Comparison of Two Models Used to Estimate In Situ Nitrogen Disappearance[1]

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ABSTRACT

Inclusion of lag time into a model describing in situ N disappearance influenced the parameter estimates describing N degradability and improved the goodness-of-fit of the model to data describing aeschynomene hay and alfalfa meal digestion. Ruminal N disappearance (%) was described using two models that assumed: 1) digestion of the potentially digestible but insoluble N fraction was initiated immediately upon exposure to ruminal contents, or 2) digestion of this fraction was preceded by a lag period during which hydration and microbial attachment is presumed to occur prior to the initiation of digestion. Digestion was assumed to occur from a homogeneous potentially digestible but insoluble N fraction by a first order process described by a rate constant, k. Incorporating a lag term resulted in an increase in the rapidly soluble N fraction, a decrease in the potentially digestible but insoluble N fraction, and a decrease in the total digestible N fraction. The two forages had different k values when fit to the nonlagged model but not when fit to the lagged model. The residual standard deviation was smaller for the lagged model, and the fit of predicted values to observed values was improved.

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INTRODUCTION

There is considerable interest in the concept of metabolizable protein as an alternative to CP for use in formulating diets of productive ruminants (8). Calculation of metabolizable protein requires information about the ruminal degradability of dietary protein sources. The National Research Council recognizes that this information is best generated with techniques designed to separate the contribution of microbial protein to total protein entering the duodenum from that of integrated dietary protein (8). Alternative methodologies to separate dietary protein into ruminally degradable and undegradable fractions are sought because of the time and expense involved in the use of markers to obtain this information. In situ experimentation is presented as an alternative methodology to estimate ruminal N degradability of a protein source (8).

There are many problems associated with the use of the in situ method to measure ruminal N degradability (8). The most serious problem is the lack of a consistent, standardized approach to calculation of ruminal degradability from in situ data. There are two other major problems with some models: 1) disregard for that time period (lag) prior to the initiation of degradation of the potentially digestible but insoluble (B) protein fraction of the feedstuff, and 2) the assumption that the B fraction is a single, homogeneous pool, the digestion of which proceeds by a first order process.

Previous research indicated increased N retention of lambs fed a basal diet of limpograss hay supplemented with alfalfa meal (AM) as compared with aeschynomene hay (AH) (3). Nitrogen degradability of each supplement was determined using an in situ approach, but interpretation was hindered because different conclusions were reached depending on choice of model used to describe the resulting data.

Ruminal N disappearance was described using two models that assumed that digestion of the potentially digestible but insoluble N fraction was initiated immediately upon exposure to ruminal contents, or alternatively, that digestion of this fraction was preceded by a lag period during which hydration and microbial attachment occurs (1). Failure to account for lag can lead to conceptual and statistical difficulties in describing in situ N disappearance from these legume protein supplements. The objective is to compare the in situ N degradability of two legume protein supplements, AM and AN, that were utilized in an earlier study (3).

MATERIALS AND METHODS

The tropical legume aeschynomene (*Aeschynomene americana* L.) was seeded on June 6, 1984 at the rate of 17 kg/ha and fertilized with 45 kg P_2O_5 and 90 kg K_2O/ha. It was harvested August 6, 1984 and mechanically dried in a heated drying wagon with a perforated metal floor. During the drying process hot air (60°C) was blown through the floor of the wagon. The dried material was ground through a hammermill (3.2-mm screen) and stored in woven plastic bags. Alfalfa meal was obtained from a commercial source. Chemical composition of each supplement was determined as reported (3) and is shown in Table 1.

Three ruminally fistulated animals (2 steers and 1 cow) were maintained on a diet consisting of Coastal bermudagrass hay fed ad libitum and .82 kg of soybean meal/animal/d starting 2 wk prior to and continuing throughout the experiment. Samples of AH and AM were ground through a 1-mm screen and then ruminally incubated for 2 min or 2, 6, 12, 24, 36, 48, or 72 h during each of three consecutive 3-d experimental periods. Each cow received each legume supplement in each period.

TABLE 1. Chemical composition[1] and in vitro organic matter digestibility (IVOMD) of legume supplements.

