



COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Item 9.2 of the Provisional Agenda

Nineteenth Regular Session

Rome, 17–21 July 2023

SUBMISSIONS BY MEMBERS ON THE DRAFT STUDY ON THE SUSTAINABLE USE AND CONSERVATION OF MICROORGANISMS OF RELEVANCE TO RUMINANT DIGESTION

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I. INTRODUCTION

1. The Commission on Genetic Resources for Food and Agriculture (Commission), at its Seventeenth Regular Session, in February 2019, adopted the Work Plan for the Sustainable Use and Conservation of Microorganism and Invertebrate Genetic Resources for Food and Agriculture.¹ The Work Plan addresses microorganisms and invertebrates as functional groups and foresees that microorganisms of relevance to ruminant digestion, building upon Background Study Paper 61,² is considered by the Commission at its Nineteenth Regular Session.

2. At its Eighteenth Regular Session, the Commission decided that work on micro-organisms of relevance to ruminant digestion should be addressed by the next session of the Commission's Intergovernmental Technical Working Group on Animal Genetic Resources for Food and Agriculture (Working Group).³ The Working Group addressed the topic at its Twelfth Session, took note a draft study⁴ commissioned by the Secretariat and invited Members and observers to submit concrete comments on and inputs to the draft study by 1 April 2023, on the understanding that the Secretariat would compile and make available all submissions in the languages in which they were received, and consolidate the draft study in the light of the comments received, for the information of the Commission.⁵

3. This document contains the submissions received from Members. The consolidated draft study is contained in the document *Draft study on the sustainable use and conservation of microorganisms of relevance to ruminant digestion*.⁶ The document *Micro-organisms relevant to ruminant digestion*⁷ seeks the Commission's guidance as to the finalization of the study and future action on the topic.

II. SUBMISSIONS BY COUNTRIES

A. CANADA

The Intergovernmental Governmental Technical Group on Animal Genetic Resources invited Members to submit concrete comments on, and inputs to the "Draft study on the sustainable use and conservation of microorganisms of relevance to ruminant digestion" at its 12th Regular Session held last January. In addition, Canada stated at this 12th Session that it reserves the right to comment on the draft study and proposed recommendations at a later date. Canada consulted its experts and gathered all comments and inputs on the draft Study and proposed recommendations.

The draft study provides an accurate overview of the state of rumen microbial characterization, classification and preservation, as well as gives excellent examples of how understanding rumen microbial diversity can be implicated in climate change, antimicrobial resistance, and animal production efficiency. However, due to the vast amount of data and topics covered, there are some points in the draft study where details are required to improve the clarity of a general audience. These points are addressed in annex 1. Thus, Canada would support the recommendation that the Commission request FAO to finalize and disseminate the study with caution and after consideration of changes from the various Commission members.

Following the findings and recommendations of the study, Canada would suggest that the Commission consider the Global Rumen Alliance as an expert group but that recruitment of experts from less well-represented regions is necessary and that the expert group communicate with the Commission on key

¹ CGRFA-17/19/Report, *Appendix E*.

² Islam, Z., Heong, K.L., Catling, D. & Kritani, K. 2012. *Invertebrates in rice production systems: status and trends*. Background study paper. No 62. FAO. Rome. <https://www.fao.org/3/ap630e/ap630e.pdf>

³ CGRFA-18/21/Report, paragraph 92.

⁴ CGRFA/WG-AnGR-12/23/6/Inf.1.

⁵ CGRFA-19/23/10.1, paragraph 28.

⁶ CGRFA-19/23/9.2/Inf.1.

⁷ CGRFA-19/23/9.2.

topics/issues as needed. So, our working group on Animal Genetic Resources would not need to address this topic regularly.

Canada supports the recommendation that the Commission request FAO to ensure that the findings of the study are taken into consideration in its work relevant to microorganisms of relevance to ruminant digestion. In addition, Canada recommends that the Commission invite Members to include characterization and accessibility of isolates as an objective for financing of studies for organisms of interest to rumen microbial efficiency and ruminant digestion.

Overall, Canada considers the genetic diversity of microorganisms relevant to ruminant digestion an important topic to be considered by Members of the Commission, but not on a regular basis. A committee of experts should communicate with the FAO when it is needed.

