ABSTRACT: Relative feed value (RFV) was evaluated relative to in situ degradation parameters of grass and legume forages. Early-cut alfalfa (n = 20), late-cut alfalfa (n = 26), cool-season grass (n = 11), warm-season grass (n = 4), and grass-legume (n = 20) samples were collected from duplicate hay bales submitted to the 2002 and 2003 Missouri State Fair Hay Contests. Subsamples were incubated in the rumen of 2 lactating Holstein cows for 0, 6 or 8, 12, 24, and 48 h to determine in situ degradation of DM, ADF, NDF, CP, and hemicellulose over time. Degradation data were fit to a variety of candidate models to estimate degradation parameters. Correlation coefficients between degradation parameter estimates [sorted according to forage (early-cut alfalfa, late-cut alfalfa, grass, or grass-legume)] and RFV were determined. For further comparison, correlations between NDF degradation parameter estimates and digestible DMI were determined with data from a previous study. Degradation data were best fit to a single, gamma 2-distributed pool model without a lag phase. Relative feed value was significantly correlated \((P < 0.05)\) with potentially digestible DM and CP for early-cut alfalfa, potentially digestible DM for late-cut alfalfa, and potentially digestible DM, NDF, and hemicellulose for grass-legume. The percentage of significant correlations (10.7%) across the entire data set was low and no correlations were significant for grass. Relative feed value did not account for the variation in degradation parameters, especially for grasses. A further correlation analysis, which compared digestible DMI with degradation parameter estimates reported from another data set, revealed that digestible DMI and degradation parameter estimates were related for grass but not for alfalfa forages. These results suggest that RFV is limited by its failure to include degradation parameters.

Key words: grass, in situ degradation, legume, relative feed value

INTRODUCTION

Numerous systems have been developed to predict the quality of forages fed to ruminants (Moore, 1994). Relative feed value (RFV; Rohweder et al., 1978) is the most widely used. Relative feed value grades forages according to their predicted digestible DMI (DDMI), the product of DMI and percentage of digestible DM (DDM). Predicted DDMI is divided by a base DDMI to establish an index with a typical full-bloom legume hay scoring 100. To parameterize the RFV system, the National Forage Testing Association selected equations that relate forage NDF and ADF to DMI and DDM, with a base DDMI of 1.29% of BW daily (Linn and Martin, 1989).

Despite the extensive use of the RFV system parameterized according to the National Forage Testing Association recommendations, RFV has been criticized. In their summary, Moore and Undersander (2002) demonstrated that NDF and ADF are inconsistent and poor predictors of DDMI. Sanson and Kercher (1996) found that RFV prediction equations accounted for less than 1% of total variation in DDMI for 20 alfalfa hays fed to lambs.

Although the poor performance of RFV has been identified statistically, further work is needed to ascertain the underlying biological reasons for this. One area that deserves attention is degradation characteristics. Degradation characteristics, such as degradation rate and extent, are linked to DMI and DDM (Mertens, 1973), the 2 factors on which RFV is based. Many studies have measured degradation characteristics, but only for a limited number of forages or chemical fractions. Furthermore, degradation characteristics have rarely been collected to evaluate RFV; only Canbolat...
et al. (2006) has done so, finding variable correlations between in vitro gas production characteristics and RFV for 1 alfalfa sample collected over 3 maturities. To further evaluate RFV, this study was designed to determine degradation characteristics of legume and grass hays that are representative of those graded by the RFV system.

MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Care and Use Committee, University of Missouri-Columbia.

Hay Types and Sampling Procedures

Hay samples were obtained from entries submitted to the Missouri State Fair Hay Contest in 2002 and 2003. The entries came from across the state of Missouri and included early-cut alfalfa (ECA), late-cut alfalfa (LCA), cool-season grass (CSG), warm-season grass (WSG), and grass-legume (GL) samples. A detailed description of the forages, including number of samples collected by year of harvest, class, variety, and cutting within year, is reported in Table 1.

Each entry was submitted as duplicate hay bales. Each bale was cored with a hay probe (Penn State Forage Sampler, Nasco, Ft. Atkinson, CO), and the core samples of duplicate bales were combined to give a representative sample of each entry. Samples were ground in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 2-mm screen. Ground samples were placed in sealed plastic bags and stored at room temperature for further analysis.

In Situ and Chemical Analysis

In situ degradation characteristics were determined for all samples. The samples were analyzed over three 2-d time periods: two 2-d periods for 2002 samples and one 2-d period for 2003 samples (as discussed below). Air-dry Dacron bags (10 × 20 cm; 50 ± 15-μm pore size; Ankom Technology, Macedon, NY) were filled with 5 ± 0.1 g of air-dried sample for 2002 hay samples and 4 ± 0.1 g of sample for 2003 hay samples; the sample mass-to-surface area ratio was approximately 12.5 and 10 mg/cm² for 2002 and 2003 samples, respectively, which are within those suggested by Nocek (1988). Duplicate bags were prepared for insertion into 2 cows, giving a total of 4 bags per sample at each incubation period.
time. Bags were heat-sealed (AIE-200, American International Electric, Wittier, CA), secured to plastic cable ties, and tied in bundles to nylon retrieval cords according to their incubation time.