Item	Aeschynomene hay	Alfalfa meal
Organic matter, %	91.4	88.9
Crude protein, %	19.6	17.4
NDF, %[2]	54.6	49.0
ADF, %[2]	40.4	30.2
Lignin,[2] %	10.4	8.1
IVOMD, %	69.3	64.2

[1] As % DM.

[2] Ash-free.

Dacron bags measuring 18 × 17.5 cm (mean pore size of 52 μm were used. Seams were sewn twice with nylon thread and edges also were sealed with waterproof glue. Dacron was purchased from Erlanger, Blumgardt & Co., Inc., New York, NY. Waterproof glue for sealing dacron bags was from Advance Color Corp., Los Angeles, CA.

Eight-gram samples of each supplement were placed into bags, which were closed and tied with nylon twine (18 to 77-kg test). Bags were suspended in the ventral portion of the rumen at each time interval and removed at the same time. They were rinsed immediately after removal and machine washed in the delicate rinse cycle for 15 min. Samples then were dried at 60°C for 48 h. Initial and residual N were determined by the Kjeldahl method (2).

The kinetics of N disappearance were estimated with two similar models. The first, referred to as nonlagged (NL), was proposed by Orskov and McDonald (11) and describes the amount of nutrient disappearance (%) at time t as follows:

$$D = A + B\,(1 - \exp(-kt)) \qquad [1]$$

where: D = percentage of nutrient disappearance at time t, A = percentage of rapidly soluble fraction, B = percentage of potentially digestible insoluble fraction, k = rate constant of digestion of the potentially digestible insoluble fraction (h^{-1}), and t = time of ruminal incubation (h).

The second model, referred to as lagged (L), is based on the models of McDonald (6) and Mertens (7) and describes the amount of nutrient disappearance (%) at time t as follows:

$$D = A, \text{ for } t < T \qquad [2a]$$

$$D = A + B(1 - \exp(-k\,(t - T))), \text{ for } t \geq T \qquad [2b]$$

where D, A, B, k, and t as are in Equation [1], and T is a lag period (h).

Parameter values for Equations [1] and [2] were obtained by fitting the data using a derivative-free nonlinear regression procedure performed by the NLIN procedure of SAS (13). Within a given method, estimates of A, B, k (for both equations), and T (for equation [2] only) for AH and AM were compared using analysis of variance with the GLM procedure of SAS (13). The experimental design was a split plot in time in which forage × animal interaction (2 df) served as the error term for main effects in the main plot (forage and animal), and the three-way interaction (forage × animal × period) was used as the error term for the subplots defined by period. Main effect means were separated by Duncan-Waller k test, which was applicable only when a significant F test ($P<.05$) existed.

Differences between methods were compared in a variety of ways. First, the residual standard deviation ($S_{y \cdot x}$) of the two methods was compared. A smaller $S_{y \cdot x}$ indicated that a given model fit the data more "closely". Second, an asymptotic likelihood ratio test was used to test the reduction in the sum of squares for error [SSE; (5)]. This tested the null hypothesis that inclusion of additional information in the equation form yielded no improvement in SSE, and was equivalent to a goodness-of-fit test. Third, corresponding parameters from the two models (A, B, and k) were compared by pairing within treatment combinations. Finally, the predicted values generated by each model were regressed against the observed values, and the regression coefficients tested under the multivariate null hypothesis of equality and univariate null hypothesis: $H_0 = 1$. Further, the r^2 of the regressions of predicted on observed values were compared between the two methods (14).

RESULTS

When data were fit to the NL model, AH had a greater proportion of N in the rapidly solubilized A fraction than did AM ($P = .004$), but less in the potentially but slowly degradable B fraction ($P = .002$; Table 2). The rate constant describing N disappearance from the B fraction was greater for AH than for AM ($P = .009$).

When data were fit to the model L, results were similar: AH had a greater A fraction than AM ($P = .018$) and a lesser B fraction ($P = .027$). However, there were no differences between forages in rate constant, k ($P = .32$) or lag time, T ($P = .27$).