- P4, Para. 4 : “Defaunated ruminants are also more productive in terms of average daily weight gain or milk production.” There is a need to clarify the negative impacts of defaunation.
- P5, Para. 1. : “Respondents to a survey of members of the Global Research’s Alliance’s (GRA’s) Rumen Microbial Genomics (RMG) network conducted for the present study mentioned this risk of losing microbial diversity”. Needs to be more clearly detailed. What reduction in rumen diversity is being referenced, The document to this point does not clearly demonstrate the loss of biodiversity.
- P5, Para. 1. : ”Respondents to a survey of members of the Global Research Alliance (GRA) Rumen Microbial Genomics (RMG) network contacted for the present study mentioned this risk of losing microbial diversity.” Clearer indication of the diversity and global representation of the experts surveyed for the report.
- Figure 1. Global Methane Emissions: Why were Canadas values not included in the FAO enteric methane data?
- P9, Para. 1.: “Sourcing soya protein from land that has undergone land-use change, particularly deforestation, is another major contributor to global warming (CIEL, 2022). “The reasons why soya is singled out are unclear, and the importance on ruminant nutrition should be clarified. Potentially more examples are need to justify the arguments detailed here.
- P14, Para. 2: The role of methanogens in association with the host tissue is not included, incorporation of this lack of functional understanding is a gap in knowledge necessary for application of biotechnology in methane mitigation.
- P20, last para: “Breeding and dietary strategies have shown potential to reduce methane emissions via beneficial changes in the rumen microbiome.” Authors should clarify the level of adoption of practices with regards to this technology and the industry use and acceptance.
- P22, 2nd para: “ 3-NOP is now approved for use in Australia, Brazil, Chile and the European Union, and is under consideration by regulating bodies in many other countries”. Which countries? Why USA and Canada did not adopt it yet?
- P25, Box 2: “They also noted that there are not enough isolates to allow effective study of microbial function.” If a publically available culture collection would be present, how would this impact the characterization of isolates? Are there the resources and facilities to advance isolate research if access barriers are removed and biobanks are funded.
- P28, last para.: “of the 410 microbes whose genomes were sequenced and made openly accessible by the Hungate Collection, only about 40 are attributed to cultures available in DSMZ or ATCC, with 370 remaining in individual freezers.” Would increased funding to promote microbial characterization and culture preservation increase the number of strains in culture collections or are there other barriers that need to be addressed. How do we improve this percentage, or is it just “willingness to share”.
- P29, Table 2: The title of this table is unclear - the recommendation is to state that these are institutions/groups capable of expanding research in microbial function and maintain culture collections for microbial genetic diversity. Information is missing for the scientist capable of maintain culture collections for the University of Illinois Urbana.

- P30, Para.2: “low communities.“ Please change this wording to be more clear on what the authors are trying to address.
- P31, Section 9.1, Para.2: “Likewise isolates are important for assessing AMR risks and enable the discovery and exploitation of bioactive compounds”. The need to maintain microbial diversity for the future of One Health should be more highlighted in this research gap. Understanding the current diversity so that we are able to tract decreases in global microbial diversity is a research gap that will feed into 9.2, the conservation and culture collections.
- P32, Section 10.: “Ensuring adequate resourcing global research initiatives for the culture cataloguing and management of rumen microbes” Why is the characterization of microbes not an objective, or is this included under management? The recommendation is that microbial diversity is characterized and then maintained in culture collection. Global genetic diversity characterization should be a key part of the culture collection program.
- Annex 1: The authors should consider adding more information about the expert survey, such as what qualifies to be an “expert” and why there are low numbers for some areas and high “unknown”. Why are there no experts from Asia? Additional references to the annex should be added throughout the document.

B. THE KINGDOM OF THE NETHERLANDS

Comments page 4:

2nd paragraph: It would be good to know what extra understanding is needed relative to what is known already.

3rd paragraph: Isn't lipolytic capacity rather limited in the rumen ? Lipids are generally considered to be rather inert to being fermented as an energy source (often more lipid leaves the rumen than actually entering due to added microbial lipid).

4th paragraph: Most space means also most metabolic activity ?

