SIMILARITY OF RUMEN DEGRADABLE PROTEIN (RDP) DEGRADATION RATES COMPARING *IN VITRO* AND *IN SITU* TECHNIQUES.

Matthew A. Brooks, MS,* Nichole F. Johnson, Robyn M. Harvey, Monty S. Kerley, PhD

Department of Animal Science, University of Missouri, 920 E. Campus Dr., Columbia, MO 65211, USA

Abstract

Protein provided to ruminants can be divided into rumen degradable protein (RDP) and rumen undegradable protein (RUP). The RDP provides amino acids and ammonia-nitrogen which are vital to the growth of rumen microbes. An imbalance of the RDP supply and microbial RDP requirement can alter ruminal fermentation. The imbalance could lead to reproductive inefficiencies, inadequate growth, and ruminal acidosis. To better estimate RDP provided to the rumen, it is necessary to know the rate at which the RDP degrades. One *in situ* and two *in vitro* experiments were performed measuring degradation rates of varying protein sources (bloodmeal, BM; dried distillers grain, DDG; dried distillers grain with solubles, DDGS; casein, C; soybean meal, SBM; corn gluten feed, CGF; and a RUP mix) using dairy cow rumen fluid. The *in situ* method measured remaining nitrogen over a time course of 0 to 72 h, while the *in vitro* methods measured ammonia release as a measure of nitrogen degradation over a time course of 0 to 48 h. The data were analyzed to determine the proportion of RDP degraded over time when taken to complete degradation (extent). The plotted proportions of degraded RDP over time result in a linear decline with slope a measure of degradation rate (K_d). The K_d ranged from 1.91-2.84% h^{-1}. Bloodmeal had the lowest rates at 2%, and the SBM had the rates at 2.8%; however, BM, DDG, DDGS, C and the RUP mix were similar (P>0.01). There was no difference between *in situ* and *in vitro* methods (P=0.26). These similar rates will help improve estimation of protein release in the rumen, thus helping accurately balance RDP with microbial requirements.

Introduction

The unique digestive physiology of ruminant animals and their symbiosis with rumen microbes requires nutritionists to formulate diets that meet the requirements for both the microbes and the animal. The microbes in the rumen are allowed to have first access to any feeds the animal ingests. These microbes ferment the carbohydrates in the feed to generate the ATP they need for maintenance and growth. The end products of this fermentation come in the form of short chain fatty acids, which are absorbed across the rumen wall to be used as an energy substrate for the animal. The animal also depends on the higher quality microbial protein leaving the rumen, which the animal digests and uses as amino acids for its own growth and maintenance. The bacteria depend on nitrogen entering the rumen to synthesize amino acids. Starch fermenting bacteria mainly rely on peptides for their main source of nitrogen, while fiber fermenting bacteria rely mostly on ammonia for their nitrogen.[6] If there is inadequate supply of nitrogen to the rumen, microbial fermentation decreases, leading to a decrease in microbial growth, which decreases amino acid supply to the animal. Conversely, excess peptides and nitrogen to the rumen cause a rapid increase in short chain fatty acids and ammonia (NH_3), as can be seen in Table 1.[5] The increase in volatile fatty acids and lactic acid leads to a decrease in ruminal pH and

increased risk of subacute to acute ruminal acidosis, which can lead to a keratinized rumen and possibly death of the animal.[4] The subsequent increase in rumen NH_3 concentration also leads to an increased absorption across the rumen wall and increased blood urea nitrogen (BUN) level in the animal. These events have been shown to negatively impact fertility and conception rates.[7] Therefore, the balance of RDP and microbial nitrogen requirement needs to be maintained close to zero. To do this, the amount of RDP being fed and its rate of release into soluble fraction of the rumen contents needs to be accurately predicted. The aim of this study was to determine K_d of protein in differing feed stuffs to allow diet formulation that provides the required amount of RDP.

Methods

All feedstuffs were collected from the University of Missouri feed mill. All samples were dried at 55°C for 24 h and ground through a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 1-mm screen. All samples were then analyzed for 100% dry matter at 105°C for 24 h and for total nitrogen by combustion analysis (LECO FP-428; LECO Corporation, St. Joseph, MI).

Three separate experiments were performed for this study. The first experiment was an *in vitro* digestion of two sources of bloodmeal (BM-B & BM-C), dried distillers grains with solubles (DDGS), and soybean meal (SBM-A). Rumen fluid was obtained from two ruminally fistulated multiparous lactating Holstein cows provided *ad libitum* access to a lactation diet (240 g corn silage, 123 g alfalfa hay, 150 g alfalfa haylage, 467 g concentrate and 190 g CP, 240 g acid detergent fiber and 410 g neutral detergent fiber/kg DM) formulated to meet dairy cattle requirements[3] housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Center. The fluid was then strained through four layers of cheese cloth and mixed in a 1:4 ratio of strained fluid to McDougall's buffer to make inoculum. Three grams of each feed was then digested in 150mL of the inoculum in triplicate over the course of 48 hours. Samples were taken at hours 0, 4, 8, 12, 16, 20, 28, 36, and 44 for later analysis of NH_3 concentration. Ammonia was measured using the phenol-hypochlorite assay.[1]

A second *in vitro* experiment was conducted with another bloodmeal source (BM-A), casein (C), corn gluten feed (CGF), dried distillers grains (DDG-B), and soybean meal (SBM-B). This experiment was conducted with the method previously described with only 0.5 g of feed degraded in 25 mL of inoculum in triplicate and samples taken at hours 0, 4, 8, 12, 16, 24, 36, and 48 for later analysis of NH_3.

