

Comparison of In Situ and In Vitro Techniques for Measuring Ruminal Degradation of Animal By-Product Proteins¹

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ABSTRACT

Ruminally undegraded protein (RUP) values of blood meal (n = 2), hydrolyzed feather meal (n = 2), fish meal (n = 2), meat and bone meal, and soybean meal were estimated using an in situ method, an inhibitor in vitro method, and an inhibitor in vitro technique applying Michaelis-Menten saturation kinetics. Degradation rates for in situ and inhibitor in vitro methods were calculated by regression of the natural log of the proportion of crude protein (CP) remaining undegraded versus time. Nonlinear regression analysis of the integrated Michaelis-Menten equation was used to determine maximum velocity, the Michaelis constant, and degradation rate (the ratio of maximum velocity to the Michaelis constant). A ruminal passage rate of 0.06/h was assumed in the calculation of RUP. The in situ and inhibitor in vitro techniques yielded similar estimates of ruminal degradation. Mean RUP estimated for soybean meal, blood meal, hydrolyzed feather meal, fish meal, and meat and bone meal were, respectively, 28.6, 86.0, 77.4, 52.9, and 52.6% of CP by the in situ method and 26.4, 86.1, 76.0, 59.6, and 49.5% of CP by the inhibitor in vitro technique. The Michaelis-Menten inhibitor in vitro technique yielded more rapid CP degradation rates and decreased estimates of RUP. The inhibitor in vitro method required less time and labor than did the other two techniques to estimate the RUP values of animal by-product proteins. Results from in vitro incubations with pepsin-HCl suggested that low postruminal digestibility of hydrolyzed

feather meal may impair its value as a source of RUP. (**Key words:** in vitro, in situ, ruminal protein degradation)

Abbreviation key: **BM** = blood meal, **FM** = fish meal, **HFM** = hydrolyzed feather meal, **IIV** = inhibitor in vitro, **IS** = in situ, **MBM** = meat and bone meal, **MMI** = Michaelis-Menten saturation kinetics applied to IIV procedure, **SBM** = soybean meal, **SRF** = strained ruminal fluid, **TAA** = total AA.

INTRODUCTION

Formulation of diets for RUP requires accurate estimation of the ruminal CP degradation of feedstuffs. Numerous in vitro and in vivo techniques have been developed to estimate ruminal CP degradation (17). In situ (**IS**) procedures are used most commonly to evaluate ruminal CP degradation because they utilize the actual ruminal environment (17). However, IS procedures are time-consuming and labor intensive. The inhibitor in vitro (**IIV**) procedure (2) yielded estimates of ruminal degradation of plant protein supplements that were similar to those yielded by IS procedures (7). Proteins in animal by-products often are very resistant to ruminal degradation (21) but can vary substantially in ruminal CP degradability (3). This study evaluated IS and IIV procedures (2), including a new approach in which Michaelis-Menten saturation kinetics were applied to the IIV procedure (**MMI**) (5), to assess ruminal CP degradation of several animal by-product proteins.

MATERIALS AND METHODS

Composite samples were prepared from two batches of flash-dried blood meal (**BM**; 1 and 2), two batches of hydrolyzed feather meal (**HFM**; 1 and 2), and one batch of meat and bone meal (**MBM**), all of which were obtained from National By-Products, Inc. (Des Moines, IA). Two batches of menhaden fish meal (**FM**), one of nonruminant grade FM with high

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solubles (Special Select™; **FM 1**) and another of ruminant grade FM with low solubles (Sea Lac™; **FM 2**), were obtained from Zapata-Haynie Co. (Hammond, LA). One batch of solvent-extracted soybean meal (**SBM**) was obtained locally (Madison, WI). These protein sources, which were fed in a lactation study reported elsewhere (10), were analyzed for DM and total Kjeldahl N (1). The proteins were evaluated for ruminal degradability using an IS method, an IIV method using limited substrate, and the MMI technique and for post-ruminal digestibility using a modified pepsin-HCl method.

