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Evaluation of Selected Mathematical Approaches to the Kinetics of Protein Degradation In Situ¹

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ABSTRACT: A linear model, two mathematical nonlinear models, and a curve-peeling procedure were used to estimate rate and extent of ruminal CP degradation of meat and bone meal (MBM) and soybean meal (SBM) from data obtained using the in situ Dacron polyester bag technique. Most of the values for extent of CP degradation of MBM were lowest when determined using curve peeling or the nonlinear models. In general, rates and extents of CP degradation of MBM estimated using the linear model and including ruminal incubations up to 12 h were greater than those obtained with the linear model and including ruminal incubations up to 24 h or up to 72 h. In addition, the models ranked the MBM samples differently for rate and extent of CP degradation. The results of the lack-of-fit test indicated that the linear model was inappropriate for estimating rate of degradation of MBM. However, CP degradation for SBM could be described by the linear model if long ruminal incubation times (greater than 48 h) were included in the calculations. Regression analyses were conducted

to evaluate various compositional characteristics as predictors of CP degradation for MBM. Most of the correlation coefficients between CP degradation and the same independent variables were greater when the nonlinear models and curve peeling were used compared with the linear model. In general, the correlation coefficients between extent of CP degradation and the independent variables obtained with the linear model increased as the duration of ruminal incubations included in the model increased. Lysine concentrations, followed by CP solubility and ash content, were the best predictors of ruminal degradation of MBM protein. When using a specific mathematical model to predict CP degradation, analysis of residuals vs fitted and lack-of-fit tests should be performed to assess the validity of the model to describe the degradation patterns of the protein source under consideration. Also, long (at least 48 h) ruminal incubation times may be needed to correctly describe the pattern of CP degradation for MBM.

Key Words: Mathematical Models, Meat and Bone Meal, Soybean Oilmeal, Protein Degradation, Rumen

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Introduction

Current guidelines recommend formulation of diets for ruminal undegradable protein (**RUP**) to increase amino acid flow to the duodenum. However, positive responses to RUP supplementation have not always been found (McCarthy et al., 1989; Hussein et al.,

1991). Lack of animal response to RUP supplementation has been attributed to various factors, such as poor amino acid profile of the RUP supplements fed or depression in microbial N flow. Moreover, the technique and mathematical models used for estimating RUP can affect the values obtained (Nocek and English, 1986). The in situ technique is the most commonly used procedure for estimating ruminal degradation of protein supplements. This technique involves the incubation of feed samples in polyester or nylon bags in the rumen and determination of CP disappearance from the bags after various incubation times. The data are then commonly fitted to linear (Mathers and Miller, 1981) or nonlinear (Ørskov and McDonald, 1979; Mertens and Lofton, 1980) models to estimate rate of CP degradation. Curve peeling (Shipley and Clark, 1972) has also been used to describe kinetics of CP degradation. Even though these mathematical approaches are widely applied to

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Table 1. Source of raw material and processing conditions used for various meat and bone meal samples

| Sample number | Raw materials | Temperature (°C)/Time (h) ^a |
|---------------|---------------------------------|--|
| 1 | 40% beef, 25% pork, 35% poultry | 138–152 / 1.5 |
| 2 | 45% beef, 55% poultry | beef: 130 / 2.4; poultry: 130 / 4.0 |
| 3 | 100% beef | 130 / continuous flow |
| 4 | 90% beef, 10% fallen animal | 130 / .75 |
| 5 | 100% cattle | 100 / .75 |
| 6 | 100% pork | 138 / 2.0 |
| 7 | 90% beef, 10% pork | 143–149/.75–1 |
| 8 | 37% beef, 63% pork | 121 / 2.5 |
| 9 | Not available | Not available |
| 10 | 99% beef | 116 / .75–1 |
| 11 | 100% cattle | 93 / 1–1.25 |
| 12 | 70% beef, 20% pork, 10% poultry | 130 / continuous flow |
| 13 | 50% pork, 40% beef, 10% misc. | 121–138 / 1.5–2.0 |
| 14 | 80% dairy, 10% beef, 10% pork | 121 / .5 |
| 15 | 60% pork, 40% beef | 125 / .25 under vacuum |

^aTemperature refers to the temperature of the heating unit of the batch cooker. Time refers to the average duration of the heat treatment during the rendering process. Continuous flow indicates that the inflow rate of the raw material was the same as the outflow rate of the processed material.

describe ruminal protein degradation, there is no indication in the literature that they have been tested for goodness of fit when used.

In the present study, meat and bone meal (**MBM**) and soybean meal (**SBM**) were used as examples of slowly and rapidly degradable proteins, respectively, with the following objectives: 1) to evaluate mathematical models used to determine CP degradation for goodness of fit; 2) to assess whether the mathematical model selected has any effect on estimated RUP values; and 3) to evaluate various compositional characteristics of MBM as predictors of ruminal degradation of MBM protein.

