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Heterogeneity of the digestible insoluble fiber of selected forages *in situ*

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ABSTRACT

Long-term *in situ* incubations were performed to verify the likelihood of the heterogeneity concept of the potentially digestible fraction of the insoluble fiber (NDFom) by fitting both heterogeneous and homogeneous potentially digestible NDFom versions of a generalized compartmental model of digestion (GCMD). Corn silage and eleven tropical grasses and alfalfa hay were studied. Data were gathered from a study in which forage samples in nylon bags were incubated in rumen cannulated steers so that three profiles per forage were generated. The incubation endpoint was used to form sets of time profiles. The original set consisted of profiles ending at 1440 h, and the other two were formed by using 96 and 240 h as the incubation endpoints, respectively. The indigestible residue was estimated using nonlinear least squares or by assuming it to be 2.4 times lignin determined by the sulphuric acid method (Lignin (sa)). Therefore, eight different models were evaluated by combining end points of digestion, and the homogeneous and heterogeneous versions of GCMD with the two ways of estimating the indigestible residue. The likelihood of the models was assessed by computing Akaike information criteria. The effects of forage, model, and their interaction were analyzed by taking model as a repeated measurement. Heterogeneity of the potentially degradable fraction for NDFom was detected with long-term incubation trials (up to 1440 h) for some forages, and the introduction of the 2.4×Lignin (sa) as a direct measure of the indigestible residue improved the likelihood of the heterogeneous version of GCMD. The forage by model interaction was significant for many comparable parameter estimates, which means that specific and inconsistent results for models within forages were produced depending on the definition of the incubation end-point. The indigestible residue was overestimated with short-term incubation profiles, but the overestimation was lower for the profiles ending at 240 h whether compared to profiles ending at 96 h. Given the likelihood of the heterogeneous version of GCMD fitted to profiles ending at 1440 h and at 240 h for some forages, the heterogeneity concept should be investigated whenever the research interest relies on estimating the kinetic attributes of the degradation profiles of the NDFom *in situ*.

Abbreviations: *AICc*, corrected Akaike information criterion; BW, body weight; CP, crude protein; DM, dry matter; GCMD, generalized compartmental model of digestion; LSM, least squares mean(s); max, maximum value; min, minimum value; NDFom, insoluble fiber as neutral detergent fiber expressed exclusive of residual ash; Lignin (sa), lignin determined by solubilization of cellulose with sulphuric acid; NLS, nonlinear least squares.

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1. Introduction

The *in situ* technique provides quantitative means to obtain parameter estimates of the anaerobic digestion of substrates by rumen microbes. There are several recommendations as well as several drawbacks and limitations reported for the *in situ* trials (Huntington and Givens, 1995). Nevertheless, this technique is worldwide used and researchers have generated profiles that can be kinetically described by mathematical models. The insoluble fibrous residues obtained after incubating samples *in situ* (or *in vitro*) at increasing time intervals produce degradation profiles that can be interpreted quantitatively as a first-order process in which only the substrate is limiting (Waldo et al., 1972; Mertens, 1977, 2005). Sometimes, however, the profiles exhibit lag times and more than one inflection points followed by an asymptotic phase; the latter is assumed to represent the indigestible or unavailable fraction (Mertens, 1977; Robinson et al., 1986; Ellis et al., 2005). To quantitatively assess the kinetic attributes of those profiles, researchers have applied semi-logarithmic plots to look for the discrimination of a lag phase and inflection points of the decreasing curve that can be visually separated in two or more linear sections, a technique long used to describe radioactive decay of isotope mixtures known as "curve peeling" (Mertens, 2005). Nevertheless, because of the widespread use of powerful computers and continuous development and refinement of statistical software, the use of the curve peeling technique is rather difficult to justify nowadays.

The mathematical models used to describe degradation profiles of the insoluble fiber (NDFom) are based on an intrinsic fractionation of the NDFom that require a proper characterization of the indigestible residue (the asymptotic phase), which depend on time that samples remain in the rumen (Mertens, 1977; Robinson et al., 1986; van Milgen et al., 1993). This incubation time can alter the number of potentially digestible fractions, and the estimates of the fractional rate or rates related to the digestion process (Ellis et al., 2005; Van Soest et al., 2005). The indigestible residue can only be accurately estimated by biological assays with long-term (90–120 d) anaerobic incubations (Chandler et al., 1980). The use of different end-points of digestion, particularly short-term incubations lasting for 72–96 h, has produced profiles with overestimated indigestible residues and no more than one detectable inflection point (Nocek and English, 1986; Robinson et al., 1986). In addition, more complex models require data points in quantity and quality to avoid during the estimation method numerical artifact estimates, which are not likely to represent true biological values (Bard, 1974; Robinson et al., 1986; Ellis et al., 2005). Therefore, inconsistent results have been found in the literature regarding the heterogeneous nature of the potentially degradable fraction of NDFom *in situ* (Nocek and English, 1986; Robinson et al., 1986; van Milgen et al., 1992a,b, 1993).

A generalized compartmental model of digestion (GCMD) modified to account for heterogeneity in the potentially digestible fraction of NDFom has recently been proposed by Vieira et al. (2008) to deal with degradation profiles that exhibit sigmoid shape and possibly an additional slow digesting sub-fraction. This model could be applied to describe *in situ* data and is based on the concept that the potentially digestible substrate, *i.e.*, the feed or forage particle containing the digestible substrate, must be prepared prior to digestion in a sequential process (Akin et al., 1974; van Milgen et al., 1991; Mertens, 2005). This preparation is characterized by a gamma time dependency distribution, and the subsequent digestion process is assumed to follow first-order decay.

The goal of the present research was to describe with the GCMD *in situ* degradation profiles of NDFom generated by varying the end-point of digestion in order to check the likelihood of the heterogeneity concept applied to the potentially digestible fraction of the insoluble fiber of selected forages.

2. Materials and methods

Data used in this study were gathered from the work of Campos (2010), who studied the nutritive value of 12 forages based on their chemical composition and *in situ* digestion kinetics of NDFom. The following forage species were used: (1) *Acroceras macrum* Stapf., (2) *Urochloa mutica* (Forssk.) T.Q. Nguyen, (3) *Pennisetum purpureum* Schum. cv. Cameroon, (4) *Saccharum* spp., (5) *Pennisetum purpureum* Schum. clone CNPGL 92-79-02, (6) *Pennisetum purpureum* Schum. clone CNPGL 91-06-02, (7) *Hemarthria altissima* (Poir.) Stapf. & C.E. Hubbard, (8) *Urochloa maxima* (Jacq.) R. Webster cv. Mombasa, (9) *Pennisetum purpureum* Schum. cv. Napier, (10) *Setaria sphacelata* cv. Kzungula, (11), *Zea mays* L. as corn silage, and (12) *Medicago sativa* L. as commercial alfalfa hay. The forage species were cultivated in the Northern Rio de Janeiro State (21°45′14″S and 41°19′26″W), Brazil, at 15 m of altitude, a region where an Aw climate (according to the Köppen standards) predominates with an annual rainfall of 800 mm. The exception was the alfalfa hay which was from Southern Brazil, where a Cf climate (Köppen standards) prevails. Forage samples were analyzed for dry matter (DM, AOAC 967.03; AOAC, 1990), crude protein (CP, AOAC 984.13; AOAC, 1990), fat (AOAC 2003.06; Thiex et al., 2003), ash (AOAC 942.05; AOAC, 1990), NDFom (without sodium sulphite and amylase and with ash excluded; Van Soest et al., 1991), and lignin by the sulphuric acid method (Lignin (sa), AOAC 973.18; AOAC, 1990) after a sequential neutral-acid detergent extraction (Van Soest et al., 1991); the reported results are listed in Table 1.