IS

The IS procedure used in this study was recommended by Nocek (17). Dacron bags, 9 × 12 cm (52- μ m pore size; Marvelaire; Ankom, Fairport, NY), were filled with 2 g of ground (2-mm screen; Wiley mill; Arthur H. Thomas Co., Philadelphia, PA) sample. Duplicate bags at each time point were incubated in the ventral rumen of two cows in reverse order (i.e., bags were inserted into the rumen such that all were removed simultaneously) for 4, 8, 12, 16, 20, 24, 36, 48, 72, and 96 h. After removal from the rumen, bags were immediately soaked in ice water and transferred to a washing machine for rinsing (8). Zero-hour bags were soaked in tepid tap water for 30 min and were washed with the other bags to estimate the soluble (degraded) CP fraction (A). In situ incubations were replicated three times (twice in one cow and once in the other). Bags were dried for 48 h at 60°C in a forced-air oven and weighed; the entire bag was then placed into a Kjeldahl flask for CP analysis (1). The undegradable CP fraction (C) was defined as the CP remaining in the bag after 96 h. The potentially degradable CP (fraction B) was defined as 100 - (A + C). Fractional degradation rate (k_d) was determined as the slope of the regression on time of the natural logarithm of CP remaining in the bag minus fraction C. Ruminal CP escape (RUP) was calculated accordingly:

$$\text{RUP (percentage)} = B(k_p / (k_d + k_p)) + C \quad [1]$$

where ruminal passage rate (k_p) was assumed to be 0.06/h, which was similar to that reported for dairy cows in an early stage of lactation (11, 19).

Limited Substrate IIV

Samples were ground (1-mm screen; Wiley mill) and weighed into duplicate 50-ml centrifuge tubes so that each tube contained 1.875 ± 0.025 mg of N. Five

milliliters of McDougall's buffer (14) were added to each sample and allowed to soak for 60 to 90 min at 39°C. The IIV procedure was modified from that used earlier (2) in that the strained ruminal fluid (**SRF**) plus buffer mixture was placed in dialysis tubing and dialyzed at 39°C for 2 h in a saline bath to remove most of the background NH_3 and total AA (**TAA**) that were present initially. Duplicate samples were incubated for 0 and 4 h at 39°C after addition of 10 ml of SRF buffer inoculum. Inhibitor concentrations were 1.0 mM hydrazine and 30 μ g of chloramphenicol/ml, which were added to suppress microbial uptake of NH_3 and TAA and to allow quantitative recovery of the end products from CP degradation. Incubations were stopped by the addition of 5% (wt/vol) TCA and placement of the tubes on ice for 30 min. Zero-hour incubations were treated with TCA after the samples were soaked in buffer, but before inoculum was added, to prevent microbial protein degradation. After TCA treatment, samples were centrifuged ($15,300 \times g$ at 4°C for 15 min), and supernatant fractions were stored at 4°C until analyzed the next day for NH_3 and TAA by a semiautomated method (6) modified to include dialysis in both manifolds and automated data collection and analysis. Standard curves for both NH_3 and TAA were prepared by the addition of known amounts of NH_3 and leucine to separate 0-h blank tubes. Four blank tubes were incubated at both 0 and 4 h for every 50 tubes that contained added protein. Net release of NH_3 and TAA was defined as the concentration difference between tubes containing added protein and the mean blank. The degraded CP fraction (A_0), defined as the proportion of total N present as NH_3 and TAA at 0 h, was computed using a ratio of TAA to total N of 50 μ mol/mg of N for all protein sources (5). The potentially degradable CP fraction present at 0 h (B_0) was defined as 100 - A_0 . The CP fraction remaining undegraded at 4 h (B_4) was defined as 100 - A_4 , where A_4 was computed from the net release at 4 h of NH_3 and TAA as was defined for A_0 . Undegraded fractions were not corrected for the undegradable fraction (C). The degradation rate (k_d ; expressed per hour) was calculated as

$$k_d = (\ln B_4 - \ln B_0) / 4 \text{ h} \quad [2]$$

Estimated ruminal CP escape values were computed by substituting B_0 for B in Equation [1] and assuming C = 0. The IIV incubations were replicated on 3 different d with three separate batches of ruminal inoculum.

