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Simplified in situ method for estimating ruminal dry matter and protein degradability of concentrates[1]

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ABSTRACT: In this study, dry matter and crude protein in situ degradation data from different concentrate feeds were used to test the accuracy of effective degradability (ED) measures when using reduced ruminal incubation times compared with models based on seven or eight incubation times. The ED was estimated both with and without correction for nylon bag particle loss. The crude protein ED corrected for particle loss of the calibration data set was widely distributed in a range from 16 to 90% with an overall mean value of 60.4%, and the dry matter ED was distributed in the range from 22.7 to 80.7%, with a mean value of 56.9%. The simplified method was developed based on bilinear regression models where all combinations of one to three disappearance values were tested to find the optimal time point combinations to estimate ED. Bilinear regression models based on two and three ruminal incubation times gave similar estimates to a standard in situ method over a wide range of passage rates both for the data set used to parameterize the models and the independent data set used to evaluate the models. Using two incubation times, the bilinear model based on 4 and 24 h gave the most accurate estimates, and the models based on 2, 8, and 24 h for uncorrected data and 4, 8, and 24 h for corrected data were most accurate of the three time points bilinear models. The number of nylon bags used by these models was reduced by 58 to 78% compared with the standard in situ method, and the total incubation time needed was substantially reduced.

Key Words: Cattle, Degradation, Dry Matter, Mathematical Model, Protein, Rumen

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Introduction

Several methods are used to estimate ruminal degradation of DM and CP (Hvelplund and Weisbjerg, 1998). The in situ method has achieved the widest use. In spite of a number of limitations, no in vitro method has been generally accepted as a satisfactory alternative (Stern et al., 1997; Hvelplund and Weisbjerg, 1998). Therefore, the in situ technique has been chosen as the reference method to measure rumen CP degradation in several protein evaluation systems for ruminants (Madsen, 1985; Vérité and Peyraud, 1989; Tamminga et al., 1994). However, the method requires a large number of nylon bags to be ruminally incubated for each feed sample and, in turn, a substantial amount of human work. Therefore, a need exists for simpler in situ techniques—in particular, for routine analysis (Vanzant et al., 1996). Some simplified in situ techniques have been proposed for degradability measurements. For example, Wilkerson et al. (1995) used only a 16-h ruminal incubation time to estimate the ruminal CP degradability in roughages by assuming that the 16-h sample directly estimated the escape CP, whereas Broderick (1994) and Calsamiglia et al. (1994) calculated effective degradability (**ED**) by estimating the ruminal degradation rate from double-point incubations. However, questions remain as to whether it is possible to develop a simplified in situ method for concentrate feeds that can be used to estimate ED directly without losing accuracy. Therefore, the objective of this study was to evaluate a novel mathematical approach to calculate ED of CP and DM in concentrate feeds from in situ studies using a minimal number of incubation times. We evaluated these models for in situ data both corrected and not corrected for small particle loss of the wash fraction.

Materials and Methods

Feeds

Data from an earlier study by Volden and Harstad (1995) and from different unpublished in situ studies

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Table 1. Water solubility of dry matter, in situ dry matter wash fraction, and uncorrected and corrected effective degradability (ED) for different concentrates

Feed	No.	Water solubility, % of DM[a]	Wash fraction, % of DM[a]	ED, uncorrected, % of DM[a]	ED, corrected, % of DM[a]
Fishmeal	14	24.0 ± 2.3	34.8 ± 4.3	44.2 ± 4.0	35.0 ± 2.5
Barley	15	10.2 ± 1.4	30.8 ± 1.6	77.2 ± 1.8	70.7 ± 1.5
Oats	15	6.9 ± 0.7	52.4 ± 2.0	72.3 ± 0.7	44.4 ± 2.2
Soymeal, extracted	8	30.1 ± 0.6	31.1 ± 0.7	68.7 ± 0.8	68.1 ± 0.9
Rape seed meal	12	21.2 ± 1.5	30.3 ± 2.3	65.8 ± 2.1	61.9 ± 1.1
Rape seeds	4	11.7 ± 2.0	17.4 ± 5.5	53.7 ± 8.5	51.0 ± 7.1
Lupine seeds	5	34.8 ± 3.5	41.3 ± 4.7	77.8 ± 2.5	75.4 ± 2.2
Concentrate mixtures	54	16.1 ± 0.5	47.3 ± 1.0	74.3 ± 0.3	58.3 ± 0.8
Peas	2	26.8	54.7	76.4	61.4
Maize	1	8.2	14.3	48.0	44.2
Maize gluten meal	2	8.6	14.6	32.4	27.7
Dried beet pulp[b]	3	24.0	20.0	60.0	
Guar meal[b]	1	28.2	27.9	74.4	

[a]Standard deviation not listed for feeds with three or fewer samples.
[b]Wash fraction was smaller than water solubility, therefore there was no corrected degradability.

at the Agricultural University of Norway were used to develop the models. Descriptions of the feeds used in the present study to develop the simplified models are shown in Tables 1 and 2. The material consisted of 81 individual feed ingredients and 54 concentrate mixtures. The concentrate mixtures consisted mainly of barley and oats treated with an annular gap expander (OE expander; Amandus Kahl, Reinbek, Germany) in the range of 110 to 130°C.