Material and Methods

Samples and Analyses

Fifteen MBM and two SBM samples were obtained from commercial sources. Meat and bone meal samples differed in raw material composition, temperature and duration of heat treatment, and rendering process used to separate the fat from the raw materials (Table 1). Meat and bone meal samples were analyzed for N, DM, ash, lysine, and OH-proline contents (Table 2). Nitrogen analyses were performed using a Tecator 1030 Autoanalyzer (Tecator, Herndon, VA). Lysine and OH-proline analyses were conducted on a Beckman 119CL Amino Acid Analyzer (Beckman Instruments, Fullerton, CA), following acid hydrolysis, which was conducted by placing 150 mg of sample and 15 mL of 6 N HCl in a N₂-purged screw-cap vial and heating for 22 h at 110°C. Hydroxy-proline contents were used to estimate total collagen in the MBM samples (AOAC, 1990).

Ruminal Degradation

A ruminally cannulated cow fed a 70:30 forage:concentrate diet was used for the in situ incubations. An average of $.5 \pm .06$ g of each protein supplement was weighed into Dacron polyester (Erlanger, Blumgart and Co., New York) bags (6 × 10 cm) with an average pore size of 52 ± 16 μm. Samples were incubated in the rumen for 2, 4, 8, 12, 16, 24, 48, and 72 h after being soaked in distilled water (38°C) for 10 min. Each sample was evaluated in duplicate bags at each time on two consecutive days. A pair of bags was also incubated in only distilled water at 38°C for 10 min to estimate washout at time zero for each

Table 2. Chemical composition (% of DM) of various meat and bone meal samples

| Sample | CP | Ash | Collagen | Lysine |
|--------|------|------|----------|--------|
| 1 | 57.6 | 23.3 | 21.5 | 3.1 |
| 2 | 51.8 | 32.7 | 25.6 | 2.8 |
| 3 | 51.8 | 26.9 | 20.7 | 3.0 |
| 4 | 51.7 | 30.6 | 22.4 | 2.9 |
| 5 | 48.8 | 35.6 | 23.5 | 2.5 |
| 6 | 48.8 | 37.5 | 22.3 | 2.8 |
| 7 | 47.2 | 35.3 | 24.7 | 2.6 |
| 8 | 53.1 | 26.8 | 21.9 | 2.8 |
| 9 | 52.7 | 33.8 | 25.9 | 2.7 |
| 10 | 50.6 | 34.2 | 22.3 | 3.0 |
| 11 | 45.0 | 44.6 | 38.7 | 2.2 |
| 12 | 50.6 | 36.1 | 30.9 | 2.8 |
| 13 | 53.8 | 22.7 | 23.8 | 3.0 |
| 14 | 52.5 | 26.8 | 24.7 | 3.0 |
| 15 | 52.5 | 29.1 | 28.5 | 2.8 |
| Mean | 51.2 | 31.7 | 25.2 | 2.8 |
| SE | 2.9 | 5.9 | 4.6 | .23 |

sample. After ruminal incubation, each bag was rinsed with cold tap water for at least 20 min. The bags that were only soaked in distilled water were rinsed following the same procedure.

Linear, nonlinear, and curve-peeling approaches were used to predict rate of ruminal CP degradation. The linear model used was that described by Mathers and Miller (1981), in which rate of degradation (K_d) of the potentially degradable protein was calculated as the slope of the regression line of the natural logarithm of residual N vs incubation time. To describe the effect of duration of ruminal incubation on RUP values obtained with the linear model, three data sets were created: one that included ruminal incubation times up to 12 h (data set A; considering the residue at 12 h to be completely resistant to ruminal degradation), another that included incubation times up to 24 h (data set B; considering the residue at 24 h to be completely resistant to ruminal degradation), and a third that included ruminal incubation times up to 72 h (data set C; considering the average of the residue at 48 and 72 h to be completely resistant to ruminal degradation). The Mathers and Miller (1981) model assumes that there is no C fraction (a fraction completely resistant to ruminal degradation) or that it is insignificant. Therefore, the N remaining after 12 h, 24 h, and the average of 48 and 72 h of ruminal incubation was subtracted before natural logarithm transformation and regression with data set A, data set B, and data set C, respectively. Nitrogen remaining at 0 h (after washout) was not included in the regression.

One of the nonlinear approaches used was described by Ørskov and McDonald (1979). Using this method, ruminal CP disappearance follows first-order kinetics defined by the equation

$$\text{CP disappearance} = A + B \times (1 - e^{-K_d \times t}) \quad [1]$$

where A is the soluble CP fraction (% of CP), B is the potentially degradable CP fraction (% of CP), K_d is the degradation rate constant (h^{-1}), and t is the ruminal incubation time (h).