4th paragraph: With respect to defaunated animals, also because relatively less fermentation / a more efficient microbial growth ?

5th paragraph: Some sentences match with those in 10.3389/fmicb.2018.02161 it seems. Desired ?

5th paragraph: Gottschalki in SMT clade? Authors of 10.3389/fmicb.2018.02161 have included gottschalki in SMT clade.

6th paragraph: next to species and breeds, just a well individual differences of course.

6th paragraph: Is there really sound evidence of causal relationships in scientific literature ? Maybe the message can be ‘reduced’ somewhat by stating these are basically the most important traits studied in the recent literature and that influence of the rumen microbiome was concluded on the basis of regression analyses, such as heritability estimation (host-microbe axis) and microbiability (Microbe-trait axis) estimations ? With results of regression analyses overlapping across studies, and sample sizes increasing, the probability for causal relationships between host- microbe and microbe-trait axes increases.

6th paragraph: Is there clear evidence for this ? Are causal relationships and potential to breed proven ? Are associations reported sufficient proof for this ?

Comments page 5:

1st paragraph: Do we have enough scientific evidences and facts to support this sentence? In particular, is a desired result for feed efficiency and emission linked to the reduction in rumen microbiome diversity?

Also, are sufficient scientific facts available for fungi, protozoa and viruses to support such a statement?

1st paragraph: So one could also consider this as a trade-off of ongoing breeding efforts, particularly with respect to methane ?

1st paragraph: Why would these species not always remain to be available from natural host species as these will keep having this core microbiome with all its diversity ?

2nd paragraph: "optimum" too vague and contextual perhaps ? Is an optimal microbiome associated with a disease or phenotype discovered or understood for humans, with microbiome research in biomedical field being light years ahead compared to microbiome research in animals or livestock

2nd paragraph: predictions based on regression models from the literature presumably ? These first two paragraphs are perhaps too enthusiastic about the state of the art for breeding with microbial phenotypes. There is a huge lack in appropriate databases for microbial functional annotation. Omics studies which use pathway analyses for microbes are massively increasing (similar to RNAseq pathway analyses). The annotation to the function is done in databases which are based on cultivation studies. Here I see the lack (similar to RNA studies): Due to the rare knowledge on functions of single microbes and much more important: their function in the entire community these databases are not sufficient yet but are used for publication in more and more studies- intending we know a lot, but indeed that is based on very bare knowledge. Perhaps money and effort in cultivation studies, should include cultivation of entire microbial communities (rumen simulation fermenters for example) to increase accurate databases, which can be subsequently used by breeders. I think then we will prevent losses of diversity (which may arise by 'black box' breeding). Functional understanding of the rumen microbiome is needed (cultivation of entire communities) provided in a worldwide available database, instead of perhaps cultivation to save individual species ?

2nd paragraph: CH₄ emission (g/d), CH₄ yield (g/kg feed) or CH₄ intensity (g/kg product) ?

3rd paragraph: This paragraph is limited to feed additives/supplements only, and does not handle nutrition in general.

3rd paragraph: More importantly this is achieved for methane yield.

3rd paragraph: this statement on production gains is too decisive and would leave this sentence out. They actually might be some production gains (and sometimes these are shown) but not to forget that studies also have to be designed properly to demonstrate this.

4th paragraph: this is due to the bromoform. Which needs to be added as the same seaweed without substantial amounts of bromoform will not do the trick.

4th paragraph: this reduction % totally depends on type of animal, dosing, and diet. E.g. finishing feedlot beef may also have only a third of methane emission compared to that in grazing cattle. The point here are the trade-offs and associated other GHG emission sources. Also with Asparagopsis causing such extreme reduction percentages such trade-offs exist.

Comments Page 6:

2nd paragraph: Indeed knowledge to be brought together, but perhaps write this is 'the' instead of 'a' major challenge ?

4th paragraph: an expert group is good, but the major focus perhaps should be the management of infrastructure (data sharing) as the most important tool, as well as the development of sustainable cultivation methods which are similar to natural systems (rumen simulation techniques) instead of picking specific cultivates and store them.

If we want to work with dietary interventions and breed for/ with microbial phenotypes we need to understand the balance of the microbial systems.