To evaluate the simplified models, we used an independent data set (H. Volden, unpublished data) consisting of 32 individual feed ingredients (Table 3). In these feeds, there was no correction for small particle loss of the wash fraction.

In situ Measurements

Three nonlactating cows fed a diet consisting of two-thirds hay and one-third concentrate mixture on DM basis at maintenance level (Madsen et al., 1995) were used to obtain the in situ disappearance profiles of DM and CP. Feed samples were ground through a 1.5-mm screen. Approximately 2 g of feed was placed into nylon bags (6 × 12 cm, 13 to 15 mg/cm^2) with a pore size of 36 μm (ZBF AG, CH 8803, Rüschlicon, Switzerland). Nylon bags were incubated in the rumen for 2, 4, 8, 16, 24, and 48 h. For 44 feed samples, nylon bags were additionally incubated for 72 h. The number of nylon bags used varied by incubation time and cow: three (0 and 2 h), four (4, 8, 16, and 24 h), six (48 h), and eight (72 h). The rationale for increasing the number of bags with increased incubation time was to ensure adequate residue for subsequent analysis. Both DM and CP disappearance values were measured for each cow. The in situ 0-h wash fraction of DM and CP was measured by washing nylon bags containing samples in a domestic washing machine for 3 × 10 min in cold water. Water solubility of DM and CP was obtained by soaking a 1-

Table 2. Water solubility of crude protein, in situ crude protein wash fraction, and uncorrected and corrected effective degradability (ED) for different concentrates

Feed	No.	Water solubility, % of CP[a]	Wash fraction % of CP[a]	ED, uncorrected, % of CP[a]	ED, corrected, % of CP[a]
Fishmeal	14	19.6 ± 3.2	31.6 ± 4.4	46.0 ± 4.1	36.7 ± 3.6
Barley	15	18.6 ± 4.0	31.5 ± 4.1	74.2 ± 1.2	66.9 ± 1.4
Oats	15	9.4 ± 0.9	58.0 ± 2.7	91.2 ± 0.9	81.1 ± 1.1
Soymeal, extracted	8	6.3 ± 1.1	13.9 ± 2.1	62.7 ± 1.7	59.0 ± 1.8
Rape seed meal	12	12.8 ± 1.7	29.7 ± 3.4	71.6 ± 2.1	64.8 ± 1.9
Rape seeds	4	15.6 ± 4.3	21.4 ± 4.3	63.0 ± 9.6	59.2 ± 11.5
Lupine seeds	5	35.3 ± 8.2	49.8 ± 6.7	86.5 ± 2.8	82.7 ± 3.1
Concentrate mixtures	54	13.4 ± 0.9	41.0 ± 1.4	71.0 ± 0.9	56.9 ± 1.3
Peas	2	55.3	67.6	86.4	81.0
Maize	1	8.9	13.8	32.5	28.7
Maize gluten meal	2	5.3	10.0	23.6	19.6
Dried sugar beet pulp	3	25.0	27.7	56.8	55.3
Guar meal	1	8.1	13.3	68.3	66.4

[a]Standard deviation not listed for feeds with three or fewer samples.

g sample of feed prepared for in situ measurements in 40 mL of distilled water in a 100-mL centrifuge tube at 20°C for 1 h with shaking every 10 min. The sample was then filtered through a Schleicher & Schuell 589[1] black ribbon filter (Ref. No. 300-011) and further washed with 3 × 40 mL of distilled water. After washing, the filter was dried at 45°C for 48 h and then weighed and analyzed for N in the feed residue. The ruminal incubation program and washing and drying procedures were completed as described by Volden and Harstad (1995).

All cows were cared for according to laws and regulations controlling experiments on live animals in Norway (i.e., the Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance concerning Experiments on Animals of January 15, 1996).