No animal effects were found within either model ($P>.1$). There were period differences ($P<.05$); period 2 differed ($P<.05$) from the other periods in the partitioning to the A and B fractions, and period 3 differed ($P<.05$) from the first two periods in k. The differences may indicate a disruption in rumen function due to consecutive periods. As both models found these differences, they are more likely due to changes in rumen function than to the model used to fit the data.

The time course for N disappearance for AH and AM is shown in Figures 1 and 2, respectively. Points represent means (with 95% confidence intervals) calculated across animals and periods. Curves were generated from mean parameter estimates for each of the forages. Individual points are not monotonically increasing over the whole interval, whereas both curves are. This behavior indicates that both models are incomplete in their description of N disappearance.

Residual standard deviations and the results of the asymptotic likelihood ratio test for all treatment combinations are presented in Table 3. In only one of 18 treatment combinations was $S_{y \cdot x}$ less for NL than for L (AM, animal 3, period 3). The reduction in $S_{y \cdot x}$ due to inclusion of a lag term in the model ranged up to

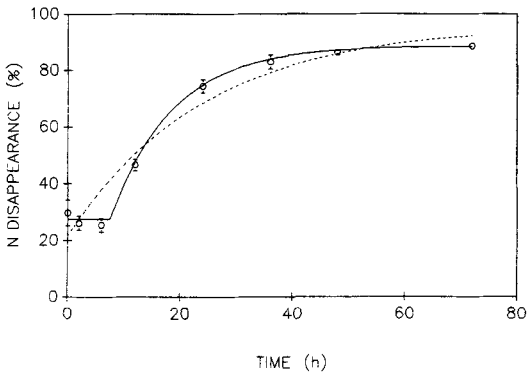

Figure 1. Time course of N disappearance (%) for aeschynomene hay. Points represent means across all other treatment combinations; error bars are 95% confidence intervals. Solid lines represent lagged model, dashed lines represent nonlagged model.

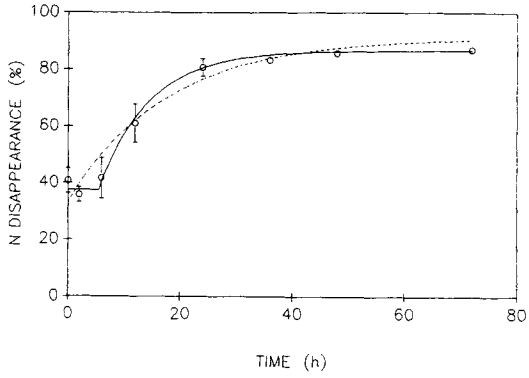

Figure 2. Time course of N disappearance (%) for alfalfa meal. Points represent means across all other treatment combinations; error bars are 95% confidence intervals. Solid lines represent lagged model, dashed lines represent nonlagged model.

10-fold, but rates of convergence to a minimum residual sum of squares and parameter correlation were unaffected (data not presented). Results of the likelihood ratio test show that in 15 of the 18 treatment combinations, L resulted in a superior fit to the data than did NL.

Pairwise comparisons of parameters common to both models are shown in Table 4. These values represent the mean difference between L and NL for each of the parameters. As all are different from zero ($P<.0001$), it is highly probable that the two models give consistently different parameter values. Analysis of the differences using the split-plot ANOVA described above revealed no animal or forage effects ($P>.2$). Although a period effect for the difference in A values was found ($P = .009$), none of the differences between other parameters showed the effect of period or of interactions of the main plot effects with period. Thus, choice of model was not influenced by the animals or forages used in this study, and only a minimal effect on choice of model was seen due to period.

When the observed data are plotted against the predicted data from models NL and L, the

TABLE 2. Main effect means of in situ nitrogen disappearance characteristics as estimated by two non-linear models.