The nonlinear model described by Mertens and Loften (1980) was also evaluated as defined by the equation

$$\text{CP remaining} = D \times (e^{-K_d \times t}) + U \quad [2]$$

where D is the degradable CP fraction (% of CP), U is the undegradable CP fraction (% of CP), and K_d and t are as defined above, for Eq. [1].

The nonlinear models were solved by nonlinear regression using the compromise of Marquardt (1963) to fit the curves with Mathematica (Wolfram Research, Champaign, IL; Wolfram, 1991).

The fourth mathematical approach tested was curve peeling (Shipley and Clark, 1972). This procedure was used to estimate pool sizes and degradation rates of the rapidly and slowly degradable fractions of MBM and SBM proteins. For this procedure, the natural logarithm of the CP residue after ruminal incubation was plotted vs time. From this plot, it could be easily determined whether the data had some curvature. Next, a line was drawn through the linear portion of the data with the longest incubation times. The exact point of including data in this line was subjective, but plotting the residuals vs the fitted values and testing for lack of fit were helpful in assessing whether the line included data with curvature. This first line represented the undegradable CP pool, and it had a slope equal or very close to zero. After this line was drawn, it was peeled from the composite curve by subtracting its value at each time point from the value of the composite curve. The subtraction was done on a linear scale. This peeling process was repeated until the line resulting from the subtraction was linear. The slope of each line corresponded to the fractional degradation rate for that pool, and the intercept of each line corresponded to the size of that pool. To obtain a single rate constant for the different pools, a weighted average of the degradation rates of the slowly and rapidly degradable CP pools was then calculated.

Independently of the mathematical model used to estimate rate of degradation, the extent of ruminal CP degradation (% of CP) was calculated as follows:

$$\text{Extent of ruminal CP degradation} = \text{soluble CP fraction} + \text{degradable CP fraction} \times \left[\frac{K_d}{(K_d + K_p)} \right]$$

where soluble CP fraction is the percentage of CP disappearing at time zero when using the linear model or curve peeling, A when using Eq. [1] or $100 - (D + U)$ when using Eq. [2]; degradable CP fraction = $100 - \text{soluble CP fraction}$ when using the linear model, B when using Eq. [1], D when using Eq. [2], or the sum of the rapidly and slowly degradable CP pools when using curve peeling; and K_p is the rate of passage (assumed to be $.06 \text{ h}^{-1}$ in all cases).

Statistical Analysis

The linear and the nonlinear mathematical models as well as each of the peeled lines obtained with curve peeling were tested for goodness of fit by the lack-of-fit test of Sokal and Rohlf (1969). Further, to assess whether the linear fit was appropriate to estimate K_d for MBM and SBM, residuals vs fitted were plotted using R-Code2 (Cook and Weisberg, 1994) and a score test (Chen, 1983) was performed on the residuals from the linear models to statistically determine the presence of curvature.

Several factors that could potentially affect ruminal degradation of MBM were studied by simple linear regression analyses. The linear model used for these analyses was

$$Y = \beta_0 + \beta_1 \text{factor}_i + \epsilon_i$$

where Y was ruminal CP degradation, factor_i was each of the factors considered to potentially affect ruminal degradation of MBM protein, and ϵ_i was the random error.

Results and Discussion

Ruminal Degradation

Table 3 shows CP solubility and rate and extent of CP degradation obtained from the various mathematical approaches. The two nonlinear models generated identical values for CP solubility and rate and extent of CP degradation. In fact, the theoretical concept behind each nonlinear model is the same. The only difference is that one model (Mertens and Loften, 1980) looks at CP remaining and includes the undegradable CP fraction in the equation, whereas the other (Ørskov and McDonald, 1979) looks at CP disappearance and includes CP solubility but not the undegradable CP fraction in the equation. Therefore, no distinction is made between the two models in Tables 3 and 4.

In general, rates and extents of CP degradation of MBM estimated using the linear model with data set A (incubations up to 12 h) were greater (Table 3) than those obtained with data sets B (incubations up to 24 h) or C (incubations up to 72 h). Curve peeling and the nonlinear model resulted in the lowest estimates of extent of CP degradation for most of the MBM samples. However, in the case of SBM, estimates of rate and extent of CP degradation obtained with the linear model followed a quadratic pattern, being highest with data set A (incubations up to 12 h), intermediate with data set C (incubations up to 72 h), and lowest with data set B (incubations up to 24 h). The extent of CP degradation for the SBM samples was not as affected by the mathematical model and the duration of ruminal incubations as it was for the MBM samples. This could probably be attributed to the smaller completely undegradable CP fraction of SBM compared with MBM. We conclude that long ruminal incubation times (48 h or more) are necessary to correctly describe the degradation patterns of MBM.

Aside from the differences in the absolute values of estimates of rate and extent of CP degradation among mathematical models, it is worth noting that relative differences among estimates within the same MBM sample obtained from the models were greater than 10

percentage units in many instances. Moreover, the ranking of the various MBM samples by extent of CP degradation was different depending on the mathematical model used (Table 3). This could have profound implications for diet formulation, and it might be one of the reasons why RUP supplementation in some studies does not result in improved animal performance.

