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Comparison of two mathematical models in the description of *in situ* degradability*

Comparação de dois modelos matemáticos na descrição de degradabilidade *in situ*

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Abstract

With the purpose of comparing the ruminal degradation models, proposed by Waldo et al. (1972) and Mertens and Loften (1980), the data of in situ degradability were employed. The experiment evaluated the potentially degradable residue of neutral detergent fiber (NDF) of grass Tifton 85 (*Cynodon spp*) submitted to twelve cutting ages (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 days), in a randomized block design with three replicates. At each cutting age, NDF degradation was investigated by utilizing nine incubation times (0, 3, 6, 12, 24, 48, 72, 96 and 120 hours). The analysis was done by taking into account a strip experiment and the factors studied were cutting ages and the incubation times of the grass. Each plot comprised a non-lactating cow, with a permanent ruminal fistula. The quality of the fit of each model was evaluated by the respective fitted determination coefficients, test for 'lack of fit' and also the variances of the estimators of the parameters, by proposing expressions for estimate of the confidence interval for the parameters of the models. The results showed a better fit of the model by Waldo et al. (1972) to the data of neutral detergent fiber of grass Tifton 85.

Keywords: degradability model, fit quality, non-linear regression, colonization time.

Resumo

Com o objetivo de comparar os modelos de degradação ruminal, proposto por Waldo et al. (1972) e Mertens e Loften (1980), utilizaram-se os dados de um ensaio de degradabilidade *in situ*. O experimento avaliou o resíduo potencialmente degradável da fibra em detergente neutro (FDN) da gramínea Tifton 85 (*Cynodon spp*) submetida a 12 idades de corte (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 e 360 dias), em um delineamento em blocos casualizados com três repetições. Em cada idade de corte a degradação da FDN foi estudada utilizando nove tempos de incubação (0, 3, 6, 12, 24, 48, 72, 96 e 120 horas). A análise foi feita considerando um experimento em faixas e os fatores estudados foram as idades de corte e os tempos de incubação da gramínea. Cada parcela foi constituída por uma vaca, não-lactante, com fístula ruminal permanente. A qualidade do ajuste de cada modelo foi avaliada pelos respectivos coeficientes de determinação ajustados, teste para 'falta de ajustamento' e obtiveram-se também as variâncias dos estimadores dos parâmetros, propondo-se expressões para a estimação do intervalo de confiança para os parâmetros dos modelos. Os resultados mostraram um melhor ajuste do modelo de Waldo et al. (1972) aos dados de fibra em detergente neutro da gramínea Tifton 85.

Palavras-chave: modelo de degradabilidade, qualidade de ajuste, regressão não-linear, tempo de colonização.

Introduction

Information on quantitative knowledge of the factors which control the digestive processes have directed the investigation in the field of Ruminant Nutrition, aiming to reach the best performances for the herds, thus the handling of the diets has focused as a way to increase animal production.

The "*in vitro* and *in situ"* trials are reliable techniques to supply estimates of the nutritive value of ruminant feeds. Nevertheless, the ideal would be a method which would be able to estimate with a reasonable accuracy *in vivo* digestibility, without the need of conducting a conventional trial, since it is extremely tiring and limited in its use, when there is a need for fast responses.

The in situ degradability technique has been adopted by the AFRC (1992) as a standard method of characterization of the ruminal degradability of nitrogen, being able to be utilized to report the characteristic degradation of dietary fibers (Aerts et

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al., 1977; Navaratree, Ibrahin and Shiere, 1990) and protein (Crawford et al., 1978; Stern e Satter, 1984; Poos-Floyd, Klopfenstein and Britton, 1985). The use of this technique has the advantage to provide a fast and simple estimate of nutrient degradation in the rumen, in addition to enabling the accompanying of this over time (Mehrez e Orskov, 1977).

Evaluating a possible relationship between a dependent variable and an independent variable is a common task in statistic analyses and may be done through regression models, which according to Draper and Smith (1998), can be linear, linearizable and non-linear. The studies of growth of animal and plants, as well as the nutrient degradation studies over time are reported by non-linear, presenting some difficulties in the process of estimating parameters.

