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Methods to Evaluate Rumen Protected Lysine for Dairy Cows

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Abstract

There are many rumen protected amino acid products available for dairy cattle feeding. However, feed formulation programs require values related to rumen solubility, rate of disappearance in the rumen and total tract digestibility and often such values are not available. *In vivo* testing procedures are complex, time consuming and expensive. This study was conducted to determine if a newrapid, lower cost *in vitro* method developed for feed ingredients could be applied to a rumen protected lysine product (DairynatLys-30®, Jefo Nutrition Inc). *In vivo* determination of the rapidly solubilized protein fraction, rate of degradation of the slowly solubilized fraction and total tract digestibility studies were compared to the *in vitro* method in use in many ingredient analysis laboratories for feed ingredients such as forages, protein supplements and grains. Results showed that the rapidly soluble fraction (8.33% and 8.66% of total N for *in vivo* and *in vitro* methods) and rates of disappearance in the rumen (2.64%/h and 2.43%/h for *in vivo* and *in vitro* procedures) compared favorably between the two methods for the rumen protected product. Total tract digestibility values were slightly higher (84.4%) with the *in vivo* method used than with the *in vitro* method (75.9%), and both are in the expected calculated range of digestibility of 80%. In conclusion the *in vitro* method appears to be an acceptable alternative for evaluating rumen protected amino acids.

Keywords

Rumen Protected Amino Acids, Lysine, *In Vitro* Testing

1. Introduction

Formulating diets on the basis of amino acids rather than protein is rapidly becoming standard practice in dairy

cattle nutrition. There is pressure to optimize protein and amino acid use in dairy cows as excess nitrogen (N), resulting from over formulating the protein of the diet, is a burden to both the environment and to the cow. With respect to the animals, cows must consume energy to convert excess amino acids to urea for elimination. As to the environment, many jurisdictions have introduced strict guidelines that limit the use of manure application upon land. Diets need to be formulated in order to reduce oxidation of amino acids to the extent possible, by feeding the correct amounts of amino acids whenever possible.

Lysine is an essential amino acid needed for the synthesis of protein in growth, milk production, tissue maintenance and repair and gestation. Lysine is additionally a constituent of carnitine, a compound needed to transport fat into cellular structures [1]. Animal by-products are rich sources of lysine, while vegetable sources are not.

To take advantage of advanced formulation technology, the formulator must be able to predict the amount of amino acids derived from feed ingredients. Animal tests required to assign the values to nutrients can be expensive and time-consuming and many suppliers do not provide the values needed. A new laboratory method for the determination of rate of digestion and protein digestibility have been published and adopted by feed ingredient analysis laboratories in North America [2]. To our knowledge, results have not been compared to biological tests for products such as this one. If this method can be applied to the analyses of rumen protected amino acids, then there would be substantial savings in cost, and much-needed uniform results could be obtained. This study was conducted to generate values for a rumen-protected lysine product (Dairynat Lys-30®, Jefo Nutrition Inc), and to determine if the newer laboratory method can be used to replace animal testing for this product and similar products.

2. Materials and Methods

2.1. Description of Test Product

The product tested was rumen protected lysine (DairynatLys-30®) developed by Jefo Nutrition, Inc. St. Hyacinthe, QC, Canada. The product contained 30% lysine from lysine HCL, protected in a lipid matrix.

2.2. Animal Studies

The following animal testing procedures were conducted by the Atlantic Dairy and Forage Institute, Fredericton Junction, NB, Canada. Cows used in the evaluations were handled in accordance with guidelines as outlined by FASS [3]. Cows were housed in stanchion stalls and allowed ad-libitum access to feed. Feed was mixed and allocated twice daily at approximately 600 hours (h) and 1800 h. The cows were milked twice daily at approximately 700 h and 1700 h. The ingredient and nutrient composition of the diet given to the cows is provided in **Table 1**.

2.2.1. Determination of Ruminal Rate of Disappearance of Lysine from Rumen Protected Lysine

Two rumen cannulated mid-lactation Holstein cows were used in this study. The procedure was conducted over

Table 1. Composition of the diet used during the study period (% of dry matter, DM).

Ingredient Composition	% of DM	Nutrient Composition	% of DM
Grass silage	34.68	Dry matter	51.8
Corn silage	15.31	Crude protein	16.7
Canola meal	20.38	Acid detergent fiber	23.2
Barley grain	18.72	Neutral detergent fiber	37.4
Corn grain	7.25	Ash	8.81
Premix	2.34	Fat	3.93
Fat	0.60		
Limestone	0.38		
Yeast	0.21		
Urea	0.13		

two consecutive days. Three samples of Lys 30 from 3 different manufacturing lots were studied, and provided to both cows. Analyses were conducted as suggested by Vanzant *et al*. [4] with the exception of grinding. Samples were incubated in the prilled form in which they would normally be included in the diet. The ingredient samples were placed in the nylon bags (pore size 53 microns) in the form received. For each sample, 15 replicates, weighing approximately 6 g each were incubated in the rumens of each of the cows (30 bags in total/sample). Samples were incubated in the ventral sac of the rumen,and were sequentially added so that they could all be removed at the same time. Three samples were each incubated for 2, 6, 12, 18 and 24 h. Residues were washed in tepid water five times for 3 minutes using a washing machine, dried at low temperature in a forced air oven and dry matter determined by sample, incubation time, and cow. Samples were then pooled by time within cow each day to insure sufficient sample for further analysis.

