Ruminal availability of nitrogen from liquid feeds

Evan C. Titgemeyer and James S. Drouillard

Department of Animal Sciences and Industry
Kansas State University
Manhattan, KS 66506-1600

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 where 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 following 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 the case. 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. However, feeds that have a low protein concentration or a highly fermentable carbohydrate component are difficult to evaluate because microbes will take up much of the ammonia that is produced from the feed. Although microbial inhibitors can be added to in vitro assays to prevent utilization of ammonia or amino acids by microbes, those assays are only valid over short incubation periods and may not work well for sources of non-protein nitrogen because not all of the end-products may contribute to the ammonia pool directly.

Evaluation of ruminal nitrogen availability in liquid feeds

With the above points in mind, we 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. The assay works because we ensure that 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. In our assay, buffered rumen fluid is incubated with the nitrogen source to be tested in the presence of an

excess amount of energy (starch). Following 12 hours of incubation, the amount of cytosine (a marker of microbial mass) is measured. This assay was designed for evaluating the ruminal availability of nitrogen in liquid feeds that cannot be appropriately tested with other approaches.

We have previously demonstrated linear relationships between the amount of casein (a purified protein that is considered completely available to ruminal microbes) added to fermentation tubes and the amount of microbial cytosine that was produced (Schoenholz et al., 1998). However, we also wanted to ensure that similar responses could be achieved when non-protein nitrogen sources, such as urea, were tested. Therefore, we tested graded levels of casein, urea, and an equal mix of casein and urea. Essentially, we observed that responses were similar regardless of whether the available nitrogen was provided as a true protein source or as a non-protein nitrogen source (Figure 1). Thus, the assay was demonstrated to be relatively robust with regard to the type of substrates that could be evaluated.

We were also 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 some low-protein supplements might provide enough sugar to the fermentation to impact the results. Therefore, we tested two levels of a mixture of sucrose, glucose, and fructose added to the fermentation tubes and measured the response in microbial production of cytosine in vitro. Interestingly, the lower level of sugar (0.25 g/tube) led to small increases in cytosine production, but the higher level (0.5 g/tube) did not change the amount of cytosine produced. Thus, for our studies using this approach, we add sugars to the standard curves and correct for the amount of cytosine that was produced from carbohydrate rather than nitrogen supply.

With an assay in place to assess nitrogen availability in liquid feeds, 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. For preparing the test products, we used the following base ingredients: cane molasses, steep liquor, distiller's solubles, and concentrated separator byproduct. We also made products using 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, partially 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. For assessing mineral additions, salt (NaCl) was added at 2% of the product weight and phosphoric acid was added at 4% of the product weight. Heating of the products was accomplished by placing the samples in a boiling water bath for 15 minutes, whereas unheated products were maintained at room temperature throughout the process.

For our evaluation of the effects of heating of the liquid feeds, we tested the products that contained 2% added NaCl. In this study (Table 1), we observed that the base ingredient used to manufacture the product impacted the availability of the protein. Notably, products made with concentrated separator byproduct had lower ruminal availability of nitrogen than the other products. In general, products made with typical feed ingredients had lower 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 larger depression in nitrogen availability in response to heating for products containing casein than for those containing urea. This 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) as well as the base ingredient used to make the product (data not shown). For example, the negative effect of NaCl was greater for those products made with urea than for those made with casein. In contrast, the negative effect of phosphoric acid additions was rather dramatic for products made with either casein or urea.

In summary, the data demonstrate that 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. Additionally, significant interactions between some of these variable were also present. It is clear that future research will be needed to fully characterize these effects so that negative impacts of manufacturing on protein availability can be prevented, thereby allowing the feed industry to exploit the full value of the feed ingredients that are employed.

Factors impacting ruminal nitrogen availability in cooked molasses-urea blocks

In a series of experiments (Trater et al., 2003), we evaluated the effects of several processing factors on the ruminal availability of nitrogen from cooked molasses blocks containing urea. Essentially, we wanted to know if the processing of the blocks would decrease the availability of the urea nitrogen. For these experiments, release of ammonia during in vitro fermentations was used as the response criterion. Over several experiments, we studied effects of the time of urea addition (i.e., beginning or end of cooking), molasses pH, addition of MgO after cooking to reduce block swelling, and post-cooking storage temperature.