Comments page 7:

Chapter 2, 1st paragraph: human-edible food with high nutrient value (for human beings with highly valuable amino acid patterns) is something to state as well ? And not to forget the value of ruminant manure in agricultural systems.

Comments page 8:

1st paragraph: the 6 does not hold for feedlot cattle, and high-concentrate or starchy-diets fed cattle; instead, the 12 appears abnormally high to me and wonder whether this actually occurs ?

1st paragraph, last sentence: But in the summary it is stated that this is not proven ? Actually I agree, mostly this cannot be demonstrated even though some beneficial effects are found in rare cases.

Last paragraph: a fundamental issue to be noted is that often (at least in many parts of the world) these breeding results were accompanied with enhanced nutrition practices. Something to be stated as well perhaps ?

Comments page 9:

1st paragraph: the rest being excreted via urine and faeces (N intake allocates almost equally in urine, milk and faeces)

1st paragraph: Last sentence: at lower levels (if feasible) but still optimal for their health and production

Last paragraph: Some? Probably a lot. Suggested wording: Constraints remain, f.ex... although the last decades has seen

Last paragraph: and translation to functionality at the whole rumen level is required. Efforts to demonstrate this should be made.

Comments page 10:

1st paragraph: agreed we are on the way, but still billion miles away from real understanding. Perhaps integration of DNA and culture-based methods needed for a better functional understanding and future databases ?

2nd paragraph: but breakdown of lipids is rather limited in the rumen ? Normally, more lipid leaves the rumen compared to what enters with feed due to extra microbial lipid synthesized and dietary lipid hardly disappearing ?

2nd paragraph: indeed good to note here that many research efforts were very technology-driven

2nd paragraph: so poorly understood means that we still cannot indicate what it means at whole rumen level ?

Comments page 12:

4th paragraph: “analyse the acquired data and it risks” instead of “analyse and it risks”.

Comments page 13:

1st paragraph: Understanding in what way ? Understanding at the level of whole rumen function or rather understanding of how they functioned within the rumen environment that was sampled at a certain moment ? I think the latter, which is not the same as the former and perhaps to .

2nd paragraph: Global Rumen Census in capitals to indicate naming of this flagship project ?

3rd paragraph: not sure what biotechnological exploitation would be ?

3rd paragraph: the mentioned immense potential of DFM really has to be tuned down as I have not seen them achieve a lot in an existing rumen microbiome in vivo.

3rd paragraph: why combination with additives that reduce methane emissions ? What is the interrelationship here ? Additives that impact on methanogens may deliver higher rumen H₂ levels. Is the thought that DFM would take more of this H₂ away or be more able to colonize better under such circumstances ?

3rd paragraph, last sentence: so far such efforts have not been successful, so it probably will be a major challenge to find 'better' phenotypes of microbes already known. Would this not require 'holistic' knowledge of the entire symbiont (microbes + host) first before this could be achieved ?

Comments page 14:

Last paragraph: Referred to rumen protozoal species I guess, and hence argued here from a genomics viewpoint ?

At the rumen level we have models explaining the role / functionality of the entire protozoal population which is required for understanding and steering whole rumen function.

Last paragraph: not sure why controversial ? in what way controversial ? Or is meant here that it cannot be delineated from bacterial activity ?

Comments page 15:

2nd paragraph: not to be forgotten that with defaunation also efficiency of bacterial growth synthesis goes up, and may hence lead to relatively less OM fermentation. This in itself causes less methane as well ?

2nd paragraph: "This suggests that removal of protozoa may be a way to decrease enteric methane emissions". But the OM previously fermented by protozoa will be fermented by bacteria then, also leading to H₂ and causing CH₄. Unclear how this mechanism would work here leading to less H₂ as suggested.

Perhaps a better argument could be that if the H₂ is not ending up in hydrogenosomes as a protozoal storage of H₂, it somehow ends up (through bacterial activity) in the rumen environment, for example causing bacteria to shift their fermentation profile towards propionate for example, leading to less H₂ production and hence less CH₄.

2nd paragraph: "strategy that eliminates all protozoa may not be optimal", in terms of rumen degradation of fibre ?

2nd paragraph, last sentence: It simply becomes more efficient, as can be explained through modelling exercises.