Two ruminally cannulated, multiparous Holstein cows housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Center were selected for the in situ procedures. Each animal was provided ad libitum access to a standard lactation diet. The diet was a corn silage, alfalfa hay, alfalfa haylage-based diet (19% CP, 24% ADF, and 41% NDF; DM basis) fed as a total mixed ration. Bags were inserted into the ventral rumen of the cows in reverse order. Incubation times chosen were 0, 8, 12, 24, and 48 h for the 2002 hay samples (for the original run, see below) and 0, 6, 12, 24, and 48 h for the 2003 hay samples. All samples within year (2002 or 2003) were to be incubated during a single 2-d period, giving two 2-d periods in total. However, one of the cows used to incubate the 2002 samples stopped ruminating during the 2-d period sampling period, and the samples from this cow for that period were discarded. New subsamples of the 2002 hay samples were incubated in this cow for an additional 2-d period, with incubation times of 0, 6, 12, 24, and 48 h. Samples from 2003 were incubated during a single 2-d period as originally planned, giving a total of three 2-d periods during which the forages were incubated. A standard forage was not used to correct for differences between runs because all samples within a cow × period were incubated in one run, and any systematic differences between cow × period could be detected by comparing degradation parameter values across cow × periods with an ANOVA (see below). During all runs, the 0-h bags were exposed to rumen fluid briefly (approximately 5 min) to allow hydration. All bags were removed simultaneously, as suggested by Nocek (1988).

After removal from the rumen, the bags were doused with cold (approximately 15°C) water to halt fermentation and were rinsed until the wash water ran relatively clear. Bags were then washed in a domestic washing machine until the wash water ran completely clear, as suggested by Cherney et al. (1990b). Samples were air-dried in a 55°C convection oven to a constant mass and then were completely dried in a 105°C oven. Bags were then air-equilibrated and weighed to determine their residue mass. Residues were then removed, composited by duplicates within cow, and stored at <0°C for further analysis.

Bag residue and original forage samples were ground with a Wiley mill to pass through a 1-mm screen. All material was subsequently analyzed for DM by drying at 105°C for 24 h; NDF and ADF were analyzed sequentially by using an Ankom Fiber Analyzer (Ankom Technology), and total N was analyzed by combustion analysis (Leco FP-428, Leco Corporation, St. Joseph, MI). Hemicellulose (HEM) was calculated as the difference between NDF and ADF. No assay to determine microbial contamination was made; in previous work in which bags were washed in a similar manner, Coblentz et al. (1997) reported negligible microbial contamination of the residues.

Calculations and Statistical Analysis

All degradation data were expressed as percentage disappearance. Neutral detergent fiber, HEM, and ADF degradation data at each incubation time were corrected for insoluble material washed from the bag by using a variant of an equation from Weisbjerg et al. (1990), as cited by Stensig et al. (1994). Because NDF, ADF, and HEM are insoluble entities, it was assumed that the truly soluble fraction, W, was 0, so the equation of Weisbjerg et al. (1990) was simplified and applied as follows:

\[ K(t_i) = M(t_i) - P \left(1 - \frac{M(t_i) - P}{1-P}\right) \]

where \( K(t_i) \) is the corrected degradation at time \( t_i \) (g/kg), \( M(t_i) \) is the measured (uncorrected) degradation at time \( t_i \) (g/kg), and \( P \) is the insoluble fraction washed from the 0-h bag (total fraction washed from the 0-h bag; g/kg).

A method was developed to eliminate aberrant bag observations that might be caused by undetected bag rupture or other errors. Although such observations can be identified and eliminated by using conventional statistical methods (e.g., Studentized residuals and Cook’s distance; Kaps and Lamberson, 2004), such methods require the data to be fit to a predetermined model. However, because several models were under consideration, conventional methods could not be applied, and an alternative method for removing outliers was formulated. Bag observations that satisfied any one of the following conditions were considered for removal:

1. Those whose disappearance values were 20% greater than the mean disappearance value of the next incubation time of the same cow;
2. Those whose disappearance values were 20% less than the mean disappearance value of the prior incubation time of the same cow; or
3. Those creating >15% replicate error relative to the duplicate bag at the same incubation time and from the same cow.

Condition 1 is based on the premise that degradation increases monotonically; those that fulfill condition 1 violate that premise. For illustration, Figure 1A shows a 6-h bag observation from a 2002 GL sample that fulfilled condition 1 and was removed. If a bag observation had an aberrantly low disappearance value, it could cause the bag at the next incubation time to fulfill condition 1, even if the bag at the next incubation time was not aberrant. For this reason, condition 2 was enacted to identify such bags with aberrantly low dis-
appearance values and prevent nonaberrant bags from being removed. Figure 1B shows a 12-h bag observation from a 2002 CSG sample that fulfilled condition 2 and was removed. Condition 3 was designed to remove bag observations that failed to fulfill conditions 1 and 2 yet were still grossly aberrant; most duplicate bag pairs repeated well (median replicate error was 2.4% after removing bags that fulfilled conditions 1 and 2), and a large replicate error (>15%) indicated that 1 of 2 bags in a duplicate pair was aberrant. If condition 3 was fulfilled by a duplicate bag pair, the aberrant bag of the pair was identified visually as an outlier from the degradation curve and removed. Although a subjective selection, the aberrant bag of the duplicate pair was usually identified easily; Figure 1C illustrates an example in which a bag from a poorly replicated (17% replicate error) 48-h bag pair (2002 LCA sample) was identified as aberrant because its disappearance (99%) was abnormally high, both absolutely and relative to the asymptotic disappearance value suggested by observations before 48 h.