	Model parameters						
	Nonlagged			Lagged			
Effect	A[1]	B[2]	k(h^{-1})[3]	A[1]	B[2]	k(h^{-1})[3]	T (h)[4]
	(%)			(%)			
Forage							
Aeschynomene	33.3[c]	58.0[c]	.057[c]	37.5[a]	49.2[a]	.112	5.46
Alfalfa	21.8[d]	74.3[d]	.041[d]	27.5[b]	61.1[b]	.090	7.52
SE	.54	.58	.0011	.97	1.41	.0123	.976
Animal							
1	27.6	67.2	.049	32.3	55.7	.106	6.41
2	27.0	67.4	.048	32.4	55.3	.093	6.77
3	28.1	63.9	.050	32.7	54.3	.104	6.28
SE	.66	.71	.0013	1.19	1.72	.0150	1.196
Period							
1	24.7[a]	71.6[c]	.040[c]	29.7[a]	58.5[c]	.095[a]	7.53[a]
2	32.1[b]	59.9[d]	.046[c]	35.6[b]	50.9[d]	.084[a]	5.58[b]
3	25.9[a]	67.0[cd]	.062[d]	32.1[ab]	55.9[c]	.125[b]	6.35[ab]
SE	1.35	1.22	.0016	1.08	.65	.0061	.405

[a,b]Means in the same column within measures with different superscripts differ ($P<.05$).

[c,d]Means in the same column within measures with different superscripts differ ($P<.01$).

[1] Rapidly solubilized N fraction.

[2] Slowly solubilized but potentially digestible N fraction.

[3] First order rate constant for disappearance from the B fraction.

[4] Lag time.

TABLE 3. Residual standard deviations of parameter estimates of in situ nitrogen disappearance under all treatment combinations, nonlagged vs. lagged model.

Forage[1]	Period	Animal	Nonlagged model	Lagged model	$P<$[2]
AH	1	1	10.3	5.6	.025
		2	8.1	2.7	.005
		3	9.2	3.2	.005
	2	1	8.4	7.0	.25
		2	5.5	3.7	.10
		3	4.5	2.5	.05
	3	1	4.2	1.4	.005
		2	3.4	1.6	.025
		3	6.7	3.4	.025
AM	1	1	10.3	4.5	.01[3]
		2	7.2	.7	.001
		3	2.0	3.1	.25
	2	1	10.0	6.5	.05
		2	8.2	3.4	.01
		3	4.7	2.5	.05
	3	1	9.1	2.1	.001
		2	7.0	1.8	.001
		3	7.4	2.0	.001

[1] AH = Aeschynomene hay, AM = alfalfa meal.

[2] Indicates the probability that the lagged model is not an improvement over the nonlagged model, based on asymptotic likelihood ratio test.

[3] The mean squares error for the nonlagged model was used as the denominator for the likelihood ratio, as the nonlagged model had the reduced sum of squares for error.

slopes are different (.941 vs. .989, NL vs. L; $P = .0029$). For this type of plot, the slopes are numerically equal to the coefficient of determination (r^2). Within forages, slopes for the two models also differed. For AH, the r^2 for NL was .945, while for L, $r^2 = .992$ ($P = .0496$). For AM, NL and L yielded r^2 of .933 and .984, respectively ($P = .0205$). A systematic deviation

TABLE 4. Differences between comparable parameters in the two models across all treatment combinations[1].

Item	Lagged − nonlagged[2]	SE[3]	$P<$
A (%), rapidly solubilized N fraction	4.88	.39	.0001
B (%), slowly solubilized but potentially digestible N fraction	−11.03	1.03	.0001
k (h^{-1}), first order rate constant for disappearance from the B fraction	.052	.005	.0001
A + B (%), digestible N fraction	−6.15	.76	.0001

[1] n = 18.

[2] Mean of differences obtained by pairing within a treatment combination.

[3] Standard error of the mean of differences.

from observed values at both high and low N disappearance was found for NL, underpredicting high values and overpredicting low values.

Testing the values for the slopes against a hypothesized value of unity, which would indicate perfect agreement between the model and the data, revealed that the slope for NL was different from 1 (P = .0036) while that for L was not (P = .2102). Within AH, the slope was not different from unity for L (P = .46) but was for NL (P = .0516). Within AM, NL gave a slope different from 1 (P = .0279), while L did not (P = .2873).

