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Mathematical models describing disappearance of Lucerne hay in the rumen using the nylon bag technique

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Abstract

It is essential to study the dynamics of rumen degradation of feeds before their potential use in formulating diets for ruminants. Various mathematical models have been developed to describe this degradation. The non-lagged exponential model (Model I), the lagged exponential model (Model II), the Gompertz model (Model III), and the generalized Mitscherlich model (Model IV) were examined using two alternative software (SAS and MATLAB) to determine their efficacy in accounting for variation in ruminal disappearance of dry matter (DM) and crude protein (CP) of lucerne hay from three cuttings. All models described DM degradability well (R^2 >0.98). Only Models I and II converged when fitted to CP degradability data (R^2 >0.98). It was concluded that any of these models could be used to describe the degradation of DM, whereas only Models I and II could be used to describe the degradation of CP from three cuttings of Lucerne hay. All the models that were fitted to the DM degradation data performed reasonably well, with only minor differences in goodness of fit. However, these models differed in values of the parameter estimates. Additionally, SAS failed to converge in the analyses of CP with Models III and IV, and MATLAB converged to nonsensical values with Model III. Model I might be recommended because it fitted the data well and required estimates of the fewest parameters.

Keywords: alfalfa hay, in situ digestion, model selection, nonlinear regression
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Introduction

Forage is an important component of rations for ruminant animals. Lucerne (*Medicago sativa* L.) is important because of its high protein content and digestibility in comparison with many other forages (Ferdinand & Jung, 2005). Identification of the quality and nutritional value of these forage plants would be effective in describing animal nutrition (Jeromela *et al*., 2017; Bi *et al*., 2018; Besharati *et al.*, 2020). Changes in digestive processes are of nutritional importance, because they determine the quantity of nutrients that are available to the animal (Sauvant & Noziere, 2016; Van Soest, 2018).

Formulating appropriate diets for ruminants requires detailed descriptions of the nutritional value of feeds and their degradation kinetics in the rumen (Tedeschi, 2019; da Costa *et al*., 2020). Therefore accurate estimates of degradation parameters are required to fit various mathematical models and to select best fit models for describing the in situ ruminal incubation of feeds. Degradation kinetics can be measured by techniques such as in situ, in vivo and in vitro (Benedeti *et al*., 2019). The nylon bag technique has been used widely to estimate ruminal nutrient degradation because it is relatively simple and low cost compared with methods that involve intestinally cannulated animals (Tassone *et al*., 2020).

Non-linear models may help to obtain more accurate descriptions about the degradability of feeds. Various mathematical models have been developed to describe the ruminal degradation kinetics of feeds from data obtained by the in situ technique. The usual model is the simple negative exponential or Mitscherlich model suggested by Ørskov & McDonald (1979). However, the segmented model (France *et al*., 1990), the inverse polynomial model (France *et al*., 1990), the lag compartment model (Van Milgen *et al*., 1991), the generalized Mitscherlich model (Dhanoa *et al*., 1995), Gompertz curves model (France *et al*., 1990), and the generalized Von Bertalanffy model (Ricker, 1979) have been used to describe aspects of digestion kinetics. The best-fit models may differ between feeds (forages and non-forages) and may depend

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on whether a lag phase in degradation is considered (Lopez *et al*., 1999). Previous studies found that lucerne varies in digestibility and intake, even if it is harvested at a constant maturity (Kawas *et al*., 1990). The chemical and physical changes in lucerne as a result of increased maturity, and the method of preservation may affect rumen digestion and passage (Nelson & Satter, 1992).

The objective of this study was to determine in situ the kinetics of DM and CP disappearance in the rumen of various cuttings of Lucerne hay. Degradation parameters were estimated using various mathematical models to identify the best model to describe the data.

Materials and Methods

Two diminishing returns and two sigmoidal models were used to describe the ruminal degradation of the DM and CP of various cuts of lucerne. Four replicate samples that were being digested in situ were withdrawn for analysis at 0, 4, 8, 12, 16, 24, and 48 hours of digestion.

Models I and II are simple negative exponential curve models (monomolecular Mitscherlich or first-order kinetics models) with and without a lag phase (Ørskov & McDonald, 1979). Model III is a Gompertz curve, asymmetrical about an inflection point M (France *et al*., 1990). Model IV is the generalized Mitscherlich model, with the addition of a square root time dependence component (Dhanoa *et al*., 1995). Mathematically, these models are specified as follows:

Model I: first-order kinetics model without lag phase: $Y = a + b(1 - e^{-ct})$

Model II: first-order kinetics model with lag phase: $Y = a + b(1 - e^{c(t+l)})$

Model III: Gompertz model: $Y = a + b(k - k^{e(ct)})/(k - 1)$

Model IV: generalized Mitscherlich model: $Y = a + b(1 - e^{-c(t-l)-d(\sqrt{t}-\sqrt{l})})$

where: Y = the quantity of either DM or CP,
a,b,c, d and
k = parameters to be estimated, and t = time.

