ReaShure®
Precision Release Choline

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SCIENTISTS SAY

Not All Rumen-Protected Choline Products Are Created Equal

Comparing ReaShure® and CholiGEM™

There are four important features to a good rumen-protected nutrient: rumen stability, feed and TMR stability, nutrient availability, and animal performance.

-Dr. Clay Zimmerman,
Director of Science and Technical Services,
Balchem Corporation
Real Science webinar, December 2023

Not all rumen-protected nutrients are equally effective, as demonstrated by multiple studies using different research methods.

-Kari Estes,
Research Manager,
Balchem Corporation
Real Science Exchange podcast, July 2024

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Measuring the bioavailability of a rumen-protected nutrient is essential for evaluating its value to dairy cows. However, there is no recognized *in vivo* procedure for measuring choline bioavailability in dairy cows. Unlike other nutrients with established biomarkers indicating intake or status, choline lacks universally accepted biomarkers, complicating its bioavailability assessment.

Three primary factors significantly affect the overall bioavailability of rumen-protected nutrients:

- **Feed Stability** – The ability of an encapsulated nutrient to protect its payload from damage and degradation during conveyance to storage, mixing into feed, or sitting in a total mixed ration (TMR).
- **Rumen Protection** – The capability of an encapsulated nutrient to protect its payload from degradation by rumen microbes.
- **Intestinal Release** – The capacity for an encapsulated nutrient to be robust enough to protect its payload in the feed and rumen while possessing the capability to release the nutrient into the small intestine for absorption by the cow.

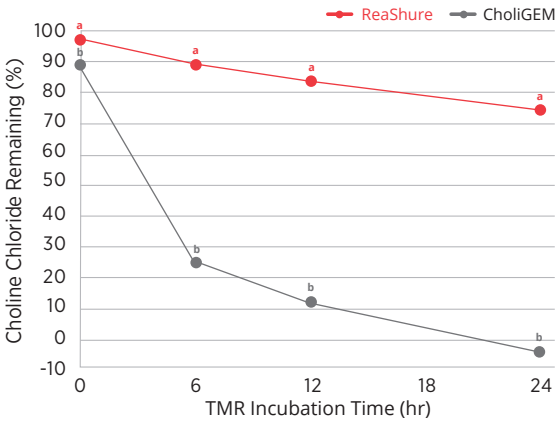
In vitro models effectively measure feed stability and rumen protection but struggle to predict intestinal absorption. Below is a summary of studies comparing two commercially available rumen-protected choline products using various *in vitro/in situ* methods to determine feed stability and rumen protection. These studies did not assess intestinal availability. Unless noted otherwise, each of these studies was funded and conducted by Balchem or its subsidiaries.

Trial 1: Total Mixed Ration (TMR) Stability Study

The study was designed to measure the stability of rumen-protected choline products in a TMR environment.

- CholiGEM lost the majority (75%) of its choline content after just 6 hours in the TMR
- CholiGEM lost 100% of its choline content after 24 hours in the TMR
- ReaShure lost only 10% of its choline content after 6 hours and only 25% after 24 hours in the TMR

Figure 1 TMR Stability of ReaShure and CholiGEM



[ab]Values with differing superscripts within a timepoint are considered statistically different (P<0.05).

Estes et al. 2024

Products were evaluated for TMR stability using a modified version of the methods developed by Ji et al. (2016) and Ishimaru et al. (2019). In this experiment, a corn silage and haylage-based TMR (DM = 38.7%; pH =4.8) was utilized. For each treatment, the TMR (200 g) and the equivalent of 1 g of CC from each product were weighed in triplicate and gently mixed for 30 seconds in a sealable Ziploc® freezer bag. Each treatment was then stored unsealed for 0, 6, 12 and 24 hr at 22°C (72°F). At each timepoint, the contents of each Ziploc® bag were transferred to a strainer bag (250 µM pore size) and were shaken for 1 min. with 1 L of distilled water to facilitate solubilization of any free CC released from the products. A sample of the solution was then taken, filtered (0.45µM disk filter) and analyzed for CC content. Any CC measured in the solution was considered to be the damaged/unstable fraction of the product.