As lysine was the only source of N in the ingredient, N determination was used to estimate rate of loss of lysine at each time point. Lysine contains 19.15% N, and lysine was calculated as 5.22 times N. Samples of the original materials were likewise included for analysis.

Rates of disappearance in the rumen were determined as the natural log of the amount remaining regressed against time. The intercept provides the amount that is rapidly released in the rumen (A fraction) and the rate function generated represents the rate for lysine disappearance (solubilization) in the rumen (Kd).

2.2.2. Determination of Intestinal Digestibility

Due to the complexity of the animal testing and the labor involved, a single sample of DairynatLys-30® was evaluated, using 6 cows. Two cows served as untreated controls, two acted as positive controls and two were used to evaluate Dairynat Lys-30®).

Blood was drawn at approximately 1 h before and again at 1 h after feeding to serve as an estimation of the baseline level of lysine for each cow. Immediately thereafter, the two positive control cows received an abomasal (1.2 L) infusion containing 50 g of lysine in the form of lysine HCl over a 30 minute period. Similarly, the two treatment cows received a solution containing 50 g of lysine in 1.2 L of water from the test product placed through the rumen and into the abomasum over a 30 minute period. The untreated control cows received water only (1.2 L) over the same time period.

Blood was then drawn from all 6 cows at 2, 4, 6, 8 and 10 hours after feeding, and these times were after the infusion was complete. All blood samples were obtained by tail vein puncture. Blood (7 - 10 ml) l was collected into tubes containing anticoagulant. Blood was centrifuged at 3000 × g for 15 minutes to separate the plasma. Plasma was stored frozen (−32˚C) until submitted for analysis of free lysine. Analyses were conducted by the University of Montreal Laboratoire de Chromatographie Faculté de médecinevétérinaire.

2.3. Laboratory Analyses

Three samples of DairynatLys-30® were submitted to a commercial laboratory, Cumberland Valley Analytical Services, Cumberland, MD., USA for *in vitro* determination of protein digestibility using the method described by Ross *et al*. [2]. These results were compared to the *in vivo* results described.

3. Results and Discussion

Results of the *in vivo* and *in vitro* tests are provided in **Table 2**. The rapidly soluble fractions, or A protein fractions were similar between the two methods of analyses, as were the determined rates of solubilization of the slow digesting fraction. Therefore the *in vitro* method appears to be quite adequate for the determination of the

Table 2. Results of the *in vivo* and *in vitro* analyses (n = 3).

	In Vivo		*In Vitro*	
Analysis	**Mean**	**St. Dev.**	**Mean**	**St. Dev.**
Lysine, %	31.5	0.70	31.0	1.91
Soluble fraction, A, %	8.33	1.99	8.86	2.31
Rate of disappearance, Kd, %/h	2.64	0.07	2.43	0.56
Total tract digestibility[1]	84.4	6.57	75.9	5.24

[1]Based on 1 sample *in vivo* and 3 samples *in vitro*.

rapidly soluble protein fraction, as well as the prediction of the rate of ruminal disappearance of the slowly soluble fraction of this rumen protected lysine product.

Total tract digestibility was somewhat higher with the *in vivo* test than with the *in vitro* test. However, only one sample could be evaluated *in vivo*, and the values are the means for the two cows. The calculated total tract digestibility value was 80%, and the values obtained *in vivo* and *in vitro* were both within the limits expected.

Very limited information is available regarding the intestinal digestibility of lipid coated, rumen protected lysine. Rossi *et al.* [5] compared rumen protected amino acids treated with different commercially available coatings using the mobile nylon bag technique. Triglycerides, fatty acids and calcium salts of fatty acids were determined to have digestibility values of 100% when used to ruminal protect lysine hydrochloride. In comparison, Wu *et al.* [6] using the same technique, determined that triglyceride protected lysine sulfate had a total tract digestibility of 86.7%. These values compare most favorably with the *in vivo* results obtained in the current study (84.4%). The *in vitro* results were somewhat lower at 75.9%.

A more commonly used procedure is to assess the relative bioavailability of products, based on plasma amino acid concentrations [7]-[9]. This method required frequent blood sampling to divine changes in plasma concentrations of the protected amino acid, and computation of the area under the curve. While such a method may permit products to be compared to each other if analyzed at the same time, it does not provide a true value for total tract digestibility, and thus the *in vitro* method would be preferred from that point of view.

The *in vitro* method of protein digestibility of Ross *et al.* [2] was developed for the routine, consistent analysis of feed ingredients. These results show that the method can also provide accurate results for rumen protected amino acid products. The advantage to the *in vitro* method is that it is rapid, and less costly. While *in vivo* methods do not lend themselves to routine analysis, as might be required for new product development or quality control, the *in vitro* procedure fits these roles perfectly.

4. Conclusion

These results show that the *in vitro* method of Ross *et al.* [2] can be used to supply much needed values for solubility, rates of digestion and the total tract digestibility of rumen protected lysine products. Such results support the provision of accurate values that are required for feed formulation platforms, and will advance the acceptance of such products.

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