The models developed to report the digestive events occurring in the rumen are numerous; some approach the quantification of total or partial processes of ruminal digestion and others are complex models of simulation which regard rumen in a global way. At present, two sorts of interest are realized which demand the evaluation of digestibility of a forage plant. The former is the need to compare different forage plants, by taking into account that the most digestible will show better economical/productive return by the animals which consume it, and the latter is the formulation of mechanistic models which express progressively and truly the dynamic phenomenon of digestion considering the circumstantial factors inherent to feeds such as composition, amount, feeding frequency and son on.

The option for one of the above-reported interest will make the choice of the mathematical model to be chased easy. The first interest recorded utilizes dynamic models as related with time of permanence of feed in the rumen and estimates its maximum degradation potential, characteristics which may be utilized for evaluation of the value of that feed. The second interest, generally, defines compartmental, mechanistic models in the sense of identifying biological compartments where feed undergoes sequentially modifications foreseen in the model.

According to Mertens (1993) the first evaluations of digestion processes, which depend on retention time, were qualitative and based upon the visual interpretation of digestion curves, these being of difficult description, since these curves showed non-linear behaviors. The author reports that Waldo was the first to suggest a conceptual innovation, which has suited as a basis for a new view of the mathematical models related with digestion kinetics, with which a real quantification of the fact was intended.

The model by Waldo et al. (1972) who reports the degradability technique for evaluation of rumen-incubated nutrients is given by the following equation:

$$R(t) = D\left(e^{-ct}\right) + I \qquad onde:$$ where

$R(t)$ is the residue after incubation in the rumen in time 't'; 'D' is the degradable fraction I (%); 'c' is the constant degradation rate; 't' incubation time in hours and I is the insoluble and non-degradable fraction.

It is known that for digestion to process, the microorganisms must penetrate the resistant barriers of the surface of particles of feeds to reach their preferred substrates and the extent to which microorganisms fix and penetrate these physical barriers is reflected in colonization time which characterizes the ruminal digestion of several feeds.

Mertens and Loften (1980) suggested the inclusion of parameter L, 'lag time' or colonization time, for the estimates of the parameters of the first-order model of Waldo et al. (1972) for *in situ* degradability of NDF, DM and N, as indicated in the equation:

$$R(t) = \begin{cases} D_0 + I & para\, 0 \le t \le L \\ D_0 e^{-c(t-L)} + I & para \quad t > L \end{cases}$$ where

D_0 is the degradable fraction (t d" L, D_0 = R -), the parameters $R(t)$, c e t , were defined in the previous equation.

The authors also suggested for the calculation of 'lag time' the equation:

$$L = \frac{\ln D_0 - \ln D_i}{-c}$$ where

$\ln D_i$ is the intercept of equation $\ln(R - I)$ with the axis of the ordinates; D_0 is the degradable potential residue in time t = 0.

The matter is summed up in how reliable the estimates obtained by the fit of degradation data of these models are, and what would be the real need to employ more complex models, with higher number of parameters and, hence, greater flexibility of fit to the data of nutrient degradability.

Methodology

The data for analysis were obtained in Reis (2000), where grass Tifton 85 (*Cynodon* spp) was submitted to twelve cutting ages (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 days), by making use of a randomized block design with three replicates, and at each age, degradation was evaluated in nine incubation times (0, 3, 6, 12, 24, 48, 72, 96 and 120 hours). The potentially degradable residue of neutral detergent fiber (NDF) of grass was analyzed. The statistical analysis was done by considering a strip experiment, according to Gill (1987), since the factor time, due to its nature, was given no randomization.

