Nonlinear Mixed Models Applied to Ruminal Degradability Studies

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Abstract

This article presents an application of three classical models to studies of ruminal degradation kinetics, namely Ørskov and McDonald's model (1979); Van Milgen, Murphy and Berger's model (1991), and Richard's model proposed in France, Dijkstra, and Dhanoa (1996). Our approach is focused on accounting for animal effects given that measurements are repeated in the same animal. The models were studied under the perspective of nonlinear mixed-effects (NLME) models. In this way, we intended to accommodate the problems of response variance heterogeneity and correlations between repeated measures. To apply the proposed method, we used data from an experiment conducted in a Latin square design to assess the dry matter degradability of the following three silages: Elephant grass (*Pennisetum purpureum* Schumach.) silage treated with bacterial inoculant, Elephant grass silage treated with enzyme-bacterial inoculant, and corn (*Zea mays L.*) silage. Samples were incubation for 0, 2, 6, 12 , 24, 48, 72 and 96 h. For these experimental data, the Van Milgen, Murphy, and Berger's model showed better performance than the others. The proposed approach indicated that inclusion of animal effects is important for obtaining more accurate information and can be considered in NLME modeling. Furthermore, it was also possible to perform an easy-to-interpret analysis of contrasts between treatments by using Tukey's test.

Keywords: Degradation kinetics, Digestibility, *In situ* degradability, Intake, Random effects, Rumen degradation.

1. Introduction

Collections that generate correlated data are common in several areas of knowledge. In many situations, such collections are carried out longitudinally, implying that observations on the same individual are correlated and not independent, which limits the use of certain statistical methods and techniques. Correlations can be accounted for by using the mixed model theory, which relates a response variable to predictor variables as fixed and random effects factors under the assumption that the residual distribution is Gaussian (J. C. Pinheiro & Bates, 2000). Most of the time, however, linear mixed models are not appropriate for explaining relationships between variables. In such analyses, nonlinear mixed effects (NLME) models arise as an extension of linear mixed models for describing nonlinear parameters.

In the case of longitudinal data, the interest commonly lies in specific individual characteristics, given that the dependent variable or response is measured several times and the effect associated with the individual/subject is included in the model as a random effects factor. That is, mixed models are often used to deal with correlated or hierarchical data. Also, recognizing that there are random effects factors influencing the observed response can increase the accuracy and precision of fixed effects estimates, minimizing seriously inflated type I error rates (Wang, 2016).

The nonlinear mixed model is based on a mean curve that is fitted to the data, such that individual curves incorporating the random effects of each individual appear as deviations from this mean curve. In literature, there are several methods proposed to model continuous, unbalanced, and multilevel longitudinal data. One of the first models, proposed by Gregoire and Schabenberger (1996) incorporates subject random effects, whereas that developed by Littell (2006) directly models correlation structure. Gregoire and Schabenberger's (1996) approach employs nonlinear fixed effects models, inducing correlations in the marginal distribution of within-subject observations and using random effects that vary across subjects to reduce the impact of autocorrelation. The second procedure (Littell, 2006) uses a covariance structure and generalized least squares estimators, which are considered the best unbiased estimators (Tasissa & Burkhart, 1997).

In the field of cattle research, it is common to find articles that discuss longitudinal data without taking into account possible animal correlations (among observation in the same subject). An example is seen in rumen degradability studies using the model proposed by Mehrez and Ørskov (1977). This approach may lead to inappropriate conclusions because it ignores important effects and makes assumptions that are inconsistent with the reality of the data.

Rossi, Martins, Guedes, and Jobim (2010) noted that alternative and/or more innovative methods can provide a more parsimonious explanation for data of this nature. The authors emphasized the importance of Bayesian inference to make comparisons between parameters while considering different experimental treatments in a coherent manner, without having to resort, for example, to asymptotic procedures.

Frequentist methods can be applied to data sets with a longitudinal structure, as performed byMedeiros, Lima, Savian, Malheiros, and Werner (2020). The authors sought to address the problems of variance heterogeneity and correlations between repeated longitudinal measurements in in situ ruminal degradation kinetic studies by using NLME. From a statistical point of view, similar problems encountered in different contexts and areas can be properly addressed through the use of mixed models, such as seen in J. C. Pinheiro and Bates (2000); Sartrio (2013); Luwanda and Mwambi (2016); Wyzykowski, Custdio, Custdio, Gomes, and Morais (2015); Calama and Montero (2004); and Xu et al. (2014). The proposal to apply a mixed effects methodology involving fixed and random effects parameters and the construction of a data (co)variance matrix (Yang, Huang, Trincado, & Meng, 2009) seems adequate to capture between- and within-animal variabilities and allows modeling the degradability of each animal (subject-specific) as the average degradability of all animals (population specific) (Schabenberger & Pierce, 2002).