Comments page 16:

2nd paragraph: why is fungi activity enhanced ? By consuming / taking away H₂ produced by fungi, and thereby allowing more/better fungal activity ?

2nd paragraph: 20% of what ? Of biomass, or abundance ?

4th paragraph: Viruses are microbes ?

Comments page 17:

2nd paragraph: "a source of nutrients for the ruminant following their outflow", cannot see why this would be different from non-EPS related nutrient and microbe outflow ? Or intention to say EPS stimulates outflow as a particle ?

2nd paragraph: the contribution of EPS but compared to what ? Or EPS as a protein source in itself meant here ?

2nd paragraph: recognizing the EPS phenotype is important, but focus also has to be on overall microbial activity and feed degradation and could be mentioned here as well in some manner.

Comments page 19:

3rd paragraph: Also the diet of importance, i.e. the solid food given to these animals, or are you strictly referring to non-solid feed fed animals ? If so, I would say the environment may have a main impact as well ?

4th paragraph: replace "incidence" by abundance ?

4th paragraph: perhaps change into "that the host's genotype partly determines the establishment of these(...)microbial abundances"

4th paragraph: change "The organisms associated with" into the abundances of organisms associated with...

4th paragraph: change "various rumen microorganism associations with host attributes and performance" into various association studies between microbial abundances and host attributes as well as between microbial abundances and performance traits ?

4th paragraph: Are enough scientific evidences are gathered to prove those microorganism associations with host's attributes ? These are all associations that have been found, but any study will find associations within the study itself.
I wonder whether there are any independent evaluations whether these associations found were indeed predictive and hence appear to be causal ?

Suggest to make the first sentence of the paragraph clearer; instead of "evidence has emerged" wording could be 'Considerable heritability estimates for specific taxa abundances let assume a host genetic influence on the rumen microbial community'. The last sentence could be 'However evidence is based on descriptive results from DNA based studies. For proofs of causality integrative holistic studies including cultivation and DNA based studies are needed. '

5th paragraph: "reduced diversity" is being portrayed as an eminent threat or danger by the authors. Threat to whom and how? Missing the scientific arguments here. A higher fermentation rate indeed will narrow the diversity, promoting those that are best capable to take advantage of this (i.e. less niches remain to be occupied as is

commonly seen in all ecosystems when increasing 'input'), and might even happen directly following a large meal ?

5th paragraph: 'intensive' instead of "extensive" ?

5th paragraph: "lower bacterial diversity and lower concentrations of fibrolytic microbes" also occurs with higher concentrate intake in early lactation cows, but changes again later on.

<https://www.journalofdairyscience.org/action/showPdf?pii=S0022-0302%2817%2930150-9>

5th paragraph: "which may drive further homogeneity globally in ruminant microbiomes", not sure whether this argument holds. As soon as one stops with an additive, with increased concentrate, or returns to the previous diet, the community changes again and become more diverse again, actually proving these rumen microbes do not have to be considered lost.

5th paragraph: Is there actual proof of diversity loss ? It reads a little as advocating the proposed work. Comments page 20:

Box 1: Agreed to the state of the art, but the aim of these cultivation approaches might be much more in the usage for the right decisions in animal nutrition and breeding instead of preserving species, because we exclude them by our livestock systems. We should aim to establish diversity again into breeding/ feeding systems by general decisions for which we need more knowledge on microbes, host and their interaction.

1st paragraph Box 1: double use of word "number"

2nd paragraph Box 1: Not sure what is meant here in last sentence. The additives focus on methanogens only, or have a different mode-of-action for which I wonder whether microbial diversity is really an issue. Perhaps for the plant-based additives ?

Chapter 5, 1st paragraph: But *Leucaena* has been used in Brazil in sugar-cane diets for a long time to serves as extra N source. Not sure whether they used this bacterium and had toxic effects ? text seems to imply that there are no other microbes capable of degrading DHP ?

Chapter 5, 2nd paragraph: methane is expressed how ? per unit of product or per unit of feed ?

Chapter 5, 2nd paragraph, last sentence: breeding to a large extent still to be demonstrated as achievement in reality. NZ has demonstrated this for sheep, although projected further decline the coming decades remains to be demonstrated after all (there may be trade-offs occurring). Is this info already there for cattle ? It also has to be verified independently that cattle bred for low methane indeed achieves less methane per unit of feed, irrespective of the increased productivity and reduction of methane intensity through increased yield of animal product.

