore, should the authors not come up with λ_1 and λ_3 that are both positively related to hydrogen, and next compute λ_2 by subtracting λ_1 and λ_3 from 1? This will still result in a greater propionate proportion in response to elevated hydrogen. Fifth, the proposed modeling framework accounts for inhibition of glucose utilization by hydrogen (Eq. 6). I encourage the authors to defend this choice by a reference and discuss if this is actually the case in the rumen. For example, Van Gastelen et al 2020 (https://doi.org/10.3168/jds.2019-17936) in their in vivo study observed increased digestibility in response to higher doses of 3-NOP/higher hydrogen in the rumen. Therefore, it might be questionable if increased hydrogen result in decreased microbial activity.

General modeling approach

It seems the same data were used for model calibration and evaluation. This means that the model evaluation statistics are biased and likely overestimate the model performance. These practises should be avoided and I the authors should perform a more independent model evaluation. The requires either independent data, or a cross-validation for which the data is split into multiple subsets. If the verdict of the authors is that both of these options are not practically feasible, I strongly recommend to remove

Table 2 from the manuscript and include in the discussion that the present model yet requires independent evaluation.

I suggest the authors perform a global sensitivity analysis to evaluate if model output is largely affected by the present set of parameters that was optimized, or parameters that were not optimized, but simply assigned a certain value. I also wonder if the authors considered an identifiability analysis before they performed their model calibration procedure.

What are the implications of setting the pH at 6.6? Has the impact of this choice been explored?

Other general comments/questions

Figure 2: I recommend to show the entire dose-response picture for all metabolites as done in for hydrogen in Fig 6.

The mechanism of methanic inhibition by *A. taxiformis* is considered bromoform in this paper. Do unsaturated fatty not play any role in methanogenic inhibition by macroalgae (it seems some papers suggest this)?

Fig 6: Please be more specific about hydrogen. Is this hydrogen in the headspace, dissolved hydrogen or hydrogen emission? Furthermore, results of this Figure are not described in the results section of the paper. I suggest to add this and also discuss whether these simulated values are in line with observations in other studies.

Specific minor comments

Abstract: "…adverse effect of…" and "…identifying optimal conditions on…". Adverse effects of *A. taxiformis* and optimal conditions on its use are barely discussed. I would suggest to reword these phrases in the abstract or to extend the discussion section of the present manuscript.

Abstract: "…multi-experiment estimation approach…". It looks like multiple treatments from one experiment to me, not multi-experiment. Please clarify or reword.

Abstract: Penultimate sentence of the abstract "We are … in vivo conditions" does not seem a conclusion to me. Suggest to remove.

Line 14-16: Why not be more specific and staying closer to Beauchemin et al: "The safety of feeding bromoform-containing macroalgae to livestock will also need to be investigated, as bromoform can be toxic to the environment (i.e. ozone depletion) and can impair human health"

Lines 23-24: "fermentation profile" and "structure of the rumen microbiota". Please be more specific. Do the authors refer to dynamic profiles of certain metabolites and the relative abundance of microbiota at a certain taxonomic level, respectively?

Lines 32: “metabolic hydrogen” appears only once in this paper. From other papers I understand this means atomic hydrogen [H], but I still wonder what the physiological relevance is of metabolic hydrogen. Suggest to remove “metabolic”.

Line 43: “basal diet” instead of “basal”?

Line 50-51: Was the in vitro system set up as a batch culture? If so, please state.

Line 52-53: Suggest to replace “along the fermentation” by “throughout the incubation period”.

Line 61: Three instead of four microbial groups?

Line 103: Not sure if detailed is a verb.

Line 159-165: My understanding is the present model could be considered a slightly more empirical approach than one in which the NAD is incorporated. I recommend the authors state this in a few words/sentences.

Line 249: “Methanogenic” not “Methanogenesis”. Incorporate throughout.

Line 250: Not sure if distribution is the right word here as there is no uncertainty estimate (i.e. standard deviation or variance). Would partitioning not be more appropriate?

Line 310: “the linearity … be valid”. What is the evidence for stating this?

Line 311-315: To be crystal clear, stating that VFA proportions are unaffected within the rumen physiological range of hydrogen partial pressure only makes sense if you look at the plain glucose fermentation pathways, i.e. when staying away from NAD dynamics.

Lines 329-331: See previous comments about identifiability analysis. Have the authors performed an identifiability analysis? If so, the authors could simple state whether or not this was the case.

Line 338-339: What is the rationale behind incorporating the impact of hydrogen on amino acid fermentation? Improved prediction of methane production?

Line 364-365: See comment on penultimate sentence of abstract

Line 365-367: This sentence is not a conclusion drawn from simulation results or model development. Suggest to remove. Including this in the discussion is fine, though.

Fig 1: Suggest to show all numbers as subscript.

Fig 2: bar, not bars; incorporate throughout.

Fig 3: intercepts for 4 of the plots are missing. Do the points represent all the different macroalgae inclusion rates?

References: Dijkstra et al. 1992 is missing.