# EVALUATION OF NITROGEN AVAILABILITY IN LIQUID FEEDSTUFFS

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## Summary

We developed an in vitro assay to assess ruminal availability of protein in liquid feeds containing soluble protein/nitrogen. Microbial mass accumulating as a result of assimilation of dietary nitrogen by ruminal microbes during an in vitro fermentation is measured. In the assay, microbial growth is most limited by the availability of protein/nitrogen, so microbial mass is proportional to the amount of available nitrogen in the sample. In liquid feeds that we generated in the laboratory, ruminal nitrogen availability decreased in response to mild heating, and the decline was greater for feedstuffs containing true protein rather than urea. Addition of salt to the products decreased nitrogen availability by an average of 21%, whereas addition of 4% phosphoric acid decreased nitrogen availability by 50%. Future research will be needed to fully characterize these effects so that negative impacts of manufacturing on protein availability can be prevented.

## Introduction

Under many conditions, protein supplements provided to ruminants are of optimal value when the protein (nitrogen) is completely available for use by the ruminal microbes. Although nitrogen from urea is considered to be completely available to the ruminal microbes, its availability can be reduced during processing as a result of various reactions that can occur. Similarly, true proteins that are predominantly available in the raw form may be unavailable to ruminal microflora when incorporated into various types of feeds.

Various approaches are available to assess the ruminal availability of proteins in feedstuffs. These include measures conducted in live animals, in situ disappearance of nitrogen from Dacron bags, and in vitro ammonia release (and similar assays in which other end-products of protein degradation are measured).

Live animal evaluations are ideal, but are too slow and costly for routine evaluation of a wide range of feeds. In situ incubation of substrates in Dacron bags provides a good measure for many feedstuffs, but it measures the insoluble proteins that remain after fermentation and, as such, is of little value in measuring availability of soluble nitrogenous compounds in liquid feeds. Moreover, in situ methodologies are based on the premise that protein solubility is synonymous with degradability, but this is not true. Many soluble nitrogen compounds are not ruminally degraded, and, conversely, nitrogen in some insoluble compounds can be degraded by ruminal microbes. Test-tube assays measuring ammonia release work well for some feeds, but feeds that have a low protein concentration or an easily fermentable carbohydrate component are difficult to evaluate because microbes will take up much of the ammonia that is produced from the feed.

The objective of this research was to evaluate how several processing characteristics alter the ruminal availability of nitrogen from liquid feeds by using a microbial growth assay developed for this purpose.

## Experimental Procedures

We previously developed an in vitro assay to assess ruminal availability of protein in liquid feeds containing soluble protein/nitrogen. In this assay, we measure the microbial mass that accumulates as a result of assimilation of dietary nitrogen by ruminal microbes during an in vitro fermentation. In the assay, microbial growth is most limited by the availability of protein/nitrogen and, therefore, the microbial mass is proportional to the amount of available nitrogen in the sample. Buffered rumen fluid is incubated with the nitrogen source to be tested in the presence of an excess amount of energy (starch). After 12 hours of incubation, the amount of cytosine (a marker of microbial mass) is measured.

In initial work with this assay, similar responses were achieved when true proteins and non-protein nitrogen sources, such as urea, were tested. Thus, the assay is relatively robust with regard to the type of substrates that can be evaluated.

We also were concerned that the addition of carbohydrates other than the starch, which we purposely added as an energy source, could impact the relationship between available nitrogen and microbial cell mass. The practical concern was that sugars provided by some low-protein supplements might impact the results. In tests, addition of sugars to the fermentations led to small, but significant, increases in cytosine production. Thus, we added sugars to the standard curves and corrected for the amount of cytosine that was produced from carbohydrate rather than nitrogen supply.

In these experiments, we evaluated some of the processing variables that might impact the ruminal availability of nitrogen in liquid feeds. We tested the effects of base ingredient, heating, and addition of minerals. We prepared the test products from the following base ingredients: cane molasses, steep liquor, distiller's solubles, and concentrated separator byproduct. We also made products from purified components to mimic the base ingredients but with "contaminants" removed. The goal of using the purified components was to model the effects of individual components that might be provided by the various base ingredients. The purified components included 1) 55% sucrose, 2) 33% sucrose plus 11% glucose plus 11% fructose, 3) 30% starch, partly hydrolyzed by amylase, plus 4.5% lactic acid, and 4) 5% soluble starch. Products were made by adding either casein or urea as the nitrogen source to mimic true protein and non-protein nitrogen in feeds, respectively. Most of the crude protein contained in the products was supplied by the casein or the urea. To assess mineral additions, we added salt (NaCl) at 2% of the product weight, and phosphoric acid was added at 4% of the product weight. The products were heated by placing the samples in a boiling water bath for 15 minutes, whereas unheated products were maintained at room temperature throughout the process. The samples were stored frozen between the time they were produced and the time they were analyzed.