In this study we aimed to evaluate the ruminal degradation kinetics through the nonlinear models of Ørskov and McDonald (1979), Van Milgen, Murphy, and Berger (1991), and France, Dijkstra, and Dhanoa (1996) and compare them in order to determine the best berformance. For that, we will consider the three nonlinear models with the inclusion of mixed effects. So that the models include fixed effects and random effects, allowing the variability between animals to be evaluated, with different structures for the (co)variance matrix of errors and random effects. In addition, we will discuss the comparison of experimental fixed effects treatments.

In this study, we aimed to evaluate ruminal degradation kinetics using the nonlinear models proposed by Ørskov and McDonald (1979), Van Milgen, Murphy, and Berger (1991), and France, Dijkstra, and Dhanoa (1996) and compare the results in order to determine the model with the best performance. For this, we considered the three nonlinear models with the inclusion of mixed effects; that is, the models contain both fixed and random effects. Such an approach allowed us to assess between-animal variability using different structures for the (co)variance matrix of errors and random effects. We also provide a discussion of fixed effects treatments.

2. Materials and Methods

2.1 Material

For model comparison (Table 2), we used a set of observations from a ruminal degradability experiment carried out in the Dairy Cattle Sector of the Iguatemi Experimental Farm (FEI), State University of Maringá, Maringá, Paraná, Brazil. Ruminal degradation kinetics were assessed according to Rossi et al. (2010). Treatments consisted of Elephant grass (*Pennisetum purpureum* Schumach.) silage with bacterial inoculant (SCE-IBC) (Propiolact MS01), Elephant grass silage with enzyme-bacterial inoculant (SCE-IEZ) (Bacto Silo), and corn silage (SMI) (*Zea mays L.*), hereafter referred to as T_1, T_2 and T_3, respectively. Silages were stored in trench silos, without coating, with a capacity of approximately 20 t.

A 3×3 Latin square experimental design was used, with cows treated as a nuisance factor (three lactating Holstein cows, C_1, C_2 and C_3) and periods (P_1, P_2 and P_3) and treatments (T_1, T_2 and T_3) considered as factors of interest. For each animal/period/treatment combination, hereafter referred to as subject or individual (ind), ruminal degradation was evaluated at the following incubation times: 0, 2, 6, 12, 24, 48, 72, and 96 h (Table 1). The observed and analyzed response was dry matter (DM) disappearance. For more details on the experiment, see Rossi et al. (2010).

Table 1. Dataset structure.

			Time (h)							
ind	Combination	Treatment	0	2	6	12	24	48	72	96
1	C_1P_1	T_1	y_{11}				$\cdots$			y_{18}
2	C_1P_2	T_3	y_{21}				$\cdots$			y_{28}
3	C_1P_3	T_2	y_{31}				$\cdots$			y_{38}
4	C_2P_1	T_2	y_{41}				$\cdots$			y_{48}
5	C_2P_2	T_1	y_{51}				$\cdots$			y_{58}
6	C_2P_3	T_3	y_{61}				$\cdots$			y_{68}
7	C_3P_1	T_3	y_{71}				$\cdots$			y_{78}
8	C_3P_2	T_2	y_{81}				$\cdots$			y_{88}
9	C_3P_3	T_1	y_{91}				$\cdots$			y_{98}

ind: animal/period/treatment combination.

2.2 Methods

The sampling structure induces a correlation among observations of the same subject. When faced with this type of problem, several authors adopted a mixed effects modeling approach(Medeiros et al., 2020; Xu et al., 2014; Calama & Montero, 2004; J. C. Pinheiro & Bates, 2000). A mixed nonlinear model (1) considering the i-th subject in the j-th evaluation time, according to J. C. Pinheiro and Bates (2000), is such that:

$$\boldsymbol{y}_i = f(\boldsymbol{\phi}_i, \boldsymbol{v}_i) + \boldsymbol{\varepsilon}_i \quad i = 1, \ldots, N = 9 \tag{1}$$

where, $\boldsymbol{y}_i = [y_{i1}, \ldots, y_{ij}, \ldots, y_{in_i}]'$ denotes the vector of measurements from i-th subject (animal/period/treatment) in the j-th observation time, f is the differentiable function of parameter vector $\boldsymbol{\phi}_i$ $(k \times 1)$, k is the number of parameters in the model, $\boldsymbol{v}_i = [v_{i1}, \ldots, v_{ij}, \ldots, v_{in_i}]'$ is the predictor vector, and $\boldsymbol{\varepsilon}_i = [\varepsilon_{i1}, \ldots, \varepsilon_{ij}, \ldots, \varepsilon_{in_i}]'$ is the vector residual terms. Still according to J. C. Pinheiro and Bates (2000) (2):

$$\boldsymbol{\phi}_i = \boldsymbol{X}_i\boldsymbol{\beta} + \boldsymbol{Z}_i\boldsymbol{b}_i \tag{2}$$

where $\boldsymbol{X}_i$ and $\boldsymbol{Z}_i$ are, the incidence matrix (or design) for fixed and random effects, respectively, with the respective parameter vectors $\boldsymbol{\beta}$ and $\boldsymbol{b}_i$. As demonstrated by Calama and Montero (2004), the NLME model has as its basic assumptions:

$$\boldsymbol{b}_i \overset{iid}{\sim} \mathbf{N}_q(\mathbf{0}, \boldsymbol{D})$$
$$\boldsymbol{\varepsilon}_i \overset{iid}{\sim} \mathbf{N}_J(\mathbf{0}, \boldsymbol{R}_i(\boldsymbol{\beta}, \boldsymbol{b}_i, \boldsymbol{\rho}))$$

here $\mathbf{N}$ denotes a multivariate normal distribution with a null mean vector and $\boldsymbol{D}$ is the $q \times q$ positive-definite varianceCcovariance matrix for random effects, representing among subject variability. In this formulation $\boldsymbol{R}_i(\boldsymbol{\beta}, \boldsymbol{b}_i, \boldsymbol{\rho})$ is the $n_i \times n_i$ intraindividual varianceCcovariance matrix defining within-subject variability. $\boldsymbol{R}_i$ is allowed to depend on both random and fixed effects, and $\boldsymbol{\rho}$ represents a set of common but unknown parameters. The $\boldsymbol{R}_i$ matrix is able to describe within-subject heteroscedasticity and autocorrelation by including both correlation effects and weighting factors. It can be decomposed and written as (3):

$$\boldsymbol{R}_i(\boldsymbol{\beta}, \boldsymbol{b}_i, \boldsymbol{\rho}) = \sigma^2 \boldsymbol{G}_i^{1/2} \boldsymbol{\Gamma}_i \boldsymbol{G}_i^{1/2} \tag{3}$$

where, for the i-th subject with n_i measurements, σ^2 is the scaling factor for the error dispersion, $\boldsymbol{G}_i$ is the $n_i \times n_i$ diagonal matrix that accommodates the variability of the error due to time, and $\boldsymbol{\Gamma}_i$ is the $n_i \times n_i$ of within-time error autocorrelation (Crecente-Campo, Tom, Soares, & Diguez-Aranda, 2010; Davidian & Giltinan, 2003).

Among the nonlinear f functions proposed in the literature, we focused on the exponential model (Ørskov & McDonald, 1979), Van Milgen's model (Van Milgen, Murphy, & Berger, 1991), and Richards model (France, Dijkstra, & Dhanoa, 1996), as depicted in Table 2. We used the parameterization presented in Teixeira et al. (2016).

Table 2. Candidate statistical models for describing ruminal degradability

Model	Statistical expression
Ørskov (OR)	$y_{ij} = \beta_1 + \beta_2(1 - e^{-\beta_3 t_{ij}/2}) + \varepsilon_{ij}$
Van Milgen (VM)	$y_{ij} = \beta_1 + \beta_2[1 - (1 + \beta_3 t_{ij})e^{-\beta_3 t_{ij}}] + \varepsilon_{ij}$
Richard's (RI)	$y_{ij} = \beta_1 + \beta_1\beta_2[\beta_1^{\beta_4} + (\beta_2^{\beta_4} - \beta_1^{\beta_4})e^{-\beta_3 t_{ij}/2}]^{-1/\beta_4} + \varepsilon_{ij}$

β_1: soluble fraction (%) ($\beta_1 \geq 0$);
β_2: potentially degraded insoluble fraction (%) ($\beta_2 \geq 0$);
β_3: joint fractional rate of latency and degradation ($\beta_3 \geq 0$);
β_4: parameter without biological meaning ($\beta_4 \geq -1$).

To adjust the models and analyze the data, we used the resources available in the `nlme` package (linear and nonlinear mixed effects models) (J. Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2021) of the R statistical environment (R Core Team, 2021). The following steps were taken:

- nonlinear models were fitted to individual curves considering only fixed effects, using the `nlsList` function to determine whether this approach would be sufficient to explain ruminal degradation kinetics. In the analyzed case, this model structure was not sufficient;
- random effects were added to all model parameters for selection of the $\boldsymbol{D}$ matrix;

- after the $\boldsymbol{D}$ matrix was chosen, models were adjusted by incorporating different random effects components into parameters;
- the best structure for the varianceCcovariance matrix of residuals ($\boldsymbol{R}_i$) was defined;
- the variance components of all three adjusted models were defined, and the final model was selected; and
- parameter estimates for the final model and pairwise contrasts were analyzed to compare the performance of treatments.

We considered `pdSymm` (positive definite matrix), `pdDiag` (diagonal matrix), and `pdIdent` (identity matrix) as structures for the (co)variance matrix ($\boldsymbol{D}$) of random effects of parameters (e.g. J. C. Pinheiro and Bates (2000), section 4.2.2).