2.1. Details of incubations and end-point characterization of the time series data

The *in situ* incubations of the forage samples performed by Campos (2010) followed the general recommendations provided by Nocek (1988). The forage samples were ground to pass through a 5-mm sieve; the average pore size of the nylon bags tissue was 50 μm, and the ratio of sample DM to bag surface area set to 15 mg/cm^2. Three degradation profiles per forage were produced by incubating bags *in situ* without replications per time point and in reverse sequence at 1440, 912, 528, 336,

Table 1
Chemical composition of the forages studied.

Forage[a]	Chemical fraction					
	DM[b]	Ash[c]	CP[c]	Fat[c]	NDFom[c]	Lignin (sa)[c]
1	253	36	104	35	719	45
2	212	93	78	14	695	87
3	164	75	124	13	716	49
4	277	37	21	13	472	55
5	215	91	102	11	699	49
6	217	67	125	10	681	45
7	198	71	88	12	757	52
8	254	116	170	17	652	30
9	185	98	136	7	645	30
10	162	90	89	21	702	67
11	326	64	69	30	609	46
12	874	88	184	26	465	95

[a] Numbers are related to: *A. macrum* (1), *U. mutica* (2), *P. purpureum* cv. Cameroon (3), *Saccharum* spp. (4), *P. purpureum* clone CNPGL 92-79-02 (5), *P. purpureum* clone CNPGL 91-06-02 (6), *H. altissima* (7), *U. maxima* cv. Mombasa (8), *P. purpureum* cv. Napier (9), *S. sphacelata* cv. Kzungula (10), *Z. mays* as silage (11), and *M. sativa* as hay (12).
[b] g/kg.
[c] g/kg DM.

240, 192, 168, 156, 144, 120, 108, 96, 84, 72, 60, 48, 42, 36, 30, 24, 18, 12, 9, 6, and 3 h until their joint removal at time zero. To avoid overwhelming rumen capacity due to the large number of samples to be incubated, the forages were divided in two groups. The group one was formed by *A. macrum* (forage one), *U. mutica* (forage two), *P. purpureum* cv. Cameroon (forage three), *Sacharum* spp. (forage four), *P. purpureum* cv. Napier (forage nine), and *Z. mays* (forage 11). The group two was formed by *P. purpureum* clone 92-79-02 (forage five), *P. purpureum* clone 91-06-02 (forage six), *H. altissima* (forage seven), *U. maxima* (forage eight), *S. sphacelata* (forage 10), and *M. sativa* (forage 12). Therefore, profiles were produced by incubating the time series bags in rumen cannulated steers (Holstein–Zebu crosses) by using three animals per group of forages. In group one, the individual BW of the animals were 690, 493, and 410 kg; in group two, BW were 587, 565, and 451 kg. Animals were fed on average 19 g DM/kg BW/d equally divided in two meals (at 7:00 am and 05:00 pm) of a diet consisted of, on a DM basis, 0.60 parts of Tyfton-85 hay, 0.07 parts of chopped Elephant grass (*P. purpureum* cv. Cameroon with 270 g DM/kg, and harvested at 180 cm height, approximately), 0.18 parts of grounded corn, and 0.15 parts of soybean meal.

After their joint removal, bags were grouped by forage and washed with tap water in a laundry machine by applying three washing-cycles of 20 min each. Three replicates of the zero-time bags per forage were not incubated in the rumen but were washed together with the incubated bags. Afterwards, bags were dried in an air circulating oven at 55 °C for 72 h and their contents were ground to pass through a 1-mm sieve and analyzed for DM (AOAC 967.03; AOAC, 1990) and NDFom (without sodium sulphite and amylase; Van Soest et al., 1991).

The incubation end point was used to form sets of time profiles. All time points were used to form the first set, *i.e.*, all residues from time zero to 1440 h were considered. Because 96 h has been used as a recommended end point for degradation studies (Mertens, 1977, 2005), it was selected to generate a set of profiles by accounting for all incubation residues until this point in the time series data. In addition, a profile set was generated by considering the end-point of digestion at 240 h. This end point was selected to avoid substantial contamination by mineral deposits in the bags after 10 d of *in situ* incubations (van Milgen et al., 1992a,b). As a result, two sets of 36 profiles each formed due to the different end-points (96 and 240 h) were added to the original set of 36 profiles (three per forage) with all data points (from zero to 1440 h) in the time series.

2.2. Compartmental models of digestion

The heterogeneity concept for the potentially digestible fraction of NDFom was introduced in the GCMD as suggested by Vieira et al. (2008) and shown in Eq. (1).

$$R(t) = \tilde{A}_{NDF}\left\{A_1\left\{\delta_1^N \exp(-k_1 t) + \exp(-\lambda t)\sum_{i=1}^{N-1}\frac{(1-\delta_1^{N-i})(\lambda t)^i}{i!}\right\}\right.$$
$$\left. + A_2\left\{\delta_2^N \exp(-k_2 t) + \exp(-\lambda t)\sum_{i=1}^{N-1}\frac{(1-\delta_2^{N-i})(\lambda t)^i}{i!}\right\}\right\} + U_{NDF} \quad (1)$$

The function $R(t)$ represents the NDFom incubation residue at a given time, t; $\tilde{A}_{NDF}$ is the potentially digestible substrate initially unavailable to the digestive actions of the rumen microbes, and that must be prepared for digestion; N is a positive integer representing the order of time dependency; $\lambda(1/h)$ is the asymptote of the rate of $\tilde{A}_{NDF}$ preparation for digestion; A_1 and A_2 are the dimensionless fast and slow potentially digestible sub fractions of $\tilde{A}_{NDF}$, with k_1 and k_2 as their respective fractional digestion rates $(1/h)$; $\delta_1 = \lambda/(\lambda - k_1)$ and $\delta_2 = \lambda/(\lambda - k_2)$ are constants; and U_{NDF} represents the indigestible

Table 2
Characteristics of the models studied.

Model	Equation	Definition of the end point time (h)	Estimation of U_{NDF}
MOD1	(1)	1440	Convergence
MOD2	(1)	1440	2.4×Lignin (sa)
MOD3	(1)	96	2.4×Lignin (sa)
MOD4	(2)	96	Convergence
MOD5	(2)	96	2.4×Lignin (sa)
MOD6	(1)	240	Convergence
MOD7	(1)	240	2.4×Lignin (sa)
MOD8	(2)	240	Convergence

or unavailable NDFom fraction. The constraint $A_2 = 1 - A_1$, being A_1 and A_2, was applied to reduce by one the number of parameters to be estimated in the model, and thus saving one error term degree of freedom for an improved precision in the parameter estimation process. Eq. (1) describes the initial smooth delay observed in degradation profiles of many tropical and temperate forages, as well as sigmoid shape patterns with two detectable inflection points. The initial smooth delay quantitatively described by parameter λ as the asymptotic rate of substrate preparation, is the net result of the processes of hydration, solubilization of digestion inhibitors, and preparation of the bacterial cell machinery to synthesize enzymes and other cell structures, and to form biofilms that subsequently result in the effective digestion of the insoluble fibrous matrix (Mertens, 2005; van Milgen et al., 1991; Vieira et al., 2008).