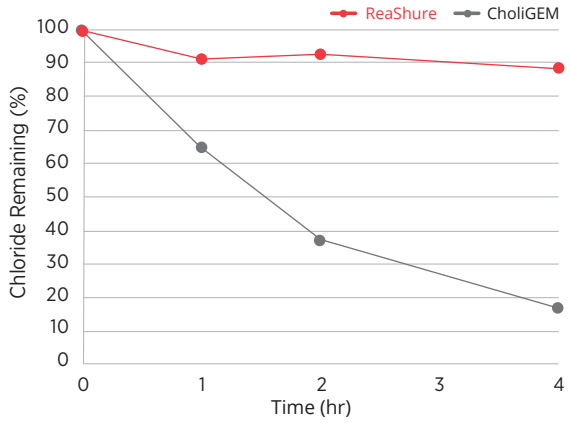
Data were analyzed using the MIXED procedure of SAS with the fixed effects of treatment, time and their interactions, and the random effect of bag. Means were separated using the Tukey adjustment.

Trial 2: *In Vitro* Rumen Stability Assay – Water Medium

The study was designed to simulate the rumen stability of rumen-protected choline products in a water medium.

- CholiGEM lost 83% of its choline content after just 4 hours in the water
- ReaShure lost only 12% of its choline content in 4 hours

Figure 2 Water Stability of ReaShure and CholiGEM



Balchem Laboratories, 2023

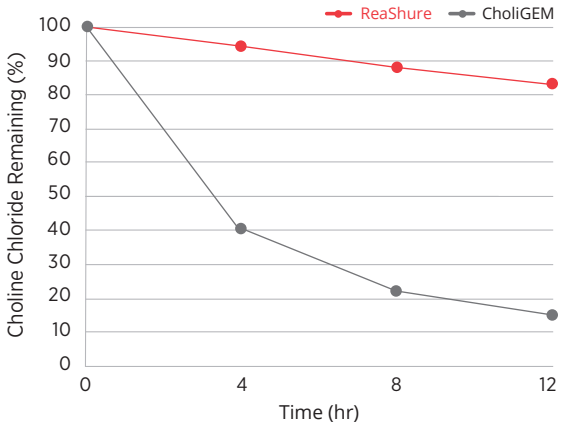
The water test is a relatively quick and easy procedure to illustrate the stability of RP-choline products. In this procedure, one sample of each product (two grams) is individually incubated in 100 mL of room temperature, distilled water for 1, 2 and 4 hr. Chloride content (proxy for choline) of the water is measured at each incubation timepoint. Any chloride released in the solution at each timepoint is considered to be the unstable fraction of each product.

Trial 3: *In Vitro* Rumen Stability Assay – Buffer Lipase Solution

This test was designed to more closely simulate the enzymatic environment in the rumen.

- CholiGEM lost the majority (60%) of its choline content after just 4 hours in the buffered lipase solution
- CholiGEM lost 85% of its choline content after 12 hours in the buffered lipase solution
- ReaShure lost only 17% of its choline content after 12 hours in the buffered lipase solution

