In Vitro and In Vivo Ammonia Release Studies on Various Slow - Release Urea Products

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Story in Brief

The rate of release of ammonia from various commercial slow-release urea products was studied, both in vitro and in vivo. The rate of release of ammonia from urea-gelatinized starch products was found to be the same as for urea in in vitro incubations as well as in an animal trial. A slow-release liquid supplement had a faster rate of ammonia release than did a molasses-urea mixture; however, only forty to fifty percent of the total nitrogen that was present as urea was released as ammonia from the slow-release product. No further ammonia nitrogen was released from the slow-release product after twelve hours of incubation.

Sulfur coated urea fertilizers have also been studied in vitro and the ammonia accumulation from the product with the fastest rate of release was only thirty percent of the total non-protein nitrogen after twelve hours. The products tested to date have not proven to be satisfactory in meeting the criteria of a sustained-release urea supplement which we established.

Introduction

Utilization of non-protein nitrogen (NPN) sources as a protein supplement for winter range cattle is relatively poor due to the low energy availability of dry winter range grasses. Related research reported in this publication indicates that utilization of urea can be equivalent to natural protein if the urea is fed hourly. Since this is an impractical management procedure, efforts have been intensified to find a sustained-release urea product.

The criteria for an ideal sustained-release urea supplement must be more exacting than to just prevent ammonia toxicity with a lower ammonia release. In order to be used as a protein supplement on winter range, a sustained-release urea product should have a slow continual

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release of ammonia over a twelve to twenty-four hour period. There should be no sharp peak of ammonia concentration, but a steady level over a period of time.

The purpose of this trial was to study the rate of ammonia release from existing urea supplements, which are advertised as slow-release products, to determine if any of them meet the above criteria.

Materials and Methods

The products studied were two urea-gelatinized starch mixtures (GS 1 and GS 2), a slow-release urea-molasses liquid supplement (SLC), three sulfur coated urea fertilizers formulated to release 15.0, 39 or 70 percent of the urea in seven days (SC 15, SC 39 and SC 70) and a resin coated urea (RC). These were compared to urea (U) and a molasses-urea (MU) mixture.

Trial 1.

Ammonia release rate was measured in a buffered urease solution. Jack Bean urease was dissolved in a pH 7.0 phosphate buffer to give a concentration of urease of 1.0 mg/ml. The control U and MU solutions and test products were measured isonitrogenously into 8 tubes (20 mg N/tube) and all were incubated at 39°C. Individual tubes were removed and 10 ml samples were taken at 0, 10 min., 20 min., 30 min., 1 hr., 2 hr., 4 hr. and 8 hr. In this trial and all further trials NH_3-N was determined by distillation over magnesium oxide.

Trial 2.

In vitro fermentations in rumen fluid were used to more closely simulate animal conditions. The Ohio in vitro fermentation system was used with each flask containing 20 percent rumen fluid taken from fistulated steers on either a high concentrate or high roughage ration depending on whether corn or hay served as the in vitro substrate. Ten ml samples were taken at 0, ½, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. for ammonia determination.

Trial 3.

Twelve fistulated wethers, were allotted into three groups in a 3 x 3 latin square. 1500 g of rations 2, 3 and 4 (Table 1) were fed each day except the morning when sheep were to be sampled. On sample days sheep were fed ration 1 and the appropriate supplement was poured into the rumen through the fistula. Sheep were sampled on day 5, 10 and 16 after being switched to a ration. On day 5 of period 1, 150 g of supple-

Table 1. Ration Composition

Ingredient	Ration (Kg/100 Kg)			
	1	2	3	4
Cottonseed hulls	82.5	74.5	74.5	74.5
Dehydrated alfalfa meal	17.0	15.0	15.0	15.0
Corn-urea mix (20.5 percent urea)	--	10.0	--	--
Gelatinized starch urea-2 (66 percent C.P.)	--	--	10.0	--
Gelatinized starch urea-1 (64.5 percent C.P.)	--	--	--	10.0
Trace mineral salt	0.5	0.5	0.5	0.5

ment were added to the rumen. Four sheep developed symptoms of ammonia toxicity within 1 hr. of adding the supplement. On all other sample days sheep received only 75 g of supplement. Sheep were sampled for rumen ammonia at 0, 1, 2, 4, and 8 hrs.