Eq. (2) is a simple version of Eq. (1), but with a homogeneous $\tilde{A}_{NDF}$. This model was devised by Vieira et al. (2008) via generalization of the model proposed by van Milgen et al. (1991). In fact, despite conceptually proposed for digestion kinetics the solution is mathematically the same as that suggested by Matis et al. (1989) for digesta flow kinetics.

$$R(t) = \tilde{A}_{NDF}\left\{\delta^N \exp(-kt) + \exp(-\lambda t)\sum_{i=1}^{N-1}\frac{(1-\delta^{N-i})(\lambda t)^i}{i!}\right\} + U_{NDF}(s) \quad (2)$$

The terms $R(t)$, $\tilde{A}_{NDF}$, N, λ, and U_{NDF} have the same meanings as previously described; $\delta = \lambda/(\lambda - k)$ is a constant to simplify the expression, and $k(1/h)$ is the fractional digestion or degradation rate of the assumed homogeneous potentially digestible fraction of NDFom.

2.3. Treatment description and additional assumptions

The U_{NDF} was directly estimated by NLS, but was also estimated as $U_{NDF} = 2.4\times$Lignin (sa) (Van Soest et al., 2005), with Lignin (sa) computed as g Lignin (sa)/g NDFom for this particular reason. Therefore, we fitted Eqs. (1) and (2) with all parameters estimated directly by NLS, and both equations after replacing U_{NDF} by 2.4×Lignin (sa) as the asymptotic end-point of digestion. Possible biases in the time residues due to long incubation times were avoided by fitting Eqs. (1) and (2) to profiles with end-points at 240 h for all forages. A descriptive summary of the models studied is listed in Table 2.

Because NDFom has no soluble component in the rumen fluid phase the estimated parameter coefficients were normalized by assuming the correction proposed by Waldo et al. (1972), *i.e.*, $A_n = \hat{\tilde{A}}_{NDF}/(\hat{\tilde{A}}_{NDF} + \hat{U}_{NDF})$, and $U_n = \hat{U}_{NDF}/(\hat{\tilde{A}}_{NDF} + \hat{U}_{NDF})$. Given that $A_n = 1 - U_n$, and by assuming that U_n can be equivalent to 2.4×Lignin (sa), it can be demonstrated that $U_{NDF} = \tilde{A}_{NDF}U_n/(1 - U_n)$, and the second member of this equality was used in place of U_{NDF} in Eq. (1). The resulting model was fitted to profiles with 240 h as an end-point of digestion and treated as MOD7. The original Eqs. (1) and (2) fitted with all parameters to profiles ending at 240 h are henceforth referred to as MOD6 and as MOD8, respectively (Table 2).

2.4. Statistical procedures and model evaluation criteria

The parameters of the different models were estimated with the NLIN procedure of SAS, version 9.1 (SAS System Inc., Cary, NC, USA). The methods of Newton and Marquardt were used. The former presents a good performance and easily reaches convergence but struggled when correlations among estimates of the parameters were high and whenever the Hessian matrix was not positive definite. The Marquardt's algorithm usually overcome these problems (Bard, 1974), and was used to fit the models when the Newton's method did not perform well. The SAS programs for fitting the GCMD are provided in Appendix A.

The selection of the best version concerning the order of time dependency (N) for all models (MOD1–MOD8) was based on the criterion suggested by Vieira et al. (2008). Once chosen the most suitable N version of the models, their likelihood was evaluated by computing Akaike's information criterion ($AICc_r$; Akaike, 1974; Burnham and Anderson, 2004; Motulsky and Cristopoulos, 2003). The corrected $AICc_r$ was calculated from the sum of squares of the error (SSE_r), number of estimated parameters including the error variance (Θ_r), and sample size (n_r) for the rth different models whichever $r = 1, 2, \ldots, 8$; in other words, $r = 1$ for MOD1, $r = 2$ for MOD2, and so on. The differences among $AICc_r$ values (Δ_r), the Akaike weights or

likelihood probabilities (w_r), and the evidence ratio or relative likelihood (ER_r) were also computed by using the following equations:

$$AICc_r = n_r \ln\left(\frac{SSE_r}{n_r}\right) + 2\Theta_r + 2\Theta_r \frac{\Theta_r + 1}{n_r - \Theta_r - 1} \quad (3)$$

$$\Delta_r = AICc_r - \min AICc_r \quad (4)$$

$$w_r = \frac{\exp(-\Delta_r/2)}{\sum_{r=1}^{R} \exp(-\Delta_r/2)} \quad (5)$$

$$ER_r = \frac{\max w_r}{w_r} \quad (6)$$

The number of time points (n_r) in the profiles dictates the valid comparisons that can be made among models. In this regard, valid comparisons concerning information criteria could only be made for models sharing the same n_r, *i.e.*, between MOD1 and MOD2, among MOD3, MOD4, and MOD5, and among MOD6, MOD7, and MOD8; other model comparisons have no interest (e.g., MOD8 vs. MOD1, or MOD7 vs. MOD3).

The computed values of the information criteria were categorized into arbitrary classes analogous to those reported by Raftery (1995) and Burnham and Anderson (2004). The rationale behind the classes lies in the computation of the individual probability for each model, which equals to the numerator of Eq. (5). For Δ_r values belonging to the interval [0,2], the computed probability decrease from one to 0.368; for $\Delta_r = 10$, the probability decreases further to approximately 0.007, *i.e.*, lower than 1/100. For $\Delta_r > 10$ the probability is lower than 0.007. Therefore, Δ_r values belonging to the interval [0,2] indicate that models were equivalent in minimizing information loss and considered more likely in mimicking observed data, and whenever this happened the model with a lower Θ_r was preferred. Models with $\Delta_r \subset (2, 10]$ were considered to have less support, and models with $\Delta_r > 10$ considered to have no support. The same concepts were applied to w_r and ER_r. For example, if within a set of three models one achieved $\Delta_r = 0$, and the other two models $\Delta_r = 2.8$ and $\Delta_r = 10$, the w_r values for the three models would be 0.800, 0.247, and 0.005, respectively. Hence, for $w_r > 0.8$ models were considered likelihood representations of reality; they were considered less likely for $w_r \subset (0.5, 0.8]$; and considered unlikely for $w_r \leq 0.5$. If we consider Eq. (6) and the hypothetical values for Δ_r equal to 0, 6, and 10, this would yield ER_r values equal to one, 20, and 148, respectively. For this reason, models that had $ER_r = 1$ were considered the best choice; less likely models those ones with $ER_r \subset (1, 20]$; and models that had $ER_r > 20$ were considered the poorest choice in the set, given the data. Therefore, the appraisal of the combined criteria is a reasonable tool for model selection, and the simple ones were considered the best choice if there was no evidence of superiority in favor of more complex models.