Table 3. Dry matter and crude protein in situ effective degradability of the feeds used to evaluate the simplified models

Feed	No.	DM	CP
Wheat	5	83.8 ± 1.5	77.7 ± 1.7
Barley	5	78.0 ± 1.8	74.0 ± 2.3
Oats	5	71.1 ± 1.6	88.7 ± 1.2
Rape seeds	7	56.6 ± 2.4	63.6 ± 3.3
Soymeal, extracted	1	67.8	64.0
SoyPass	1	54.4	39.5
Peas	2	73.9	83.5
Maize	1	52.3	38.3
Sorghum	1	48.9	31.0
Rye	2	80.0	78.6
Wheat bran	1	63.2	73.8
Dried sugar beet pulp	1	56.6	42.9

Estimating Effective Degradability by the Reference Method

Effective degradability was estimated with or without correction for particle loss of the wash fraction. The wash fraction was assumed to be totally and instantaneously degraded when not corrected for particulate loss. The particulate loss was measured as the difference between the in situ 0-h wash fraction and the water-soluble fraction. Three assumptions were made for the properties of the particle loss fraction (Dhanoa et al., 1999): 1) it consists of a degradable and undegradable fraction of the same ratio as the particles remaining in the bag after washing; 2) the degradation rate was the same for the particle loss fraction as for the particles remaining in the bag; and 3) the passage rate was the same for the particle loss fraction as for the particles remaining in the bag.

These three assumptions give the following equation to adjust the experimental disappearance values when correcting for particle loss in the wash fraction:

$$D^{corr}(t_i) = \frac{D(t_i)(100 - s) - (w - s)}{100 - w} \quad [1]$$

where $D(t_i)$ and $D^{corr}(t_i)$ are the measured and corrected percentage disappearance at time t_i, s is the water solubility (%), and w is the wash fraction (%).

The in situ residues were not corrected for microbial contamination. Although this will affect the absolute degradation values, it will not change the principles in development of simplified in situ methods and their evaluation.

In the reference method, with a full-time incubation program, the kinetics of the in situ DM and CP disappearance were calculated by a generalized Von Bertalanaffy model (López et al., 1999) expressed as:

$$D(t) = A + B(1 - e^{-ct})^{1/v} \quad [2]$$

where $D(t)$ is the percentage of disappearance from the bags at time t, A and B are the soluble and potentially degradable fractions, and c is the first-order degradation rate. The v parameter will adjust the shape of the curve where low values result in a sigmoid shape (Figure 1, SoyPass), high values result in an initially very steep curve (Figure 1, barley), and a v = 1 gives a first-order kinetics. This model was used both for the uncorrected and corrected experimental disappearance values. The nonlinear parameters in both models were estimated from a Levenberg-Marquardt algorithm implemented in MATLAB (The MathWorks, Inc., Natick, MA).

The ED was calculated by Fogler (1992) as:

$$ED_r = \int_0^\infty D(t) \cdot E(t)dt \quad [3]$$

where the r subscript denotes the reference method and $E(t)$ is the rumen residence time distribution function (**RTD**) of the feed particles. The RTD function is a normalized, dimensionless ruminal outflow concentration profile, where the total area under the curve is equal to 1. Equation [3] may be used for any combination of rumen degradation ($D[t]$) and RTD model ($E[t]$). We used the RTD for an ideally mixing compartment, $E(t) = k_p \cdot e^{-k_p t}$, where k_p is the passage rate (h^{-1}) of feed particles out of the rumen. In the calculations of ED, the passage rate was set at 0.06 h^{-1} (if no other value was specified), which is used for concentrates in several protein-evaluating systems (Vérité and Peyraud, 1989; Tamminga et al., 1994). The disappearance and RTD models were inserted into Eq. [3] and integrated numerically to estimate effective degradability.

Simplified Method: Estimating Effective Degradability Using a Bilinear Regression Model

Effective Degradability was estimated by simple bilinear models, generally described by:

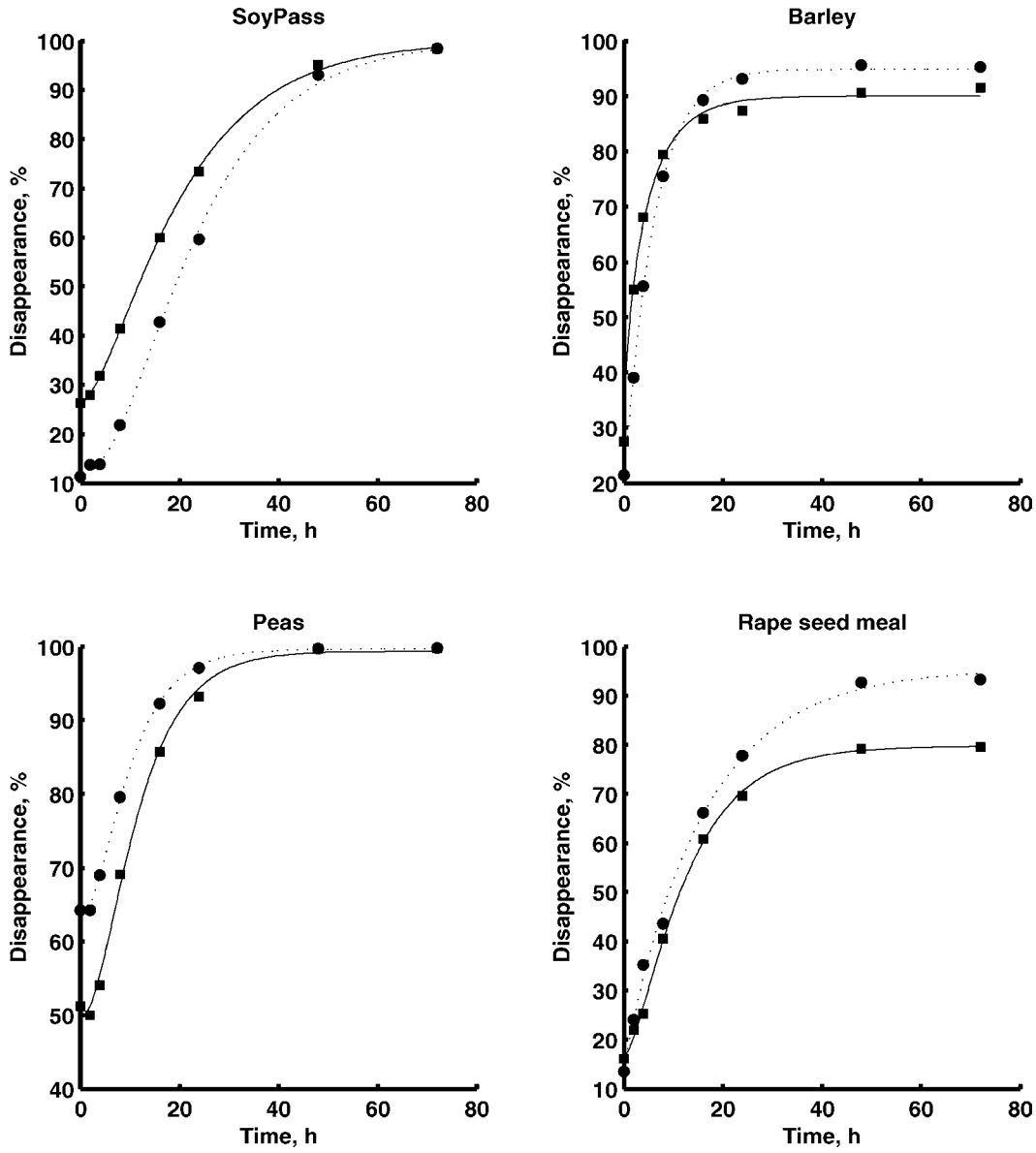

Figure 1. Dry matter (■) and crude protein (•) disappearance values (not corrected for particle loss of the wash fraction) and their respective curves fitted with the equation $D(t) = A + B(1 - e^{-ct})^{1/v}$.

$$ED_b = a_0 + \sum_{i=1}^{n} a_i \cdot D(t_i) \quad [4]$$

$$ED_b = a_0 + a_1 s + \sum_{i=2}^{n} a_i \cdot D^{corr}(t_i) \quad [5]$$

where the b subscript denotes the bilinear model, a_0 and a_i are the parameters, $D(t_i)$ is the percentage of DM or CP disappearance from the bags at the times t_i, and n is the number of incubation times used in the model. For uncorrected data, this general model was used to test all one- (7), two- (21), and three-timepoint (35) model combinations from 0 (wash sample) to 48 h to determine the timepoints that were optimal for estimating ED. Both the wash and the water-soluble fractions had to be measured when using corrected disappearance values (Eq. [1]), therefore, the water solubility was incorporated in all bilinear models using disappearance values corrected for particle loss of the wash fraction:

where $D^{corr}(t_i)$ is the corrected percentage of DM or CP disappearance, a_0, a_1, and a_i are the parameters, and s is the water solubility (%). The bilinear models were parameterized separately for the DM and CP $D(t)$ data sets and for a data set containing both the DM and CP $D(t)$ values.

The accuracy relative to the reference model of the different bilinear models was evaluated by calculating the root mean square error (**RMSE**):

$$\text{RMSE} = \sqrt{\frac{\sum(\text{ED}_r - \text{ED}_b)^2}{n}} \quad [6]$$

Table 4. Root mean square error (RMSE), coefficient of determination, and percentage reduction in the number of nylon bags by different bilinear regression models

Uncorrected				Corrected			
Incubation times, h	RMSE	r^2, %	Percentage reduction[a]	Incubation times, h	RMSE	r^2, %	Percentage reduction[a]
8	3.08	97.5	89	8	3.38	97.2	81
4 and 24	1.47	99.5	78	4 and 24	1.67	99.3	69
2, 8, and 24	0.96	99.8	69	4, 8, and 24	1.07	99.7	58

[a]Percentage reduction in number of nylon bags compared to the reference method, with 72 h as longest incubation time.