The statistical model was the following:

$$y_{ijk} = \mu + \delta_i + a_j + \delta a_{ij} + \beta_k + a\beta_{jk} + \delta\beta_{ik} + E_{ijk}$$

so: μ a constant associated with all the observations;

δ_i the effect of cutting age i, with i =1,...,12;

a_j the effect of animal j, with j =1, 2, 3;

δa_{ij} the effect of the interaction of the i-th cutting age with the j-th animal, regarded as error (a), with a normal distribution of mean zero and variance σ_a^2;

β_k the effect of incubation time k, with k =1,..., 9;

$a\beta_{jk}$ the effect of the interaction of the o k-th incubation time with the j-th animal, regarded as error(b), with a normal distribution of mean zero and variance σ_b^2;

$\delta\beta_{ik}$ the effect of the interaction of the k-th incubation time with the i-th cutting age;

E_{ijk} regarded as error(c), with a normal distribution of mean zero and variance σ^2.

The data were submitted to the analysis of regression by utilizing routines of the software Statistical Analysis System (SAS, 1991), using the non linear procedure of the method of Gauss-Newton (Neter, Wasserman and Kutner, 1985) to proceed the estimate of parameters ‘D’, ‘I’, ‘c’ e ‘L’, of the models of Waldo et al (1972) – M1 and Mertens and Loften (1980) – M2, considering a initial estimate and seeking to minimize the sum of squares of errors. In estimating the parameters, the iterative process was utilized till the improvement in the fit of data was negligible.

According to Souza (1998), on the contrary of the linear model, determination of the estimate of the parameters may be problematic in the non-linear case. The success in the utilization of Gauss-Newton’s algorithm is going to depend upon the appropriate choice of the response function and upon good initial values. Although, there are some general orientations to the determination of initial values, the choice procedure is a procedure decided by the researcher. A number of alternatives for determination of those values are presented in Draper and Smith (1998) and Gallant (1987).

In fitting the model by means of the NLIN procedure of SAS, the asymptotic estimate of the variance and covariance matrix of the estimates of the parameters was obtained, according to Souza (1998), considering a strip experiment. The matrix terms are expressed by:

$$\begin{bmatrix} SE_a^2 & \hat{\rho}_{ab}SE_aSE_b & \hat{\rho}_{ac}SE_aSE_c \\ \hat{\rho}_{ab}SE_aSE_b & SE_b^2 & \hat{\rho}_{bc}SE_bSE_c \\ \hat{\rho}_{ac}SE_aSE_c & \hat{\rho}_{bc}SE_bSE_c & SE_c^2 \end{bmatrix} x \frac{QME_{combined}}{rQMDR}$$

where: SE_a, SE_b, SE_c are the standard errors of the estimates of parameters a, b, and c considering only the nine incubation times; $\hat{\rho}_{ab}$, $\hat{\rho}_{ac}$ and $\hat{\rho}_{bc}$ are the asymptotic correlation coefficients among the parameters; QMDR is the mean square of the regression deviation based upon means; r is the number of animals where the incubation times were evaluated; $QME_{combined}$ is the mean square of error obtained by a linear combination of the mean squares of errors b and c of the analysis of variance, according to Satterthwaite (1946).

The main diagonal of the covariances matrix furnishes the expressions of variances adequate to the calculation of the asymptotic confidence intervals:

$$\hat{V}(\hat{a}) = SE_a^2 \frac{QME_{combined}}{r\,QMDR}$$

$$\hat{V}(\hat{b}) = SE_b^2 \frac{QME_{combined}}{r\,QMDR}$$

$$\hat{V}(\hat{c}) = SE_c^2 \frac{QME_{combined}}{r\,QMDR}$$

With the goal of evaluating the goodness of the models were considered the fitted determination coefficients, F test for ‘lack of fit’ presented by Hoffmann and Vieira (1998); and of the asymptotic confidence intervals for the estimates of the parameters for the two models, at each cutting age.

Results and discussion

The cutting age of the grass influenced the NDF degradation of Tifton 85 (P< 0.01) and the results of the analysis of variance are presented in Table 1.