However, for some 0-h bags, the aberrant bag could not be identified because disappearance values of both bags were reasonable based on the behavior of the degradation curve >6 h; Figure 2 gives an example of a 0-h bag pair from which an aberrant bag could not be identified for removal despite poor repeatability (31% replicate error) of disappearance values within the pair. In this case, no correction could be made, and both bag observations were retained.

Models based on the gamma distribution (Pond et al., 1988; Ellis et al., 1994; Ellis et al., 2005) were considered for describing the in situ degradation data. Because of the limited number of incubation times, only the single pool models reported in Table 2 could be considered. The G1 and G1L models are equivalent to the
commonly used first-order kinetics models of Ørskov and McDonald (1979) and McDonald (1981), respectively, and were considered with less frequently used, age-dependent G2, G2L, G3, and G3L models.

The NLIN procedure (SAS Inst. Inc., Cary, NC) was used to estimate parameters in the models for DM, NDF, ADF, HEM, and CP degradation data. Parameters estimated included the age-dependent degradation rate, \( \lambda_d \) (%/h); fraction degraded at \( t = 0 \), \( a \) (%); fraction not degraded at \( t = 0 \) that was potentially degradable, \( b \) (%); potential extent of degradation, \( a + b \) (%); and the discrete lag time before the onset of degradation, \( \tau \) (h).

Note that most degradation rates reported in the literature are age-independent rates; that is, they refer to specific degradation rates (g of substrate degraded/g of total substrate-h⁻¹) that remain constant over time. The specific degradation rate associated with \( \lambda_d \) (an age-dependent rate) increases asymptotically to \( \lambda_d \) (Ellis et al., 1994). For this reason, comparison between age-independent rates reported in most literature and the age-dependent rate, \( \lambda_d \), is not commensurate and leads to the finding that age-dependent rates are greater than age-independent rates. To allow a more commensurate comparison, the mean degradation rate of \( \lambda_d \), \( k_l \), was calculated as 0.59635 × \( \lambda_d \) (Pond et al., 1988). This mean rate is the specific degradation rate associated with \( \lambda_d \), averaged over time. It is effectively an age-independent equivalent of an age-dependent rate and can be compared with age-independent rates commonly reported in the literature.

Because NDF, ADF, and HEM degradation data had been corrected to make disappearance 0 at \( t = 0 \), the value of \( a \) was constrained to 0 for fitting procedures for these fractions. For all fractions, the value of \( a + b \) was bounded between 0 and 100%, which are the theoretical limits of degradation.

By using the criteria for eliminating aberrant bag observations, nearly all NDF, ADF, and HEM bag observations had to be eliminated for one 2003 ECA sample. So few bag observations were left after this elimination that NDF, ADF, and HEM degradation data could not be fit to a model for this forage sample.

At first, degradation data on each cow were kept separate for the fitting procedure. However, after a preliminary ANOVA indicated that degradation parameter values did not differ consistently across cow × period, the cow × period data were pooled by year of forage (2002 or 2003) so that the data on 2 cows (n = 2, 2002 and n = 2, 2003) were used to construct each degradation curve. The results of model fitting using this pooled data set were used in all subsequent statistical analysis.

Models were evaluated by using residual sums of squares (SSRES), residual mean square (MSRES), and Akaike’s information criterion (AIC) values for DM, CP, and NDF degradation data. The model with the lowest numerical value for each test was considered best (Kaps and Lamberson, 2004).

Relative feed value and forage degradation parameter estimates (\( \lambda_d \), \( k_l \), \( a \), \( b \), and \( a + b \)) were sorted according to forage class (ECA, LCA, grass, or GL). The CORR procedure of SAS was used to determine the correlation coefficients between RFV and the degradation parameter estimates. Correlations with \( P < 0.05 \) were considered significant.

### Table 2. Degradation models considered for describing the in situ data

| Model | Description | Equation¹ |
|-------|-------------|-----------|
| G1    | Single, gamma 1-distributed pool model without lag phase | \( Y(t) = a + b(1 - e^{-\lambda_d \cdot t}) \) |
| G1L   | Single, gamma 1-distributed pool model with lag phase | \( Y(t) = a + b[1 - e^{-\lambda_d \cdot (t - \tau)}] \) |
| G2    | Single, gamma 2-distributed pool model without lag phase | \( Y(t) = a + b[1 - e^{-\lambda_d \cdot t}(1 + \lambda_d \cdot (t - \tau))] \) |
| G2L   | Single, gamma 2-distributed pool model with lag phase | \( Y(t) = a + b[1 - e^{-\lambda_d \cdot (t - \tau)}(1 + \lambda_d \cdot (t - \tau))] \) |
| G3    | Single, gamma 3-distributed pool model without lag phase | \( Y(t) = a + b[1 - e^{-\lambda_d \cdot t}(1 + \lambda_d \cdot (t - \tau)](\lambda_d \cdot (t - \tau)] / 2) \) |
| G3L   | Single, gamma 3-distributed pool model with lag phase | \( Y(t) = a + b[1 - e^{-\lambda_d \cdot (t - \tau)}(1 + \lambda_d \cdot (t - \tau)](\lambda_d \cdot (t - \tau)] / 2) \) |