MMI

The MMI method was used as described previously (5). Samples were ground (1-mm screen; Wiley mill) and weighed into 50-ml centrifuge tubes such that increasing amounts of N were in consecutive tubes. Samples equivalent to 1.1, 2.2, 4.5, 9.0, 13.5, and 18.0 \pm 0.02 mg of N per tube were weighed into duplicate tubes; two 0-h tubes with 200 \pm 2 mg of air-dried sample also were weighed to estimate the degraded CP fraction (A_0); B_0 was defined as described for the IIV method. Incubations were carried out for 2 h and analyzed for NH_3 and TAA as described previously for the IIV method. Velocity of protein degradation, v , was computed at each N level for each protein source:

$$v(\text{milligrams of N per hour per milliliter of SRF}) = [\text{milligrams of } NH_3 \text{ N} + \text{micromoles of TAA}/(50 \mu\text{mol/mg of N})]/(2 \times 4.5) \quad [3]$$

where 2 is the time (hours of incubation, and 4.5 = equivalent volume (milliliters) of SRF in 10 ml of inoculum. Fractional degradation rate (k_d), the tangent through the origin of the plot of v versus total N concentration, is given by the ratio of maximum velocity to Michaelis constant: $k_d = V_{\max}/K_m$. This ratio was estimated using nonlinear regression methods described previously (5). As with the IIV method, estimated ruminal protein escape was computed by substitution of B_0 for B in Equation [1] and assuming $C = 0$. A total of 14 tubes was used per sample in each incubation; MMI incubations were replicated on each of 3 d with three separate batches of ruminal inoculum.

Pepsin-HCl

The AOAC (1) acid pepsin method was used to estimate postruminal protein digestion. Five grams (air-dried basis) of ground sample (1-mm screen; Wiley mill) were weighed in duplicate into folded, labeled Whatman number 4 filter papers (18.5 cm; Arthur H. Thomas); placed into ether extraction cylinders; and extracted for 72 h to remove lipid (1). After samples were dried for 24 h in a 60°C forced-air oven, they were cooled, and triplicate 0.5-g aliquots were weighed into 200-ml teflon-capped jars. Fresh, prewarmed (42 to 45°C) pepsin solution [150 ml of 0.002% (wt/vol) pepsin (activity, 1:10,000) in 0.075N HCl] was added to each jar. Jars were laid on their sides in a 45°C incubator-shaker and continuously agitated ($8 \times g$) for 16 h. After incubation, jars were removed and allowed to sit for 15 min. Residues were filtered through Whatman number 4 filter paper

(12.5 cm) using vacuum filtration. Residues and filter papers were rinsed with acetone, dried overnight in a 60°C forced-air oven, and then transferred directly to Kjeldahl flasks for determination of CP (1). Pepsin CP digestibilities (percentages) were calculated as

$$\text{digestible CP} = [1 - (\text{residual CP}/\text{total CP})] \times 100. \quad [4]$$

Statistical Analyses

The general linear models procedure of SAS (18) was used for ANOVA on results from the IS, IIV, and MMI incubations. The statistical model included protein source and incubation run. When effects caused by protein source were significant ($P < 0.01$), mean separation was by least significant difference at $P = 0.05$. Regression analyses (18) of IS on IIV degradation rates and IIV on MMI degradation rates also were conducted.

RESULTS AND DISCUSSION

The DM and CP concentrations of the samples are presented in Table 1. The CP values were similar to those of NRC (16) except for BM samples, which were 8 and 10 percentage units higher. Mansfield et al. (13) found similar CP contents in BM, FM, and HFM. Protein escape values estimated by the IS method were comparable to the RUP reported by the

TABLE 1. Composition and estimated digestibilities of animal by-product proteins that were compared using three methods of estimating RUP.

| Protein source ¹ | DM | CP | Digestible protein ² | |
|-----------------------------|------|-----------|---------------------------------|-----|
| | (%) | (% of DM) | \bar{X} (% of CP) | SD |
| SBM ³ | 89.9 | 45.3 | 92.9 ^a | 1.1 |
| MBM | 90.0 | 59.3 | 76.8 ^b | 0.5 |
| BM 1 | 88.7 | 95.2 | 97.9 ^a | 0.0 |
| BM 2 | 89.0 | 97.2 | 98.7 ^a | 0.1 |
| FM 1 | 87.8 | 72.1 | 95.0 ^a | 1.1 |
| FM 2 | 89.0 | 71.6 | 95.3 ^a | 0.4 |
| HFM 1 | 92.9 | 80.7 | 8.5 ^d | 0.8 |
| HFM 2 | 94.5 | 88.0 | 33.2 ^c | 0.2 |

^{a,b,c,d}Means in columns with different superscripts differ ($P < 0.05$).

