**Application**

The development and *in-vivo* assessment of novel oxygen-releasing feed additives, to reduce enteric ruminant methane emissions, in both intensive and pasture-based production settings.

**Introduction”**

As the reduction of enteric methane (CH4) production commands global attention, coupled with emerging acceptance that current available mitigative solutions fall short, there is a growing demand for alternative feed additive approaches. The rumen oxidation reduction potential (ORP) parameter influences CH4 production rates, as methanogens are typically active only at ORPs below -300 millivolts. Thus, a controlled elevation of rumen ORP offers a potentially benign means of CH4 suppression, where extending the period of elevated rumen ORP would serve to inhibit the activity and growth of methanogens. Previous *in-vitro* work demonstrated successful CH4 mitigation, with consistent CH4 reductions of >50% observed when supplementing with various oxygen-releasing compounds, including calcium peroxide and magnesium peroxide (CaO2, MgO2). CaO2 was proposed as a potentially viable rumen ORP modulator, pending further *in-vitro* and *in-vivo* assessment (Graham et al., 2024).

**Materials and methods**

This research evaluated the *in-vivo* effects of various doses of CaO2 on animal performance for the first time in beef cattle. Nine cannulated Aberdeen Angus × Friesian steers (739 ± 67 kg BW) were used in an 84-day Latin Square design trial (n=9). Several CaO2 inclusion rates were evaluated during once-a-day and twice-a-day feeding, providing valuable insights into animal response to varying CaO2 concentrations. *In-vivo* ORP (continuous), static ORP, static pH, ammonia, volatile fatty acids (VFAs), *in-sacco* diet digestibility, feed intake, palatability, and rumen microbiome were all monitored, while a portable CH4 analyser provided preliminary CH4 emission indications.

**Results**

No significant negative impacts of inclusion rates of CaO2 up to 1.7% of dry matter intake (DMI) were observed, while positive indications of reduced enteric CH4 emissions were observed from animals receiving treatment vs. controls. Equivalent rumen ORP elevations were observed at both the initial higher and lower doses applied during once-a-day feeding, alongside similar levels of CH4 suppression. Thus, to determine the mitigative efficacy of CaO2 on enteric CH4 production at lower inclusion rates, an additional lower dose of CaO2 was introduced during the twice-a-day feeding phase. Consistently greater ORP values of >100 mV were observed in the rumen of treatment vs. control animals during twice-a-day feeding, which was again associated with reduced enteric emissions. These findings, together with the minimal palatability issues encountered at each CaO2 inclusion rate and CaO2’s capacity to withstand both heat and pressure during incorporation into a feed pellet format, suggest that it may have potential as a tangible alternative to existing ruminant feed additives with a prospective contribution to supporting global methane mitigation goals.

**Conclusions**

The potential and merit of employing ORP as a tool for the direct manipulation and modulation of the rumen environment, using CaO2, was demonstrated. The evaluation of CaO2 in larger-scale animal trials using more robust emissions monitoring, is supported by this work. The long-term goal of this research is to establish a slower-release formulation, to allow more consistent, controlled, and sustained CH4 suppression, effectively mitigating CH4 in both intensive and pasture-based production systems. Controlled-release formats of CaO2 would also serve to reduce the complexity of application on farm, allowing for continuous CH4 suppression, while animals are grazing on pasture.

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**References**

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