Comments page 21:

1st paragraph: doesn't this really have to be proven first with independent measurements / studies, with various diets and production conditions ?

1st paragraph: Maybe the scientific facts/data/rigor needs to be highlighted here that actually support the statement that 30% reduction of methane emission achieved by breeding strategies. This seems quite large and larger than individual animal variation encountered in feeding trials that cannot be attributed to variation in DM intake, digestion, and so on.

Earlier on page 5 it was stated "predictions suggest that breeding efforts could result in reductions of up to 30 percent in CH₄ emissions.". So the 30% is a prediction based on

1st paragraph: change "largely" into 'also because the host'

2nd paragraph: “linked to this heritability”, BTA linked to their abundance not to the taxa itself ?

2nd paragraph: assuming the possibility instead of “indicating the possibility” ?

2nd paragraph: “larger rumen volume”this means that lower methane involves smaller rumens, and faster passage rates, lower pH, and this cannot be pursued continuously as it will certainly lead to trade-offs such as reduced digestibility, feed intake, and so on. So, can the initially achieved 5-10% lower methane simply be extrapolated to 30% or more in the future, knowing it will be associated with these fundamental changes in the rumen ? Is this proven or understood yet ?

2nd paragraph: underlies instead of underlines ?

2nd paragraph, last sentence: it preferably is to be studied in conjunction with other functional traits such as feed intake level, rumen function and feed digestibility.

3rd paragraph: with respect to categories, also changes in the basal diet may have quite some effects on efficacy of additives/supplements. Here only additions to the basal diet are mentioned.

3rd paragraph, last sentence: chemicals, or better write microbes that use hydrogen to convert these chemicals ?

4th paragraph, last sentence: probably it is mainly related to the rate of fermentation, irrespective of the precise composition of the microbiome

5th paragraph: thought density has often been shown not to correlate with methane production rate. Comments page 22:

1st paragraph: also a meta-analysis of Congio et al. demonstrates such effects.
<https://www.sciencedirect.com/science/article/pii/S0959652621019119?via%3Dihub>

2nd paragraph: a recent meta-analysis for dairy was published by Kebreab et al.
<https://www.journalofdairyscience.org/action/showPdf?pii=S0022-0302%2822%2900710-X>

3rd paragraph: affecting atmospheric ozone if released, and the possibility of long-term detrimental effects on the ruminant have to be studied and there are indications it can be carcinogenic.

3rd paragraph: linked to a changed efficacy then of bromoform ?

4th paragraph: capturing excess H₂ always (automatically) delivers extra nutrients ?

4th paragraph: but most of the H₂ is always redirected as only a small fraction is exhaled by the host. Meaning most of the H₂ not captured is redirected anyway, also without DFM.

4th paragraph: results appear not so promising. and they should be separated from the effect of an improved silage quality. The latter will always decrease methane yield & intensity in an indirect manner, but that was not meant to be indicated here.

5th paragraph: is it more or less presumed that excess hydrogen is a problem for rumen function ? The question then is whether this is really the case if the microbiome is given sufficient time to adapt to this. At what dosing does this become a problem ? In 3NOP studies with normal dosing there were no effects on digestibility and excess hydrogen apparently was not a problem for the microbiome ? At lower dosages of bromoform perhaps the same.

Comments page 23:

1st paragraph: Ellis et al. were not able to demonstrate any CH₄ reduction with Lactobacillus in the diet of dairy cattle.

<https://www.journalofdairyscience.org/action/showPdf?pii=S0022-0302%2816%2930391-5>

2nd paragraph: few have been commercialized also because efficacy has to be demonstrable.

2nd paragraph: also a purified form of nitrate salt is commercially available.

Comments page 25:

1st paragraph: “moving towards an understanding of causation”, but also moving towards the level of whole rumen function it prerequisite. Measures have to be demonstrably effective at this level.

1st paragraph: “innovations for its management”, but also to delineate functionalities which might be useful when aiming to manipulate whole rumen function.