¹SBM = Soybean meal, MBM = meat and bone meal, BM = blood meal, FM = fish meal, and HFM = hydrolyzed feather meal.

²Abomasally plus intestinally digestible protein estimated using pepsin-HCl (1).

³Solvent-extracted.

NRC (16) for the same proteins (Table 2). Estimated RUP for the animal by-product proteins was 24 to 57 percentage units higher than that for SBM. Proportions of CP in the rapidly degradable fractions (A) were similar among SBM, MBM, FM 1, and FM 2. The animal by-product proteins exhibited ruminal CP degradation rates that were 8 to 40 times slower than those for SBM. The ruminally undegraded fractions (C) estimated by the IS method also were much larger for the animal proteins (Table 2), ranging from 16% (MBM) to 69% (BM 1) of CP; SBM had 0% CP in fraction C. Animal proteins BM 1 and HFM 1 contained the smallest fractions A and B, the largest fraction C, the slowest IS degradation rates, and, consequently, the highest estimates of RUP (Table 2). Nocek (17) reported that protein escape values estimated by IS techniques might not represent the CP truly available for postruminal digestion because IS measurements are influenced by bag porosity, particle size, ratio of sample to bag surface area, dietary effects, and microbial contamination. Despite these potential errors and the different sources of our samples, our IS estimates of RUP (Table 2) closely approximated those previously reported (16, 20) for these animal by-product proteins.

The degradation characteristics determined using IIV for the animal by-product proteins, including BM 2 and HFM 2, are presented in Table 3. Values obtained for two standard SBM, one each of solvent SBM and expeller SBM, were similar to those

reported previously for the same samples (2). Large differences existed between the potentially degradable CP fraction (B) estimated by the IS technique and the undegraded fraction (B_0) determined using the IIV method. Fraction B ranged from 17 to 67% of CP (Table 2) and fractions B_0 ranged from 97.4 to 99.7% of CP (Table 3). These fractions are not equivalent. Fraction B_0 , as calculated by the IIV method included soluble, intact protein and was determined as the difference between 100% and the fraction of total N present as degraded products, NH_3 and TAA, at 0 h (A_0). The potentially degradable CP fraction (B) calculated by the IS method was determined as CP remaining in the bag at 0 h minus residual CP at 96 h (fraction C). The undegraded CP fraction (B_0), as determined by the IIV method, and was not discounted for the undegradable fraction C. The sums of fractions B and C ranged from 59 to 87% of CP (Table 2) and were closer in magnitude to fraction B_0 (Table 3). Previously, ADIN (22) or the CP remaining undigested after long-term exposure to proteases (2, 12) was used to estimate the indigestible fraction C. However, fraction C was not determined because the IS estimate of RUP also was not discounted for fraction C.

Degradation rates determined by the IS and IIV techniques ranked the proteins similarly. Linear regression of IS rates on IIV rates for the six proteins tested by both methods yielded a significant coefficient of determination ($r^2 = 0.948$; regression sums of

TABLE 2. Crude protein fractional degradation rates (k_d), CP degradation fractions, and RUP contents of soybean meal (SBM) and selected animal protein by-products estimated by the in situ method.

| Protein source ¹ | k_d (/h) | Fraction A ² Fraction B Fraction C | | | RUP ³ |
|-----------------------------|--------------------|--|-------------------|-------------------|-------------------|
| | | (% of CP) | | | |
| SBM ⁴ | 0.080 ^a | 33.5 ^{ab} | 66.5 ^a | 0.1 ^c | 28.6 ^c |
| MBM | 0.013 ^b | 39.6 ^a | 43.9 ^b | 16.4 ^b | 52.6 ^b |
| BM 1 ⁵ | 0.002 ^c | 13.4 ^c | 18.1 ^c | 68.5 ^a | 86.0 ^a |
| FM 1 | 0.010 ^b | 41.2 ^a | 37.2 ^b | 21.6 ^b | 52.9 ^b |
| FM 2 | 0.014 ^b | 35.2 ^{ab} | 48.4 ^b | 16.5 ^b | 55.6 ^b |
| HFM 1 ⁵ | 0.002 ^c | 21.9 ^{bc} | 17.4 ^c | 60.7 ^a | 77.4 ^a |
| SEM | 0.002 | 3.8 | 4.7 | 2.9 | 3.0 |
| $P > F$ | <0.001 | 0.007 | <0.001 | <0.001 | <0.001 |