The third experiment was an *in situ* analysis of nitrogen degradation using dried distillers grains (DDG-A), soybean meal (SBM-C), and a rumen undegradable protein mix (RUP-A) consisting of several protein sources mixed to obtain approximately 50% RDP. Samples (1.0g) were weighed into 5cm x 10cm ANKOM rumen *in situ* bags (50 micron pore size, ANKOM Technology, Macedon, NY).

Samples were placed in mesh bags and briefly rinsed in warm water (37°C). Samples were placed in the rumen of two ruminally fistulated multiparous lactating dairy cows at different time intervals and then all removed at the same time to accommodate incubation times of 0, 4, 8, 12,

24, 36, 48, and 72 h. The 0 h samples were rinsed and placed in the rumen before being immediately removed with the rest of these samples. After removal, bags were run through 3 cold-water rinse cycles in a commercial washing machine for 10 min per rinse. Samples were then dried at 105°C for 48 h before being weighed and analyzed for total nitrogen.

For the *in vitro* experiments, nitrogen mass was calculated using the concentrations obtained from the NH_3 analysis. This mass was used with the initial mass of nitrogen in the sample to calculate the proportion of degraded nitrogen. The time point at which degradation reached extent (no further nitrogen was degraded) was set to 100% degradation of RDP. The amounts at each time point were then calculated as proportion of RDP degraded. The *in situ* experiment used direct measurement of remaining nitrogen in the sample to determine the degraded nitrogen and thus the proportion of RDP degraded.

Data for the experiments were analyzed using proportion of degradation as the dependent variable with flask as the experimental unit for the *in vitro* experiments and cow as the experimental unit for the *in situ* experiment. All statistical analyses were performed using the Proc GLM procedure in SAS® version 9.1.3 (SAS Institute Inc., Cary, NC) to determine the homogeneity of slope with the means adjusted to time as a covariate. Pair-wise comparisons were then done of each feed using a significance level of $P > 0.01$.

Results

Protein degradation rate was determined as slope of degradable RDP over time. The R^2 values for all regressions were greater than 0.91. Since these data were evaluated as proportions and were corrected for the extent of RDP degraded, all intercepts values were close to 1.0. The RDP K_d values ranged from 1.91-2.84% h^{-1} (Table 2). Analysis of these data showed SBM-A (2.83%), SBM-B (2.84%), and CGF (2.72%) to be at the higher range of the degradation rates. These three rates were significantly higher than all three BM feeds, which were at the lower end of measured rates (2.08%, 2.06%, & 1.99% for BM-A, B, & C, respectively). With the exception of DDG-B, all of the lower rates (< 2.22%) were animal protein sources; however, they are not significantly different from either of the DDG or DDGS. The RUP-A consists of both animal and plant proteins, and its rate fell in the middle of all feed samples with a rate not significantly different from other protein sources. Contrast estimates evaluating differences between *in situ* and *in vitro* techniques show no significant differences (P=0.26).

Discussion

In recent years, ruminant animal nutrition for domestic animals shifted to determining differing fractions of proteins based on rumen availability. This method of fractioning protein based on rumen availability has now been adopted by both the dairy and beef industries to help optimize performance and growth of their animals.[2, 3] Protein entering the rumen is fractionated into two components, rumen degradable protein (RDP) and rumen undegradable protein (RUP). RUP is resistant to degradation by the rumen and is passed out into the lower intestine to be degraded and utilized by the animal. RDP is the protein fraction which is able to be broken down and utilized by the rumen microbes.

In this study, the degradation of RDP was taken to extent, meaning the residence time in the rumen fluid was long enough to allow for the complete degradation of RDP. By measuring this disappearance over time, it was determined at which point this extent was reached. Feeds with less RDP reached extent faster than those with greater amounts of RDP. When these data were corrected for the amount of RDP, the degradation curves became linear and the plotted slopes became similar. These similar slopes suggest the protein source is immaterial to the rate of degradation, which is in opposition to the current assumptions. This means, when formulating diets for ruminants, the protein source is not as important as balancing the diet for RDP. Further, this study was able to examine the difference between *in vitro* and *in situ* methods for estimating the rate of degradation. The data show similar results can be obtained with the less laborious and faster bench top *in vitro* methods when compared to the *in situ* method, which requires multiple interactions with the rumen fluid donor animal and longer time for analysis due to washing and drying of samples. Although keeping fistulated cattle is still required to obtain the rumen inoculum, the benefits of the *in vitro* are the ability to run more samples with less effort using simpler NH_3 assays.