Results and Discussion

Rate of ammonia appearance in the buffered urease solution is shown in Figure 1 and 2. Nearly 100 percent of the urea had hydrolyzed to ammonia by 30 minutes after initiation of the incubation with the urea control and the two gelatinized starch products (Figure 1). Ammonia was released from the urea-molasses mixture more gradually, not reaching a maximum until four hours after initiation (Figure 2). The

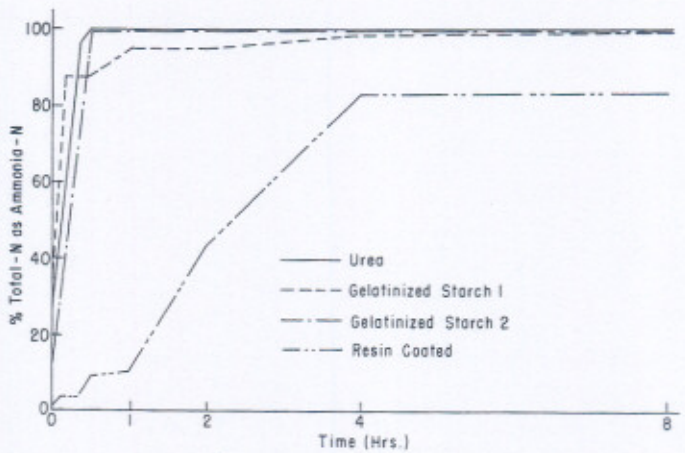

Figure 1. Percent total NPN appearing as ammonia-N in buffered urease solution.

resin coated urea had the most ideal rate of urea hydrolysis (Figure 1) while with the SLC and SC products, release of ammonia was too slow to be of benefit in the rumen.

Gelatinized starch product 1, when incubated in rumen fluid from either a concentrate fed steer (Figure 3) or a roughage feed steer

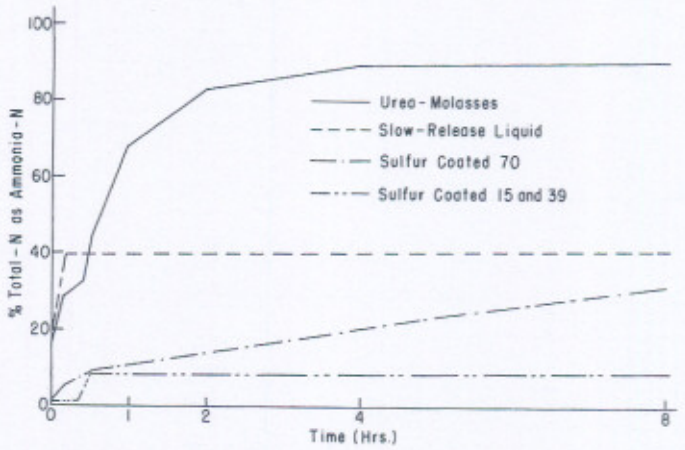

Figure 2. Percent total NPN appearing as ammonia-N in buffered urease solution.

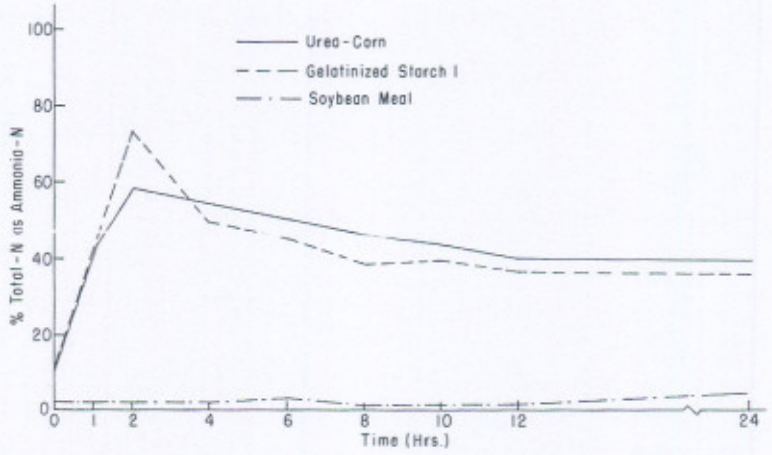

Figure 3. Percent total-N as ammonia-N in rumen fluid from concentrate fed steer with corn as in vitro substrate.

