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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,