1st paragraph: how additives work ? It is meant what is the mode-of-action at a more molecular / pathway level ?

Comments page 26:

Box 3: Perhaps 2 further actions to add here:

1. further develop tools like CowPI which are very useful when filled with more data
2. not only reduce methane emissions but also Nitrogen surpluses

The draft presented here mainly focusses on methane reduction and ideas considering the symbiotic nitrogen usage by rumen microbiome and host is missing. Symbiotic nitrogen usage enhances the nutrient value of entire systems but asks for studies on the interaction between microbiome and host. This topic is of major importance for the near future and has to be addressed together with enteric methane.

Chapter 6, 1st paragraph: is this not always the case, and why would this be required for "One Health" ? Unsure still about the concept.

Chapter 6, 2nd paragraph: erase “in” in “including in human”.

Chapter 6, 2nd paragraph, last sentence: and this is compared with what as a reference situation, moment, state ?

Comments page 27:

1st paragraph: GHs ??

Comments page 28:

1st paragraph: “guided rumen microbiome innovations”? Wondering how ?

2nd paragraph: “rumen microbiome is thus not always at the forefront” is an underestimate perhaps ? On the other hand there is the need to focus at the whole rumen level to achieve reduction.

5th paragraph: a further major problem is the lack of infrastructure – for example pipelines to implement results from cultivation studies into databases used by DNA based approaches (pathway analyses) – is there a need for a (worldwide) clear pipeline ?

5th paragraph, last sentence: “risk of losing rumen microbial diversity”, not sure whether this is a logical conclusion; “research infrastructure”, a need for (appropriate funding of) IT specialists

Comments page 29:

1st paragraph: “The labelling of products obtained ... “, sentence reads difficult

1st paragraph: “cost implication”, perhaps just as much because of lack of opportunity or control in administrating them.

Table 1: nitrate salts are allowed as feed, if in purified form; 3NOP is used in Brazil and Chile; Asparagopsis, there may be restrictions as this contains arsenic and halogen levels which may be too high according to regulation. Pure bromoform as active compound not allowed to be used, discussions / research is ongoing.

Comments page 31:

Chapter 9, 1st paragraph: “This step can be made if the isolate or a close relative is available in pure culture, as this enables hypotheses based on “omic” studies to be tested “, considering the sentence below, change ‘can be made’ and ‘enable’ into something like ‘a step forward to’

A lot of effort and funds have gone to rumen microbiome work and omics, but what clear successes can be mentioned at the whole rumen level ?

To be tested in vivo ?

Chapter 9, 1st paragraph, last sentence: hypothesis need to focus at the whole rumen level. It is at this level where success needs to be obtained/demonstrated. Functional annotation of microbes in the rumen are needed, not separated by single cultivation. Omics technologies can ‘combine’ the single performances to pathways but that is still correlation and not causation – so perhaps cultivation approaches focusing on the entire rumen are needed.

Chapter 9, 2nd paragraph: this also happens without DFM's, or is the point made here that this can be enhanced ?

Chapter 9, 2nd paragraph: “exploitation of bioactive compounds”, what would be really helpful is that observed improvements at the rumen or host level actually are demonstrated to be captured by such omics work, and that further in-depth knowledge on rumen microbial activity and metabolism is obtained this way (in terms of substrate degradation, microbial synthesis, VFA production, methanogenesis).

Comments page 32:

2nd paragraph: not costly for the farmer if the dairy sector / society simply pays for it.

2nd paragraph, last sentence: accountability will be the main issue here. The question is how to do this properly and in a justified / farm-specific manner ?

Chapter 10: to be mentioned here to invest in finding causality in all this work, and how to make possible to translate detailed knowledge at species level towards rumen fermentation and CH₄ ? Conserving and managing cultures of rumen microbes, and studying their characteristics is one thing, but the gap with rumen functionality has to be made to make it of use for nutritionists, farmers and breeders.

An action that could be taken by the expert group: address the biggest challenge of correlation to causality - on the rumen and on the holobiont (microbes+host interaction) level – to be overcome by integrating culture and DA based methods – the need for IT pipelines that make it possible to integrate the data - need to do this uniformly worldwide and easily to access.

2. simultaneously with methane, feed efficiency traits: address nitrogen efficiency.