DISCUSSION

Inclusion of a lag term into models used to describe N disappearance leads to rather profound changes in interpretation. There were differences between the supplements in readily soluble A and more slowly degraded B fractions using both models. The lagged model, however, predicted a larger A fraction and a smaller B fraction for each supplement than did the nonlagged model. In addition, the estimate of the potentially digestible fraction (A + B) for each supplements was less (than the value predicted by NL) when L was used, and more closely approximated the empirically observed asymptote of the N disappearance curve for each legume supplement. Also, there was a difference between forages in the rate constant for digestion of the B fraction when using NL, but not when using L. Calculation of the effective percentage ruminal degradation [adjusted for ruminal turnover of the B fraction; (6, 11)] indicates that, although the relative difference between the two models increases as passage rate increases, the difference between the two models is not substantial over the range of ruminal particle turnover rates for particles frequently observed in vivo (12) (Table 5). Parameter estimates for A, B, and k, however, were substantially different for the two models (Table 4).

The preponderance of statistical evidence favored L over NL. The reduced residual standard deviation indicated a substantial increase in precision, whereas comparison of observed to predicted values showed greater accuracy of prediction for L. Further, it should be recognized that NL is a specific case of L (the two models are equivalent when lag time equals zero), and that, given the sophisticated computer technologies available to most researchers today, L is no more difficult to compute than NL. If a researcher so desires, lag can be tested

TABLE 5. Effective ruminal nitrogen degradation of aeschynomene hay and alfalfa meal as determined with nonlagged[1] and lagged models.[2]

Model and feedstuff	Ruminal turnover rate[3]		
	.03 h^{-1}	.05 h^{-1}	.07 h^{-1}
	(%)		
Nonlagged			
Aeschynomene hay	64.7	55.3	49.2
Alfalfa meal	71.3	64.2	59.3
Lagged			
Aeschynomene hay	64.1	54.5	47.8
Alfalfa meal	70.4	63.4	58.2

[1] $P = A + B\,[K_{dB}/(K_{dB} + K_{pB})]$, where P = effective ruminal N degradation; A = percentage soluble, rapidly digested N fraction; B = percentage insoluble, more slowly digested nitrogen fraction; K_{dB} = rate constant describing degradation of the B fraction by a 1st order process (h^{-1}); and K_{pB} = rate constant describing passage of the B fraction from the rumen (h^{-1}) (11).

[2] $P = A + B(K_{dB}/(K_{dB} + K_{pB}))\exp(-K_{pB}T)$, where P, A, B, K_d, and K_{pB} are defined as in footnote 1, and T = lag time (6).

[3] Ruminal turnover rate (h^{-1}; equivalent to K_{pB} defined above) describes the rate at which the insoluble but potentially digestible N fraction (B) of a feedstuff passes out of the rumen.

for difference from zero through use of the standard error of that parameter's estimate. Subsequently, we recommend L over NL for description of the time course of N disappearance from other feedstuffs as well.

Still, residual analysis showed greater serial correlation for L than for NL (data not presented). This was likely due to the residuals generated in the early portion of the curve. Examination of Figures 1 and 2 revealed N appearance during the initial portion of the curve, probably due to bacterial attachment (10). Yet NL was continually increasing in this portion, whereas L remained constant. This constancy enabled L to fit the later portions of the curve more closely, resulting in the improved fits described. However, it also forced a structure onto the residuals that inflates the serial correlation.

In conclusion, lack of fit of these models (observed as the deviation of predicted from observed values) implied that the B fraction of each of these legume protein supplements changed in composition over the time period studied. Lack of fit for each model was most extreme in the early portion of the N disappearance curves for AH and AM, and may be attributed to bacterial attachment resulting in increased residual N during early incubation periods (4, 10). To more accurately describe the time course of N disappearance from these forages, more time points should have been included, especially in the early portion of the disappearance curve. Although some protein sources may not exhibit lag prior to the digestion of the insoluble but potentially digestible N fraction (9, 15), under our conditions and with these forages, L better described in situ N disappearance than did NL. The lagged model was not a greater computational challenge than the nonlagged model, and is recommended for other feedstuffs in addition to those specifically reported herein.

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