The information criteria provide enough evidence for choosing one model over the others, but some comparisons among parameter estimates might be useful and as such were computed by statistical analysis. Because different models were fitted to the same profile, a mixed model to account for repeated measures was used to verify how comparable parameter estimates were affected by models:

$$Y_{jkr} = \mu + \alpha_j + a_k + \alpha a_{jk} + \beta_r + \alpha\beta_{jr} + e_{jkr} \quad (7)$$

The term Y_{jkr} is the parameter estimate obtained by fitting the *r*th model to the degradation profile of the *j*th forage generated by incubating samples in the *k*th animal. The model was independently fitted to estimates for both forage-animal groups (see Section 2.1). The fixed effects in Eq. (7) are forage (α_j), model (β_r), and their interaction ($\alpha\beta_{jr}$); and the random effects are animal (a_k), the forage by animal interaction (αa_{jk}), and the usual error term (e_{jkr}). The statistical model was fitted by using the PROC MIXED of SAS (version 9.1) with Maximum Likelihood as the estimation method, the Kenward–Roger degrees of freedom option chosen, and the variance–covariance matrix modeled as variance components, compound symmetry with a constant correlation and homogeneous variances, compound symmetry with a constant correlation and heterogeneous variances, the banded main diagonal with heterogeneous variances and uncorrelated repeated measures, and the unrestricted variance–covariance structure (Littell et al., 1998, 2006). The subject term required in the repeated statement of the PROC MIXED procedure was the forage by animal interaction (αa_{jk}). Once the *AICc* is one of the SAS outputs, these different variance–covariance structures were evaluated on the basis of the information criteria described by Eqs. (4)–(6). Null hypotheses were rejected for comparisons having P<0.05 unless otherwise stated.

3. Results

The inclusion of the direct estimate of parameter U_{NDF} as 2.4×Lignin (sa) improved the likelihood of the models with a heterogeneous potentially degradable fraction for NDFom, *i.e.*, the loss of information was lower for MOD2 and MOD7 whether compared to their MOD1 and MOD6 versions, respectively (Table 3). The inclusion of the laboratory measure of U_{NDF}, however, did not improve the likelihood of the single pool version (MOD5), which had a poor performance in mimicking the degradation profiles; MOD4 was more likely. Although models with heterogeneous fractions (MOD2 and MOD7) performed well, a larger proportion of the time profiles was best described by the homogeneous versions MOD4 and MOD8. The inclusion of the measured U_{NDF} as 2.4×Lignin (sa) did not improve the likelihood of Eq. (1) (MOD3) in describing profiles in which the end time point was at 96 h. Nevertheless, MOD7 was more likely in describing some time profiles despite the fact that this conclusion was based on an analysis performed on profiles pooled across forage species and animals.

Table 3
Relative frequencies distributed into the arbitrary classes of the information criteria (Δ_r, w_r, and ER_r)[a] calculated after fitting the eight models. Valid comparisons can only be made between MOD1[b] and MOD2,[c] among MOD3,[d] MOD4,[e] and MOD5,[f] and among MOD6,[g] MOD7,[h] and MOD8.[i]

Model	Classes for Δ_r values			Classes for w_r values			Classes for ER_r values		
	[0,2]	(2,10]	>10	≤0.5	(0.5,0.8]	>0.8	=1	(1,20]	>20
MOD1	0.444	0.500	0.056	0.611	0.056	0.333	0.389	0.472	0.139
MOD2	0.667	0.139	0.194	0.389	0.083	0.528	0.611	0.167	0.222
MOD3	0.111	0.639	0.250	0.917	0.028	0.056	0.083	0.361	0.556
MOD4	0.833	0.139	0.028	0.278	0.139	0.583	0.722	0.194	0.083
MOD5	0.250	0.111	0.639	0.806	0.083	0.111	0.194	0.111	0.694
MOD6	0.083	0.806	0.111	0.944	0.028	0.028	0.056	0.333	0.611
MOD7	0.389	0.528	0.083	0.694	0.083	0.222	0.306	0.583	0.111
MOD8	0.639	0.194	0.167	0.361	0.194	0.444	0.639	0.083	0.278

[a] Thirty-six fits were evaluated for each model (MOD1–MOD8). See Section 2.4 for details on information criteria.
[b] MOD1, Eq. (1) fitted to profiles ending at 1440 h with indigestible residue estimated by NLS.
[c] MOD2, Eq. (1) fitted to profiles ending at 1440 h with indigestible residue estimated by 2.4×Lignin (sa).
[d] MOD3, Eq. (1) fitted to profiles ending at 96 h with indigestible residue estimated by 2.4×Lignin (sa).
[e] MOD4, Eq. (2) fitted to profiles ending at 96 h with indigestible residue estimated by NLS.
[f] MOD5, Eq. (2) fitted to profiles ending at 96 h with indigestible residue estimated by 2.4×Lignin (sa).
[g] MOD6, Eq. (1) fitted to profiles ending at 240 h with indigestible residue estimated by NLS.
[h] MOD7, Eq. (1) fitted to profiles ending at 240 h with indigestible residue estimated by 2.4×Lignin (sa).
[i] MOD8, Eq. (2) fitted to profiles ending at 240 h with indigestible residue estimated by NLS.

More specifically, MOD7 was fitted with a greater likelihood to one profile of *A. macrum* ($w_7 = 0.950$), one profile of *Saccharum* spp. ($\Delta_7 = 0$ and $w_7 = 0.679$), two profiles of *P. purpureum* clone 92-79-02 ($w_7 = 0.919$ and 0.833), two profiles of *P. purpureum* clone 91-06-02 ($w_7 = 0.909$ and 0.785), one profile of *H. altissima* ($\Delta_7 = 0$ and $w_7 = 0.789$), two profiles of *S. sphacelata* ($w_7 = 0.899$ and 0.928), and two profiles of the corn silage ($w_7 = 0.916$ and 0.996). The MOD6 was more likely in representing one profile of *P. purpureum* cv. Cameroon ($w_7 = 0.966$) and one profile of *U. maxima* cv. Mombassa ($\Delta_7 = 0$ and $w_7 = 0.769$).

3.1. Differences among parameter estimates

The use of the different models yielded significant differences among parameter estimates. Because of specific characteristics of models, the estimates of the parameters were compared within sets of models and within forage-animal groups (Table 4). Among parameters of MOD1, MOD2, and MOD3 within the group one, the mean time for substrate preparation (N/λ), the fast digesting sub-fraction (A_1), and the estimates of k_2 were all affected by the forage–model interaction; by its turn, the estimates of k_1 were affected by the single effects of forage and model. Within group two, the estimates of N/λ

Table 4
P-values of the repeated measures analysis performed on estimates of some comparable parameters obtained after fitting the eight different models[a] (MOD1–MOD8) to check the effects of forage, model, and their interaction.