Quality of Fit of the Mathematical Approaches

Despite the high coefficients of determination associated with the prediction of K_d when using the linear model (average = .81), the analysis of residuals suggested that the linear fit was not appropriate (Figure 1). Had the linear fit been appropriate, residuals should be equally distributed about the fitted line, and no pattern should be observed. However, from Figure 1, it can be seen that residuals had a U-shaped distribution about the line, which suggests inadequacy of the linear fit. Furthermore, the linear model was rejected by the lack-of-fit test for all the SBM samples and for 3 of the 15 MBM samples when using data set C (incubations up to 72 h). However, the score test (Chen, 1983) for testing the residuals for curvature was positive for 9 of the 15 MBM samples when using data set C (incubations up to 72 h), suggesting that the linear model was not appropriate for MBM. For data set B (incubations up to 24 h), the linear model was rejected by the lack-of-fit test for all the SBM and 8 of the 15 MBM samples. For data set A (incubation for up to 12 h only), the lack-of-fit test accepted the linear model as valid to describe CP degradation of all the SBM samples and 14 of the 15 MBM samples. This clearly indicates that SBM and MBM, and probably most protein supplements used in ruminant diets, have several protein fractions that are degraded at different rates, as suggested by Johnson (1976) and later included in the Cornell net carbohydrate and protein system (Russell et al., 1992). Degradation of the rapidly degraded CP fraction may be described by a linear model but, as fermentation proceeds, a different model is needed to fit the degradation patterns of the more slowly degraded CP fractions (Figure 1).

Using curve peeling, the slowly degradable CP fraction was separated from the rapidly degradable CP pool. Evaluation of the lack-of-fit test suggested that both the slowly and the rapidly degradable CP pools determined by curve peeling could be, indeed, described by linear equations. The description of CP degradation of MBM and SBM obtained with curve peeling is shown in Figure 2. Both the rapidly and slowly degradable CP pools were larger in SBM (23.8 and 54.0%, respectively) than in MBM (11.5 and 30.3%, respectively). However, the rapidly degradable CP pool was more rapidly degraded in MBM (.533 h^{-1}) than in SBM (.231 h^{-1}), but the slowly

Table 3. Crude protein solubility and rate and extent of degradation of CP from meat and bone meal (MBM) and soybean meal (SBM) estimated with different mathematical models^a