a,b,c Means in columns with different superscripts differ ($P < 0.05$).

¹MBM = Meat and bone meal, BM = blood meal, FM = fish meal, and HFM = hydrolyzed feather meal.

²Fraction A = Soluble (degraded) CP fraction, fraction B = potentially degraded CP fraction, and fraction C = undegradable CP fraction.

³Estimated RUP (percentage) = $B(k_p/(k_d + k_p)) + C$, where k_p = fraction ruminal passage rate of 0.06/h.

⁴Solvent-extracted.

⁵Only BM 1 and HFM 1 were incubated in situ.

squares = 0.0042) and an intercept (-0.006) that was not different from 0 ($P > 0.27$). However, CP degradation rates estimated by the IS method were considerably slower than those determined by the IIV method: the slope of the regression was 0.49. Previously, degradation rates estimated for seven protein concentrates by a similar IS method averaged 35% of IIV rates (7).

The CP escape values estimated using the IIV method were within 3 percentage units of the escape values estimated using the IS method for SBM, MBM, BM 1, and HFM 1. However, IIV estimates of RUP for FM 1 and FM 2 (Table 3) were 5 and 14 percentage units higher than those obtained by the IS method (Table 2). Broderick et al. (7) found the opposite trend for FM; CP escape values were 9 percentage units lower when calculated by the IIV method than when calculated by the IS method at an assumed ruminal passage rate of 0.05/h. Estimates of CP escape of 46 and 63% were reported previously for nonruminant grade FM and ruminant grade FM, respectively (3). The IS method indicated that nonruminant grade FM 1 was not different from ruminant grade FM 2 (Table 2); however, RUP of FM 2 (ruminant grade FM) was greater than the RUP of FM 1 (nonruminant grade FM) according to the IIV method (Table 3). Otherwise, these data indicated that the IIV and IS methods yielded similar estimates of RUP for animal by-product proteins.

Ruminal degradation characteristics of the animal by-product proteins obtained using the MMI technique are presented in Table 4. The MMI method yielded more rapid degradation rates for all proteins than did either the IS or IIV methods. Linear regression of IIV rates on MMI rates yielded a significant coefficient of determination ($r^2 = 0.991$; regression sum of squares = 0.019) and a slope of 0.80. Rates obtained by the IIV and MMI methods ranked the proteins identically. Degradation rates and CP escape values obtained for the standard solvent and expeller SBM were similar to those found previously by the MMI method for these same samples (5). Relative degradation rates, computed by setting the rate obtained by the IIV and MMI methods for expeller SBM to 1.0, were more similar than the actual rates (Tables 3 and 4). As a consequence of the more rapid rates, estimates of RUP were lower for the MMI method than for either the IIV or the IS method. Differences in estimated RUP between methods averaged 13 percentage units (IS minus MMI; six samples) and 16 percentage units (IIV minus MMI; eight samples). The MMI technique yielded the same relative rankings as did the IIV technique for estimated CP escape values. Differences among the proteins in observed Michaelis constants and maximum velocities were not as great as differences in degradation rates and CP escape values (Table 4). Previously, Michaelis constants differed by a factor of 20, and maximum

TABLE 3. Crude protein fractional degradation rates (k_d), degradable CP fractions (B_0), and RUP contents of proteins estimated by the inhibitor in vitro method.