Parameter	Forage		Model		Interaction	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
N/λ[b]	0.090	0.071	0.014	0.044	0.003	0.237
A_1[b]	0.203	0.023	0.933	0.111	0.004	0.228
k_1[b]	0.021	0.811	0.026	0.019	0.172	0.497
k_2[b]	<0.001	<0.001	0.106	0.042	0.002	<0.001
$\tilde{A}_{NDF}$[b]	0.001	<0.001	0.171	0.331	0.064	<0.001
N/λ[c]	0.677	0.008	<0.001	<0.001	0.508	<0.001
k[c]	0.003	<0.001	<0.001	0.339	0.003	<0.001
$\tilde{A}_{NDF}$[c]	<0.001	0.003	<0.001	0.002	<0.001	<0.001
N/λ[d]	0.042	0.042	<0.001	<0.001	0.007	<0.001
$\tilde{A}_{NDF}$[d]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
A_1[e]	0.099	0.347	0.256	0.670	0.007	0.043
k_1[f]	0.003	0.002	0.378	0.376	0.853	0.414
k_2[f]	0.153	<0.001	0.181	0.003	0.011	<0.001

[a] Models are defined by heterogeneity or not of the potentially digestible NDFom, the inclusion of a laboratory estimate for indigestible NDFom, and incubation end point. For details, see Sections 2.1–2.3.
[b] Parameters of MOD1, MOD2 and MOD3 were compared.
[c] Parameters of MOD4 and MOD5 were compared.
[d] Parameters of MOD6, MOD7 and MOD8 were compared.
[e] Parameters of MOD6 and MOD7 were compared for all forages except alfalfa because MOD7 estimates for A_1 were all equal to one (zero variance for this forage).
[f] Parameters of MOD6 and MOD7 were compared.

and k_1 were only affected by model, and the forage effect influenced the A_1 estimates. The estimates of $\tilde{A}_{NDF}$ were affected by the forage factor in group one, and by the interaction factor in group two. The slow digesting sub-fraction A_2 is the A_1 complement and their statistical analysis results were identical.

Eq. (2) fitted to profiles in which the end time point was at 96 h (MOD4 and MOD5) presented significant interaction effects for N/λ estimates within group two, and for k estimates of both groups (Table 4). The N/λ estimates for group one were only affected by model. The estimates of $\tilde{A}_{NDF}$ were affected by the forage–model interaction in both groups. The inclusion of the measured U_{NDF} strongly biased the estimates of k of MOD5 because this model was unlikely in mimicking the degradation profiles if compared to MOD4 (Table 3).

The estimates of N/λ and $\tilde{A}_{NDF}$ were affected by the forage–model interaction for MOD6, MOD7, and MOD8 in both groups (Table 4). The relationship between the measured U_{NDF} (which equals to U_n; see Section 2.3) and the unknown effects of the loss and influx/efflux of particles across the entire profile over the solution space for all parameters, and $\tilde{A}_{NDF}$ in particular, were conserved by replacing U_{NDF} on Eq. (1) by $\tilde{A}_{NDF}U_n/(1-U_n)$ in MOD7. Within both groups the A_1 estimates of the heterogeneous versions (MOD6 and MOD7) were affected by the forage–model interaction, and this result was analogous for A_2. There were strong evidence of the interaction effect over the estimates of k_2, but no evidence for the interaction effect for k_1; only the forage effect was evident for k_1 estimates in both groups.

The LSM of the MOD6, MOD7, and MOD8 parametric estimates are listed in Table 5. The estimates of $\tilde{A}_{NDF}$ were not shown. The forage–model interactions were accounted for by performing tests on effect slices; P-values related to model effects are henceforward, respectively reported within parenthesis for both forage-animal groups whenever appropriate. These tests over N/λ estimates for MOD6, MOD7, and MOD8 within forages were significant for *U. mutica* (P=0.004), *P. purpureum* cv. Cameroon (P<0.001), *Saccharum* spp. (P=0.002), *P. purpureum* clone 92-79-02 (P=0.007), corn silage (P=0.049), and alfalfa (P<0.001); conversely, by considering both forage-animal groups, the same estimates were statistically different among forages within MOD6 (P=0.026 and 0.008), MOD7 (P=0.023 and P<0.001), and with no detectable forage differences within MOD8 (P=0.831 and 0.207). The estimates of $\tilde{A}_{NDF}$ were statistically different with respect to the forage by model interaction within both forage-animal groups (Table 4). The tests on effect slices for $\tilde{A}_{NDF}$ estimates revealed that forages were different within MOD6 (P=0.002 for group one), MOD7 (P<0.001 for both forage-animal groups) and MOD8 (P<0.001 for group one), being other effect slices not significant for forages within MOD6 (P=0.179, group two), and MOD8 (P=0.058, group two). Differences between A_1 estimates of MOD6 and MOD7 were only detected for *U. mutica* (P=0.001), and *P. purpureum* clone 92-79-02 (P=0.006); there were differences among forages within MOD7 in both groups (P=0.014 and P<0.001), and among forages within MOD6 (P=0.013) in group two. The estimates of k_2 were also tested for effect slices and differences between MOD6 and MOD7 were detected only for *Saccharum* spp. (P=0.002), and alfalfa hay (P<0.001). Differences among k_2 estimates for forages were non-significant within MOD6 (P=0.050 and 0.615). There were differences among forages within MOD7 (P<0.001) for group two, and no differences among forages for group one (P=0.078). In Table 5 were reported the A_n and U_n estimates for MOD6 and MOD8 only; the estimates for MOD7 were not presented because they can be directly computed from the equality $A_n = 1 - U_n$, given that $U_n = 2.4 \times$Lignin (sa), with Lignin (sa) expressed as g/g NDFom.

The estimates of the normalized indigestible fractions (U_n) of MOD1, MOD4, MOD6, and MOD8 were analyzed according to the statistical model (Eq. (7)). The results of the analysis for A_n were identical to U_n and we focused on the results of the latter fraction. In this case, the more likely variance–covariance matrices were the heterogeneous variances with compound symmetry for group one, and the unrestricted structure for group two; their likelihood probabilities were 0.788 and 1.000, respectively. There were differences among forages (P=0.002 and 0.007), and among models (P<0.001 for both groups), with no interaction effect for group one (P=0.481), and a significant interaction effect for group two (P<0.001). Models differed in the description of *P. purpureum* clone 92-79-02 (P=0.030), and the corn silage (P<0.001) degradation profiles. Differences among forages of group two within MOD1 were observed for estimates of U_n (P=0.015).

4. Discussion

The attack and degradation of forage cell walls are actions performed by a myriad of microorganisms in the rumen. Some microbes adhere to the forage cell walls, particularly to those tissues that exhibit the more resilient structures to be digested; other microbes, including some of the same organisms that digest the less digestible parts, are able to degrade the more digestible tissues of the plant cell walls even without prior attachment (Akin et al., 1974; Akin and Amos, 1975; Akin and Rigsby, 1985). The amount of available specific surface area and accessibility of microbes to digestion sites have been listed as constraints to the microbial digestion of forage particles (Wilson, 1993). Some authors have argued that prior to adherence, hydration, solubilization of digestion inhibitors, and the synthesis of glycocalyx and enzymes are mandatory; in sequence, daughter bacterial cells must colonize adjacent digestion sites and pass through all previous cited processes (Mertens, 1977, 2005; van Milgen et al., 1991; Russell, 2002). However, researchers have shown that the invasion stages to inner tissues by rumen bacteria do not take place simultaneously in all regions of the forage particle (Cheng et al., 1980; Hastert et al., 1983). These events confer a time dependency (N) to the process of substrate preparation which is represented by the mean time for substrate preparation estimated as N/λ (Vieira et al., 2008), with their estimates shown in Table 5. Once the initial steps for substrate preparation are gradually overcome, the subsequent digestion takes over, but plant tissues are more likely to be digested at different rates and extents possibly due to differences in the proportions of tissues in the forage particle such as the ratio of, e.g., mesophyll-phloem to vascular tissues (Akin et al., 1974; Akin and Amos, 1975; Hastert et al.,

Table 5
Least squares means of the estimates of the *in situ* digestion parameters obtained for each forage by fitting MOD6, MOD7, and MOD8 to the degradation profiles with 240 h as the incubation end point.