Blocks were manufactured in a scraped-surface, steam-jacketed cooking vessel with a functional volume of 25 liters. The vessel was stainless steel and could withstand vacuum pressure lower than 635 mm Hg. The vessel contained a central shaft with four nylon paddles that turned at 32 rpm. Cane molasses (10 kg dry matter) was added to the vessel (adjustments to the pH were made at this point), then urea (if added before cooking, at a level of 12%) and tallow (at a level of 6%) were added, and cooking was initiated. Blocks with urea added before cooking were cooked to a temperature of 126°C, which allowed sufficient removal of water from the mixture for hardening of the molasses without scorching. After cooking, vacuum was increased to approximately 635 mm Hg for 3 minutes to remove additional water. For blocks with urea added after cooking, the final temperature of the product was 120°C, at which time urea was added and mixed, and then vacuum was increased to approximately 635 mm Hg for 3 minutes. Final cooking temperature was lower when urea was added after cooking to prevent scorching

and premature hardening of the block before discharge from the vessel. All mixtures were cooked for about 21 minutes. Samples of blocks were collected during discharge and immediately cooled.

The same amount of urea was added to each block, and the measured crude protein concentrations of the blocks were not affected by treatment. However, we also chemically measured the amount of urea nitrogen in each block as an estimate of the amount of unreacted urea that remained in the product. Thus, in blocks that were found to contain less urea, more of the added urea apparently was converted to another form during processing.

For our ammonia release assay, ruminal fluid was collected from steers fed prairie hay. Preliminary studies had demonstrated that we achieved complete hydrolysis of urea in 1 hour and that molasses did not interfere with this release. Thus, for the limited range of samples being investigated, ammonia release was a valid method of estimating ruminal nitrogen availability. For the assay, molasses blocks were crushed to a coarse powder, mixed with buffer and ruminal fluid, and fermented for 1, 8, and 24 hours. The 8 and 24 hour timepoints were used to determine if, during processing, some of the urea was converted into a form that was available to ruminal microbes at a rate slower than that of unprocessed urea. Although a few interactions between treatment and fermentation time occurred, responses were generally similar among the three fermentation times and only an average value is discussed.

Effect of time of urea addition to blocks. Urea was added to the cooking vessel before or after cooking, but prior to vacuum application. Urea concentrations were higher for blocks with urea added after rather than before cooking (Table 4). Ammonia release was higher when urea was added after cooking than when it was added before cooking. These differences may be due to differences in reaction time as well as the fact that much of the water was removed from the products during cooking, such that there was much less water available for chemical reaction when urea was added after cooking.

Effect of molasses pH. Prior to cooking, the pH of the molasses was adjusted to four levels (pH of 4, 6, 7, and 8) using either hydrochloric acid or potassium hydroxide. Molasses at its native pH of 5 also was evaluated. The different pH's were expected to change the chemical reactions that might take place during cooking as well as to change the amount of sucrose that was hydrolyzed to glucose and fructose, which could also subsequently alter chemical reactions involving urea. Concentration of urea in blocks decreased linearly as initial molasses pH was reduced (Table 4), suggesting that at the lower pH either more sucrose was hydrolyzed and(or) that sucrose or the resultant reducing sugars were more reactive. Final block pH differed from the beginning molasses pH. There was a smaller range in final block pH (5.03 to 6.42) than in the beginning molasses pH values (4 to 8), although there was a good relationship between molasses and final block pH values. As pH increased from 4 to 8, there was a linear increase in ammonia release, likely a result of more sucrose being hydrolyzed at the lower pH to the more reactive glucose and fructose, which could react with urea to form compounds unavailable to the ruminal microbes.

Effect of adding magnesium oxide to blocks after cooking. Molasses blocks were manufactured with either 0 or 0.14 kg MgO added after cooking, but prior to vacuum application, to prevent swelling of blocks during storage. For these blocks, molasses was adjusted to a pH of 8 using potassium hydroxide prior to cooking. Addition of magnesium oxide did moderately reduce urea concentration of blocks and increase pH (Table 4). However, in vitro ammonia release was not affected by the MgO addition.

Effect of storing blocks at elevated temperatures. Samples of blocks were collected during discharge and subsequently stored at different temperatures. One sample was allowed to cool immediately at 25°C (77°F). Other samples were held at 66°C (150°F) or 82°C (180°F) for 12 or 24 hours before cooling. These storage temperatures mimicked block temperatures that could occur during storage under different environmental conditions and(or) storage configurations. Heated storage decreased the urea concentration in the blocks, and blocks that were held at 66°C during storage had lower urea concentrations than blocks that were held at 82°C. Although the initial pH of the molasses was around 7.7 for all blocks, the final block pH varied from 6.7 to 8.5. Blocks that were held at 66°C during storage had higher pH than those held at 82°C, suggesting that the reactions occurring at 66°C raised pH during storage. Blocks that were immediately cooled had greater nitrogen availability than those that were stored at elevated temperatures, and the decrease in ammonia release was greater for those held at 66°C than those held at 82°C. Blocks held at 66°C during storage tended to form a hard outer crust, which trapped gases produced within the blocks, and swelled more and were more sponge-like than blocks that were immediately cooled or those held at 82°C. Although some crust formed near the end of storage for blocks held at 82°C, they generally remained in a somewhat liquid state during a majority of the 24-hour holding time.