Table 1: Analyses of variance of the NDF degradation for the models of Waldo et al. (M1) and Mertens and Loften (M2), of grass Tifton 85, considering a strip experiment

Sources of variation	Tifton85			
	M – 1		M –2	
	GL	QM	GL	QM
Animal (A)	2	2080,1050**	2	2136.4163**
Age (I)	11	2255.9148**	11	2347.2542**
Error (a)	22	337.1578	22	316.8002
Time (T)	8	10241.4337**	7	8793.1778**
Error (b)	16	31.8786	14	22.1256
I x T	88	93.3759**	77	86.0688**
Error (c)	176	21.6046	154	21.7393
CV 1 (%)	28.26		28.62	
CV 2 (%)	8.69		7.56	
CV 3 (%)	7.15		7.50	

** P < 0.01

The significant interaction (P< 0.01) shows that the cutting ages of the grass differ relative to the incubation times, and thus, it was opted by its unfolding, doing the fit of models M1 (Table2) and M2 (Table 3) for each cutting age.

The results presented in tables 2 and 3 show that F test for the regression deviation was not significant (P> 0.05) at all cutting ages, both for the model of Waldo et al. (M1) and for the model of Mertens and Loften (M2), showing that both are appropriate in reporting neutral detergent fiber degradation of grass Tifton 85 and that the medium squares of the regression deviations estimate the respective residual variances.

In Tables 2 and 3 are also presented the results of the determination coefficients fitted to the models of Waldo et al. (M1) and Mertens and Loften (M2) respectively. The value of the determination coefficient depends upon the number of observations, tending to increase when the number of observations decreases. To overcome this drawback, Hoffmann and Vieira (1998) defined the determination coefficient fitted to this number of observations. Sampaio (1997) further reports that the great number of collections, although physiologically has a greater biologic meaning, interferes in the digestive process by the constant removal of the nylon bag out of the rumen. The results in tables 2 and 3, show that in fitting the model of Mertens and Loftens (M2) to the data, there was a decrease in the determination coefficients fitted to most of the cutting ages, excepting the cutting of the grass at 60 e 330 days old.

In Table 4, the estimates of the parameters of the models of Waldo et al. (M1) and Mertens and Loften (M2) at each cutting age are viewed as well as the estimate of the variance of the estimates of these parameters, adequate to the calculation of the asymptotic confidence intervals. The fit of the model of Mertens and Loften (M2) determined greater estimates of variance of the estimate of the potentially degradable residues (parameter D) in most of ages, except for cutting at 30, 120

Table 2: Analysis of variance for neutral detergent fiber (NDF), studying the effect of time within each cutting age, for the model of Waldo et al. (M1)

Source of variation	GL	QM					
		30 days	R^2_{aj}	60 days	R^2_{aj}	90 days	R^2_{aj}
Time/ Cutting Age	8	705.5578		289.2791		653.7791	
Non-corrected model	3	31456.3138		57666.7123		42421.5022	
Constant (a)	1	88785.7640		170720.6482		122251.4247	
Corrected model	2	2791.5887	98.82	1139.7444	98.38	2506.5410	95.50
Deviation of regression	6	10.0875 ^{ns}		5.7786 ^{ns}		36.1613 ^{ns}	
Combined error	189	22.4607		22.4607		22.4607	
		120 days	R^2_{aj}	150 days	R^2_{aj}	180 days	R^2_{aj}
Time/ Cutting Age	8	656.2946		535.5542		454.5625	
Non-corrected model o	3	46229.3323		51107.0248		48675.3423	
Constant (a)	1	133470.0070		149061.3668		142465.2078	
Corrected model	2	2608.9949	99.33	2129.8538	99.37	1780.4095	97.75
Regression deviation	6	5.4644 ^{ns}		4.1102 ^{ns}		12.6711 ^{ns}	
Combined error	189	22.4607		22.4607		22.4607	
		210 days	R^2_{aj}	240 days	R^2_{aj}	270 days	R^2_{aj}
Time/ Cutting Age	8	1723.2567		2244.4848		1471.6421	
Non-corrected model	3	35087.6764		39152.5506		25172.1671	
Constant (a)	1	91598.9352		99645.0814		63813.9019	
Corrected Model	2	6832.0471	99.03	8906.2852	99.13	5851.2997	99.35
Regression Model	6	20.3702 ^{ns}		24.0004 ^{ns}		11.7604 ^{ns}	
Combined error	189	22.4607		22.4607		22.4607	
		300 days	R^2_{aj}	330 days	R^2_{aj}	360 days	R^2_{aj}
Time/ Cutting Age	8	859.3705		1125.0248		549.7542	
Non-corrected model	3	33482.9001		34178.0844		49466.3937	
Constant (a)	1	93672.8186		93586.5402		144011.2430	
Corrected Model	2	3387.9409	98.44	4473.8565	99.37	2193.9691	99.75
Regression deviation	6	16.5906 ^{ns}		8.6871 ^{ns}		1.6086 ^{ns}	
Combined error	189	22.4607		22.4607		22.4607	