| Protein source ¹ | k_d (/h) | Relative rate ² | Fraction B_0 —— (% of CP) —— | RUP ³ |
|-----------------------------|--------------------|----------------------------|-----------------------------------|--------------------|
| Standard protein | | | | |
| Solvent SBM | 0.158 | 5.45 | 99.1 | 27.3 |
| Expeller SBM | 0.029 | 1.00 | 98.7 | 66.5 |
| Experimental protein | | | | |
| SBM ⁴ | 0.167 ^a | 5.78 ^a | 98.5 ^c | 26.0 ^g |
| MBM | 0.061 ^b | 2.13 ^b | 98.1 ^d | 48.6 ^f |
| BM 1 | 0.010 ^e | 0.33 ^f | 99.7 ^a | 86.0 ^b |
| BM 2 | 0.004 ^e | 0.13 ^f | 99.6 ^a | 94.3 ^a |
| FM 1 | 0.041 ^c | 1.43 ^c | 97.4 ^e | 58.1 ^e |
| FM 2 | 0.024 ^d | 0.84 ^{de} | 97.9 ^d | 69.9 ^{cd} |
| HFM 1 | 0.019 ^d | 0.66 ^e | 99.0 ^b | 75.3 ^c |
| HFM 2 | 0.027 ^d | 0.94 ^d | 98.9 ^b | 68.2 ^d |
| SEM | 0.003 | 0.84 | 0.1 | 1.9 |
| $P > F$ | <0.001 | <0.001 | <0.001 | <0.001 |

^{a,b,c,d,e,f,g}Means in columns for the eight experimental proteins with different superscripts differ ($P < 0.05$).

¹SBM = Soybean meal, MBM = meat and bone meal, BM = blood meal, FM = fish meal, and HFM = hydrolyzed feather meal.

²Degradation rate expressed as a proportion of that observed for expeller SBM, which was set = 1.0.

³Estimated RUP (percentage) = $B_0(k_p/(k_d + k_p))$, where k_p = the ruminal passage rate of 0.06/h.

⁴Solvent-extracted.

velocities differed only by a factor of 7, among proteins with MMI degradation rates ranging from 0.047 to 0.989/h (5).

The MMI approach was developed to aid the quantification of very slow degradation rates. For example, proteins in two forages containing tannin, lespedeza and sainfoin, which had degradation rates <0.01/h according to the IIV method had rates of 0.03 to 0.05/h by the MMI method (4). Similar results were obtained in the present study. Degradation rates for BM 1 and BM 2 were, respectively, 0.010 and 0.004/h by the IIV method and 0.022 and 0.016/h by the MMI method.

From our results, it cannot be determined whether the MMI, IIV, or IS technique best predicted in vivo RUP or animal response; however, the IS method may be criticized on theoretical grounds. At 0 h and later, substantial amounts of intact (undegraded) protein that is soluble or present in small particles may wash out of the IS bag, leading to overestimation of CP degradation. Microbial contamination of the IS residue results in an underestimation of CP degradation. Although these two sources of error tend to cancel each other out, the magnitude of this compensation likely will not be quantitatively correct. The abbreviated IIV method, for which the extent of degradation was determined at 0 and 4 h only, yielded similar estimates of RUP as did the IS method. The IIV technique was much more rapid and

required substantially less labor than did the IS method and, thus, may be more useful for screening animal by-product proteins for RUP value. The MMI technique required 3.5 times more analyses than the IIV method (14 vs. 4 tubes per protein). It is not clear from this work whether the more rapid degradation rates obtained by the MMI technique would yield more accurate estimates of in vivo RUP values for the animal by-product proteins than the estimates made by the IIV method.

Substantial disparity was observed between the pepsin-HCl residue (an estimate of CP that is indigestible in the abomasum and small intestine) and the ruminal undegradable fraction (C; residual N after 96 h of IS incubation). Four of the protein sources (SBM, BM 1, FM 1, and FM 2) showed little relationship between the size of fraction C (range, 0 to 69%; Table 2) and the pepsin-HCl residue (range, 1 to 7%; Table 1); MBM had an intermediate pepsin-HCl residue (23%) and an intermediate fraction C (17%). However, the second largest fraction C was found for HFM 1 (61%) and corresponded to the largest pepsin-HCl residue (91%). Protein HFM 2, for which fraction C was not determined, had the second lowest pepsin-HCl digestibility. Menden and Cremer (15) reported that a similar pepsin-HCl in vitro method was effective for predicting apparent protein digestibility in the rat. Craig and Broderick (9) observed, for heat-treated cottonseed meals, a high