Regarding the residual matrix ($\boldsymbol{R}$), three other correlation structures were considered for $\boldsymbol{\Gamma}$, namely `corAR1` (autoregressive of order 1 - AR1), `corCompSymm` (compound symmetry), and `corLin` (General Linear). The `nlm` package implements the `corARMA` function (autoregressive moving average - ARMA), which can be useful to decide between an AR1 and ARMA(p,q) correlation structures. A useful approach is to generate all possible combinations of ARMA models (for $p = 0$ to $p = 2$ and $q = 0$ to $q = 2$) and choose the one with the lowest Akaike information criterion (AIC) and/or Bayesian information criterion (BIC). The three matrices were considered with and without the `varIdent` class matrix ($\boldsymbol{G}$) to correct for possible heteroscedasticity within groups (treatments or times) (J. C. Pinheiro & Bates, 2000). To obtain the estimates presented in Tables 4, 5, 6, and 8, we used restricted maximum likelihood estimation (`method = "REML"` of the `nlme` function).

Several authors do not recommend the use of the determination of coefficient (R^2) to select an NLME model, according to Spiess and Neumeyer (2010). Therefore, of the models described in Table 2, that with the best performance in predicting DM was determined using the following criteria: intercept, slope, residual sum of squares (RSS), mean squared error (MSE), root-mean-square error (RMSE), and (R^2) of the simple linear model fit between DM values observed and DM values predicted by NLME model. Furthermore, model efficiency (ME), normalized model efficiency (NME), correlation between observed and fitted values (Corr), and concordance correlation (ConCorr) were determined after adjusting the models. These statistics were obtained by using the `IA_tab` function of the `nlraa` package (Miguez, 2021; Miguez, Archontoulis, & Dokoohaki, 2018).

3. Results

The observed response (DM) for each subject (animal/period/treatment) is displayed in Figure 1. The curves indicate that the proposed models are plausible and suggest differences between treatments.

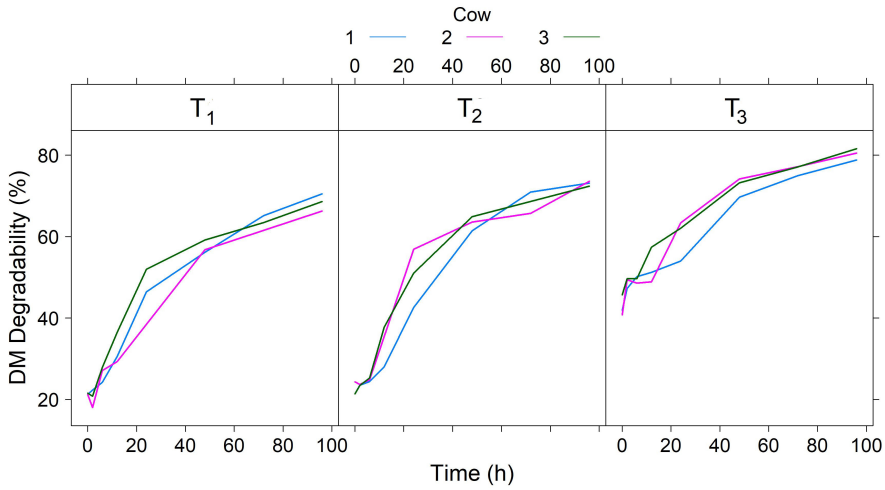


Figure 1. Rumen degradability of dry matter (DM) per animal and treatment

The models presented in Table 2 were adjusted for each individual (Table 1), totaling nine adjustments for each model. Adjustments were made using the `nlsList` function of the `nlme` package, which uses a nonlinear least squares procedure. Because of the difficulty in estimating Richards model parameters, we opted for a first fit considering $\beta_4 = 1$. With this adjustment, we obtained initial values for the simultaneous adjustment of the four parameters. A summary of parameter

estimates is presented in Table 3. For all models, the coefficient of variation (CV) was high (Table 3), indicating a large variation in parameter estimates. This suggests that an effect can be added through a probability distribution, that is, by including random effects components in the model. Such a procedure affords a mixed effects model.

Table 3. Summary of parameter estimates for models fitted to each curve (ind)

		Parameter			
Model	Statistic	β_1	β_2	β_3	β_4
	mean	27.6786	52.0225	0.0523	-
OR	SD	12.5968	8.8589	0.0177	-
	CV(%)	45.5108	17.0290	33.8497	-
	mean	30.0505	42.9881	0.0711	-
VM	SD	12.3312	7.2544	0.0190	-
	CV(%)	41.0349	16.8754	26.7380	-
	mean	14.6044	58.2513	0.1431	1
RI*	SD	5.9844	2.5824	0.0538	1
	CV(%)	40.9764	4.4332	37.6296	1
	mean	14.7507	60.2679	0.0990	0.4716
RI	SD	6.4449	3.2281	0.0342	1.5911
	CV(%)	43.6919	5.3562	34.5448	337.3897

RI*: Richard's model with $\beta_4 = 1$; SD: standard deviation of parameter estimates; and CV(%): coefficient of variation of parameter estimates.

In this next step, we made adjustments considering mixed effects models. First, we considered that all model parameters (Table 2) were associated with a random effect of subjects and a fixed effect of treatments.

To determine the best varianceCcovariance matrix for random effects ($\boldsymbol{D}$), we considered three matrix structures: multiple of the identity (`pdIdent`), diagonal (`pdDiag`), and general positive-definite (`pdSymm`).