Results and Discussion

Effective Degradability Estimated by the Reference Method

Tables 1 and 2 show the mean values for DM and CP water solubility, in situ wash fraction, and estimated ED for the feeds used to parameterize the bilinear models, respectively. There was a large difference between water solubility and wash fraction for many of the feeds—in particular, for oats where the wash machine loss for DM and CP was more than 45 percentage units higher than the water solubility. These large differences also resulted in large differences between corrected and uncorrected degradability estimates, in particular, for oats, concentrate mixtures, and peas. The mean ED varied largely between the different feeds. Calculated with a passage rate of 0.06 h^{-1}, the uncorrected CP ED values in the data set used to parameterize the models ranged from 23 to 95%, with a mean value of 70.2%. The corrected CP ED values ranged from 16 to 90%, with a mean value of 60.4%. Also, the shape of the disappearance curves varied widely, and Figure 1 illustrates some of the disappearance curve variation in the data set. The *v* parameter in the generalized Van Bertalanaffy model (Eq. [2]) made this model very flexible, and curves with both slow and steep initial degradation rate were fitted well by this model (Figure 1).

Table 3 shows DM and CP uncorrected mean ED in the independent data set used to test the bilinear models. The total mean CP ED of this data set was 70.4% and ranged from 31.0 to 89.8%, which was similar to the data set used to parameterize the models.

Effective Degradability Estimated by Bilinear Regression Models

Table 4 shows RMSE and r^2 values for the one, two, and three ruminal incubation time models with the lowest RMSE values compared to the reference method. Using a passage rate of 0.06 h^{-1}, more than 97% of the variation in ED was explained when the one- incubation time (8 h) bilinear model was used. For passage rates lower than 0.05 h^{-1}, a model based on the 16-h incubation time showed the lowest RMSE value of the one-timepoint models (data not shown). However, with 17% of the residuals larger than 4 percentage units (uncorrected model) in absolute value, the errors seemed to be too large for using only a single timepoint model in routine analyses. Using a two-timepoint model, only 2% of the residuals were larger than 4 percentage units in absolute value, and the model based on 4- and 24-h incubation times generally had the lowest RMSE of the two-point model across the passage rates tested (Figure 2). Using uncorrected data, the model based on 2-, 8-, and 24-h incubation times had the lowest RMSE of the three-timepoint models for passage rates higher than 0.047 h^{-} (Figures 2 and 3), and the model based on 2, 16, and 48 h had lowest RMSE for slower passage rates. Using data corrected for particle loss, the model based on 4-, 8-, and 24-h incubation times had the lowest RMSE of the three-timepoint models. The parameters in the two- and three-timepoint models varied with the passage rate and are presented in Tables 5 and 6. These

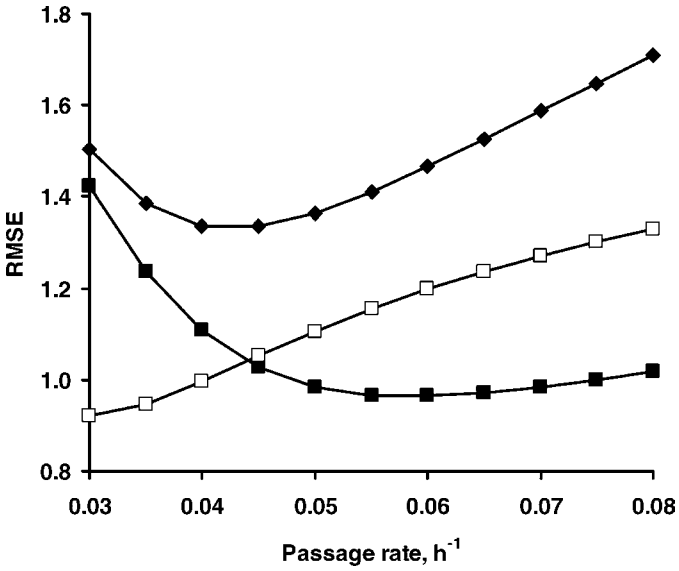

Figure 2. Root mean square error of fit relative to the standard method (RMSE, percentage units) as a function of passage rate for different two and three incubation times models not corrected for particle loss of wash fraction. ♦ = 4 and 24 h; ■ = 2, 8, and 24 h; □ = 2, 16, and 48 h.

