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**Application**

Ruminant livestock contribute significantly to agricultural greenhouse gas emissions. Direct abatement using antimethanogenic compounds could readily reduce emissions but must be stable and effective over time. Providing compounds in a stable form or carrier that can be routinely incorporated into diets provides a pathway to tangible mitigation outcomes.

**Introduction**

In Australia ruminant livestock contribute significantly to agricultural greenhouse gas emissions. Halogenated compounds such as bromoform (CHBr3) have been shown to have potential as a feed additive for mitigating enteric methane but are inherently volatile due to their chemical characteristics. Nevertheless, if complexed into a stable carrier these compounds could be incorporated into various feed preparations and released in the rumen to achieve a direct abatement outcome and so contribute to reductions of agricultural emissions. The requirement for daily oral administration of compounds may limit utility in extensive production systems but readily aligns with intensive systems such as dairy and feedlot. The aim of this study was to identify a solid inert carrier used to stabilise the antimethanogenic compound bromoform that would deliver *in vitro* abatement and could progress to *in vivo* application.

**Materials and Methods**

Two solid inert carriers [activated carbon (AC), polymer resin (PR)] containing bromoform (15% w/w) were dosed daily *in vitro* at a low (L) or high (H) concentration, creating four treatments; ACL, ACH, PRL and PRH equivalent to 12.6 and 25.2 mg CHBr3/kg DM for low and high doses, respectively. A substrate only treatment of 30 g ground oaten chaff was used as a control. Treatments were incubated in triplicate in 50 µm pore size nylon bags using an artificial rumen simulation technique (RUSITEC) for 14 days to assess methane mitigation. The RUSITEC system was initiated and maintained as described by Garcia *et al.,* (2019). A constant flow of buffer into each fermenter maintained pH of 6.0 – 7.0. Gas produced by each fermenter was measured daily, sub sampled for methane quantification, fermentation fluid collected for analysis of VFA at three time points over 14 days and *in vitro* NDF, ADF and DM degradability calculated. Data were analysed per timepoint using one-way ANOVA in JMP V15.20 (SAS Inst.) with treatments as fixed factors and methane concentration, production, total gas, and VFA profile as variables. A Tukey-Kramer HSD pairwise comparison was used when means differed (*P*<0.05).

**Results**

All carrier types supported significant reductions in methane concentration and yield (*P*<0.05). The AC carrier delivered the greatest and most persistent mitigation effect regardless of concentration with mean reductions of 89-91% in the first week and despite withdrawal of ACH during the second week, 97-99%, indicating a carry-over effect. Treatment PRH demonstrated a mean reduction in methane yield of 76%, during the first week, however, this diminished to 41%, in the second week. There was a delay of at least 72 h to achieve 16-18% methane reductions for PRL compared with other carrier combinations that demonstrated an *in vitro* effect within 48 h. Carrier type did not influence (p>0.05) NDF, ADF or DM degradation compared with the control across sampling timepoints. Total mean VFA concentrations were similar (p>0.05) between all treatments (104 mmol/L) and control (101 mmol/L) across sampling timepoints. An antimethanogenic activity of all treatments was apparent without a decrease in total gas production, total VFA concentration or substrate degradability *in vitro.* These preliminary results indicate that carrier type will influence the mitigation outcome. A 15% (w/w) CHBr3 for the PR may have exceeded its actual carrying capacity thereby explain the diminishing effect over time. The polymer resin in this study was less effective and less consistent in terms of effects on methane production *in vitro*. Conversely the concentration of CHBr3 on AC could be decreased given almost complete inhibition was achieved at the low concentration.

**Conclusions**

Bromoform stabilised onto an AC carrier demonstrated greater and more consistent effects on methane mitigation than PR, indicating it is a more promising candidate for progression to *in vivo* trials. The utility of AC to complex CHBr3 in a stable form requires further validation beyond 14 days. Ultimately the commercial use of inert solid carriers such as AC is dependent on the demonstration of consistent and minimal level of methane mitigation over an extended time period for either a feedlot or dairy application.

**References**

Garcia, F., Vercoe, P., Martínez, M., Durmic, Z., Brunetti, M., Moreno, M., Colombatto, D., Lucini, E., & Ferrer, J., 2019. Animal Production Science, 59(4), 709-720.