According to the likelihood ratio test results, matrices were not considered to have significant differences, despite $p = 0.0485$ (see Table 4). AIC values of OR and VM models indicated better results for the identity matrix. For the RI model, the diagonal matrix was indicated as the best; however, the values obtained for identity and diagonal matrices were very similar. Considering the lowest BIC value, we concluded that the identity matrix afforded the best results, in addition to requiring the estimation of fewer parameters.

Table 4. Assessment of different $\boldsymbol{D}$ matrix structures for the three models

Model	$\boldsymbol{D}$ Matrix	df	AIC	BIC	logLik	LRT	p−value
	pdIdent	11	361.31	385.72	-169.66		
OR	pdDiag	13	365.31	394.16	-169.66	0.0000	1.0000
	pdSymm	16	365.81	401.32	-166.91	5.4994	0.1387
	pdIdent	11	348.99	373.40	-163.49		
VM	pdDiag	13	352.99	381.84	-163.49	0.0006	0.9997
	pdSymm	16	354.15	389.66	-161.07	4.8402	0.1839
	pdIdent	14	355.77	386.84	-163.88		
RI	pdDiag	17	353.88	391.62	-159.94	7.8838	0.0485
	pdSymm	23	365.01	416.06	-159.50	0.8710	0.9900

df: degrees of freedom; logLik: log-likelihood value; LRT: likelihood ratio statistic.

VarianceCcovariance identity matrices of the fitted models had the following estimates: $\widehat{\boldsymbol{D}}_{OR} = 2.12 \times 10^{-5}\mathbf{I}_{(3)}$; $\widehat{\boldsymbol{D}}_{VM} = 10.27 \times 10^{-5}\mathbf{I}_{(3)}$; $\widehat{\boldsymbol{D}}_{RI} = 9.48 \times 10^{-5}\mathbf{I}_{(4)}$ (where $\mathbf{I}$ is a matrix of ones on the main diagonal and zero otherwise).

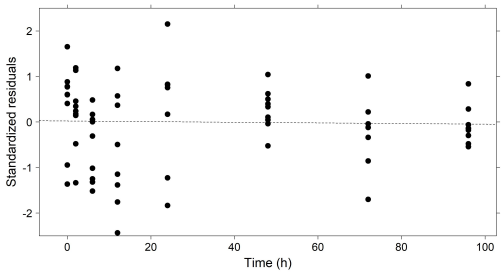
A random effect was considered for each model parameter with a varianceCcovariance identity matrix. In Table 5, we present the results of model adjustments. All possibilities for incorporating random effects were tested. Ørskovs and Van Milgens models had the lowest AIC, BIC, and logLik values when random effects were attributed to parameter β_3 only, being hereafter referred to as OR_3 and VM_3, respectively. For Richards model, this behavior was observed when random effects were attributed to β_4 only (RI_4). For these models, the estimated varianceCcovariance matrices were $\widehat{\boldsymbol{D}}_{OR_3} = 2.12 \times 10^{-5}\mathbf{I}_{(1)}$, $\widehat{\boldsymbol{D}}_{VM_3} = 10.27 \times 10^{-5}\mathbf{I}_{(1)}$, and $\widehat{\boldsymbol{D}}_{RI_4} = 0.1188\mathbf{I}_{(1)}$.

Table 5. Evaluation of the mixed effects of each model parameter using the identity varianceCcovariance matrix ($\boldsymbol{D}$)