TABLE 4. Crude protein fractional degradation rates (k_d), degradable CP fractions (B_0), RUP contents, Michaelis constants (K_m), and maximum velocities (V_{max}) of proteins estimated by the Michaelis-Menten inhibitor in vitro method.

| Protein source ¹ | k_d (/h) | Relative rate ² | Fraction B_0 ————— (% of CP) ————— | RUP ³ | K_m (mg of N/h) | V_{max} (mg of N/h per ml) |
|-----------------------------|---------------------|----------------------------|---|-------------------|----------------------|---------------------------------|
| Standard protein | | | | | | |
| Solvent SBM | 0.208 | 5.59 | 99.0 | 22.3 | 0.54 | 0.112 |
| Expeller SBM | 0.037 | 1.00 | 99.4 | 61.2 | 3.92 | 0.145 |
| Experimental protein | | | | | | |
| SBM ⁴ | 0.219 ^a | 5.88 ^a | 98.8 ^c | 21.4 ^g | 0.53 ^c | 0.115 ^a |
| MBM | 0.099 ^b | 2.65 ^b | 98.0 ^d | 37.3 ^f | 1.12 ^c | 0.111 ^a |
| BM 1 | 0.022 ^{ef} | 0.59 ^{ef} | 99.7 ^a | 73.0 ^b | 3.26 ^a | 0.071 ^c |
| BM 2 | 0.016 ^f | 0.42 ^f | 99.8 ^a | 79.2 ^a | 2.71 ^{ab} | 0.042 ^d |
| FM 1 | 0.076 ^c | 2.04 ^{bc} | 97.2 ^e | 43.4 ^e | 1.19 ^c | 0.087 ^{bc} |
| FM 2 | 0.042 ^{de} | 1.14 ^{de} | 97.9 ^f | 57.5 ^c | 1.99 ^b | 0.083 ^{bc} |
| HFM 1 | 0.044 ^d | 1.17 ^{de} | 99.2 ^b | 57.5 ^c | 2.24 ^b | 0.095 ^{ab} |
| HFM 2 | 0.056 ^{cd} | 1.51 ^{cd} | 99.3 ^b | 51.3 ^d | 1.98 ^b | 0.111 ^a |
| SEM | 0.007 | 0.21 | 0.03 | 1.3 | 0.25 | 0.007 |
| $P > F$ | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

a,b,c,d,e,f,g Means in columns for the eight experimental proteins with different superscripts differ ($P < 0.05$).

¹SBM = Soybean meal, MBM = meat and bone meal, BM = blood meal, FM = fish meal, and HFM = hydrolyzed feather meal.

²Degradation rate expressed as a proportion of that observed for expeller SBM, which was set = 1.0.

³Estimated RUP (percentage) = $B_0(k_p/(k_d + k_p))$, where k_p = the ruminal passage rate of 0.06/h.

⁴Solvent-extracted.

coefficient of determination ($r^2 = 0.996$) for true digestibility of CP in the rat and AA release in an *in vitro* pepsin-pancreatin assay. Mansfield et al. (13) found that a mixture of animal by-product proteins, in which HFM provided 46% of CP, resulted in lower NH_3 and greater NAN flow when infused into continuous culture ruminal fermentors. However, compared with SBM, this dietary mixture lowered milk protein content and yield of lactating cows. Waltz et al. (21) observed that ruminal escape values were greater, but intestinal AA absorption was lower, for HFM protein than for SBM or BM. Poor effectiveness of HFM as a RUP source, despite substantial resistance to ruminal degradation, might have been due to low intestinal digestibility.

CONCLUSIONS

Ruminal CP degradabilities of SBM and four animal by-product proteins (MBM, BM, HFM, and FM) were estimated by the IS method, and the IIV technique using limited substrate, and the MMI technique. The most rapid degradation rates were obtained using the MMI method and the least rapid rates were obtained using the IS method; IIV rates were intermediate. Linear regression indicated that degradation rates estimated by either the IIV or MMI technique were highly correlated with those estimated by the IS method. Ruminal protein escape values by the IS and IIV methods were comparable; the MMI technique yielded lower estimates of ruminal escape. All three procedures ranked the animal by-product proteins similarly for degradation rate and ruminal escape. Of these three methods, the IIV method was the most rapid and required the least labor. *In vitro* pepsin-HCl incubations suggested that low intestinal digestibility of HFM might impair its usefulness as an RUP source.