¹\( Y(t) \) = disappearance (%); \( t \) = time (h); \( \tau \) = discrete lag time before onset of degradation (h); \( k_d \) = age-independent degradation rate (%/h); \( \lambda_d \) = age-dependent degradation rate (%/h); \( a \) = fraction degraded at \( t = 0 \) (%); \( a + b \) = potential extent of degradation (%); \( b \) = fraction not degraded at \( t = 0 \) that is potentially degradable (%).
foi, which was not examined in this study. Digestible DMI was reported for sheep, cattle, or both; if DDMI was reported for both animals, then the average DDMI was calculated and used.

Mertens (1973) fit the degradation data to a model similar to the G1L model (Table 2), which included the degradation parameters \( k_d \) and \( \tau \) but not \( \lambda_d \). Parameter \( \lambda_d \) was estimated from mean lifetime (Ellis et al., 2005), which is related to \( k_d \), \( \tau \), and \( n \) as follows:

\[
\text{mean lifetime} = n/(\lambda_d/100) = 1/(k_d/100 + \tau);
\]

hence,

\[
\lambda_d = n \cdot k_d(1 + k_d/100) \cdot \tau,
\]

where \( n \) is the order of the gamma distribution associated with \( \lambda_d \).

Relative feed value, DDMI, and forage degradation parameter estimates \( (\lambda_d, k_d, a, b, a + b, \tau) \), were sorted according to forage class (alfalfa or grass). The CORR procedure of SAS was used to determine the correlation coefficients between RFV and the degradation parameters. Correlations with \( P < 0.05 \) were considered significant.

## RESULTS AND DISCUSSION

### Chemical Composition

The chemical composition and RFV of the forages is reported in Table 3. The mean and SD of the chemical composition data were generally similar to those summarized by the Dairy NRC (2001), indicating that a representative range of forages was included in this study. However, WSG generally had lower NDF, ADF, and HEM as well as greater CP than bermudagrass reported in the NRC (2001). Warm-season grasses in this study were generally of better quality than those summarized by the NRC (2001). This difference is related to contestants of the Missouri State Fair Hay Contest submitting only higher quality WSG.

### Model Selection

Table 4 illustrates values of SS\(_{RES}\), MS\(_{RES}\), and AIC obtained when fitting the G1, G1L, G2, G2L, G3, and G3L models to the DM, CP, and NDF degradation data of all forages. These values were used as criteria for model fit, with lower values for a given model indicating better fit (Kaps and Lamberson, 2004). On this basis, models (G1, G1L, G2, G2L, G3, and G3L) were assigned a rank of model fit (1 to 6, where 1 indicates best fit and 6 the worst) relative to other models within each chemical fraction (DM, CP, and NDF). Table 5 reports the mean and range of these rankings across fractions (DM, CP, and NDF). Across chemical fractions, values of SS\(_{RES}\) were lowest for the G1L model, and values of SS\(_{RES}\) were lower for lagged (G1L, GL2, GL3) models than for their nonlagged counterparts (G1, G2, G3; Table 4). As such, the G1L model was ranked best and lagged models ranked better than nonlagged models according to the SS\(_{RES}\) criterion (Table 5). However, the use of SS\(_{RES}\) as a model selection criterion was not commensurate in this case because SS\(_{RES}\) decreases with increasing parameters, and the lagged models contained 1 more parameter than the nonlagged models.

As the ratio of SS\(_{RES}\) to error df, MS\(_{RES}\) accounts for the number of parameters in the model and is a more appropriate selection criterion than SS\(_{RES}\) where the number of parameters varies across models, as is the case in the present study. However, MS\(_{RES}\) still tends to decrease with an increasing number of parameters (Kaps and Lamberson, 2004); thus, there is a risk of biased selection of larger models when using MS\(_{RES}\) as a selection criterion. The expression for AIC \([n \log(\text{SS}_{RES}/n) + 2p\] where \( n \) is the number of observations and \( p \) is the number of parameters\] penalizes for excessive parameters in a given model to avoid such biased selection, and for this reason, AIC is the most preferable model selection criterion in this study.

Values of both MS\(_{RES}\) and AIC were lowest for the G2 models and, with the exception of the MS\(_{RES}\) for the G1 model, were lower for nonlagged (G1, G2, G3) models than their lagged counterparts (G1L, G2L, G3L; Table 4). Consequently, the G2 model was ranked best and nonlagged models ranked better than their lagged counterparts. These results indicated that the G2 model was optimal. They also suggest that the decrease in total error (SS\(_{RES}\)) by the inclusion of a lag term was not justified by the addition of a model parameter in so doing.

