Review of: "Ruminal CO2 Holdup Monitoring, Acidosis Might Be Caused by CO2 Poisoning"

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Potential competing interests: No potential competing interests to declare.

Introduction

Paper section: *According to Arrhenius' theory […] greater dCO_2 availability and lower ruminal pH.*

Reviewer comment: Addressing the possible role of a new variable CO_2 is an interesting idea and in accordance with the approach outlined by Stewart (1983).

Paper section: *In fact, the relationship between SCFA formation and ruminal pH may simply be a consequence of CO_2 release during fermentation (Wolin, 1960; Dijkstra et al., 2012). Therefore, increased SCFA production leads to greater dCO_2 availability and lower ruminal pH.*

Reviewer comment: The above sentence is not in accordance with the paper cited above, which classifies both pCO_2 and total weak acids as **independent variables**. You are here suggesting that one of the two previously classified independent variables is dependent on the other. This study does not demonstrate this interdependence, so I would not write, or I would reformulate, the above sentence.

Paper section: *The risk of subacute ruminal acidosis (SARA) is attributed […], resulting in CO_2*

poisoning.

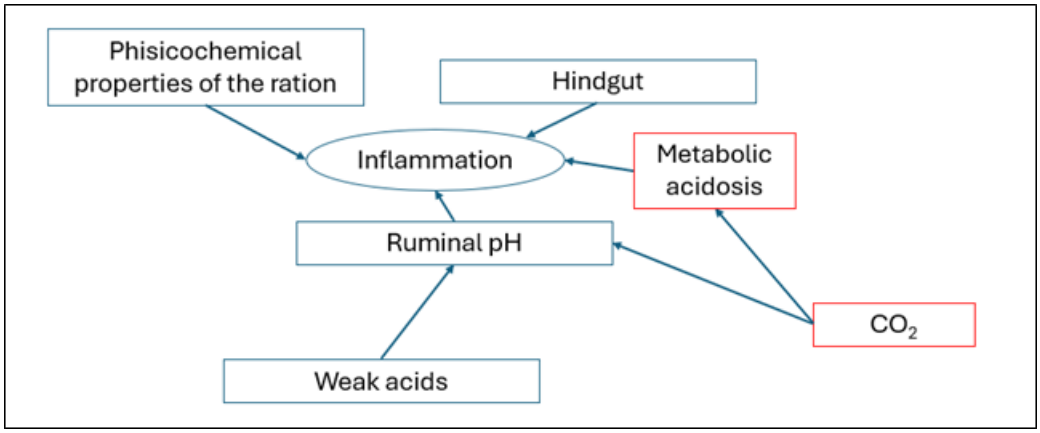
Reviewer comment: I believe this section should be largely expanded. Since the focus of the article is SARA and a possible new contributor to its pathogenesis, the current state of art should be clearly summarised, including the following:

- Current pH thresholds are determined by their reflection on inflammatory status (Gozho*et al.*, 2005, 2007)
- Possible contribution of the lower gastrointestinal tract to the pathogenesis (Li*et al.*, 2012)
- Different effects of different physical and chemical characteristics of the diet on inflammatory status (Khafipour*et al.*, 2009a, b; Francesio *et al.*, 2020)

The objective of this is to highlight how important it is that the inflammatory status, alongside the pH, is taken into consideration when talking about SARA and that other variables (and pCO_2 induced acidosis might be one of them, although results obtained by Gianasella et al. (2010) do not really strongly push in this direction) influence the inflammatory status independently of ruminal pH. Figure 1 shows what would be, in my opinion, an effective way to sketch

out the subject.

Fig. 1 Possible contribution of pCO_2 in SARA pathogenesis



Material and methods

Paper section: *The diets and cattle performance were described previously […] used as references.*

Reviewer comment: To improve paper readability, I would include the table with the diet composition in this paper as it was done in (Laporte-Uribe, 2019). Furthermore, the hypothesised DMI for each diet is not stated in the paper, nor in the reference paper cited. It is difficult later on to evaluate the experimental diets' effects on DMI, so I would also add this piece of information to the table.

Paper section: ml of 5 M sodium hydroxide (NaOH) solution and was frozen for subsequent TIC analysis

Reviewer comment: Make the TIC acronym explicit, as if I'm not mistaken, this is the 1^{st} time this acronym is appearing.

Paper section: *For instance, cattle with pH values lower than 5.4 and 5.8 for 3 to 5 h/d have a high risk of ruminal acidosis and SARA (Dohme et al., 2008; Villot et al., 2018).*

Reviewer comment: Dohme used 5.5 as the lower threshold, while Villot used 5.6.

Paper section: *The area under the curve for ruminal pH (AUC, pH units per min) emphasises the duration of the […] around 6.4 are in the upper range in cattle given a TMR and are optimal for fermentation in pasture-based diets (Russell, 1998; De Veth and Kolver, 2001).*

Reviewer comment: My apologies, but this entire section is not very clear to me. I understood the 4 categorical variables you used to categorise the animals, but what ruminal pH parameter did you use? In Fig. 2, you used AUC, but I did not understand, and it may be my fault, whether it is absolute or relative AUC. I also could not access the reference you cited:

Gibbs, S. J., and J. Laporte Uribe. 2009. Diurnal patterns of rumen pH and function in dairy cows on high-quality temperate pastures of the South Island of New Zealand. J. Dairy Sci. 92(ESuppl. 1):585.