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REFERENCES

- 1 Association of Official Analytical Chemists. 1990. Official Methods of Analysis, Vol. I. 15th ed. AOAC, Arlington, VA.
- 2 Broderick, G. A. 1987. Determination of protein degradation rates using a rumen *in vitro* system containing inhibitors of microbial nitrogen metabolism. *Br. J. Nutr.* 58:463.
- 3 Broderick, G. A. 1992. Relative value of fish meal versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. *J. Dairy Sci.* 75:174.
- 4 Broderick, G. A., and K. A. Albrecht. 1997. Ruminal *in vitro* degradation of protein in tannin-free and tannin-containing forage legume species. *Crop Sci.* 37:1884.
- 5 Broderick, G. A., and M. K. Clayton. 1992. Rumen protein degradation rates estimated by non-linear regression analysis of Michaelis-Menten *in vitro* data. *Br. J. Nutr.* 67:27.
- 6 Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* data. *J. Dairy Sci.* 63:64.
- 7 Broderick, G. A., R. J. Wallace, E. R. Ørskov, and L. Hansen. 1988. Comparison of estimates of ruminal protein degradation by *in vitro* and *in situ* methods. *J. Anim. Sci.* 66:1739.
- 8 Cherney, D.J.R., J. A. Patterson, and R. P. Lemenager. 1990. Influence of *in situ* bag rinsing technique on determination of dry matter disappearance. *J. Dairy Sci.* 73:391.
- 9 Craig, W. M., and G. A. Broderick. 1981. Effect of heat treatment on true digestibility in the rat, *in vitro* proteolysis and available lysine content of cottonseed meal protein. *J. Anim. Sci.* 52:292.
- 10 England, M. L. 1993. Utilization of animal protein by-products by lactating dairy cows. M.S. Thesis, Univ. Wisconsin, Madison.
- 11 Hartnell, G. F., and L. D. Satter. 1979. Extent of particulate marker (samarium, lanthanum and cerium) movement from one digesta particle to another. *J. Anim. Sci.* 48:381.
- 12 Krishnamoorthy, U., C. J. Sniffen, M. D. Stern, and P. J. Van Soest. 1983. Evaluation of a mathematical model of rumen digestion and an *in vitro* simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. *Br. J. Nutr.* 50:555.
- 13 Mansfield, H. R., M. D. Stern, and D. E. Otterby. 1994. Effects of beet pulp and animal by-products on milk yield and *in vitro* fermentation by rumen microorganisms. *J. Dairy Sci.* 77:205.
- 14 McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. *Biochem. J.* 43:99.
- 15 Menden, E., and H. D. Cremer. 1970. Laboratory methods for the evaluation of changes in protein quality. Page 123 *in* *Newer Methods of Nutritional Biochemistry*. Vol. IV. A. A. Albanese, ed. Acad. Press, New York, NY.
- 16 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Update. Natl. Acad. Sci., Washington, DC.
- 17 Nocek, J. E. 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. *J. Dairy Sci.* 71:2051.
- 18 SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.
- 19 Shaver, R. D., A. J. Nytes, L. D. Satter, and N. A. Jorgenson. 1986. Influence of amount of feed intake and forage physical form on digestion and passage of prebloom alfalfa hay in dairy cows. *J. Dairy Sci.* 69:1545.
- 20 Stern, M. D., and H. R. Mansfield. 1989. Animal byproducts as protein supplements for ruminants. Page 118 *in* Proc. 50th Minnesota Nutr. Conf., Univ. Minnesota, St. Paul.
- 21 Waltz, D. M., M. D. Stern, and D. J. Illg. 1989. Effect of ruminal protein degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. *J. Dairy Sci.* 72:1509.
- 22 Yang, J. H., G. A. Broderick, and R. G. Koegel. 1993. Effect of heat treating alfalfa hay on chemical composition and ruminal *in vitro* protein degradation. *J. Dairy Sci.* 76:154.