The above discussion refers to mean SS\(_{RES}\), MS\(_{RES}\), and AIC values and rankings. Values of SS\(_{RES}\), MS\(_{RES}\), and AIC values differed by chemical fraction and so did the rankings in some cases (Tables 4 and 5). Nevertheless, the rankings based on AIC, the most preferred criterion, showed unequivocally that the G2 model was best, because it was ranked 1 across all chemical fractions.

Differences in performance among models may be understood by considering model shapes. Figure 3 shows the fit of the G2 vs. G1 (A), G1L (B), and G3 (C) models to DM disappearance data of a 2003 LCA sample (where the 2 observations at each incubation time represent mean values from each of the 2 cows) to illustrate these shapes. The G2 and other age-dependent models represent degradation as following a sigmoidal curve, with an increasingly more protracted sigmoidal shape as the order increases from G2 to G3 (where \( N \) represents the order of the model; see Figure 3C; Pond et al., 1988; Ellis et al., 1994). The G1 model, by contrast, represents degradation as an abrupt first-order decay process lacking the smooth sigmoidal shape of the age-dependent models (Figure 3A). A lag phase is often added to the G1 model, yielding the G1L model (Figure 3B), because degradation does not begin in-
stantaneously but rather shows a phase of slow degradation at early time points, followed by more rapid degradation later (see Figure 3 of Van Milgen et al., 1991); this lag phase may also be added to the age-dependent models, as was done in this study for comparative purposes, as discussed below.

In this study, the sigmoidal shape of the G2 model appeared to accommodate the transition between these slow and fast degradation phases better than a G1 model, as indicated by the better fit to degradation data between 6 and 12 h (Figure 3A) and the lower error (SSRES) for the G2 model relative to the G1 model. A similar analysis suggested that the G3 model (and higher order models) appeared to possess too protracted a sigmoidal shape to properly model degradation data (Figure 3C).

Although the addition of a lag phase in the G1L model decreased total error (SSRES) relative to the G1 model, it did not improve fit to match the parsimony of the G2 model, as indicated by lower values of MSRES and AIC for the G2 model. Figure 3B shows a typical example in which error of the G1L model fit (SSRES = 0.0829) was indeed lower than that of the G2 model (SSRES = 0.0836) but was so marginal that the increased complexity of the G1L model (where model complexity was measured in terms of number of parameters) was not justified. The addition of a lag term in the G2L and G3L models did not appreciably lower total error relative to non-lagged counterparts and caused MSRES and AIC values to rise. The sigmoidal shape of the age-dependent curves already represented the transition between slow and fast degradation phases; thus, the addition of a lag phase was redundant and reduced parsimony. Often, as in the case of the LCA sample shown in Figure 3, the estimated value of the lag phase for the G2L and G3L models was 0 h, yielding a model shape identical those of the G2 and G3 models and demonstrating that the lag term was a superfluous addition.

The conclusion that a 2-compartmental model such as the G2 model performed better than the G1L agrees with Van Milgen et al. (1991). This report along with that of Ellis et al. (2005) suggest that multicompart-mental age-dependent models often perform better than the often-used G1 and G1L models and should be considered in future in situ and in vitro degradation experiments.

### Table 3. Chemical composition (DM basis) and relative feed value (RFV) of forages

| Item                  | n  | Mean | Minimum | Maximum | SD   |
|-----------------------|----|------|---------|---------|------|
| Early cutting alfalfa |    |      |         |         |      |
| DM, %                 | 20 | 87.0 | 84.2    | 89.6    | 1.4  |
| NDF, %                | 20 | 43.1 | 32.9    | 50.2    | 4.5  |
| ADF, %                | 20 | 30.4 | 21.8    | 36.9    | 4.5  |
| Hemicellulose, %      | 20 | 12.7 | 9.5     | 18.4    | 2.8  |
| CP, %                 | 20 | 20.8 | 15.0    | 29.3    | 3.8  |
| RFV                   | 20 | 143  | 119     | 203     | 23   |
| Late cutting alfalfa  |    |      |         |         |      |
| DM, %                 | 26 | 85.9 | 82.5    | 88.0    | 1.5  |
| NDF, %                | 26 | 38.4 | 26.8    | 46.3    | 5.1  |
| ADF, %                | 26 | 26.5 | 19.5    | 35.4    | 4.3  |
| Hemicellulose, %      | 26 | 11.9 | 6.6     | 18.5    | 3.7  |
| CP, %                 | 26 | 22.2 | 19.4    | 26.0    | 2.0  |
| RFV                   | 26 | 169  | 125     | 254     | 30   |
| Cool-season grass     |    |      |         |         |      |
| DM, %                 | 11 | 87.6 | 86.7    | 89.2    | 0.7  |
| NDF, %                | 11 | 65.8 | 45.2    | 77.2    | 8.1  |
| ADF, %                | 11 | 33.8 | 29.9    | 38.0    | 2.8  |
| Hemicellulose, %      | 11 | 32.0 | 12.3    | 39.2    | 7.3  |
| CP, %                 | 11 | 12.3 | 6.0     | 17.4    | 3.3  |
| RFV                   | 11 | 90   | 71.5    | 130     | 15   |
| Warm-season grass     |    |      |         |         |      |
| DM, %                 | 4  | 86.7 | 84.5    | 88.6    | 1.7  |
| NDF, %                | 4  | 62.3 | 39.5    | 73.2    | 15.5 |
| ADF, %                | 4  | 26.6 | 23.3    | 34.3    | 5.2  |
| Hemicellulose, %      | 4  | 35.7 | 15.5    | 48.4    | 15.4 |
| CP, %                 | 4  | 18.0 | 10.4    | 23.3    | 5.4  |
| RFV                   | 4  | 109  | 88      | 165     | 38   |
| Grass-legume mix      |    |      |         |         |      |
| DM, %                 | 20 | 86.8 | 81.9    | 89.0    | 1.8  |
| NDF, %                | 20 | 45.3 | 35.5    | 61.3    | 5.2  |
| ADF, %                | 20 | 30.4 | 24.1    | 38.4    | 3.6  |
| Hemicellulose, %      | 20 | 14.9 | 10.1    | 27.2    | 4.1  |
| CP, %                 | 20 | 20.4 | 12.4    | 30.8    | 4.0  |
| RFV                   | 20 | 136  | 95      | 184     | 20   |
Influence of Incubation Times on Model Selection and Parameter Estimates