| Sample | Solubility, % of CP | | Rate of degradation, h ⁻¹ | | | | | Extent of degradation, % of CP ^b | | | | |
|--------------------|---------------------|-----------|--------------------------------------|-----------------------|-----------------------|-----------|---------|---|-----------|-----------|-----------|-----------|
| | Linear and peeling | Nonlinear | | | | | | Linear A | Linear B | Linear C | Nonlinear | Peeling |
| | | | Linear A ^c | Linear B ^d | Linear C ^e | Nonlinear | Peeling | | | | | |
| MBM 1 | 29.8 | 34.0 | .279 | .108 | .038 | .049 | .261 | 86.4 (12) | 74.6 (15) | 56.6 (11) | 52.2 (13) | 56.6 (12) |
| MBM 2 | 28.3 | 32.6 | .200 | .053 | .046 | .048 | .087 | 83.3 (6) | 61.9 (1) | 58.9 (14) | 51.1 (11) | 50.8 (8) |
| MBM 3 | 25.8 | 28.5 | .357 | .080 | .048 | .045 | .127 | 88.3 (14) | 67.7 (10) | 58.0 (13) | 49.9 (8) | 48.1 (6) |
| MBM 4 | 19.3 | 23.8 | .286 | .089 | .048 | .064 | .275 | 86.0 (11) | 67.5 (9) | 54.5 (8) | 44.4 (4) | 46.0 (4) |
| MBM 5 | 17.1 | 19.5 | .287 | .099 | .051 | .051 | .092 | 81.7 (4) | 68.5 (13) | 54.2 (7) | 40.0 (2) | 37.0 (2) |
| MBM 6 | 22.1 | 27.4 | .385 | .086 | .045 | .058 | .238 | 89.2 (15) | 68.0 (11) | 55.2 (9) | 50.9 (10) | 53.1 (9) |
| MBM 7 | 14.2 | 20.4 | .353 | .091 | .036 | .056 | .269 | 87.5 (13) | 64.6 (3) | 45.9 (2) | 41.5 (3) | 42.2 (3) |
| MBM 8 | 31.7 | 35.0 | .196 | .089 | .015 | .066 | .129 | 83.4 (7) | 72.5 (14) | 60.0 (15) | 55.0 (14) | 60.5 (14) |
| MBM 9 | 16.6 | 18.1 | .307 | .094 | .027 | .068 | .070 | 84.9 (8) | 67.4 (8) | 47.9 (5) | 45.2 (5) | 46.8 (5) |
| MBM 10 | 22.1 | 28.5 | .266 | .081 | .005 | .078 | .231 | 85.0 (9) | 66.7 (4) | 57.1 (12) | 49.6 (7) | 55.8 (10) |
| MBM 11 | 10.6 | 10.3 | .257 | .104 | .009 | .044 | .038 | 81.7 (3) | 67.3 (6) | 46.6 (4) | 31.4 (1) | 29.2 (1) |
| MBM 12 | 23.4 | 27.2 | .222 | .081 | .015 | .103 | .036 | 83.2 (5) | 67.2 (5) | 55.7 (10) | 51.6 (12) | 56.3 (11) |
| MBM 13 | 20.3 | 25.8 | .133 | .086 | .008 | .055 | .129 | 75.3 (1) | 67.3 (7) | 49.4 (6) | 50.0 (9) | 57.6 (13) |
| MBM 14 | 21.3 | 24.8 | .259 | .088 | .042 | .089 | .159 | 85.0 (10) | 68.2 (12) | 46.4 (3) | 56.7 (15) | 63.2 (15) |
| MBM 15 | 17.5 | 19.8 | .209 | .079 | .028 | .059 | .073 | 81.6 (2) | 64.5 (2) | 37.9 (1) | 48.9 (6) | 50.4 (7) |
| SMB 1 | 14.4 | 13.8 | .294 | .107 | .171 | .109 | .166 | 84.9 | 69.2 | 77.7 | 69.4 | 72.2 |
| SBM 2 | 17.0 | 15.6 | .201 | .158 | .205 | .129 | .188 | 79.9 | 76.8 | 81.2 | 73.4 | 75.9 |
| Mean | | | | | | | | | | | | |
| MBM | 21.3 | 25.0 | .266 | .087 | .030 | .062 | .148 | 84.2 | 67.6 | 52.3 | 47.9 | 50.2 |
| SBM | 15.7 | 14.7 | .248 | .133 | .188 | .119 | .177 | 82.4 | 73.0 | 79.5 | 71.4 | 74.1 |
| Standard deviation | | | | | | | | | | | | |
| MBM | 5.9 | 6.7 | .068 | .013 | .017 | .017 | .086 | 3.4 | 3.0 | 6.3 | 6.5 | 9.1 |
| SBM | 1.9 | 1.7 | .066 | .036 | .024 | .014 | .016 | 3.6 | 5.4 | 2.5 | 2.8 | 2.6 |

^aThe linear model was of Mathers and Miller (1981) and the nonlinear was of Ørskov and McDonald (1979).

^bNumbers within parentheses indicate the ranking of MBM samples for extent of CP degradation.

^cThe model included ruminal incubation times up to 12 h only (data set A).

^dThe model included ruminal incubation times up to 24 h only (data set B).

^eAll of the ruminal incubation points were included in the model (data set C).

