

Peer Review

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

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This manuscript presents a bold and provocative hypothesis: that subacute ruminal acidosis (SARA) may be driven not primarily by low pH per se, but by prolonged exposure to elevated dissolved CO₂ (dCO₂) and “CO₂ holdup,” potentially amounting to a form of ruminal CO₂ poisoning. The study introduces continuous in situ monitoring of ruminal dCO₂ using ATR-IR spectroscopy, which represents a clear methodological innovation. The work is conceptually original and challenges entrenched pH-centric interpretations of ruminal acidosis.

Below is a structured evaluation.

Major Concerns

Despite its originality, several critical limitations must be addressed.

1. Sample Size and Experimental Design

Only **three cows** were used.

Continuous dCO₂ monitoring was performed in **one sentinel cow per run**.

Diets were sequential rather than randomized.

This severely limits inferential power. Observed associations between CO₂ holdup and SARA signs may reflect:

Individual variation,

Carryover effects,

Time-related confounders.

The manuscript frequently draws mechanistic conclusions that exceed what an observational pilot study with n=3 can support.

2. Causality vs Association

The central claim that SARA may be caused by CO₂ poisoning is intriguing but remains speculative.

Evidence provided:

High dCO₂ coincides with SARA signs.

Hypercapnia in lungs causes inflammation.

High dCO₂ may alter osmolarity and epithelial transport.

However:

No direct measurements of ruminal epithelial CO₂ flux were made.

No blood gas measurements were reported.

No markers of systemic hypercapnia or acidosis were assessed.

No direct epithelial injury markers were measured.

Thus, the data demonstrate **association**, not causation.

The manuscript should soften claims such as:

“SARA might be triggered by CO₂ poisoning”

and instead frame them as a mechanistic hypothesis requiring targeted testing.

Declarations

Potential competing interests: No potential competing interests to declare.