^{ns} non significant (P>0.05)

and 240 days, but for the estimate of the insoluble and non-degradable residue (parameter I), considering the same model, smaller estimates of the variance occurred for the cutting at 30, 240 and 330 days.

The model of Mertens and Loften (M2) showed sensitivity in detecting time of particle colonization (lag time) only at the cutting ages of 240 and 330 days, these estimates being of 1.02 and 1.15 hours, respectively.

According to Feitosa (1999) this lack of sensitivity of the model in the detection of lag time was also observed by Vieira (1995) standing out the need for elucidation as to the correct use of this parameter. According to Sampaio (1997) only the period comprised between lag time and a reasonably long incubation time should be submitted to modeling and that this interval would be from 6 to 96 hours for forage plants and from 4 to 64 hours for concentrates or most rapidly degrading industrial residues.

Sampaio (1997) concluded also that considering this restrict period where degradation is taking place on the incubated material, the model to be defined, generally non-linear, should contain the least possible number of parameters.

Table 3: Analysis of variance for neutral detergent fiber (NDF), studying the effect of time within each cutting age for the model of Mertens and Loften (M2).

Source of variation	GL	QM					
		30 days	R^2_{aj}	60 days	R^2_{aj}	90 days	R^2_{aj}
Time/ Cutting Age	7	542,8926		243.1339		547.8295	
Non-corrected model	3	24946.5749		49026.0634		34752.9223	
Constant (a)	1	71083.4218		145393.8591		100614.5250	
Corrected model	2	1878.1514	98.73	842.1656	98.86	1822.1208	94.56
Regression model	5	8.7025 ^{ns}		3.5210 ^{ns}		38.1450 ^{ns}	
Combined Error	168	21.7715		21.7715		21.7715	
		120 days	R^2_{aj}	150 days	R^2_{aj}	180 days	R^2_{aj}
Time/ Cutting Age	7	586.5603		476.9282		414.6565	
Non-corrected model	3	38357.2498		42818.6866		41039.2054	
Constant (a)	1	110998.3587		125141.4458		120290.5233	
Corrected model	2	2036.6953	99.13	1657.3070	99.21	1413.5464	97.15
Regression model Regression model	5	6.5526 ^{ns}		4.8111 ^{ns}		15.5968 ^{ns}	
Combined error	168	21.7715		21.7715		21.7715	
		210 days	R^2_{aj}	240 days	R^2_{aj}	270 days	R^2_{aj}
Time/ Cutting age	7	1481.8287		2121.3162		1186.6985	
Non-corrected model	3	26982.0684		30863.5431		18672.3355	
Constant (a)	1	70686.8679		77858.9961		47760.8007	
Corrected model	2	5129.6687	98.81	7365.8166	96.47	4128.1028	99.33
Regression model	5	22.7615 ^{ns}		23.5586 ^{ns}		10.1772 ^{ns}	
Combined error	168	21.7715		21.7715		21.7715	
		300 days	R^2_{aj}	330 days	R^2_{aj}	360 days	R^2_{aj}
Time/ Cutting Age	7	662.1616		1001.9613		473.9668	
Non corrected model	3	26320.9457		27275.1720		41200.2978	
Constant (a)	1	74409.6718		74839.1848		120291.2951	
Corrected model	2	2276.5826	98.06	3493.1655	99.57	1654.7992	99.73
Regression model	5	16.5255 ^{ns}		5.4477 ^{ns}		1.5969 ^{ns}	
Combined error	168	21.7715		21.7715		21.7715	