(Figure 4), had the same rate of urea hydrolysis as the corn-urea control. Figure 5 shows the ammonia curve obtained when GS-2, corn-urea and RC were incubated in concentrate rumen fluid. The RC had a much lower rate of urea hydrolysis than the other two products. The

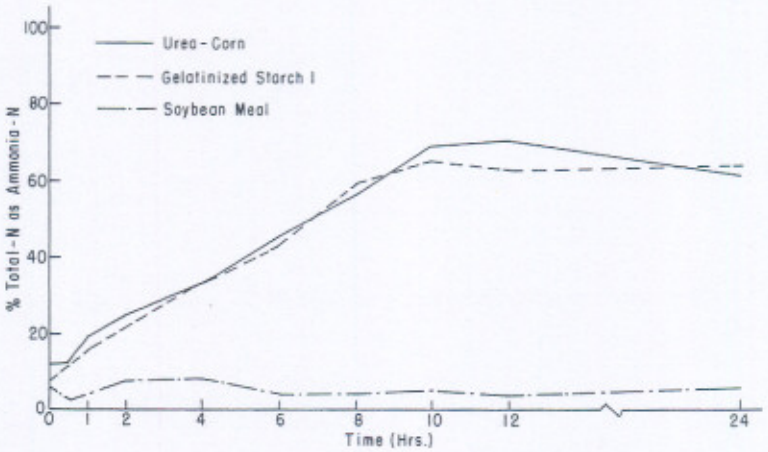

Figure 4. Percent total-N as ammonia-N in rumen fluid from roughage fed steer with hay as in vitro substrate.

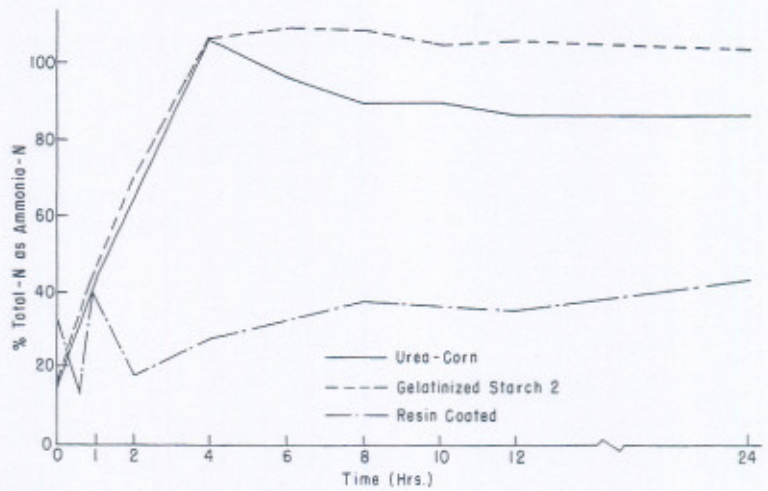

Figure 5. Percent total NPN as ammonia-N in rumen fluid from concentrate fed steer with corn as in vitro substrate.

three SC products also had a much slower rate of urea hydrolysis than the corn-urea control (Figure 6); however, the SC-70 did have a higher rate of appearance of ammonia than did the other sulfur coated products.

Liquid supplements incubated in concentrate rumen liquor had lower ammonia concentrations than the corn-urea control (Figure 7) as was the case in the buffered urease incubation. The SLC product again had the same initial rate of ammonia release as the two urea controls, but after 50 percent of the total NPN had been released as ammonia there was no further urea hydrolysis.

There were no significant differences in rumen pH in the animal trial on any of the sampling days or in the overall means (Table 2). Rumen ammonia-N levels for the whole trial are shown in Table 3. Mean ammonia-N with GS 2 was significantly lower than the corn-urea control ($P<.05$) and the GS-1 product ($P<.01$). This is shown graphically in Figure 8. Although the peak concentration of ammonia from GS-2 was lower, the rate at which it was released was similar to the corn-urea control and GS-1. The rate of hydrolysis is too rapid to get maximum utilization of the urea on a low quality roughage ration.

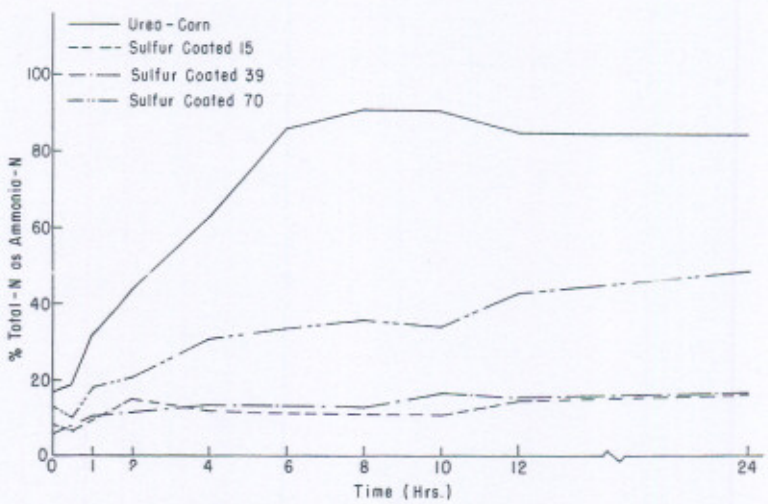

Figure 6. Percent total NPN as ammonia-N in rumen fluid from concentrate fed steer with corn as in vitro substrate.