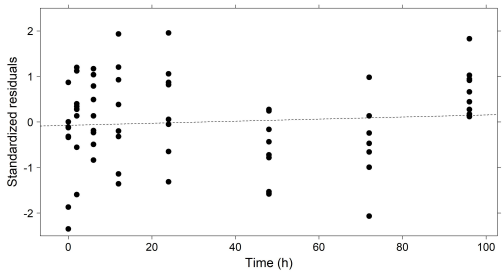
Model	i	Mixed parameters	AIC	BIC	logLik
	1	β_1	$362.9761^{(5)}$	$387.3906^{(5)}$	-170.4880
	2	β_2	$364.2511^{(7)}$	$388.6657^{(7)}$	-171.1255
	3	β_3	$361.3103^{(1)}$	$385.7249^{(1)}$	-169.6552
OR_i	4	$\beta_1\beta_2$	$363.5209^{(6)}$	$387.9354^{(6)}$	-170.7604
	5	$\beta_1\beta_3$	$361.3112^{(4)}$	$385.7258^{(4)}$	-169.6556
	6	$\beta_2\beta_3$	$361.3112^{(3)}$	$385.7258^{(3)}$	-169.6556
	7	$\beta_1\beta_2\beta_3$	$361.3104^{(2)}$	$385.7249^{(2)}$	-169.6552
	1	β_1	$359.7734^{(5)}$	$384.1880^{(5)}$	-168.8867
	2	β_2	$360.9057^{(7)}$	$385.3203^{(7)}$	-169.4528
	3	β_3	$348.9860^{(1)}$	$373.4005^{(1)}$	-163.4930
VM_i	4	$\beta_1\beta_2$	$360.4013^{(6)}$	$384.8159^{(6)}$	-169.2007
	5	$\beta_1\beta_3$	$348.9864^{(3)}$	$373.4010^{(3)}$	-163.4932
	6	$\beta_2\beta_3$	$348.9861^{(2)}$	$373.4007^{(2)}$	-163.4931
	7	$\beta_1\beta_2\beta_3$	$348.9866^{(4)}$	$373.4012^{(4)}$	-163.4933
	1	β_1	$359.6773^{(13)}$	$390.7504^{(13)}$	-165.8386
	2	β_2	$363.1060^{(15)}$	$394.1791^{(15)}$	-167.5530
	3	β_3	$355.7812^{(10)}$	$386.8543^{(10)}$	-163.8906
	4	β_4	$347.8875^{(1)}$	$378.9606^{(1)}$	-159.9437
	5	$\beta_1\beta_2$	$360.2088^{(14)}$	$391.2819^{(14)}$	-166.1044
	6	$\beta_1\beta_3$	$355.7872^{(12)}$	$386.8603^{(12)}$	-163.8936
	7	$\beta_1\beta_4$	$348.7045^{(3)}$	$379.7776^{(3)}$	-160.3522
RI_i	8	$\beta_2\beta_3$	$355.7867^{(11)}$	$386.8598^{(11)}$	-163.8933
	9	$\beta_2\beta_4$	$348.0757^{(2)}$	$379.1488^{(2)}$	-160.0378
	10	$\beta_3\beta_4$	$355.7630^{(8)}$	$386.8361^{(8)}$	-163.8815
	11	$\beta_1\beta_2\beta_3$	$355.7809^{(9)}$	$386.8540^{(9)}$	-163.8905
	12	$\beta_1\beta_2\beta_4$	$348.8564^{(4)}$	$379.9295^{(4)}$	-160.4282
	13	$\beta_1\beta_3\beta_4$	$355.7562^{(6)}$	$386.8293^{(6)}$	-163.8781
	14	$\beta_2\beta_3\beta_4$	$355.7557^{(5)}$	$386.8288^{(5)}$	-163.8779
	15	$\beta_1\beta_2\beta_3\beta_4$	$355.7579^{(7)}$	$386.8310^{(7)}$	-163.8790

$^{(\cdot)}$:column values ranks by model

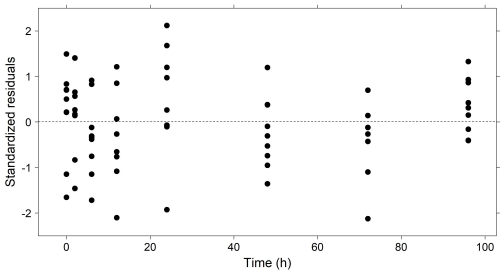
Having decided in which parameters to use random effects and their varianceCcovariance structure, we then applied the within-subject variance-covariance structure $\boldsymbol{R}_i$, in Eq. (3). The graphs depicted in Figure 2 show that the variability of residuals differs between models but not over time.



(a) Ørskov's Model



(b) Vam Milgen's Model



(c) Richard's Model

Figure 2. Time versus Standardized residuals

In an attempt to remove such effect, we modeled residual variance as a function of time. For this, we considered different variances for each time period using the `varIdent` variance function class of the `nlme` package in $\boldsymbol{G}_i$ (3). For the autocorrelation $\boldsymbol{\Gamma}_i$ matrix, we used three standard structures from the `nlme` package, namely autoregressive of order 1 (AR), compound symmetry (CS), and general linear (GL). We fitted the three models using the proposed $\boldsymbol{\Gamma}_i$ matrices with and without `varIdent` (ID). The results demonstrated that CS associated with ID produced the best results for all models (Table 6).

Table 6. Comparison of model performance for different alternatives of the $\boldsymbol{R}$ matrix

Model	$\boldsymbol{R}$	df	AIC	BIC	logLik	Test	LRT	$p-$value
		11	361.3103	385.7249	-169.6552			
	AR	12	363.3103	389.9444	-169.6552	1 X 2	0.0000	0.9999
	AR+ID	19	349.3578	391.5285	-155.6789	2 X 3	27.9525	0.0002
OR_3	CS	12	362.9669	389.6010	-169.4835	3 X 4	27.6091	0.0003
	CS+ID	19	345.8534	388.0241	-153.9267	4 X 5	31.1135	0.0001
	GL	12	363.3103	389.9444	-169.6552	5 X 6	31.4569	0.0001
	GL+ID	19	349.3578	391.5285	-155.6789	6 X 7	27.9525	0.0002
		11	348.9860	373.4005	-163.4930			
	AR	12	350.9860	377.6200	-163.4930	1 X 2	0.0000	1.0000
	AR+ID	19	346.3521	388.5227	-154.1760	2 X 3	18.6339	0.0094
VM_3	CS	12	347.9360	374.5701	-161.9680	3 X 4	15.5839	0.0292
	CS+ID	19	346.0176	388.1883	-154.0088	4 X 5	15.9184	0.0259
	GL	12	350.9860	377.6200	-163.4930	5 X 6	18.9683	0.0083
	GL+ID	not convergence						
		14	347.8875	378.9606	-159.9437			
	AR	15	349.8874	383.1800	-159.9437	1 X 2	0.000075	0.9931
	AR+ID	22	340.7743	389.6035	-148.3872	2 X 3	23.113090	0.0016
RI_4	CS	15	353.9218	387.2145	-161.9609	3 X 4	27.147531	0.0003
	CS+ID	22	340.7083	389.5375	-148.3542	4 X 5	27.213515	0.0003
	GL	15	349.8836	383.1763	-159.9418	5 X 6	23.175322	0.0016
	GL+ID	22	340.7605	389.5897	-148.3803	6 X 7	23.123121	0.0016