Figure 3 In vitro Rumen Stability of ReaShure and CholiGEM



Balchem Laboratories, 2021

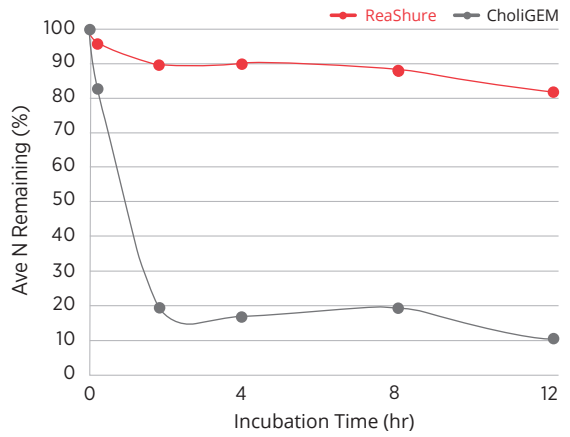
Products were evaluated for rumen stability using an internally developed *in vitro* procedure that consisted of a buffered solution with lipase enzymes that mimics the rumen environment. Duplicate samples of each product were individually sealed into Dacron® bags and suspended in the buffer solution maintained at 39°C (102.2°F) under constant agitation (160 rpm mixing rate) for 4, 8, and 12 hr. Aliquots of the solution at each timepoint were collected and analyzed for choline chloride content via a biochemical analyzer (YSI Incorporated, Yellow Springs, OH). Choline measured in the solution was considered to be the rumen degradable fraction of the product.

Trial 4: *In Situ* Rumen Stability Assay

This test is conducted in the rumen of a cow.

- CholiGEM lost the majority (80%) of its choline content after just 2 hours in the rumen
- CholiGEM lost 90% of its choline content after 12 hours in the rumen
- ReaShure lost only 18% of its choline content after 12 hours in the rumen

Figure 4 In situ Rumen Stability of ReaShure and CholiGEM



Conducted at Cumberland Valley Analytical Services, 2021

Products were evaluated at a third-party commercial laboratory (Cumberland Valley Analytical Services [Waynesboro, PA]) for rumen stability using an *in situ* procedure. For the *in situ* analysis, duplicate samples of both products were individually sealed into dacron bags (50 µm pore size, Ankom Technology) and were suspended in the rumen of three cannulated lactating cows for 0.25, 2, 4, 8 and 12 hr. Nitrogen (N; proxy for choline) and dry matter (DM) content were determined for each product at each timepoint. Samples of each product that were not rumen-incubated (0 hr) were also analyzed for N and DM content.

Summary

In this research CholiGEM failed to protect its choline payload from degradation, resulting in its loss in the TMR or simulated rumen, suggesting that it may not be available for absorption by the cow.

Conversely, ReaShure showed excellent stability in all studies, indicating that most of its choline payload could be available in the lower gut for effective absorption.

While *in vitro* and *in situ* models measure feed stability and rumen protection, they don't indicate true bioavailability. However, ReaShure has a long history of delivering results and is backed by 41 peer-reviewed papers that demonstrate its positive effects on transition cow health, calf health and growth, and milk and colostrum yield.

Download the complete research summary featuring four studies by snapping the QR code below.

"SCIENTISTS SAY

Choline is a Required Nutrient for Essentially Every Cow

Zenobi et al., 2018a
Zenobi et al., 2018b
Arshad et al., 2020
Bollatti et al., 2020a
Bollatti et al., 2020b
Potts et al., 2020
Swartz et al., 2022
Holdorf et al., 2023
Swartz et al., 2023
Poindexter et al., 2023
Arshad & Santos, 2024

Milk Production

Zenobi et al., 2018a
Arshad et al., 2020
Bollatti et al., 2020a
Holdorf et al., 2023
Swartz et al., 2023
Lima et al., 2024

Healthy Transition

Lima et al., 2012
Zenobi et al., 2018b
Arshad et al., 2020
Arshad et al., 2022
Poindexter et al., 2023
Arshad et al., 2023

Calf Health & Growth

Zenobi et al., 2018a
Zenobi et al., 2022
Holdorf et al., 2023

Improved Colostrum Quantity

Zenobi et al. 2018a
Bollatti et al. 2020b
Swartz et al. 2022
Holdorf et al. 2023

ReaShure®
Precision Release Choline

This new science changes everything we thought we knew about choline's impact on the cow and her calf. ReaShure® *Precision Release Choline* is the original and most researched rumen-protected choline source, so you can be sure you're getting the benefits you expect. Trust ReaShure and Balchem to impact her for generations. **Visit Balchem.com/ScientistsSay to learn more.**

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