Forage[a]	MOD6[b]							MOD7[c]					MOD8[d]			
	N/λ	k_1 [e]	k_2 [f]	A_n [e]	A_1 [e]	A_2 [e]	U_n [e]	N/λ	k_1 [e]	k_2 [f]	A_1 [e]	A_2 [e]	N/λ	k[e]	A_n [e]	U_n [e]
1	1.7	6.68	6.41	80.28	71.26	28.76	19.72	1.6	6.69	4.78	66.66	33.34	0.8	4.23	69.26	30.74
2	4.9	6.59	3.16	91.40	67.53	32.46	8.60	2.0	6.07	10.4	87.65	16.90	1.2	4.91	68.59	31.41
3	6.7	27.9	11.7	81.83	63.71	36.28	18.17	6.8	18.7	6.21	66.44	33.65	1.3	4.92	71.80	28.20
4	4.8	22.3	16.3	57.21	75.09	32.94	42.79	4.7	18.4	2.27	69.42	30.77	1.5	8.84	53.51	46.49
5	4.6	14.2	10.1	85.23	59.67	40.31	14.77	3.6	9.54	4.68	70.53	29.56	1.3	5.24	69.58	30.42
6	3.2	10.3	9.46	81.29	70.52	29.50	18.71	3.0	9.67	3.32	72.91	27.15	2.4	7.20	69.04	30.96
7	3.8	14.5	7.46	73.48	80.43	19.57	26.52	3.6	12.3	2.02	74.37	25.63	1.6	7.34	67.92	32.08
8	7.0	13.1	9.78	78.07	79.36	20.63	21.93	6.9	10.5	3.05	74.56	25.44	5.2	7.26	73.40	26.60
9	1.0	5.48	3.47	80.37	83.24	17.28	19.63	1.0	5.38	1.27	76.28	23.72	0.8	4.93	71.64	28.36
10	1.6	8.09	3.85	89.34	67.76	31.66	10.66	1.7	8.53	7.01	76.12	23.88	0.2	5.21	69.97	30.03
11	0.4	4.28	2.80	84.71	75.27	24.69	15.29	0.5	4.21	2.33	76.55	24.47	2.6	3.86	69.37	30.63
12	1.7	6.48	3.57	75.89	98.01	1.99	24.11	9.9	10.4	60.9	100	0	1.6	6.17	71.28	28.72

[a] Studied forages were: *A. macrum* (1), *U. mutica* (2), *P. purpureum* cv. Cameroon (3), *Saccharum* spp. (4), *P. purpureum* clone CNPGL 92-79-02 (5), *P. purpureum* clone CNPGL 91-06-02 (6), *H. altissima* (7), *U. maxima* cv. Mombasa (8), *P. purpureum* cv. Napier (9), *S. sphacelata* cv. Kzungula (10), *Z. mays* as silage (11), and *M. sativa* as hay (12).
[b] Model with a heterogeneous potentially degradable fraction (Eq. (1)) with incubation end point at 240 h.
[c] Model with a heterogeneous potentially degradable fraction (Eq. (1)) with incubation end point at 240 h, and $U_n = 2.4 \times \text{Lignin (sa)}$.
[d] Model with a homogeneous potentially degradable fraction (Eq. (2)) with incubation end point at 240 h.
[e] Original values were multiplied by 100. In MOD7, A_n and U_n were not reported because $A_n = 1 - U_n$, and $U_n = 2.4 \times \text{Lignin (sa)}$.
[f] Original values were multiplied by 1000.

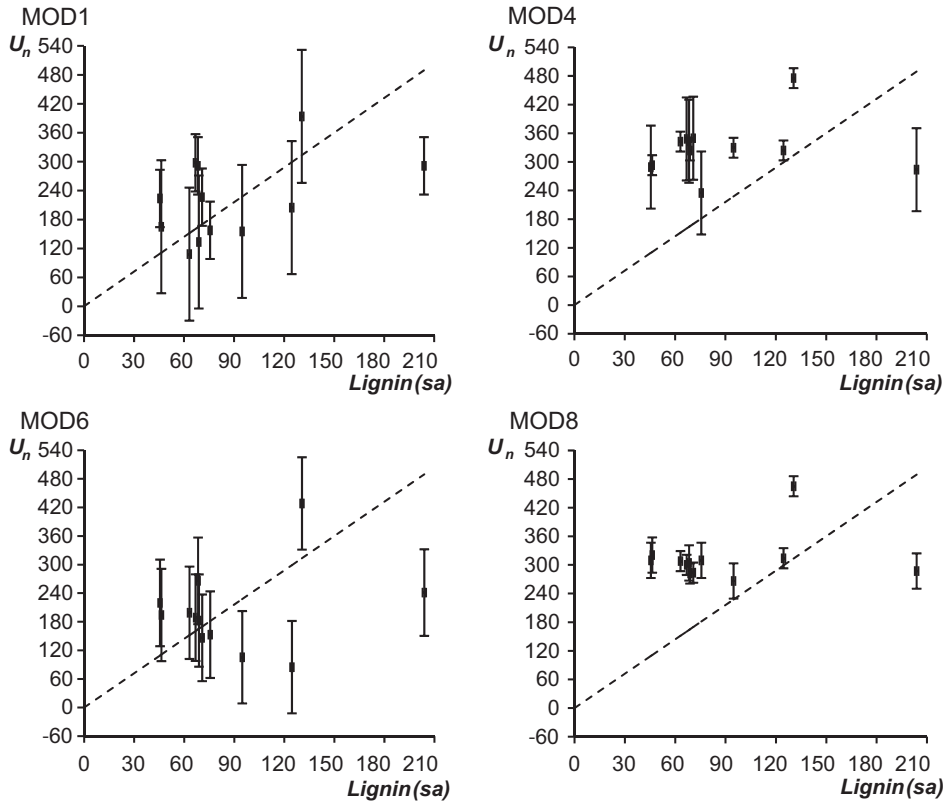

Fig. 1. On the *Y*-axis of all panels (MOD1, MOD4, MOD6, and MOD8) are point and 95% confidence interval estimates of the normalized indigestible parameter (U_n, g/kg NDFom) for the 12 forages estimated by nonlinear least squares. The *X*-axis is Lignin (sa), expressed as g/kg NDFom. The dashed lines represent the assumed $U_n = 2.4 \times$ Lignin (sa). MOD1 is Eq. (1) fitted to profiles ending at 1440 h; MOD4 is Eq. (2) fitted to profiles ending at 96 h; and MOD6 and MOD8 are Eqs. (1) and (2) fitted to profiles ending at 240 h, respectively.