^{ns} non significant (P>0.05)

Conclusions

The indicators of quality, although have shown a good fit of the models of Waldo et al. (1972) and Mertens and Loften (1980), to the data of the experiment, a decrease in the determination coefficient of the variance of the estimate of the parameters was found in fitting the model of Mertens and Loften (1980) in most of cutting ages.

The model of Mertens and Loften (1980) showed no sensitivity in the detection of colonization time (lag time) for most cutting ages studied and when it was estimated, it proved inferior to that found in the literature, stressing the need for elucidation concerning the correct use of this parameter.

Table 4: Estimate of the parameters of the model of Waldo et al. (M1) and Mertens and Loften (M2), with the respective estimates of variance

Cutting age (days)		Estimates (Variance)		Cutting Age (days)		Estimates (Variance)	
		M1	M2			M1	M2
	D	38.3553 (1.987896)	34.3914 (0.854969)		D	62.6220 (3.070597)	62.3568 (3.486102)
30	C	0.0409 (0.000022)	0.0276 (0.000007)	210	C	0.0262 (0.000005)	0.0246 (0.000006)
	I	40.8284 (1.284988)	38.6818 (0.780087)		I	26.2343 (3.310515)	25.3461 (4.245564)
	L		-----		L		-----
	D	27.6536 (7.352469)	28.6567 (16.961323)		D	77.4753 (7.528495)	77.4319 (5.770819)
60	C	0.0189 (0.000024)	0.0149 (0.000027)	240	C	0.0187 (0.000003)	0.0202 (0.000004)
	I	63.5437 (8.823736)	61.1852 (21.723679)		I	15.8803 (9.035844)	17.5325 (7.747532)
	L		-----		L		1.0177
	D	38.3622 (3.493391)	38.9501 (6.713096)		D	56.0101 (2.139839)	54.8631 (2.660594)
90	C	0.0247 (0.000014)	0.0192 (0.000015)	270	C	0.0346 (0.000009)	0.0317 (0.000010)
	I	47.2174 (3.915197)	44.4822 (9.038595)		I	22.8749 (1.733082)	22.1400 (2.214380)
	L		-----		L		-----
	D	39.7891 (4.217638)	39.7850 (4.173521)		D	42.2221 (1.976852)	40.7930 (2.694033)
120	C	0.0228 (0.000012)	0.0227 (0.000015)	300	C	0.0422 (0.000019)	0.0367 (0.000023)
	I	48.8711 (4.897822)	48.8184 (5.395706)		I	40.9485 (1.222535)	40.1651 (1.632288)
	L		-----		L		-----
	D	36.3898 (4.840462)	36.3864 (5.071860)		D	49.0897 (2.187366)	50.5547 (2.707171)
150	C	0.0217 (0.000015)	0.0211 (0.000017)	330	C	0.0336 (0.000011)	0.0371 (0.000015)
	I	54.3182 (5.709592)	54.0502 (6.752165)		I	36.0620 (1.837148)	36.7295 (1.595936)
	L		-----		L		1.1464
	D	34.5817 (7.395547)	34.6432 (7.415862)		D	35.4857 (3.070362)	35.3225 (3.354114)
180	C	0.0188 (0.000016)	0.0185 (0.000018)	360	C	0.0262 (0.000017)	0.0252 (0.000020)
	I	52.6510 (8.875696)	52.4623 (9.968649)		I	54.8941 (3.309823)	54.5752 (4.003351)
	L		-----		L		-----

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