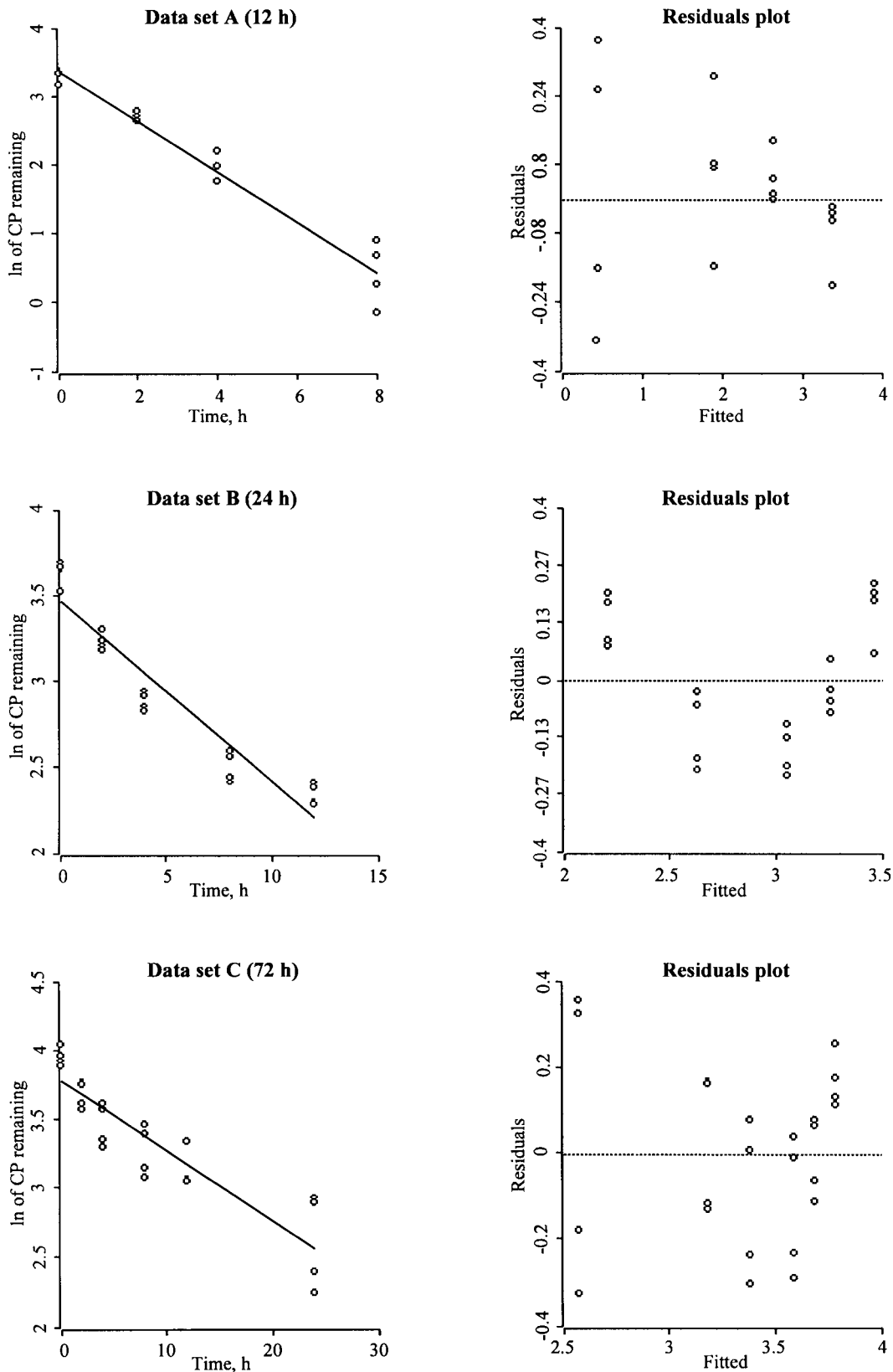


Figure 1. Pattern of CP degradation of a meat and bone meal sample as described by a linear model (Mathers and Miller, 1981) and as affected by the ruminal incubation times included in the model.

degradable CP pool was more rapidly degraded in SBM ($.080 \text{ h}^{-1}$) than in MBM ($.054 \text{ h}^{-1}$).

The nonlinear models (Figure 3) did not show lack of fit when used to evaluate CP degradation of SBM. This was expected, because SBM was the protein source used by Ørskov and McDonald (1979) to develop their nonlinear model. However, the nonlinear model did not fit the degradation pattern of MBM for 3

of the 15 MBM samples. This demonstrates that any mathematical equation should be tested for goodness of fit every time it is used to describe the degradation patterns of any given protein source.