1983). These facts may support the heterogeneity concept of the potentially degradable NDFom fraction. The sigmoid-shape patterns of the NDFom degradation profiles observed in this study are probably the net result of these actions, which include a smooth initial delay for degradation of the substrate and more than one inflection points during digestion (Mertens, 1977; Robinson et al., 1986; van Milgen et al., 1992a,b, 1993). However, all these events put together might impose a limitation on the assumption that a single λ quantitatively express the dynamics of lag mechanisms related to the fast and slow digesting NDFom fractions, but a compromise between parameterization of the compartmental model and the usefulness of the information to be generated is necessary (Vieira et al., 2008).

The decomposition of the organic matter is limited or incomplete in anoxic ecosystems such as the rumen; as a corollary, indigestible residues are the remnants of the referred process. Studies were conducted to investigate the extent of the organic matter degradation and results have had a profound impact in many applied fields (Chandler et al., 1980; Traxler et al., 1998; Van Soest et al., 2005). Although substantial contamination may occur *in situ* after prolonged times of incubation (van Milgen et al., 1992a,b), we performed long-term incubations (up to 1440 h) to investigate possible biases over parameter estimates of the digestion kinetics. By assuming the indigestible NDFom residue equal to 2.4×Lignin (sa) as a possible estimator for U_n (Traxler et al., 1998; Van Soest et al., 2005), the results for MOD1 and MOD6 are evidences that the NLS estimates for U_{NDF} normalized to U_n were biased to some extent (Fig. 1). Regarding MOD1, the *P. purpureum* clones 92-79-02 and 91-06-02, *H. altissima*, and alfalfa hay presented 95% confidence intervals not containing the 2.4×Lignin (sa) as the assumed indigestible value (Fig. 1). The interval estimate of the indigestible residue for MOD6 did not contain the 2.4×Lignin (sa) value for *U. mutica*, *Saccharum* spp., *H. altissima*, *U. maxima*, *S. sphacelata*, and alfalfa (Fig. 1). There are biases related to the *in situ* technique such as those introduced by particle size, the loss of fine particles of the test feed from the bags, the bag influx/efflux of digesta particles, the mass accumulation in the nylon bag tissues due to mineral deposits (Nocek, 1988; van Milgen et al., 1992a,b; Huntington and Givens, 1995; Mertens, 2005), and additional biases due to the NLS estimation of parameters yielding numerical artifact estimates that possibly do not represent true biological values (Mertens and Loften, 1980; Robinson et al., 1986; Vieira et al., 2008). However, despite the improved likelihood of MOD2 in relation to MOD1 as a result of the inclusion of the 2.4×Lignin (sa) as a direct estimate for U_n (Table 3), the "truth" contained in the profiles generated with such long-term ($t > 10$ d) *in situ* incubations is doubtful.

Short-term incubations ($t \leq 96$ h) do not suffice to establish the indigestible fiber residue. This fact has been recognized as a limitation for detecting the asymptotic end-point of digestion (Mertens, 1977; Ellis et al., 2005; Van Soest et al., 2005;

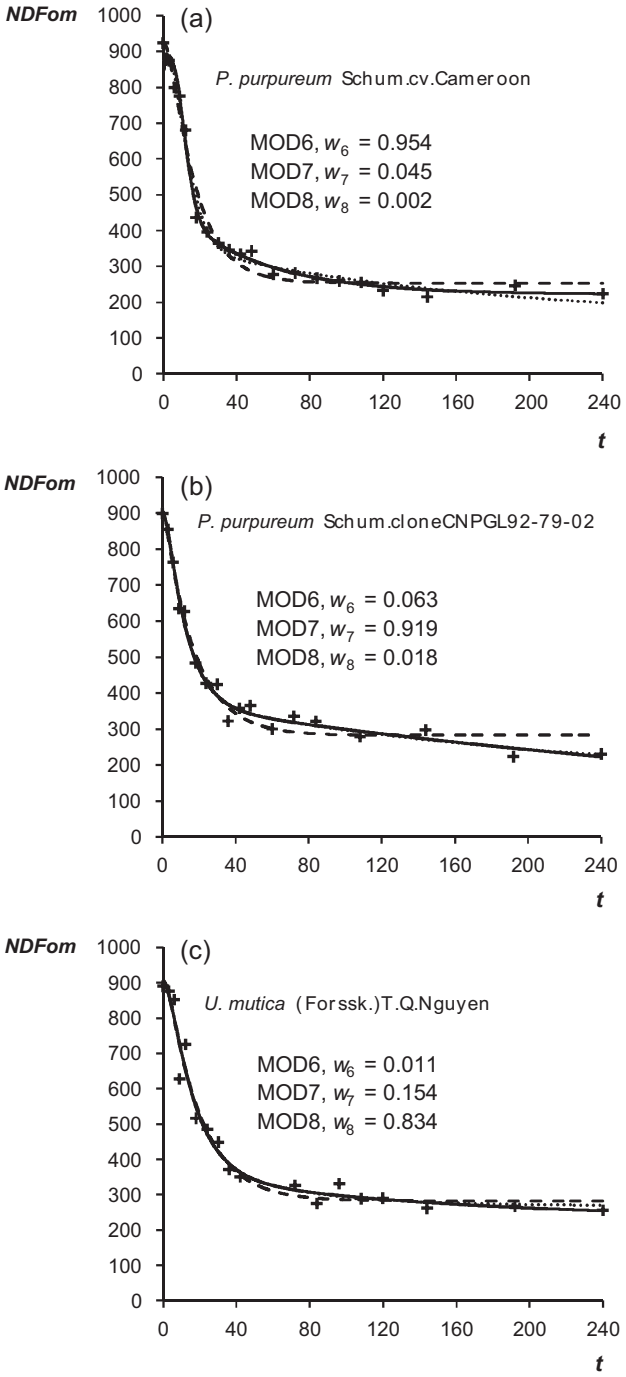

Fig. 2. Time (t, h) sequence plots lasting 240 h of incubation containing observed (+) and expected (lines) fiber residues at Y-axis (NDFom, g/kg NDFom) of some selected forages. The degradation profiles on panels (a), (b), and (c) were better represented by MOD6 (solid lines), MOD7 (dotted lines), and MOD8 (dashed lines), respectively, on the basis of greater likelihood probabilities (w_r, $\Delta_r = 6, 7, 8$). For details see Section 2.3.

Huhtanen et al., 2007; Casali et al., 2008). However, most of the estimates of digestion parameters in the literature arose from short-term incubations performed both *in situ* and *in vitro* (Van Soest et al., 2005; Huhtanen et al., 2006; Vieira et al., 2008). Therefore, we introduced the laboratory estimate of the indigestible NDFom with the expectation of an improvement in the likelihood of MOD3 estimates because long-term incubations are time consuming and prone to experimental errors. Nevertheless, there was no strength of evidence that MOD3 was superior to MOD4, meaning that no additional inflection point due to heterogeneity of $\tilde{A}_{NDF}$ could be detected with 96 h incubations. Despite its higher precise estimates for U_n compared to MOD1 (Fig. 1) and greater likelihood than MOD3 (Table 3), it was clear that MOD4 biased the parameter

estimates of digestion kinetics for the majority of the forages studied due to the lack of definition of the asymptotic phase in the degradation profiles (Fig. 1). The inconsistency of results due to the lack of an accurate definition of the asymptotic phase was evidenced by the significant forage by model interaction for *k* estimates of MOD4 and MOD5 (Table 4).