The DM and CP data were fitted to each model by nonlinear regression using the NLIN procedure of SAS (SAS Institute, Inc., Cary, North Carolina, USA). Several probable initial values were provided to calculate an initial residual sum of squares to determine the starting point for iteration. Since some of these models did not converge in SAS, the same models were fitted using MATLAB (The MathWorks Inc., Natick, Massachusetts, USA). An optimization method was used that combined the MATLAB curve fitting toolbox and the numerical algorithm based on the Levenberg-Marquardt method. The models were identified through the editor ToolStrip, and the starting points and ranges required for the models were defined. A goodness of fit measure based on the error values of the fitted curves was used to assess the adequacy of the models. The mean square error and pseudo R^2 = 1 – (residual sum of squares / corrected total sum of squares) were used to describe the general goodness-of-fit for each model. Effective degradability (ED) was calculated as:

$$ED = a + (\frac{bc}{c+k})$$ (Ørskov *et al*., 1980)

where: a, b and c are estimates of the parameters from fitting the models and
k is the fractional rate of outflow from the rumen (0.02/h, 0.03/h, 0.04/h, 0.05/h, and 0.06/h).

Results and Discussion

The variation in DM increased from the first to the third cuttings. The variation in CP was least in the lucerne from the third cutting and most in the second cutting, with the first cutting samples being intermediate.

A comparison of these fitted models for DM degradability of various cuttings of lucerne hay based on R^2 showed that Models IV, III and II were best fit for the data from the first, second and third cuttings, respectively (Table 1). Thus, except for the results from Model I for the third cutting of lucerne, models were all equivalent (P >0.3). The high coefficients of determination indicated the adequacy of their performance. The parameter estimates for time lag in DM digestion from the third cutting were greater than those of the first cutting. Therefore, it could be said that the degradability of third-cut hay takes nine hours to start. This may be because of higher air temperatures in summer, leading to an increase in structural compounds and

enhanced lignin content, which hinders the launch of microbial degradation. No lag time was observed for the second cutting of lucerne hay, therefore lag times of forages and their composition have a relationship.

Table 1 Estimated parameters describing dry matter degradation of lucerne hay harvested from three cuttings using mathematical models fitted with PROC NLIN of SAS

	Parameters						df_e	MS_e	R^2	I
	a	*b*	*c*	*L*	*d*	*k*				
First cutting										
Model I	21.44	42.64	0.0254	-	-	-	25	1.12	0.9832	7
Model II	23.35	39.11	0.0295	3.3003	-	-	24	0.85	0.9877	10
Model III	22.93	36.80	0.0509	-	-	0.1361	24	0.82	0.9882	11
Model IV	23.35	37.64	0.0421	0.4238	0.0831	-	23	0.84	0.9883	6
Second cutting										
Model I	22.99	45.47	0.0259	-	-	-	25	1.07	0.9856	6
Model II	22.83	45.63	0.0259	0.1375	-	-	24	1.11	0.9856	9
Model III	23.76	41.87	0.0392	-	-	0.3483	24	1.02	0.9871	9
Model IV	22.48	42.93	0.0307	0.0263	0.0099	-	23	1.43	0.9828	18
Third cutting										
Model I	19.47	53.29	0.0197	-	-	-	25	1.76	0.9801	7
Model II	22.98	44.19	0.0284	6.8487	-	-	24	0.77	0.9917	7
Model III	22.00	40.98	0.0564	-	-	0.0422	24	0.78	0.9916	17
Model IV	23.86	42.29	0.0300	8.9265	0.0155	-	23	0.85	0.9912	12

a: rapidly soluble fraction (%), *b*: slowly degradable fraction (%), *c*: degradation rate constant (%/h) of fraction b; *L*: lag time (h), *d*: the parameter pertaining to the variable fractional rate of degradation *k*: slope, or degradation rate coefficient (h^{-1}), df_e: degrees of freedom for error, MS_e: mean square error, I: number of iterations, Model I: first-order kinetics model without lag phase, Model II: first-order kinetics model with lag phase, Model III: Gompertz model, Model IV: generalized Mitscherlich model