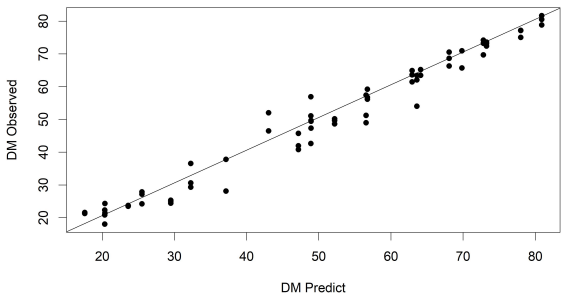
After analyzing the predictive capacity of models, as shown in Table 7 and Figures 3 and 4, we concluded that the

VM_3+CS+ID model had the best performance. The graph of observed versus predicted values of the model showed high linearity, with low RSS, MSE, and RMSE values. Furthermore, the high statistics for the NLME model corroborate this result.

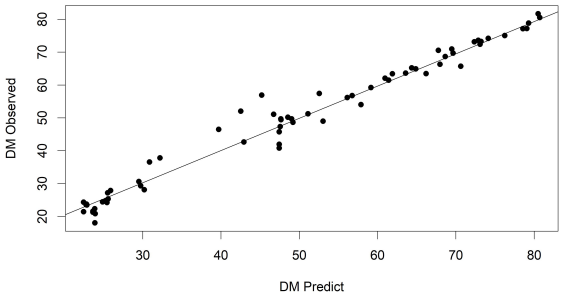
Table 7. Comparison of model performance

		Models		
	Statistics	OR_3+CS+ID	VM_3+CS+ID	RI_4+CS+ID
DM	intercept	0.8130	0.4242	1.4514
	slope	0.9716	0.9941	0.9785
	RSS	764.1455	638.3976	686.9378
	MSE	11.2374	9.3882	10.1020
	RMSE	3.3522	3.0640	3.1784
	R^2	0.9707	0.9756	0.9737
NLME	ME	0.9690	0.9755	0.9728
	NME	0.9699	0.9761	0.9735
	Corr	0.9853	0.9877	0.9868
	ConCorr	0.9847	0.9877	0.9865

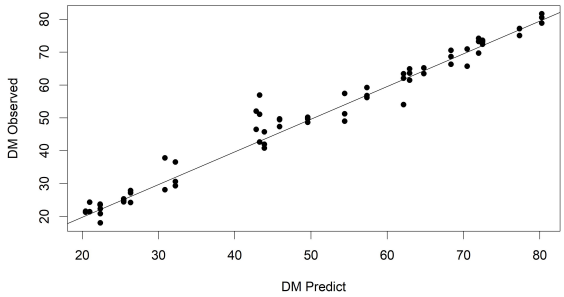
DM, curve of observed versus predicted dry matter degradability values;
RSS, residual sum of squares; MSE, mean squared error; RMSE, root-mean-square error; ME, model efficiency; NME, normalized model efficiency.



(a) Ørskov's Model

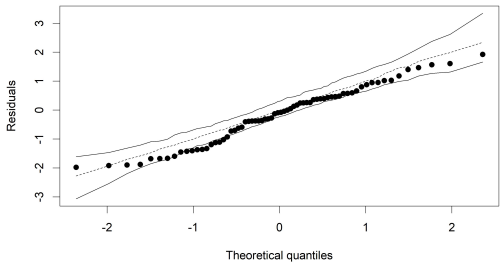


(b) Van Milgen's Model

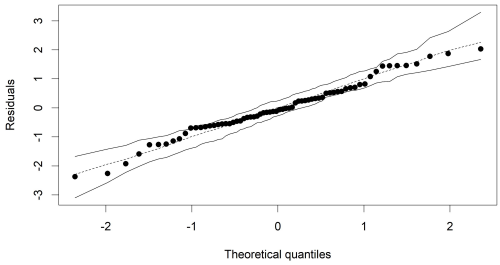


(c) Richard's Model

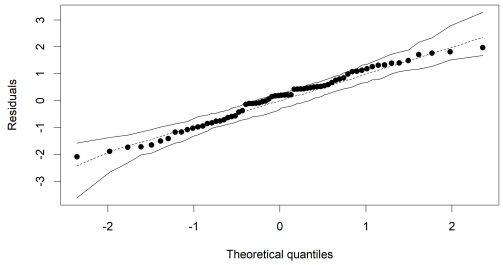
Figure 3. Observed dry matter (DM) degradability values versus DM values predicted using the with SC+ID



