

Inhibition of Ruminal Urease
(Project 596)

1. The Intracellular Nature of Urease:

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Rumen bacteria elaborate an enzyme, urease. Urease is capable of breaking down urea to ammonia and carbon dioxide. Rumen bacteria then incorporate the ammonia into new amino acids and bacterial protein. Thus, urea can be used as a non-nitrogen source for ruminants.

Unfortunately, urease often makes ammonia available faster than it can be used by rumen bacteria. That leads to poor utilization of urea or, in extreme cases, to toxicity.

Recent studies at Kansas State have been aimed at increasing the efficiency of urea utilization by slowing down, or inhibiting ruminal urease. Studies by Loper et al. reported in Bulletin 493, showed that urease could be inhibited by certain minerals and several antibiotics. The inhibitors were, however, not specific. That is, they inhibited other necessary enzymes in addition to urease.

To more clearly define urease inhibition, it was necessary to find if urease was located inside or outside the microbial cells.

Experimental Procedure

A known inhibitor of jackbean meal urease, [(S-2-carboxyethyl 3-thiosulfopropionate (3,3-dithiodipropionic acid S,S-dioxide)] (CTDD) did not inhibit urease in in vitro (artificial rumen) fermentations.

Cells were removed from rumen fluid by high speed centrifugation. Urease activity was determined in the cell-free fluid and in the resuspended cells. Rumen bacteria were disrupted using either high speed homogenization with glass beads, or with ultrasonic sound. After removing debris by centrifugation, we determined urease activity on the cell-free fluid, which contained soluble material from inside ruptured cells. CTDD was added to each preparation to see if it inhibited urease.

Results and Discussion

No urease activity was found in the cell-free rumen fluid. But whole rumen fluid and resuspended rumen bacteria showed urease activity, which could not be inhibited by CTDD. After bacteria were disrupted and cell contents were released into the surrounding fluid, urease activity, which could be inhibited by CTDD, was present in the cell-free fluid.

The results indicate that (1) rumen urease is inside microbial cells, and (2) since CTDD did not inhibit urease in intact cells, but did once urease was freed from the cells, it appears that an inhibitor to operate in the rumen, must have a molecular weight and configuration that will allow it to enter intact cells.

2. Effect of Acetohydroxamic Acid on Rumen Urease.

Acetohydroxamic acid (AHA) (molecular weight 75) has been proposed as the kind of inhibitor needed. It has been studied both in vivo and in intact fistulated animals. So we added acetohydroxamic acid at various levels to rumen fluid in in vitro fermentation systems to observe its effect on urease inhibition in intact bacterial cells. Results are shown in table 17. Decrease ammonia levels indicate urease was inhibited.

Table 17

Effect of Acetohydroxamic Acid On (AHA)
Ruminal Urease in Intact Cells, In Vitro
(mcg. $\text{NH}_3\text{-N/ml.}$)

AHA, mcg.ml.	0	Hours	1	2
		$\frac{1}{2}$		
0 (control)	8.4	102	166	232
5	6.7	67	111	170
10	6.7	53	84	149
20	6.7	32	54	104
50	7.8	18	39	71
100	8.8	15	25	46
200	7.4	12	23	34

Since Acetohydroxamic acid was effective in vitro on intact rumen microbes, it was studied in intact twin fistulated Hereford steers. AHA was administered directly into the rumen, 3gm. per feeding. Reduction of ammonia levels below control values is shown in table 18.

Table 18

Rumen Ammonia Levels (mcg. NH₃-N/ml.)
At Various Times After Feeding A Urea Containing
Diet, With and Without 3 gm. AHA

	0	$\frac{1}{2}$	1	2	3	4	6	8
Control	29	196	304	192	141	65	24	12
3 gm. AHA	35	95	180	201	129	86	34	25

Table 18 shows that rumen ammonia was depressed during the first 2 hours, when urea and AHA were both at high concentrations in the rumen. AHA apparently disappears from the rumen rapidly, perhaps via simple transfer through the rumen wall.

AHA appears to be a promising urease inhibitor. However, it is unavailable commercially, and must be synthesized in the laboratory. Our laboratory is preparing the product and the studies are being continued.

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