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## **IN VITRO DEGRADATION OF BETAINE BY RUMINAL MICROBES**

*C. A. Löest, C. K. Armendariz, and E. C. Titgemeyer*

### **Summary**

An in vitro study was conducted to evaluate the degradation of betaine sources by rumen microbes. Five sources of betaine (anhydrous betaine, betaine-HCl, feed-grade betaine, lipid-coated betaine, and concentrated separator by-product) were incubated in rumen fluid collected from steers fed grain- or forage-based diets. In vitro degradation of betaine was slower with the high roughage diet than the grain diet. Betaine from concentrated separator by-product was degraded most rapidly, but no large differences occurred among the other four sources. The disappearance of betaine from lipid-coated product indicates that it did not resist ruminal degradation. Although betaine from all sources was degraded, some still remained after 24 hours of incubation, suggesting that some betaine may bypass the rumen.

(Key Words: Rumen, Betaine, Degradation.)

### **Introduction**

Research at Kansas State University has demonstrated that cattle may respond to supplemental betaine, possibly because of its role as a methyl group donor. However, betaine is likely to be degraded extensively by rumen microbes and may need to be protected from ruminal fermentation in order for appreciable amounts to reach the small intestine.

Our objective was to estimate how much betaine from various sources may escape ruminal degradation.

### **Experimental Procedures**

Ruminal contents were collected from four ruminally cannulated Holstein steers. Two were fed a high-grain (corn) diet, and two were fed a forage-based (prairie hay) diet. Ruminal contents were strained through cheesecloth, maintained at 39°F, and mixed with an equal volume of warm McDougall's buffer. The buffer-ruminal fluid mixture (20 milliliters) was added to in vitro incubation tubes containing .2 grams (dry basis) of an energy source (corn for the grain diet and prairie hay for the forage diet) and 10 milligrams of betaine from one of five sources. The sources and their analyzed betaine concentrations were: 1) anhydrous betaine (95.7%); 2) betaine-HCl (75.3%); 3) feed-grade betaine (Finnsugar Bioproducts, Helsinki, Finland) (81.8%); 4) lipid-coated betaine (Finnsugar Bioproducts) (59.9%); and 5) concentrated separator by-product (American Crystal Sugar, Moorhead, MN) (6.2%). This by-product results when additional sugar is extracted from beet molasses. Tubes without betaine also were prepared to correct for background quantities of betaine in ruminal fluid and the energy source (corn or prairie hay). Tubes were prepared in duplicate, flushed with carbon dioxide, and sealed with one-way-valve rubber stoppers to maintain anaerobic conditions before being incubated for 2, 4, 8, 24, or 48 hours at 39°C. To stop fermentation at the end of each incubation period, ethanol (5 ml) was added, and tubes were placed in a boiling water bath and boiled for 5 minutes. Tubes were centrifuged (30,000 × g for 20 minutes) and the supernatant was analyzed for residual betaine.

## Results and Discussion

The betaine concentrations in some of the sources were slightly lower than label claims, likely because of moisture accumulation. The amounts of betaine remaining in the in vitro tubes before and after 2, 4, 8, 12, 24, and 48 hours of incubation are presented in Figure 1.

The grain diet generally led to faster betaine degradation than the forage diet, probably because of a faster fermentation rate. Among betaine sources, concentrated separator by-product appeared to be degraded most rapidly for both the forage and grain diets. Little remained after 24 h of incubation. Rapid degradation may occur because this source also provides additional sugar and may stimulate fermentation.

No large differences occurred between anhydrous betaine, betaine-HCl, feed-grade

betaine, and lipid-coated betaine. The lipid-coated betaine was a mixture of a feed-grade betaine and calcium stearate designed to enhance flowability and potentially decrease ruminal degradation of betaine. The disappearance rate of betaine from lipid-coated betaine, however, was slightly greater than that from feed-grade betaine (Figure 1), indicating that lipid coating did not effectively decrease the metabolism of betaine by ruminal microorganisms.

Even though betaine was degraded by ruminal microbes, some of the betaine from all sources remained after 24 hours, which suggests that some betaine would escape ruminal degradation and thus pass to the small intestine.

**Figure 1. Betaine Remaining after Incubation in Rumen Fluid from Steers Fed Grain- and Forage-Based Diets. CSB=concentrated separator by-product. SEM=7.4.**