Because I did not find it on the JDS website, I could not check what was done in that paper. As a general comment, my opinion on this subject is that the pH analysis should have the objective of making the acidotic challenge made in this trial comparable to other experiments done previously. As such, my advice for you would be to use some metrics that have been widespread in recent years (for example, min./pH < 5.6 or min./pH<5.8). It would be nice to see some of the other pH metrics developed by Villot:

Villot, C., B. Meunier, J. Bodin, C. Martin, and M. Silberberg. 2018. Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. Animal 12(3):481-490. doi: 10.1017/S1751731117001677

Or Denwood:

Denwood MJ, Kleen JL, Jensen DB, Jonsson NN. Describing temporal variation in reticuloruminal pH using continuous monitoring data. J Dairy Sci. 2018 Jan;101(1):233-245. doi: 10.3168/jds.2017-12828. Epub 2017 Oct 18. PMID: 29055552.

And see if any of these new metrics correlate better than others with dCO_2 results.

Results and discussion

Reviewer comment: It seems to me that this section is missing a proper discussion about the effect of the experimental diets on the animals' organisms. Were all three diets successful in inducing SARA? On what result do you base this answer?

Paper section: *Moreover, the widespread use of the ruminal pH scale (Nocek et al., 2002; AlZahal et al., 2007b) has obscured the well-established significance of dCO_2 in rumen function (Ash and Dobson, 1963; Gabel et al., 1991).*

Reviewer comment: I am not exactly sure what you mean by this sentence. Strong ions, weak acids, and pCO_2 are all independent determinants of a fluid's pH, so perhaps weak acids have obscured dCO_2?

Paper section: *Manual sampling versus continuous ruminal CO_2 monitoring […] the suitability of the ATR-IR technique and sensor for continuously monitoring CO_2 holdup and dCO_2 concentrations.*

Reviewer comment: I cannot really review this section as my competence in this area is limited; a chemist may be a more appropriate figure to evaluate this. In particular, I am not sure what would be the gold standard test to determine dCO_2, and I do not know if such a gold standard test could have been applied cow-side in a way to minimise CO_2 loss. So I cannot express myself on the first aim of this paper: (1) confirm the ruminal dCO2 range.

Paper section: *Therefore, if we can confirm that critical dCO2 concentrations are sustained for extended time periods or CO2 holdup, we could suspect that CO2 poisoning is triggering SARA.*

Reviewer comment: I am not sure how sustainable this sentence is. There are a lot of works that have studied LPS

production, their reflection on LBP, and animals' health and production. As I said before, considering metabolic acidosis as a factor may be worth some more investigation, even if none of the groups in the study of Giannasella became even mildly acidaemic, but giving it a pivotal role in the whole SARA syndrome is hazardous.

Paper section: *Ruminal pH cannot predict dCO2 concentrations.*

Reviewer comment: In the whole section you just did not mention VFAs. You justified the decrease in pH with increase CO_2 and decrease HCO_3^- but I do not think that VFAs contribution to pH dynamics can be taken out of the picture. Between the two tables you have some interesting piece on information such as the fact that high starch diet has the higher VFAs concentration and the highest pH that is worth discussing in term sampling times, and other factors that can explain this counter-intuitive finding.

Paper section: *ruminal AUC map for pH (Figure 2.1b) indicated that cattle had the lowest SARA risk when fed the High-RDS diet based on the conventional definition of SARA based on the pH scale (Nocek et al., 2002; Villot et al., 2018). However, cattle consuming the High-RDS diet exhibited typical SARA symptoms: reduced feed intake and milk yield, Table 2 (Nocek et al., 2002; Dohme et al., 2008). The rumen AUC map. revealed that cattle fed the High-RDS diet experienced critical dCO2 concentrations for extended postprandial periods, or CO_2 holdup (Spikes of critical values in Figure 2.2b).*

Reviewer comment: An alternative explanation could be the one described by Khafipour. In his experiments he succeeded in reaching SARA according to pH and clinical metrics, but despite this when SARA bouts were caused by low peNDF diet, LPS did not translocate and did not cause systemic inflammation. On the other side when SARA bouts were caused by grain challenge, LPS translocate and cause systemic inflammation (Khafipour *et al.*, 2009a, b). CO_2 holdup was not monitor in that set of studies, so we cannot exclude that it would have explained those findings as well, however not having investigate this possibility is a weakness of this study. In any case I think this alternative hypothesis should be mentioned and discuss in this section.

General comment on the paper

I found the subject of this paper interesting and intriguing, however I think it leans too much toward the CO_2 as unique variable explaining both rumen acidic pH and SARA. While analysis on ruminal fluids may be enough to explore and describes pH dynamics, blood sample are definitely needed to define the effects of different diets onto the organism whether it is searching for LBP, SAA, Hp, differential leukocyte count or pCO_2 and pH. I would think that the set of data you have collected are more suitable for investigating the relative contribution of CO_2 on ruminal pH dynamics and I would focus the paper on the first two aims you had:

1. confirm the ruminal dCO_2 range,
2. unveil the relationship between dCO_2 and pH

and I would do that trying more strongly to differentiate the relative contribution of CO2 and weak acids.

For a SARA study, I would suggest a longer study and the use of a latin square in the study design and the inclusion of a control diet designed not to cause SARA bouts, so each animal will also have its own control set of data.

References

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