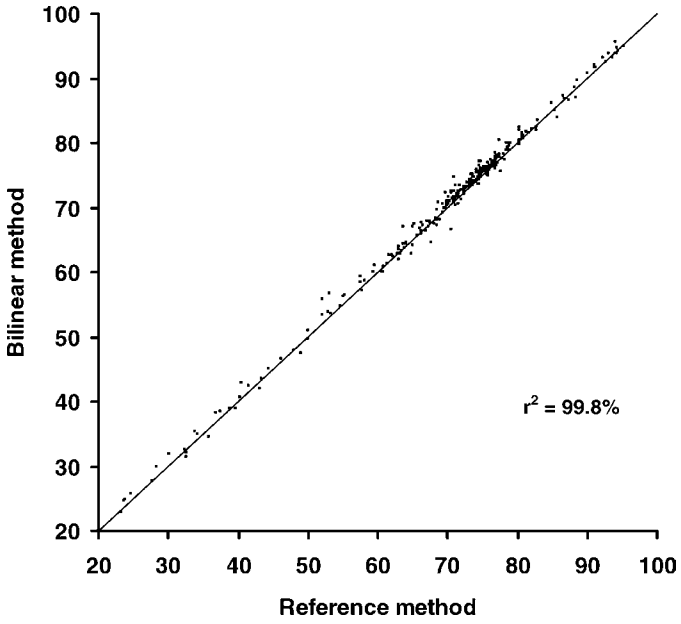

Figure 3. Correlation plot for effective degradability calculated by the reference method (ED*r*) and effective degradability calculated using the three incubation times (2, 8, and 24 h) and the bilinear regression method (ED*b*) at a passage rate of 0.06 h^{-1} for the data set used to derive the bilinear models. Data were not corrected for particle loss of wash fraction.

parameters can be combined with in situ disappearance values to estimate ED directly if using the same incubation times as in Tables 5 and 6. The parameters can be estimated by linear interpolation if the same incubation times and passage rates between the values in Tables 5 and 6 are used. These parameters can also be used for data corrected or not corrected for microbial contamination. However, new parameters must be estimated if different incubation times than those presented in Table 5 and 6 are used. These parameters were estimated using both DM and CP data. The parameters and RMSE values were only slightly different when the parameters were estimated using DM and CP data separately. As we discuss later, the bilinear regression method may be considered a simple numerical integration of the degradation Eq. [3]. Since these parameters represent the area sections under the $E(t)$ curve, it is reasonable to assume that the parameters will be similar for different feed fractions, such as DM and CP. The parameters presented in this paper can probably also be applied to other feed fractions, such as starch, NDF, and individual amino acids, although the models presented in this study were developed for DM and CP data only. The current study deals strictly with concentrate feeds. Forages have slower passage rates than concentrates (0.02 to 0.05 h^{-1}; Poncet et al., 1995). Figure 2 shows that the three-point bilinear model based on 2, 16, and 48 h gave low RMSE values at low passage rates. We therefore believe that a similar approach will work well with forages too.

Using the parameters in Table 5 on the independent data set gave ED estimates very similar to those estimated by the reference method (Figure 4). The accuracy relative to the reference method for the two- and three-timepoint bilinear models was nearly equal to the accuracy found in the data set used to parameterized the models. For the three-timepoint model, the r^2 values were even higher for the evaluation data set than the data set used to parameterize the models. The predictability of ED using the bilinear models was therefore good.

Poncet et al. (1995) reported that the passage rate of labeled concentrate particles is usually in the range of 0.04 to 0.06 h^{-1} in dairy cows. The French (Vérité and Peyraud, 1989) and Dutch (Tamminga et al., 1994) protein-evaluating system used a passage rate of 0.06 h^{-1} for concentrates, whereas the Nordic AAT/PBV system used passage rates of 0.04 to 0.08 h^{-1}, depending on the country (Madsen et al., 1995). Both two- and three-timepoint bilinear regression models fitted the data well in the actual passage rate range (0.04 to 0.08 h^{-1}). The estimates relative to the standard method were substantially more precise for the three-timepoint model than for the two-timepoint model (Figure 2), and RMSE did not vary much for the three-timepoint model within the range of realistic passage rates evaluated.

Table 5. Parameters in two and three ruminal incubation times for different passage rates of bilinear regression models not corrected for particle loss of wash fraction[a]

Passage rate, h^{-1}	Two ruminal incubation times model parameters[b]			Three ruminal incubation times model parameters[c]			
	a_0	a_1	a_2	a_0	a_1	a_2	a_3
0.04	9.1636	0.3562	0.5470	9.4615	0.1657	0.2437	0.4897
0.05	5.9113	0.4336	0.5059	6.1846	0.2102	0.2852	0.4411
0.06	3.8602	0.5001	0.4620	4.0662	0.2526	0.3145	0.3937
0.07	2.5662	0.5576	0.4184	2.6777	0.2927	0.3350	0.3493
0.08	1.7638	0.6077	0.3767	1.7630	0.3304	0.3490	0.3087

[a]The parameters were estimated from a combination of DM and CP data.
[b]ED = $a_0 + a_1 \cdot D(4\text{ h}) + a_2 \cdot D(24\text{ h})$.
[c]ED = $a_0 + a_1 \cdot D(2\text{ h}) + a_2 \cdot D(8\text{ h}) + a_3 \cdot D(24\text{ h})$.