Only Models I and II converged when using SAS to evaluate ruminal CP degradation of lucerne hay from the cuttings (Table 2). These models fit the data for CP degradation equally well (P >0.4).For Model I, the rapidly soluble fraction *(a)* of CP for the first and third cuttings of lucerne hay was greater than for Model II, whereas this pattern was reversed for the slowly degradable fraction *(b).* The values of *(b)* observed in this experiment were higher compared with Taghizadeh *et al*. (2008), but the values of *(a)* reported here agree with their results. In contrast, the values of *(a)* for CP from the current study were lower than those reported by Elizald *et al*. (1999), whereas the values of *(b)* were consistent with their data. Observations of DM and CP degradability could depend on differences in the variety of lucerne, drying conditions, climate, soil, plant maturity, sample size, the surface area of the nylon bag, and microbial contamination (Palangi & Macit, 2019).

A few important differences were noted (Table 3) in using MATLAB to fit the same nonlinear models for DM degradation as was done with SAS, First, the mean square error for from Model II for the first and third cuttings was higher than with SAS and this difference was significant for the third cutting hay (P =0.02). Second, Model III converged with an illogical negative estimate of (a) for the second cutting of lucerne. Finally, there were additional numerical that-might affect biological interpretations of the data.

Table 2 Estimated parameters describing crude protein degradation lucerne hay harvested from three cuttings using mathematical models fitted with PROC NLIN of SAS

	Parameters				df_e	MS_e	R^2	I
	a	*b*	*c*	*L*				
First cutting								
Model I	8.558	62.30	0.0186	-	25	4.30	0.9843	5
Model II	0.096	70.96	0.0186	6.99	24	4.48	0.9849	6
Second cutting								
Model I	3.389	67.34	0.0196	-	25	2.38	0.9874	6
Model II	5.020	62.92	0.0221	2.35	24	2.25	0.9901	8
Third cutting								
Model I	8.425	63.02	0.0148	-	25	3.43	0.9874	7
Model II	1.602	69.84	0.0148	6.95	24	3.58	0.9901	6

a: rapidly soluble fraction (%), *b*: slowly degradable fraction (%), *c*: degradation rate constant (%/h) of fraction *b*; L: lag time (h), df_e: degrees of freedom for error, MS_e: mean square error, I: number of iterations, Model I: first-order kinetics model without lag phase, Model II: first-order kinetics model with lag phase

Table 3 Estimated parameters describing dry matter degradation of lucerne hay harvested from three cuttings using mathematical models fitted with MATLAB

	Parameters						d_{fe}	MS_e	R^2	I
	a	*B*	*c*	*L*	*d*	*k*				
First cutting										
Model I	21.46	42.66	0.0254	-	-	-	25	1.12	0.9831	8
Model II	26.98	37.14	0.0254	0.1385	-	-	24	1.17	0.9831	7
Model III	12.27	36.79	0.0510	-	-	0.1348	24	0.81	0.9882	7
Model IV	30.09	51.15	0.0247	9. 9020	0.0460	-	23	0.87	0.9879	6
Second cutting										
Model I	22.99	45.46	0.0259	-	-	-	25	1.07	0.9859	6
Model II	29.20	39.24	0.0259	0.1470	-	-	24	1.12	0.9859	7
Model III	-13.16	41.86	0.0392	-	-	0.3481	24	1.03	0.9871	6
Model IV	29.44	41.84	0.0258	6.0340	0.0086	-	23	1.15	0.9860	6
Third cutting										
Model I	19.47	53.78	0.0195	-	-	-	25	1.77	0.9803	5
Model II	31.61	41.64	0.0195	0.2559	-	-	24	1.84	0.9803	7
Model III	18.01	41.36	0.0552	-	-	0.0461	24	0.85	0.9908	9
Model IV	32.93	65.99	0.0208	15.9400	0.0593	-	23	0.97	0.9901	4

a: rapidly soluble fraction (%), *b*: slowly degradable fraction (%), *c*: degradation rate constant (%/h) of fraction b; *L*: lag time (h), *d*: the parameter pertaining to the variable fractional rate of degradation *k*: slope, or degradation rate coefficient (h^{-1}), df_e: degrees of freedom for error, MS_e: mean square error, I: number of iterations, Model I: first-order kinetics model without lag phase, Model II: first-order kinetics model with lag phase, Model III: Gompertz model, Model IV: generalized Mitscherlich model

Anomalies were noted (Table 4) when the estimates of parameters from MATLAB that describe CP degradation were observed, First, all four models fitted the data from each of the cuttings equally (P >0.1). For Models I and II the estimates of mean square error were similar to those generated in the analyses using SAS.

Second, the estimates from Model III were consistently outside the biologically reasonable range. Model III also required an unusually large number of rounds of iteration to converge.

