



***NitroShure***<sup>™</sup>  
Precision Release Nitrogen

Balchem Research Summary

*In vitro* release of ammonia nitrogen  
from various nitrogen sources in  
batch culture

A summary of a study conducted by N. DiLorenzo and A. DiCostanzo. "In vitro release of ammonia nitrogen from various nitrogen sources in batch culture."

## Background

Meeting rumen degradable protein (RDP) requirements of cattle is necessary for optimized carbohydrate digestion in the rumen, and to maximize bacterial crude protein (CP) synthesis. Not balancing available nitrogen with available energy may cause temporary nutrient deficiencies for bacteria (Newbold and Rust, 1992). Degradation kinetics of protein sources differ greatly, even amongst NPN sources, are highly variable and are influenced by DMI. *In vivo* determination of protein degradation kinetics can be expensive, time-consuming and highly variable. A simple *in vitro* procedure to determine  $\text{NH}_3\text{-N}$  release was developed (modified from Kung et al., 2000). In spite of being a static system, relative differences amongst protein sources can provide an indication of  $\text{NH}_3\text{-N}$  release *in vivo*. This study was conducted to determine the relative rates of release of several NPN sources, including NitroShure™ Precision Release Nitrogen, compared to SBM.

## Materials and Methods

Batch culture incubations were conducted using 250-mL flasks fitted with a one-way rubber stopper gas release valve. Each flask was inoculated with 200 ml of a nutrient buffer: rumen fluid solution (4:1 ratio). Rumen fluid came from a cannulated steer fed a 95% corn silage 5% protein supplement diet. The nutrient buffer solution contained a mixture of macro and micro minerals, buffer and cysteine hydrochloride as a reducing agent.

Nitrogen sources tested included: 1. NitroShure (NTS), 2. Feed grade urea (URE), 3. Biuret (BIU), 4. Fermenten® (FER; Church & Dwight Co., Inc. Princeton, NJ), 5. Soybean meal (SBM). Flasks containing no added nitrogen sources were also incubated to serve as blanks (BLK). Four flasks per treatment were incubated per period and there were two periods in the study. All flasks received equal amounts of nitrogen from each source (155 mg; equal to 2 g of SBM) based on their nitrogen content.

During the fermentation, 10 ml samples were taken from each flask at 0, 0.5, 2, 4, 6, 8, 12 and 24 h of incubation. Each sample was analyzed for  $\text{NH}_3\text{-N}$  by Kjeldahl (Kjeltec 2300 Analyzer Tecator™, Herndon, VA).

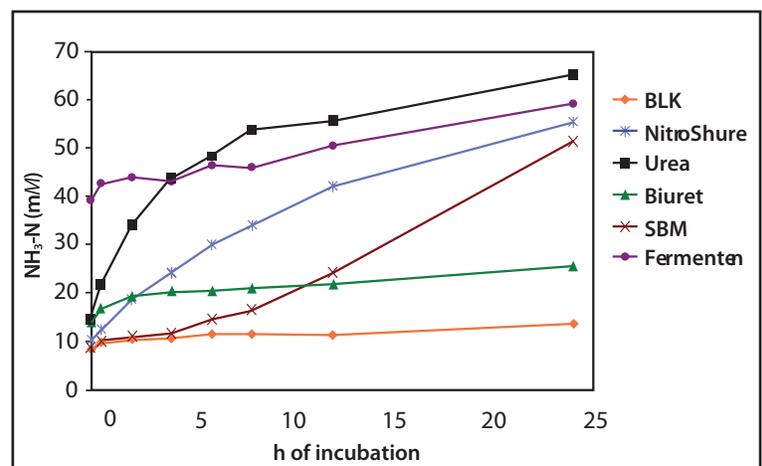
The proportion of  $\text{NH}_3\text{-N}$  released from the total incubated N (N provided by the N source + N in the nutritive solution) was calculated as follows:

$$\text{Proportion of } \text{NH}_3\text{-N released from incubated N}(\%) = \left[ \frac{\text{TRT} - \text{BLK}}{\text{mM Media}} \right] \times 100$$

Where: TRTmM was the  $\text{NH}_3\text{-N}$  value observed for each treatment at a given incubation time, BLKmM was the  $\text{NH}_3\text{-N}$  value observed for BLK at a given incubation time and mM Media was the total mM of N provided by the N source and the N in the nutritive solution.

## Results and Discussion

Figure 1 shows the results of  $\text{NH}_3\text{-N}$  concentration changes over time of incubation. There were significant ( $p < 0.01$ ) treatment x time interactions. At 6 h, deamination from SBM protein began to be significant as seen by  $\text{NH}_3\text{-N}$  accumulation relative to the BLK ( $p < 0.02$ ). This, in part, reflects a lag time for hydration, initiation of protein hydrolysis to peptides and amino acids by rumen bacteria, uptake of peptides and amino acids and the subsequent deamination of amino acids by bacteria to form volatile fatty acids and ammonia. The lag may also reflect incorporation of absorbed peptides and amino acids directly into microbial protein rather than deamination. Ammonia accumulation associated with SBM became significantly different from the BLK at six hours ( $p < 0.02$ ).