The introduction of the heterogeneity concept in Eq. (2) to devise Eq. (1) seemed to be justifiable because MOD6 and MOD7 were fitted to the degradation profiles of the selected forages at some likelihood extent (Table 3). Nevertheless, MOD8 was more likely given the data. However, there are many literature reports indicating problems of the *in situ* nylon bag technique and attempts to standardize procedures to generate *in situ* data have been made (Lindberg, 1985; Nocek, 1988; Huntington and Givens, 1995; Huhtanen et al., 2006). Some researchers have argued that the *in situ* estimates of the fractional rate constant of digestion obtained from *in situ* studies are biased and its use leads to the underestimation of the *in vivo* digestibility of the potentially digestible NDFom (Huhtanen et al., 1995, 2006, 2007). We observed that the bias due to long-term incubations ($t > 10$ d; van Milgen et al., 1992a,b) over the time series, and principally over the indigestible residue estimated for MOD1 was noticeable because some of its confidence interval estimates did not include the assumed indigestible residue as 2.4×Lignin (sa) (Fig. 1). The systematic occurrences of the forage by model interaction are reasonable evidences that the end-point definition concur for divergent and inconsistent estimates of comparable parameters (Table 4). By considering the combined likelihood of MOD6 and MOD7, we assumed that biases on this regard over incubation times up to 240 h are less prominent and the heterogeneity of NDFom should be investigated for a proper quantitative description of the NDFom degradation kinetics (Nocek and English, 1986; Robinson et al., 1986; van Milgen et al., 1993; Ellis et al., 2005; Van Soest et al., 2005).

Subtle differences were detected among models on the basis of the information criteria even though such differences appear to be visually negligible, as shown by some examples of fitted profiles presented in Fig. 2. However, the w_r estimates of MOD6 and MOD7 in panels (a) and (b) of Fig. 2 are evidences against the use of a single, uniform potentially degradable NDFom fraction as noted on previous reports with other forages (Robinson et al., 1986; Ellis et al., 2005). Nevertheless, our results were not systematic for all forages (Fig. 2, panel c), and even within forages because of the significant forage–model interaction observed (Table 4). On the basis of the information criteria, there were examples within the same forage that only one or two profiles were fitted more likely with Eq. (1) (MOD6 and MOD7) than Eq. (2) (MOD8). Although MOD8 was more likely and presented a greater precision of the NLS U_n estimates (Table 3 and Fig. 1), it was clear that the indigestible NDFom residue was overestimated for all forages excepting *Z. mays* and *M. sativa*, which in turn were underestimated in relation to their respective 2.4×Lignin (sa) values (Fig. 1). Nevertheless, despite the visual subtle differences observed among models, such differences could be quantitatively assessed to check the heterogeneity of $\tilde{A}_{NDF}$ after evaluating the likelihoods of Eqs. (1) and (2).

The estimates for U_{NDF} obtained after fitting MOD4 and MOD8 to the degradation profiles of *M. sativa* presented a clear anomalous behavior to the 2.4×Lignin (sa) values (Fig. 1). It appears that the fibrous carbohydrates in legumes are digested to a lesser extent than their counterparts in grasses (Dehority and Johnson, 1961). Generally, legume NDFom is digested at a faster rate but to a lesser extent than grass NDFom; the opposite occurs with the kinetic attributes of NDFom in grasses whether compared to legumes (Van Soest, 1994). Therefore, a single common factor 2.4×Lignin (sa) could be viewed as an oversimplification which implies that the estimation of the indigestible residue deserves further investigations and modeling refinement.

5. Conclusions

The heterogeneity of the potentially digestible NDFom fraction is not easily detected by fitting a heterogeneous model (Eq. (1)) to degradation profiles generated from short-term *in situ* incubations; in this case, Eq. (2) fitted as MOD4 and MOD8 is more likely. However, the 96 h endpoint does not suffice to establish the asymptotic phase of digestion and this is a serious source of bias. On the other hand, long-term *in situ* incubations, *i.e.*, up to 1440 h, may introduce bias on the estimates of the indigestible residue and fractional rates of the potentially digestible NDFom fractions. The use of the laboratory measured indigestible fiber residue as 2.4×Lignin (sa) appears to increase the ability of Eq. (1) as MOD7 to mimic *in situ* degradation profiles with 240 h as the incubation endpoint. Nonetheless, heterogeneity is probably not likely to be detected for all forage degradation profiles *in situ*, but its investigation could be assessed by appropriate tools whenever interest relies on the estimation of this kinetic attribute of the insoluble fiber.

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Appendix A.

The SAS program used to fit Eq. (1) was (notes within curly brackets do not belong to the program):

```
data file1;
title 'Generalized compartmental model of digestion – Heterogeneous version';
input t RNDF;
y=RNDF;
N=1; {N is allowed to vary from one to six, for instance}
datalines;
{data matrix with t and RNDF values at columns};
proc nlin data=file1 best=3 method=newton;
parms
l=0.25 to 5 by 0.25 {l represent lambda in Eq. 1}
k1=0.12 to 0.24 by 0.02
k2=0.01 to 0.1 by 0.01
A1=0.1 to 1 by 0.1
A=0.1 to 1 by 0.1
U=0.1 to 1 by 0.1;
bounds l>k1>k2>0, 1>a1>0, 1>=U>=0, 1>=A>=0;
d1=l/(l-k1);
d2=l/(l-k2);
b1=(d1**N)*exp(-k1*t);
b2=(d2**N)*exp(-k2*t);
c=exp(-l*t);
f1=0;
f2=0;
if N>0 then do i=0 to N-1;
f1=f1+(1-d1**(N-i))*((l*t)**i)/fact(i);
f2=f2+(1-d2**(N-i))*((l*t)**i)/fact(i);
end;
model y=A*(A1*(b1+c*f1)+(1-A1)*(b2+c*f2))+U;
run;
```

The SAS program used to fit Eq. (2) was:

```
data file2;
title 'Generalized compartmental model of digestion – Homogeneous version';
input t RNDF;
y=RNDF;
N=1; {N is allowed to vary from one to six, for instance}
datalines;
{data matrix with t and RNDF values at columns};
proc nlin data=file2 best=3 method=newton;
parms
l=0.2 to 5 by 0.1
k=0.01 to 0.1 by 0.01
U=0.1 to 1 by 0.1
A=0.1 to 1 by 0.1;
bounds l>k>0, 1>=U>=0, 1>=A>=0;
d=l/(l-k);
f=0;
if N>0 then do i=0 to N-1;
f=f+(1-d**(N-i))*((l*t)**i)/fact(i);
end;
model y=A*((d**N)*exp(-k*t)+exp(-l*t)*f)+U
run;
```

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