To maximize the number of forages that could be analyzed, the number of degradation observations was limited to only 5 (0, 6 or 8, 12, 24, and 48 h), with relatively few observations at early and late time points. Some may suggest that including more early degradation observations would have led to better performance of lagged vs. nonlagged models, particularly when considering that the first non-0-h observation (6 or 8 h) falls beyond most values of the lag phase (typical values ranging from 1 to 6 h; Mertens, 1973; von Keyserlingk et al., 1996). If there indeed exists a discrete lag phase before the onset of degradation, as represented by the lagged models, the omission of early degradation observations would have artificially improved the fit of nonlagged models. However, if degradation fol-

Table 4. Residual sums of squares (SSRES), residual mean square (MRES), and Akaike's information criterion (AIC) obtained when fitting DM, CP, and NDF degradation data to the various models

| Item  | G1      | G1L     | G2      | G2L     | G3      | G3L     |
|-------|---------|---------|---------|---------|---------|---------|
| SSRES |         |         |         |         |         |         |
| DM    | 1.80    | 1.61    | 1.57    | 1.43    | 1.67    | 1.32    |
| CP    | 4.41    | 3.50    | 3.96    | 3.82    | 4.15    | 3.69    |
| NDF   | 7.31    | 6.20    | 6.79    | 6.22    | 6.95    | 6.93    |
| Mean  | 4.41    | 3.77    | 4.11    | 3.82    | 4.26    | 3.98    |
| MRES  |         |         |         |         |         |         |
| DM    | 2.58    | 2.69    | 2.25    | 2.38    | 2.38    | 2.19    |
| CP    | 5.88    | 5.83    | 5.66    | 6.36    | 5.93    | 6.15    |
| NDF   | 9.13    | 8.86    | 8.49    | 8.89    | 8.69    | 9.91    |
| Mean  | 5.87    | 5.79    | 5.46    | 5.88    | 5.67    | 6.08    |
| AIC   |         |         |         |         |         |         |
| DM    | -8.21   | -6.99   | -8.54   | -6.97   | -8.46   | -7.06   |
| CP    | -6.40   | -4.90   | -6.57   | -4.74   | -6.51   | -4.79   |
| NDF   | -7.28   | -5.74   | -7.50   | -5.78   | -7.44   | -5.45   |
| Mean  | -7.30   | -5.88   | -7.54   | -5.83   | -7.47   | -5.77   |

1G1 = single, gamma 1-distributed pool model without a lag phase; G1L = single, gamma 1-distributed pool model with a lag phase; G2 = single, gamma 2-distributed pool model without a lag phase; G2L = single, gamma 2-distributed pool model with a lag phase; G3 = single, gamma 3-distributed pool model without a lag phase; G3L = single, gamma 3-distributed pool model with a lag phase. See Table 2 for the model equations.

2Values listed are the actual values multiplied by 100.

3Mean value across chemical fractions.

4Values listed are the actual values multiplied by 1,000.

Table 5. Relative ranking (1 to 6) of degradation models according to average values of residual sums of squares (SSRES), residual mean square (MRES), and Akaike's information criterion (AIC)

| Item  | G1      | G1L     | G2      | G2L     | G3      | G3L     |
|-------|---------|---------|---------|---------|---------|---------|
| SSRES rank |         |         |         |         |         |         |
| Mean  | 5.7     | 2.0     | 3.3     | 2.3     | 5.3     | 2.3     |
| Range | 5 to 6  | 1 to 4  | 3 to 4  | 2 to 3  | 5 to 6  | 1 to 4  |
| MRES rank |         |         |         |         |         |         |
| Mean  | 4.3     | 3.7     | 1.3     | 4.7     | 3.0     | 4.0     |
| Range | 3 to 5  | 2 to 6  | 1 to 2  | 4 to 6  | 2 to 4  | 1 to 6  |
| AIC rank |         |         |         |         |         |         |
| Mean  | 3.7     | 4.3     | 1.0     | 5.3     | 2.0     | 4.7     |
| Range | 3 to 5  | 3 to 5  | 1      | 4 to 6  | 2      | 4 to 6  |

1Mean and range ranks refer to rankings across fractions (DM, CP, and NDF).