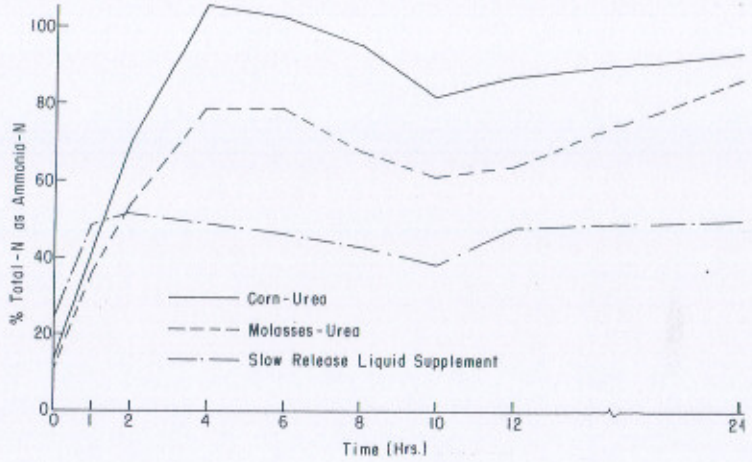

Figure 7. Percent total NPN as ammonia-N in rumen fluid fromcon-centrate fed steer with corn as in vitro substrate.

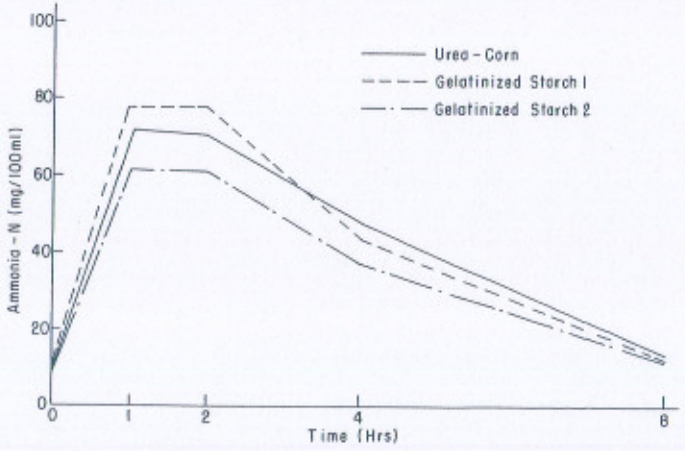

Figure 8. Rumen ammonia-N concentration from corn-urea, andtwo gelatinized starch-urea products fed with cottonseed huls.

Table 2. In Vivo Rumen Ammonia-N Levels (mg/100 ml), Means of Thirty-six Samples.

Ration	Time (hrs. after feeding)				
	T_1	T_2	T_4	T_8	Mean
Corn-urea	71.5	71.4	48.3	12.5	51.0^{2**}
Gelatinized starch-urea-2	61.7	60.8	36.9	12.4	43.0^{1}
Gelatinized starch-urea-1	76.6	77.4	44.3	12.3	52.6^{2**}

[1,2] Means with different subscripts, in same column are significantly different (* = $P<.05$; ** = $P<.01$).

Table 3. In Vivo Rumen pH, Means of Thirty-six Samples

Ration	Time (hrs. after feeding)				
	T_1	T_2	T_4	T_8	Mean
Corn-urea	7.12	7.08	6.83	6.50	6.88
Gelatinized starch-urea-2	7.01	6.92	6.69	6.49	6.78
Gelatinized starch-urea-1	7.13	7.06	6.73	6.45	6.84

Conclusions

Gelatinized starch-urea combinations have a urea hydrolysis rate in rumen fluid that is too rapid to get maximum utilization of urea with low quality roughage rations. The slow-release liquid supplement appears to have some urea so tightly bound that it is not hydrolyzed at all and its utilization is therefore low. Resin coated and sulfur coated urea products appear to have some potential as sustained release urea products; however, they need further development to find the proper coatings or combinations that will give a sustained release urea product which makes ammonia available for microbial protein synthesis over a long period of time.