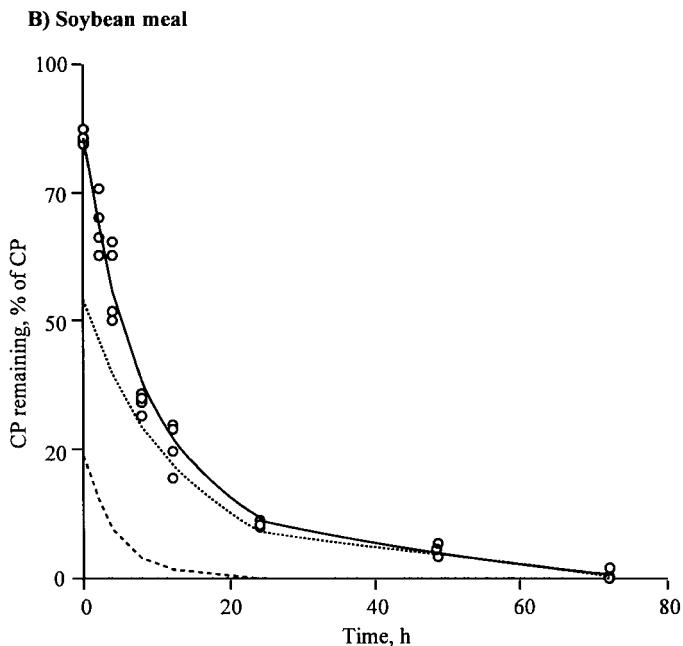
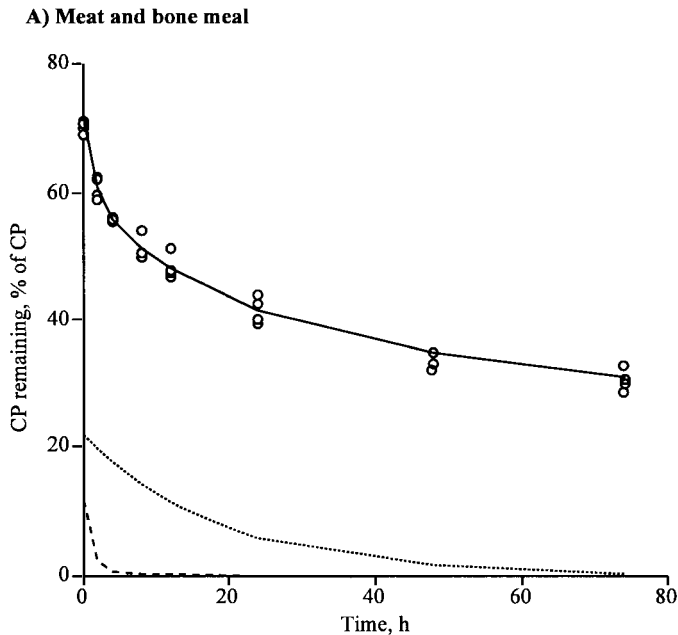
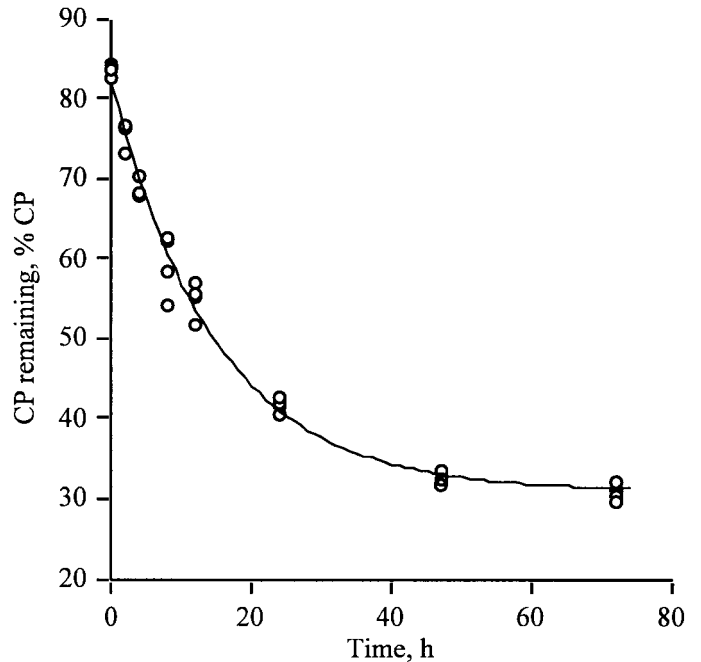


Figure 2. Description of CP degradation (solid line) of meat and bone meal (A) and soybean meal (B) using curve peeling; observed values (○), rapidly degradable CP pool (dashed line), slowly degradable CP pool (dotted line).

A) Meat and bone meal



B) Soybean meal

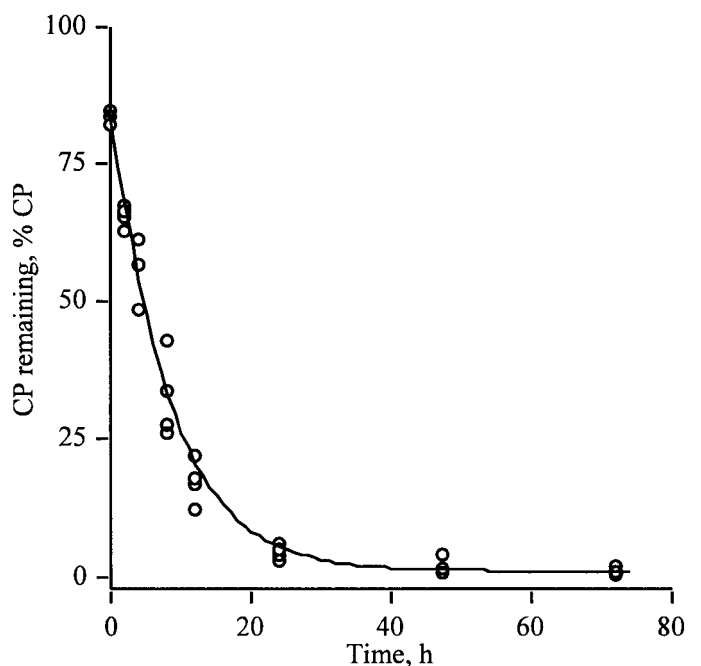


Figure 3. Description of CP degradation (solid line) of meat and bone meal (A) and soybean meal (B) using a nonlinear model (Mertens and Loftén, 1980); observed values (○).