Table 6. Parameters in two and three ruminal incubation times for different passage rates of bilinear regression models corrected for particle loss of wash fraction[a]

Passage rate, h^{-1}	Two ruminal incubation times model parameters[b]				Three ruminal incubation times model parameters[c]				
	a_0	a_1	a_2	a_3	a_0	a_1	a_2	a_3	a_4
0.04	7.1098	0.0757	0.3192	0.5712	7.5596	0.0753	0.2074	0.1545	0.5162
0.05	4.3545	0.0825	0.3886	0.5275	4.9455	0.0820	0.2416	0.2031	0.4552
0.06	2.5761	0.0909	0.4463	0.4831	3.2637	0.0903	0.2754	0.2363	0.3990
0.07	1.4173	0.1002	0.4946	0.4403	2.1711	0.0995	0.3072	0.2590	0.3481
0.08	0.6628	0.1099	0.5352	0.4003	1.4615	0.1092	0.3367	0.2745	0.3026

[a]The parameters were estimated from a combination of DM and CP data.
[b]ED = $_0 + a_1 \cdot s + _2 \cdot D(4\text{ h}) + a_2 \cdot D$D(24 h).
[c]ED = $a_0 + a_1 \cdot s + a_2 \cdot D(4\text{ h}) + a_3 \cdot D(8\text{ h}) + a_3 \cdot D(24\text{ h})$.

Therefore, the question of which model to choose is one of what to stress the most: simplicity or accuracy. The bilinear regression models substantially reduced the number of nylon bags compared to the reference in situ method (Table 4). Therefore, the amount of manual human work and, in turn, costs, can be considerably reduced. In addition, the reduction of total incubation time to 24-h will increase the number of samples that can be analyzed for each cow. Additional measurements of the wash value and water solubility have to be carried out if the degradability is to be corrected for small particle loss. Still, there will be a substantial reduction of human work compared to the reference method.

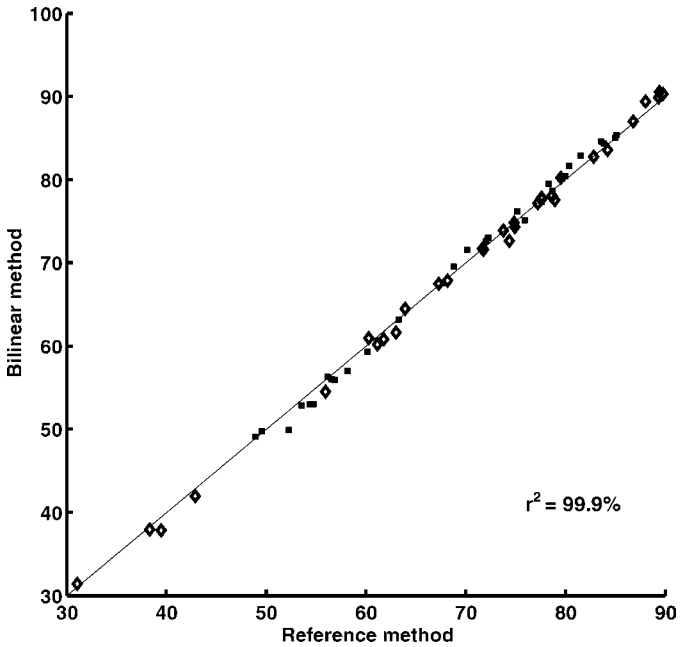

Figure 4. Correlation plot for effective degradability calculated by the reference method (ED*r*) and effective degradability calculated using the three incubation times (2, 8, and 24 h) and the bilinear regression method (ED*b*) at a passage rate of 0.06 h^{-1} for the data set used to evaluate the bilinear models (■ = DM; ◇ = CP). Data were not corrected for particle loss of wash fraction.

The bilinear regression method may be considered a simple numerical integration of the degradation equation (Eq. [3]). Integrating this equation using a mathematical model for E(t) and assuming four stepwise constant $D(t)$ values gives:

$$ED = D(t_1) \cdot \int_0^{t_i} E(t)dt + D(t_2) \cdot \int_{t_{i1}}^{t_{i2}} E(t)dt + D(t_3) \cdot \int_{t_{i2}}^{t_{i3}} E(t)dt + D(t_4) \cdot \int_{t_{i3}}^{\infty} E(t)dt \quad [7]$$

where the integration limits, t_{i1}, t_{i2}, and t_{i3}, are unknown. If we further assume that $D(t_4)$ is constant for all feeds, then the last term of Eq. [7] becomes a constant (a_0). This assumption is of course crude, but if t_{i3} is high, then the contribution to ED from this term would be small. Writing the values of the integrals as parameters results in the following equation:

$$\int_0^{t_{i1}} E(t)dt = a_1, \int_{t_{i1}}^{t_{i2}} E(t)dt = a_2, \text{ and } \int_{t_{i2}}^{t_{i3}} E(t)dt = a_3 \quad [8]$$