With the rumen passage rate estimated for an animal based on whether the animal is on a forage (~0.04% h^{-1}) or grain (~0.06% h^{-1}) based diet, the amount of protein released in the rumen over the total residence time in the rumen can be calculated. These data can then be matched with microbial protein requirement in the rumen.

LITERATURE CITED

1. G. A. Broderick and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of Dairy Science. 63:64-75
2. National Research Council. 2000. Nutrient Requirements of Beef Cattle, 7th rev. ed. Washington, DC: National Academy Press p. 120-125
3. National Research Council. 2001. Nutrient Requirements of Dairy Cattle, 7th rev. ed. Washington, DC: National Academy Press p. 43-104
4. F. N. Owens, D. S. Secrist, W. J. Hill and D. R. Gill. 1998. Acidosis in cattle: A review. Journal of Animal Science. 76:275-286
5. J. A. Pugh. 2007. Prediction of optimal rumen degradable protein levels in no-roughage, corn-based feedlot diets [M.S. Thesis]. University of Missouri, Columbia, MO.
6. J. B. Russell, J. D. O'Connor, D. G. Fox, P. J. Van Soest and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science. 70:3551-3561
7. S. Tamminga. 2006. The effect of the supply of rumen degradable protein and metabolisable protein on negative energy balance and fertility in dairy cows. Animal Reproduction Science. 96:227-239

Table 1. Effects of Rumen Degradable Protein (RDP) on fermentation characteristics.[5]

Item	% RDP			
	1.0	1.2	1.7	2.4
VFA (mM)[e]	126.2[c]	145.4[bc]	166.5[b]	201.1
Lactic Acid (mM)	50.3[a]	47.9[a]	0.7[b]	0.1[b]
OM Digestion (%)[f]	72.6[b]	71.5[b]	79.8[a]	79.6[a]
NH_3 (mM)	0.07[c]	0.22[c]	0.03[c]	12.02
MOEFF[g]	10.1[d]	11.3[cd]	14.7[b]	20.4[a]

[abcd] Means with no superscripts in common in the same row are significantly different ($P<0.05$)

[e] Total Volatile Fatty Acids

[f] Organic Matter Truly Digested

[g] Microbial Efficiency = g Bacterial N/ kg OM truly digested

Reproduced from Pugh, 2007

Table 2. Degradation rates of Rumen Degradable Protein (RDP) of different protein sources evaluated both *in situ* and *in vitro* methods.

Feed	Method	K_d[b]	Intercept	R^2	Feeds with differing K_d[b] (P<0.01)
Bloodmeal-A (BM-A)[c]	IV	-0.0208	0.9732	0.98	CGF, SBM-A, SBM-B
Bloodmeal-B (BM-B)[d]	IV	-0.0206	0.9959	0.97	CGF, SBM-A, SBM-B
Bloodmeal-C (BM-C)[e]	IV	-0.0199	0.9932	0.96	CGF, SBM-A, SBM-B
Casein (C)	IV	-0.0222	1.0544	0.98	SBM-A, SBM-B
Corn Gluten Feed (CGF)	IV	-0.0272	0.8828	0.94	BM-A, BM-B, BM-C, DDG-B
Dried Distillers Grains-A (DDG-A)	IS	-0.0261	0.9039	0.98	
Dried Distillers Grains-B (DDG-B)	IV	-0.0191	1.0377	0.94	CGF, SBM-A, SBM-B
Dried Distillers Grains w/ Solubles (DDGS)	IV	-0.0223	1.1204	1.00	SBM-B
Soybean Meal-A (SBM-A)	IV	-0.0283	0.9507	0.98	BM-A, BM-B, BM-C, C, DDG-B
Soybean Meal-B (SBM-B)	IV	-0.0284	1.0225	0.99	BM-A, BM-B, BM-C, C, DDG-B, DDGS
Soybean Meal-C (SBM-C)	IS	-0.0247	0.8985	0.91	DDG-B
RUP Mix (RUP-A)[f]	IS	-0.0237	0.8189	0.98	

[a] IV = *in vitro*; IS = *in situ*

[b] K_d = Degradation rate of Rumen Degradable Protein (RDP) = slope of the degradation curve

[c] Porcine Spray-dried Blood Cells AP 301G

[d] Porcine Spray-dried Bloodmeal (Missouri Farmers Association (MFA) Inc., Columbia, MO)

[e] Porcine Flash-dried Red Blood Cells (Hormel Foods Corporation, Austin, MN)

[f] Rumen Undegradable Protein Mix - Mixed ration of multiple protein sources with 50% RDP