## Results and Discussion

The base ingredient used to manufacture the product impacted the availability of the protein (Table 1). Notably, products made with concentrated separator byproduct had less ruminal availability of nitrogen than the other products. In general, products made with typical feed ingredients had lesser availability than those made with the purified components.

The decrease in ruminal nitrogen availability in response to heating was rather dramatic, and this response was dependent upon whether the primary source of nitrogen was casein or urea (Table 2). There was a much greater decline in nitrogen availability in response to heating for products that contained casein than for those that contained urea. This difference suggests that intact proteins are

more able to enter into heat-dependent reactions that impact availability. Interestingly, the base ingredient used to make the product did not greatly affect the response to heating (data not shown). We expected that products with more reducing sugars would be more impacted by heating, but this was not observed. For example, heating decreased the availability of nitrogen in products made with sucrose by 42%, but ruminal nitrogen availability was only decreased 33% by heating in products made with a mixture of sucrose, glucose, and fructose.

Mineral additions to unheated products (as salt or phosphoric acid) also had a large impact on nitrogen availability. Addition of salt to the products decreased nitrogen availability by an average of 21%, whereas addition of 4% phosphoric acid decreased nitrogen availability by 50%. However, the responses to the mineral additions were somewhat dependent upon the source of protein (casein vs. urea, Table 3) and the base ingredient used to make the product (data not shown). For example, the negative effect of salt was greater for those products made with urea than for those made with casein. In contrast, the negative effect of phosphoric acid additions was similar and dramatic for products made with either casein or urea. Based on our data, we cannot determine if the effects of phosphoric acid resulted from changes in acidity or from the addition of phosphate to the sample. This information would be helpful in predicting if other acids or phosphate-containing compounds would alter nitrogen availability.

In summary, processing characteristics can impact the availability of nitrogen from liquid feeds. Important variables include the base ingredient, the source of nitrogen, mineral additions, and heating. In addition, significant interactions between some of these variable were present. Future research will be needed to fully characterize these effects so that negative impacts of manufacturing on protein availability can be prevented.

**Table 1. Ruminally Available Nitrogen in Liquid Feed Products Containing Casein or Urea as the Primary Nitrogen Source and Manufactured from Different Base Ingredients**

| Base ingredient[1] | Ruminally available nitrogen |
|---|---|
| | --- % --- |
| Cane molasses | 73 |
| Concentrated separator byproduct | 47 |
| Distiller's solubles | 67 |
| Steep liquor | 72 |
| 55% sucrose | 81 |
| 33% sucrose, 11% glucose, 11% fructose | 87 |
| 30% hydrolyzed starch, 4.5% lactic acid | 69 |
| 5% soluble starch | 100 |
| Standard Error | 6.4 |

[1]Products contained 2% added salt and represent averages of unheated products and products heated in a boiling water bath for 15 minutes.

**Table 2. Effects of Heating and Nitrogen Source on Ruminally Available Nitrogen in Liquid Feeds[1]**

| Nitrogen source | Unheated | Heated[2] |
|---|---|---|
| | ---------- % available nitrogen ---------- | |
| Casein | 106 | 45 |
| Urea | 80 | 67 |
| Standard Error | 4.5 | |

[1]Values represent averages across products manufactured with a range of base ingredients.
[2]Products were heated in a boiling water bath for 15 minutes.

**Table 3. Effect of Mineral Additions and Nitrogen Source on Ruminal Nitrogen Availability in Liquid Feeds[1]**

| | Nitrogen source | |
|---|---|---|
| Mineral addition | Casein | Urea |
| | ---------- % available nitrogen ---------- | |
| None | 110 | 124 |
| 2% Salt | 106 | 80 |
| 4% Phosphoric acid | 61 | 55 |
| Standard Error | 4.8 | |

[1]Values represent averages across products manufactured with a range of base ingredients.