(a) Ørskov's Model



(b) Vam Milgen's Model



(c) Richard's Model

Figure 4. Simulated envelope for models fitted with the CS+ID

Table 8 shows the results of Tukeys test, obtained by the function `emmeans` of the package `emmeans` (Lenth, 2021). T_3 afforded a higher soluble fraction ($\hat{\beta}_1$ = 47.4471), differing significantly from treatments T_1 and T_2 at the 5% significance level. The largest degradable fraction was obtained with T_2 ($\hat{\beta}_2$ = 51.2885), and all treatments differed from each other in this parameter. Joint fractional rate of latency and degradation (β_3), however, did not differ between treatments. The results depicted in Figure 5 support the previous discussion, as T_3 starts at time zero with DM values clearly higher than that of other treatments. Total degradability was virtually equal for all treatments at 45 h; thus, the degradability rates of T_1 and T_2 were higher up to 45 h.

Table 8. Estimates and contrasts of parameters β_1, β_2 and β_3, with standard errors (SE) and 95% confidence limits (CL) of the model VM_3+CS+ID

Parameter	T_i	Estimate	SE	df	Lower CL	Upper CL
β_1	1	23.6593	0.51416	8	22.4736	24.8449
	2	22.4619	0.52545	8	21.2502	23.6735
	3	47.4471	0.50861	8	46.2742	48.6200
β_2	1	46.1181	1.11124	52	43.8882	48.3479
	2	51.2885	1.01282	52	49.2561	53.3208
	3	33.9917	1.06929	52	31.8460	36.1374
β_3	1	0.0541	0.00306	52	0.0480	0.0603
	2	0.0621	0.00320	52	0.0557	0.0686
	3	0.0547	0.00341	52	0.0479	0.0616
	T_i contrast	Estimate	SE	df	t−ratio	p−value*
β_1	1 - 3	-23.7878	0.7232	8	-32.892	0.0000
	2 - 3	-24.9852	0.7312	52	-34.166	0.0000
	2 - 1	-1.1974	0.7351	8	-1.629	0.2890
β_2	1 - 3	12.1263	1.5421	52	7.863	0.0000
	2 - 3	17.2967	1.4728	52	11.744	0.0000
	2 - 1	5.1703	1.5035	52	3.439	0.0032
β_3	1 - 3	-0.0005	0.0045	52	-0.128	0.9909
	2 - 3	0.0074	0.0046	52	1.587	0.2600
	2 - 1	0.0080	0.0044	52	1.809	0.1765

*: Tukey test

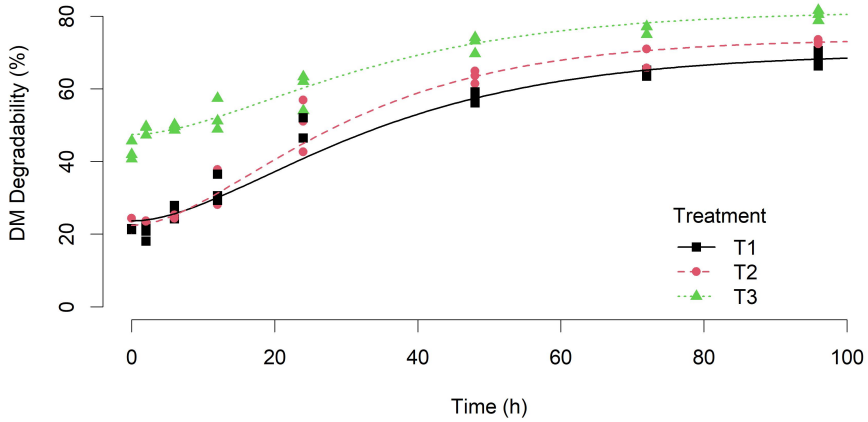


Figure 5. Prediction curves of the adjusted VM_3+CS+ID model

4. Conclusion

This study aimed to identify a nonlinear mathematical model for the study of ruminal degradability. The model proposed by Van Milgen provided better results than Ørskovs and Richards models.

Originally, these are fixed effects models that do not contemplate the addition of random effects to parameters nor the modeling of their variance and covariance structures.

However, assuming fixed effects for each animal/period/treatment combination, we observed high coefficient of variations of estimates, indicating that random effects components could improve the results. This observation was confirmed by addition of random effects of animals using NLME. This method made it possible to consider the compound symmetry autocorrelation matrix and identity covariance structure, resulting in improvements in model residuals and, consequently,

greater precision in parameter estimates.

In addition, it was possible and easy compare treatments by the parameters contrasts test using the EMM function.

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