Equation [7] can be expressed as:

$$ED = a_0 + a_1 \cdot D(t_1) + a_2 \cdot D(t_2) + a_3 \cdot D(t_3) \quad [9]$$

This equation is the three-timepoint bilinear regression model where the integration limits in Eq. [8] can be calculated from the estimated parameters a_1, a_2, and a_3. Figure 5 shows two residence time distributions with passage rates of 0.04 h and 0.08 h^{-1}, and three integration limits. The areas under the graphs from 0 to t_{i1} and from t_{i1} to t_{i2} are larger for the RTD curve with the highest passage rate (Table 5). For this curve, the area under the curve from t_{i3} to infinity is also very small; therefore, we do not make an error by assuming a constant value for the last term in Eq. [7]. However, for the slowest RTD curve, this area is rather big, and

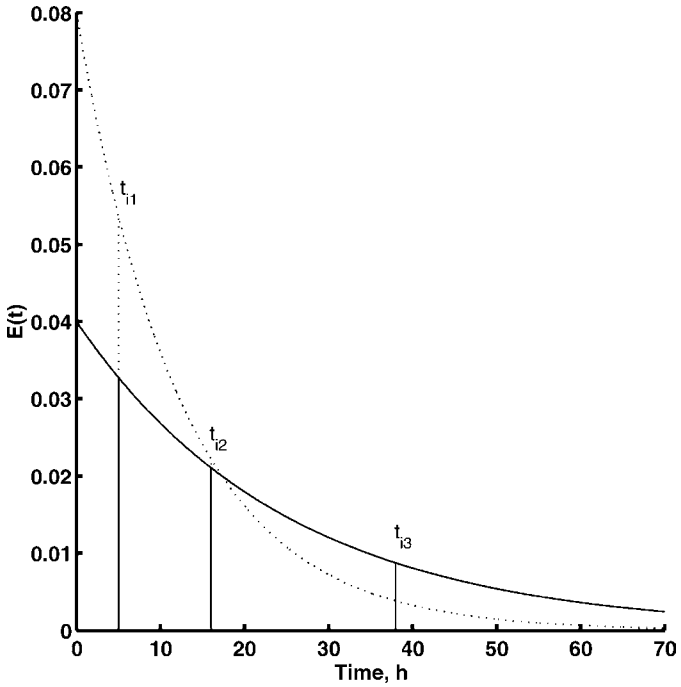

Figure 5. The residence-time distributions for passage rates of 0.04 h^{-1} (——) and 0.08 h^{-1} (·····). Three integration limits are indicated (t_{i1}, t_{i2}, and t_{i3}).

the same assumption will cause much larger errors. Figure 2 shows that the error increases as the passage rate decreases for models with 24 h as the longest incubation time.

Some simplified in situ techniques have been used for degradability measurements. Wilkerson et al. (1995) used only a 16-h ruminal incubation time to estimate the ruminal protein degradability in roughages by assuming that the 16-h sample directly estimated the escape protein. Broderick (1994) proposed a model that was based on estimating the degradation rate from the wash sample (0 h) and one sample incubated in the rumen (16 h) and by using acid detergent insoluble protein as an estimate of the total undegradable fraction. Vanzant et al. (1996) used this simplified technique to calculate the protein degradability in prairie hay and alfalfa. Using only the 0- and 16-h points to construct degradation rates resulted in similar estimates of protein degradation when compared with full time-series calculations. Calsamiglia et al. (1994) observed that degradation rate and degradability, based on double-point incubations (0 and 24 h or 2 and 24 h), explained at least 99% of the variation in measurements determined from curves based on seven timepoints in the range of 0 to 24 h. Nevertheless, in the present article, ED is calculated directly from the experimental disappearance values, which is in contrast to estimated degradation parameters, and where these are used in further calculations of degradability. Degradation parameters can be misleading when estimated from too few experimental values. Using two or three incubation times, one wrong disappearance value can result in an entire degradation curve being wrong. The approach used in the present paper is not that vulnerable for one wrong disappearance value since each value only contributes to a fraction of the calculated ED values. This makes the bilinear model approach more robust than the methods described by Broderick (1994) and Calsamiglia et al. (1994).

In this study, we have only tested models based on the discrete incubation times already used in the standard in situ method (0, 2, 4, 8, 16, 24, and 48 h). A more rigorous approach would be an optimization based on all possible incubation times. However, although somewhat limited in its approach, this study clearly demonstrated the potential of reducing the number of incubation times.

Implications

The objective of this study was to test accuracy of in situ effective degradability measures in concentrates when using reduced numbers of ruminal incubation times. The simplified method was based on bilinear regression models, where one to three disappearance values were used to estimate effective degradability directly. Bilinear regression models, based on two and three incubation times, gave similar effective degradability estimates as calculated from seven or eight incubation times. Replacing the full-timescale nylon bag procedure with a two or three ruminal incubation times model may reduce the amount of human work considerably, and this reduction may be obtained without much loss in the accuracy of the method relative to the standard in situ method. However, this model is only valid for estimation of effective degradability or escape values and not for estimation of degradation rates.

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