Table 4. Relationships between composition and processing characteristics and solubility and degradation of CP from meat and bone meal

| Independent variable | Solubility, % of CP | | | | Extent of degradation, % of CP | | | | | | | | | |
|----------------------|---------------------|----------------------|-----------|---------|--------------------------------|---------|-----------------------|---------|-----------------------|---------|-----------|---------|---------|---------|
| | Linear and peeling | | Nonlinear | | Linear A ^a | | Linear B ^b | | Linear C ^c | | Nonlinear | | Peeling | |
| | r | P-value ^d | r | P-value | r | P-value | r | P-value | r | P-value | r | P-value | r | P-value |
| Time | .70 | <.05 | .70 | <.05 | .01 | NS | .01 | NS | .68 | <.05 | .40 | NS | .33 | NS |
| Temperature | .38 | NS | .53 | <.05 | .42 | NS | .01 | NS | .09 | NS | .50 | NS | .48 | NS |
| Ash | -.66 | <.05 | -.69 | <.05 | -.04 | NS | -.40 | NS | -.20 | NS | -.74 | <.05 | -.72 | <.05 |
| Solubility | — | — | — | — | .18 | NS | .30 | NS | .73 | <.05 | .82 | <.05 | .71 | <.05 |
| Total collagen | -.57 | <.05 | -.68 | <.05 | -.27 | NS | -.32 | NS | -.41 | NS | -.58 | <.05 | -.62 | <.05 |
| Lysine | .70 | <.05 | .75 | <.05 | .15 | NS | .26 | NS | .31 | NS | .83 | <.05 | .84 | <.05 |

^aIncluded ruminal incubation times up to 12 h (data set A).

^bIncluded ruminal incubation times up to 24 h (data set B).

^cIncluded all of the ruminal incubation times (data set C).

^dNS = Not significant ($P > .05$).

Evaluation of Potential Predictors of Ruminal Degradable Protein from Meat and Bone Meal

Correlation coefficients between CP solubility values for MBM and various independent variables were in general greater when the nonlinear models were used compared with curve peeling or the linear model (Table 4). Conversely, most of the correlation coefficients between CP degradation and the same independent variables were greater when the nonlinear models and curve peeling were used, compared with the linear models. In general, the correlation coefficients between extent of CP degradation and the independent variables obtained with the linear model increased as the duration of ruminal incubations included in the model increased. This further supports the idea that the inclusion of all the protein fractions is important to describe CP degradation of a protein source.

Type of raw material (cattle, poultry, and pork) used to produce MBM was not correlated with protein degradability ($P > .05$; data not shown). Previous research showed changes in amino acid profile (Batterham et al., 1986) and lower digestibility (Leibholz, 1979) of MBM as a result of increasing heat treatment. In our study, duration of heat treatment was positively correlated ($r = .70$; $P < .05$) with CP solubility, but temperature was only significantly correlated ($r = .53$; $P < .05$) when CP solubility was estimated by the nonlinear models (Table 4). The trend for solubility to increase with duration of heat treatment may be related to the effects of heat on collagen. Eilert and Mandigo (1993) showed that moderate heat induced gelatinization of collagen and increased its solubility. Also, the correlation coefficients between collagen content and CP degradation estimated with the linear model increased as greater ruminal incubation times were included in the model. This further reinforces the presence of different protein fractions that are degraded at particular rates

and with different patterns. Crude protein degradation described using the linear model with data set A (incubations up to 12 h) corresponds primarily to the rapidly degradable fraction, which will be degraded independently of the collagen content of MBM. However, as fermentation proceeds, slowly degradable collagen from bones and ligaments will become the most predominant protein, which will have a significant impact on rate and extent of CP degradation. When all the fractions were accounted for, using curve peeling or the nonlinear models, the correlation between total collagen and CP degradation became statistically significant ($P < .05$).

Crude protein solubility values estimated by washout from the Dacron bag at time zero explained only 10% of the variation observed in CP degradation determined by the linear model (data set B), but it explained 53% of the variation observed in CP degradation determined by curve peeling and the linear model with data set C, or 67% when determined by the nonlinear models (Table 4). The estimation of CP degradation from protein solubility within the same product has been suggested elsewhere (Satter, 1986). Another good predictor of CP degradation of MBM could be ash content, which was highly correlated with CP degradation values obtained from curve peeling or the nonlinear models. Hegedús (1984) also suggested the use of ash content as a predictor of CP degradation of MBM; however, the correlation was lower than the one obtained in the present experiment. The highest correlation coefficient was found between lysine content and CP degradation determined by curve peeling or the nonlinear models. Therefore, lysine content could also be used as a predictor of CP degradation of MBM. This is most likely due to the greater CP degradation observed with samples having greater contents of muscle, because muscle protein, which is much richer in lysine than bone protein (Eastoe and Long, 1960), is more degradable than bone protein (Evans and Leibholz, 1979).

Implications

The extent of crude protein degradation values may be different depending on the mathematical approach used to calculate them and on the duration of ruminal incubations included in the model. Also, depending on the mathematical model used to estimate the extent of crude protein degradation, samples can be ranked in a different order. Because some mathematical models may not be appropriate for all types of samples, analysis of residuals vs fitted and lack-of-fit tests should be performed to assess the validity of the model to describe the degradation patterns of the protein source under study.

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