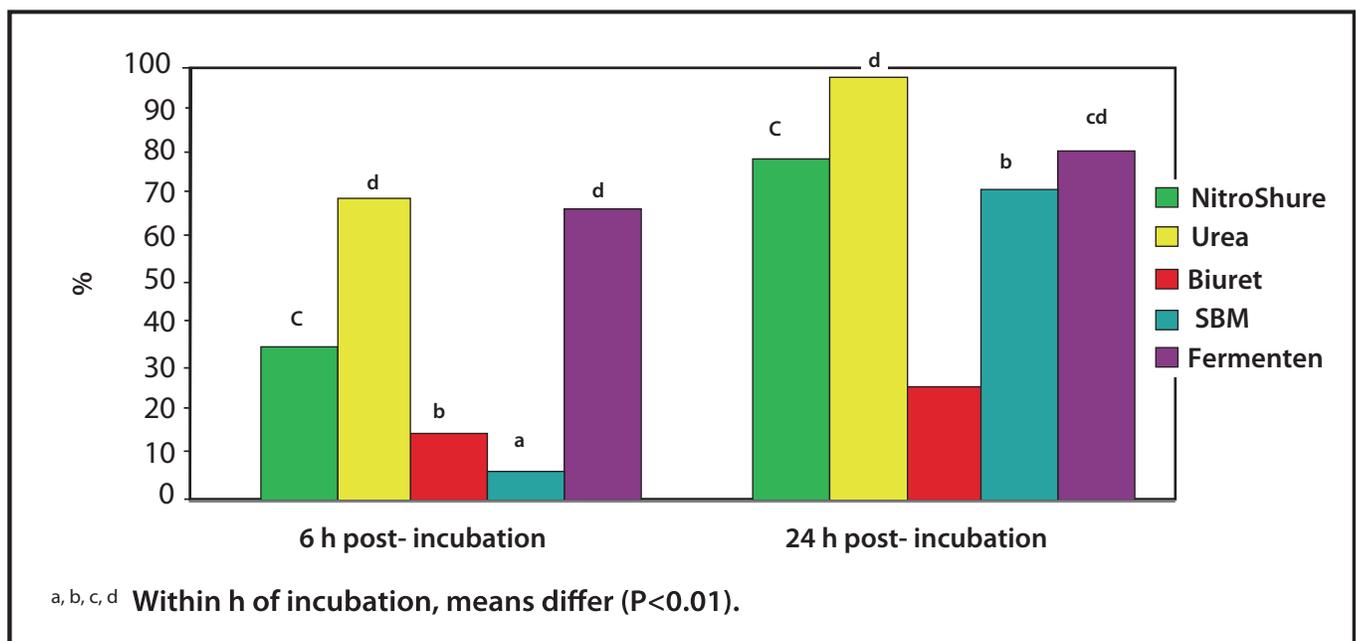


Biuret had an initial release followed by little change over the 24-hour incubation. The initial release probably reflects the fact that feed grade biuret can contain as much as 15% urea. The minimal change in ammonia release over time is not surprising since the rumen fluid donor steer was not acclimated to biuret. Studies have shown that it can take up to several weeks of feeding biuret for animals to develop a high level of biuretase enzyme needed to hydrolyze biuret to ammonia. From 4 to 24 hr Biuret had the lowest release of  $\text{NH}_3\text{-N}$  of all sources.

Urea shows a rapid release of ammonia into the media. This is not surprising as urea has high solubility and rumen fluid typically has high levels of urease enzyme. Urea and Fermenten<sup>®</sup> had the greatest ( $p < 0.01$ ) proportion of N released from incubated N at 6 hr after incubation (Figure 2). Urea had significantly higher ( $p < 0.01$ )  $\text{NH}_3\text{-N}$  release than NitroShure at all times. The lipid coating on NitroShure effectively reduced the rate of  $\text{NH}_3\text{-N}$  production compared to Urea.

Rumen ammonia levels typically peak about two to three hours post-feeding and then rapidly decline. This occurs despite good feed management practices such as frequently pushing feed up to cows (Lycos et al., 1997). Balancing energy and ammonia availability in the rumen can improve the capture and utilization of N resulting in more microbial protein, less excretion of ammonia into the environment and better utilization of carbohydrates for energy. Sources of  $\text{NH}_3\text{-N}$ , such as urea, that are rapidly released can elevate peak rumen ammonia levels resulting in increased blood urea levels which will result in increased MUN and N excretion in urine. This is not to suggest that urea does not have a place in certain dietary situations. However, feeding sources of protein with slower N release such as NitroShure can contribute to a more balanced rumen ammonia pool which will be important to maximizing microbial growth and animal performance.

Figure 2 Proportion of  $\text{NH}_3\text{-N}$  released from the total incubated N (N provided by N source + N in the nutritive solution) at 6 and 24 h post-incubation.



## References

Lycos, T., G. A. Varga and D. Casper. 1997. Varying Degradation Rates of Total Nonstructural Carbohydrates: Effects on Ruminal Fermentation, Blood Metabolites, and Milk Production and Composition in High Producing Holstein Cows. *J. Dairy Sci.* 80:3341-3355.

Newbold, J. R. and S. R. Rust. 1992. Effect of asynchronous nitrogen and energy on growth of ruminal bacteria in batch culture. *J. Anim. Sci.* 70:538-546.

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