Table 4 Estimated crude protein degradability parameters of lucerne hay harvested at three cuts using several mathematical models with MATLAB

	Parameter estimates						df_e	MS_e	R^2	I
	a	b	c	L	d	k				
First cutting										
Model I	8.556	62.30	0.0186	-	-	-	25	4.31	0.9635	24
Model II	31.40	39.45	0.0186	0.4569	-	-	24	4.49	0.9635	12
Model III	-177.4	59.87	0.0220			0.7062	24	4.58	0.9628	109
Model IV	15.61	53.60	0.0185	6.371	-0.0039		23	4.68	0.9635	5
Second cutting										
Model I	3.387	67.45	0.0196	-	-	-	25	2.35	0.9833	26
Model II	31.39	39.45	0.0196	0.5364	-	-	24	2.44	0.9833	18
Model III	-199.2	64.78	0.0232			0.7079	24	2.55	0.9826	109
Model IV	13.06	61.92	0.0198	8.107	0.0088		23	2.53	0.9836	5
Third cutting										
Model I	8.467	63.20	0.0147	-	-	-	25	3.44	0.9651	12
Model II	31.81	39.86	0.0147	0.4609	-	-	24	3.59	0.9651	15
Model III	-189.9	60.31	0.0175			0.72	24	3.70	0.9640	109
Model IV	13.44	47.46	0.0108	4.789	-0.0378		23	5.55	0.9669	7

a: rapidly soluble fraction (%), *b*: slowly degradable fraction (%), *c*: degradation rate constant (%/h) of fraction *b; L*: lag time (h), d: the parameter pertaining to the variable fractional rate of degradation *k*: slope, or degradation rate coefficient (h-1), dfe: degrees of freedom for error, MSe: mean square error, I: number of iterations, Model I: first-order kinetics model without lag phase, Model II: first-order kinetics model with lag phase, Model III: Gompertz model, Model IV: generalized Mitscherlich model

Estimates of effective degradability of the lucerne hays varied more with changes in the assumed values of ruminal rate of passage than between the mathematical models (Table 5). Significant differences in degradation of DM and CP are known to exist between samples of Lucerne hay (Von Keyserlingk *et al*., 1996).Therefore, despite the relatively small variations among the replicates that were observed in the present study, caution is advised against general interpretation of these results.

Table 5 Estimated effective degradability of dry matter and crude protein of lucerne hay harvested from three cuttings based on parameter estimates from various mathematical models fitted with PROC NLIN of SAS

	Dry matter					Crude protein				
	k=0.02	k=0.03	k=0.04	k=0.05	k=0.06	k=0.02	k=0.03	k=0.04	k=0.05	k=0.06
First cutting										
Model I	45.30	40.99	38.00	35.80	34.12	38.58	32.40	28.33	25.45	23.30
Model II	46.66	42.74	39.95	37.86	36.24	34.29	27.25	22.62	19.34	16.89
Model III	49.35	46.08	43.54	41.49	39.82					
Model IV	48.87	45.33	42.65	40.56	38.87					
Second cutting										
Model I	48.65	44.06	40.86	38.51	36.70	36.72	30.00	25.53	22.35	19.97
Model II	48.58	43.97	40.76	38.40	36.59	38.05	31.71	27.41	24.31	21.96
Model III	51.48	47.48	44.48	42.16	40.30					
Model IV	48.48	44.19	41.12	38.81	37.01					
Third cutting										
Model I	45.91	40.59	37.05	34.53	32.64	35.23	29.24	25.44	22.82	20.89
Model II	48.91	44.47	41.33	38.99	37.18	31.30	24.67	20.46	17.55	15.42
Model III	52.25	48.75	45.98	43.72	41.86					
Model IV	49.23	45.00	41.98	39.72	37.96					

k: rate of rumen passage, Model I: first-order kinetics model without lag phase, Model II: first-order kinetics model with lag phase, Model III: Gompertz model, Model IV: generalized Mitscherlich model

Conclusions

All the models that were fitted to the DM degradation data performed reasonably well, with only minor differences in goodness of fit. However, these models differed in values of the parameter estimates. Additionally, SAS failed to converge in the analyses of CP with Models III and IV, and MATLAB converged to nonsensical values with Model III. Model I might be recommended because it fitted the data well and required estimates of the fewest parameters.

Authors' contributions

VP collected the data for this study, and conducted the statistical analyses. VP and MM developed the original hypotheses and designed the experiments. ARB collaborated in interpreting the results and finalized the manuscript. All authors have read and approved the finalized manuscript

Conflict of Interest Declaration

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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