Indirect assessment of nitrogen availability from molasses-urea blocks. Cooking of molasses and urea as well as subsequent storage of the blocks at elevated temperatures yielded nitrogenous compounds that were resistant to degradation by ruminal microorganisms. A number of factors that we measured might be useful for indirectly estimating the effects of block manufacturing techniques on availability of nitrogen added as urea. The final block pH and concentration of urea appeared to be related to N availability. In almost all cases, the concentration of urea in the blocks reflected the availability of N. As urea was converted to non-urea products during the block manufacturing processes, N availability decreased. The parallelism between urea concentration and ammonia release was evident for time of urea addition, molasses pH, and storage temperature. The only deviation occurred when MgO addition slightly reduced urea concentration but did not impact ammonia release. It also may be possible to evaluate the effect of storage temperature on N availability by measuring the pH of blocks after cooking. There were large differences in final block pH due to storage temperature, and the degree of increase in block pH was related to the decrease in ammonia release.

General conclusions

A number of factors can impact the ruminal availability of nitrogen in liquid feeds. The availability of other nutrients, such as minerals, might similarly be impacted by processing characteristics. In order to fully exploit the value of feedstuffs, it is important to identify issues that impact ruminal protein availability and to subsequently design strategies that prevent degradation of the valuable qualities of feeds during processing. However, many of the methods typically used for assessing the ruminal availability of nitrogen in feeds are not appropriate for liquid feeds because some soluble nitrogen is not available to the microbes. Depending upon the sample type and the amount of information required, several different methodologies can be useful for assessing this criterion. For some products where urea is the primary nitrogen source, in vitro ammonia release may adequately reflect nitrogen availability. For more complex

samples, the ability of the product to support microbial growth is probably better for determining the ruminal availability of protein (nitrogen).

References

Schoenholz, C.K., J.S. Drouillard, E.C. Titgemeyer, and C.K. Armendariz. 1998. Development of an in vitro procedure to determine ruminal availability of protein. 1998 Cattlemen's Day, Report of Progress 804, Agric. Exp. Sta., Kansas State Univ., Manhattan. pp 86-88.

Trater, A.M., E.C. Titgemeyer, J.S. Drouillard, and J.N. Pike. 2003. Effects of processing factors on in vitro ammonia release from cooked molasses blocks containing urea. Anim. Feed Sci. Technol. 107:173-190.

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% lactate	69
5% soluble starch	100
Standard Error of the Mean	6.4

[1]Products contained 2% added NaCl 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% NaCl	106	80
4% H_3PO_4	61	55
Standard Error	4.8	

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

Table 4. Effects of ingredient and manufacturing characteristics on block pH, urea concentration, and *in vitro* ammonia release (adapted from Trater et al., 2003)

Variable	Block pH	Urea	NH_3 release
		% of DM	% of N
Urea addition[1]			
Start	5.98	9.2	64.9
End	5.75	11.5	80.5
SEM	0.016	0.12	0.6
Molasses pH[2]			
4	5.03	9.3	67.2
5	5.73	9.6	68.9
6	5.98	10.1	69.4
7	6.20	10.2	71.7
8	6.42	10.7	73.1
SEM	0.021	0.16	0.8
MgO addition[3]			
0	7.30	8.5	69.3
+	7.84	8.0	70.1
SEM	0.065	0.05	0.7
Holding temperature[4]			
Cooled	6.80	10.0	79.2
66°C (12 h)	8.05	7.5	68.1
66°C (24 h)	8.50	7.0	64.3
82°C (12 h)	6.70	9.0	72.1
82°C (24 h)	7.05	8.5	70.2
SEM	0.059	0.09	1.1

[1]Time that urea was added during the cooking process.
[2]Approximate pH of molasses used for manufacturing blocks.
[3]MgO added after cooking and before vacuum application.
[4]Temperature (time) for storage prior to cooling.

Figure 1. Relationship between available crude protein and cytosine production during in vitro fermentation.