

Two reputable reviewers have reviewed this manuscript and have recommended publication based on the quality of the work, novelty, and value to the scientific community. I agree with them. They have also pointed out a few items that must be addressed before moving forward, I recommend authors to address these issues and the point by point comments as well. Sincerely,

Reviews

Reviewed by Srinivasan Mahalingam, 21 Oct 2021 18:22

This is a fascinating study with some interesting findings. I would like to thank the authors for their unique contributions.

Initially, two controls were used for the dry matter analysis remaining experiments carry only one control? Explain it.

AUTHORS' REPLY. Both controls were used. The first control (tannin free) is mentioned in Table 4 with a footnote indicating that it is a natural grassland hay harvested in Auvergne, France. The second control is mentioned in Table 5 with a footnote indicating that it is a natural grassland hay based on *Dichanthium* spp. harvested in Guadeloupe, French West Indies. This information was in the Material and Methods section of the original manuscript. In the revised manuscript, we added information on the control used in the Results section when these controls are first mentioned. This change should add clarity for readers.

Is there any result available about the relative abundance of microbiome in respective of bacteria, archaea and protozoa for better understanding of microbiome influence on methane mitigation? Example relative abundance of microbiome (bacteria (%), archaea (%)) and protozoa (%).because not only individual microbes influence the methane production it depends on the composition of bacteria, archaea and protozoa.

AUTHORS' REPLY. In this study we adopted an amplicon sequencing approach for characterizing rumen microbes. Different primers per microbial group were used which does not allow us to accurately estimate the absolute biomass and proportions of microbial groups. We agree with the reviewer that quantitative estimations should be considered in future research, particularly when animals are fed tannin-rich diets. Also, it would be interesting to use a metatranscriptomic approach to analyze the functional changes induced by the different plants.

It is necessary to determine the effect of Hydrolysable and protein- and fibre-bound condensed tannins interaction with rumen microbiome similarities or dissimilarities (perhaps by MANOVA or PCoA, = Multidimensional scaling, MDS).

AUTHORS' REPLY. The interaction between microbes and plant variables such as chemical composition of plants including tannins and their degradability are shown in supplementary Figure 2 using PCoA. Supplementary Figure 3 of the original submission (Figure 2 of the revised version) shows correlations between microbial species and plant characteristics. We believe that the comparison suggested by the reviewer would produce similar results. No changes were made to the manuscript.

The time variation indicates variances between 3 and 12, but what about 24h?

AUTHORS' REPLY. We assume that the reviewer refers to microbial data. As stated in the manuscript we chose incubation times of 3 and 12 h based on published work by other groups. These incubation times were reported to capture the primary and secondary colonization process of feeds. Based on the results obtained, microbial data after 24 h of incubation would have been also interesting and will be considered in future studies.

Need to include estimates of error for all measures of alpha diversity. Box plots with error bars would better represent your data.

AUTHORS' REPLY. We agree that box plots figures are good for representing this kind the data but they are not adequate in this case as there are only three datapoints (Krzywinski, M. and N. Altman. 2014. Points of Significance: Visualizing samples with box plots. Nat. Methods 11:119-120. <http://doi.org/10.1038/nmeth.2813>)

Overall, data representation in the form of figures would be preferred for comprehension.

AUTHORS' REPLY. It is true that there are more tables than figures but for the most part we put them in the supplementary section. We choose to display data with numbers because they can be easily reusable by others, e.g. meta-analysis.

Reviewed by Todd Callaway, 18 Nov 2021 01:35

Rumen disappearance of tannins

The title really might be better to focus more on the attachment part, because that's what is really novel here.

AUTHORS' REPLY. The title was modified to highlight the microbial part as suggested. The revised title is 'Microbial colonization of tannin-rich tropical plants:

interplay between degradability, methane production and tannin disappearance in the rumen.'

This is a neat study looking at ruminal disappearance and impacts on the microbial populations on tropical tannin containing plants in ruminal fermentations. It is a small scale study that is fairly well focused other than an extraneous bit that needs more attachment to the rest of the study. This is a well written study, and it is solid and novel work. It's not groundbreaking but it's on the cutting edge of what we are doing today. The work adds to the body of knowledge and is good science.

Abstract runs a bit long and could be trimmed significantly

AUTHORS' REPLY. The length of the abstract was reduced by nearly 100 words. We did some minor modifications and moved a couple of lines to a different position within the abstract. This latter change was in consideration of the reviewer's comment regarding the in vitro part of the study (see below).

L Comment

85 Font size changes randomly

AUTHORS' REPLY. Corrected

105 Materials and methods

AUTHORS' REPLY. Error corrected

Figure 1 Make sure to label this as part of the in situ portion.

AUTHORS' REPLY. 'in situ' was added to the legend for clarification

There really needs to be a stronger connection between why the In vitro is attached to the in situ, it's kind of like an appendix here, that argument needs to be set up. The logic is not clear here, I see why, but it takes a lot of effort from the reader.

AUTHORS' REPLY. We appreciate the comment. We alluded the in situ/in vitro connection in the Introduction section when saying that the effect on in situ degradation and tannin disappearance was "connected ... with the microbial communities colonising feed particles in the rumen and with fermentation parameters". In line with

the suggestion, we added a short sentence in Results, 'In vitro rumen fermentation of forages' subheading that we hope brings further clarifications to readers.

I would like to see Supplementary Figure 3 included, this is some neat work to see this kind of correlation that really needs to be out there.

AUTHORS' REPLY. As suggested, Supplementary Figure 3 is now Figure 2 in the revised manuscript.