2G1 = single, gamma 1-distributed pool model without a lag phase; G1L = single, gamma 1-distributed pool model with a lag phase; G2 = single, gamma 2-distributed pool model without a lag phase; G2L = single, gamma 2-distributed pool model with a lag phase; G3 = single, gamma 3-distributed pool model without a lag phase; G3L = single, gamma 3-distributed pool model with a lag phase. See Table 2 for the model equations.
follows a more sigmoidal response, as represented by the G2 and G3 models, the fit of lagged models would have been artificially improved. Thus, the limited number of observations at early incubation times makes the selection of the G2 more uncertain but does not inherently support the G1L or other lagged model as being more appropriate. For the present purposes, the statistical procedures identified the G2 model as the best for use with this data set; thus, all degradation parameter estimates presented herein pertain to the G2 model.

Conversely, the terminal observation was less than the time required to approach asymptotic degradation (approximately 24 to 60 h for high-quality and 48 to 72 h for poor-quality forages; Ørskov et al., 1980). Asymptotic degradation was probably not approached by the 48-h terminal observation for some samples, particularly for the poorer quality WSG. At first consideration, the use of a 48-h terminal observation may seem to greatly underestimate \(a + b\), the estimate of asymptotic degradation, but note that \(a + b\) was not measured as the value of the 48-h terminal observation, but rather was estimated during the model-fitting procedure. Using nonlinear regression to estimate \(a + b\), in comparison with using a log-linear transformation, decreases the sensitivity of \(a + b\) to the value of the terminal observation and appears to report more realistic values of \(a + b\) when terminal observations of 48 h or less are used (Van Milgen et al., 1991).

The G1 and G1L models estimated the value of \(a + b\) as 100%, the upper bound set during fitting procedures, for 16 and 4 of the 402 total degradation curves, respectively; these represent a small number of instances in which \(a + b\) was clearly overestimated despite the use of nonlinear regression. These cases of overestimation may be due more to poor model fit than to the relatively early terminal incubation time per se; as Figure 3A shows, the G1 model (and G1L model to a lesser extent) was often forced to overestimate degradation values of later incubation times to better fit the sigmoidal shape of the degradation profile at early time points, which led to overestimation of \(a + b\) in some cases. The G2 and other higher order models did not appear to display this overestimating property (cf., Figure 3A, 3B, and 3C) and did not reach the 100% bound of \(a + b\) in any case. Because the G2 model was ultimately adopted to estimate all degradation parameters, because nonlinear regression has been shown to deliver more realistic estimates of \(a + b\) (Van Milgen et al., 1991), and because values of \(a + b\) were similar to those in published reports (Mertens, 1973; Brown and Pitman, 1991; von Keyserlingk et al., 1996), one may infer overall that \(a + b\) was underestimated minimally, if at all, by using a 48-h terminal incubation.

### Degradation Parameter Estimate Means

Table 6 reports means of the degradation parameter estimates \(\lambda_d\), \(k\), \(a\), \(b\), and \(a + b\) when using the G2 model. These means, with the exception of \(\lambda_d\) (see below), are similar to those presented by other reports (Smith et al., 1971; Mertens, 1973; von Keyserlingk et al., 1996), indicating they were suitable for the subsequent correlation analysis comparing degradation parameter estimates with RFV. As discussed in the Materials and Methods section, values of \(\lambda_d\) generated in this study were numerically greater than the degradation rate.
Correlation Between RFV and Degradation Parameter Values

Results of the correlation analysis between degradation parameter estimates and RFV scores are presented in Table 7. Six of the 56, or 10.7%, of tested correlations were significant. This percentage only slightly exceeds that which is expected by random chance (5%) caused by the incidence of type I error with α = 0.05. Furthermore, no correlations were significant for grasses. Correlations were thus poor overall. Statistically, this is likely because the relationship between NDF degradation characteristics and NDF concentration is fair to poor; with 275 legume and CSG forages, Mertens (1973) found the correlation coefficient between NDF and NDF degradation rate, extent, and lag to be 0.59, −0.28, and 0.22, respectively. Because RFV is essentially a reexpression of NDF (Weiss, 2002), only fair to poor correlations would be expected between RFV and degradation characteristics. Biological reasons for this poor relationship are discussed in the Shortcomings of the Conceptual Structure of RFV section below.

Despite the general lack of correlations, a few patterns were observed in the correlation analysis. The parameter $DM_{a+b}$, the potential extent of degradation, was consistently significant with RFV, the positive values of $r$ for this and other correlations indicate that RFV accounted for the correct, positive relationship between degradation parameter values and RFV scores.

Although a few patterns were observed in the correlation analysis, it must be emphasized that degrada-
tion parameter values were poorly correlated with RFV overall. Given that degradation parameters are often linked to DMI and DDM (Mertens, 1973), the factors on which RFV is based, it may be concluded tentatively that RFV was inadequate because of weak correlations between it and degradation parameters.

Assumption in Correlation Analysis

This tentative conclusion that RFV was inadequate rests on an assumption that degradation characteristics are related to DDMI. Because RFV is an index of DDMI, the most direct evaluation of RFV would involve a comparison between DDMI and RFV. Nevertheless, if degradation characteristics are related to DDMI, as assumed, RFV can be compared with degradation characteristics as if it is being directly compared with DDMI. The assumption is supported by the observation that DMI, DDM, and degradation parameters are related (Mertens, 1973). Nevertheless, it has not been tested directly because it has not been shown whether a simple linear relationship exists between DDMI and degradation values for an extensive forage data set.

Comparison with Data of Mertens (1973)

To test this assumption directly, the data of Mertens (1973) were used to determine the correlation between NDF degradation parameters and in vivo DDMI, which were reported for a wide range and number of grass and alfalfa forages. Table 8 reports correlations found between DDMI and NDF degradation parameter values reported by Mertens (1973). Correlations between DDMI and NDF degradation parameters were consistently significant for grass; correlations involving $k_d$, $\lambda_d$, and $\tau$ were all significant ($P < 0.001$, $P < 0.001$, and $P = 0.05$, respectively), and the correlation involving $b$ showed a statistical trend ($P = 0.06$; data not shown). Curiously, $NDF_b$ alone was significantly correlated with DDMI for alfalfa. These findings support the view that DDMI is linearly related to degradation parameters of grass but not alfalfa; thus, our assumption that degradation characteristics are related to DDMI was not supported for alfalfa.

Because degradation parameter values were not strongly correlated with DDMI for alfalfa, one might infer that correlations measured in this study cannot be used to demonstrate that RFV is inadequate for alfalfa. However, there are several limitations in the analysis with the data of Mertens (1973) that should caution against drawing this inference. First, the cutting time of alfalfa could not be considered because it was not reported by Mertens (1973). Because alfalfa degradation parameter values differ by cutting time (T. J. Hackmann, J. D. Sampson, and J. N. Spain, unpublished data), the relationships between degradation parameter values, DDMI, and RFV may differ by cutting time as well. Hence, examining these relationships as they pertain to a general alfalfa class only, as done in the analysis involving the data set of Mertens (1973), ignores potential differences between ECA and LCA that may significantly affect the correlation results. For example, degradation parameter values and DDMI were poorly correlated for the alfalfa class from Mertens (1973), but it is possible that stronger correlations exist for ECA and LCA when each cutting is considered separately, and these stronger correlations were simply masked in the analysis of Mertens (1973) by pooling cutting times. The analysis is also limited because degradation parameters of non-NDF chemical fractions could not be considered, because the necessary data were lacking in Mertens (1973). As such, the conclusions drawn from the analysis involving the data of Mertens do not necessarily apply to specific cutting times of alfalfa or to non-NDF degradation parameter values.

Shortcomings of the Conceptual Structure of RFV

Noting the limitations in the analysis involving the data set of Mertens (1973), and considering other studies that find RFV inadequate (Sanson and Kercher,
we suggest that RFV may be limited by its poor relationship with degradation parameter values. Because degradation parameters are related to DMI and DDM (Mertens, 1973), the lack of relationship between RFV and degradation parameters potentially limits the accuracy of RFV equations.

The lack of a relationship between degradation parameters and RFV indicates a pivotal, but not isolated, shortcoming in the conceptual structure of RFV. Relative feed value is a simple empirical prediction system that fundamentally relies on linear equations to predict DMI from NDF and DDM from ADF. It does not explicitly include terms for degradation parameters or any other factors.

In representing DMI and DDM as functions of NDF and ADF alone, RFV does not explicitly consider plant-related factors that affect DMI and DDM. A multitude of non-mutually-exclusive plant-related factors affect DMI and DDM, including forage species, growth conditions (soil type, fertilization, climate), maturity, cutting date, morphology (proportion of leaf and stem), physical properties (density, resistance to breakdown), disease, and processing method (chopping, pelleting; Van Soest et al., 1978; Minson, 1990; Van Soest, 1994).

At least in some cases, these factors may change DMI and DDM independently of NDF and ADF concentrations, contrary to the conceptual structure of RFV. For example, Cherney et al. (1990a) found that intake and DDM of 12 grass hays by sheep changed with plant morphology—intake increased with an increasing proportion of leaf blade, and intake and DDM decreased with an increasing proportion of stem—despite similar concentrations of NDF (65.8 ± 0.7%; mean ± SEM) and ADF (28.9 ± 0.8%) across the hays.

Note that most plant-related factors not accounted for by RFV affect degradation characteristics (forage species, growth conditions, maturity, cutting date, morphology; see review by Mertens, 1993). We suggest that variation in degradation characteristics may capture some variation in plant-related factors that affect DMI and DDM, such that incorporation of degradation characteristics in a forage quality prediction system may improve the prediction accuracy of specific forage classes and cuttings. Incorporating degradation parameters into a forage quality prediction system is unlikely to account for all factors influencing forage quality, such as animal-related factors that interact with forage quality (Minson, 1990), but it is a suitable first step in improving forage quality prediction systems. Whether such a forage quality system should use an empirical approach (such as RFV) or a more mechanistic approach (Baldwin, 1995) is subject to the results of future study. If incorporating degradation characteristics into a forage quality prediction system shows further promise, it must be determined how degradation characteristics should be estimated or measured, because conventional in situ or in vitro procedures are too laborious for routine analysis.

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