Chapter 4

Simple Models for Describing Ruminant Herbivory

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Additional information is available at the end of the chapter

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Abstract

The use of quantitative independent variables in experiments allows the use of regression to explore the functional relationship between treatments applied and measured responses. It provides the opportunity to not only understand the magnitude and importance of the response but also ascertain its nature. The simplest approach is to fit a polynomial. While it is often possible to obtain a very good fit using this approach, it offers in the way of providing insight into the response. At best, you can determine if the response is nonlinear and if so, if it is complex or not. The model parameters are empirical and generally cannot be interpreted as having any biological, chemical, or physical meaning—at least not directly. There are situations, however, when such a meaning can be inferred from a model fit using simple regression. In general, this is true when the relationship is truly linear or when a nonlinear model can be considered to be "intrinsically" linear; that is, it can be linearized by transforming the data in a way that can be fit using simple linear regression. A series of forage quality examples are used to illustrate these concepts in this article.

Keywords: modeling, ruminant, herbage quality, digestion, kinetics, true digestibility, intake

1. Introduction

The use of mathematical models to describe chemical, physical, and biological processes is quite common in natural sciences [1]. The best models are those with parameters that have chemical, physical, or biological meanings [2]. They go beyond being descriptive and provide a deeper understanding of the process that is being evaluated. Fitting and adapting models to experimental data are as much an art as a science, and the outcome is highly influenced by decisions made by the researcher about which models to fit, what data are needed and should

be used, and interpretation of fit statistics. In this article, models that describe the relationship between rumen escape protein and protein concentration, kinetics of fiber digestion, true digestion, and potential intake of herbage are developed and used to demonstrate how relatively simple models can be an effective tool for understanding biological processes and how they can be applied using experimental data. For each example, the underlying theory and assumptions are also presented and discussed.

It is important to make the distinction between the use of models for describing and understanding a biological response and their use to predict future outcomes. The use of models addressed herein relates to the former purpose and is most applicable to interpreting the results of designed experiments. That is, the experimental units on which the observations are made have been intentionally manipulated in some way that can be described quantitatively. Treatment responses for any experiment for which a quantitative treatment has been applied can be evaluated in this way. However, the inferences that can be made by using models fit to experimental data are limited to those appropriate to the design of the experiment. Their application to predicting results outside the bounds of the inference space associated with the experiment is not recommended.

Designed experiments often have unique features that both limit and extend the types of regression analyses that can be performed. They nearly always include multiple replications of individual treatments. When fitting a regression equation, this allows for the partitioning of residual error into pure error and lack of fit, thus providing a test for whether the linear model fits the response or not. It also allows statistical tests to be made about assumptions related to the distribution and homogeneity of residuals. These assessments can be used to refine the approach used in the regression and improve the value of the analysis.

This chapter is intended to demonstrate how relatively simple models can be used to describe important nutritional processes related to ruminant herbivory. It uses a series of examples to illustrate the principles and power of using simple mathematical models to better understand the functional relationship between important variables. The data used in the examples have been published previously, although the analyses employed here may be slightly different from those used in the original studies from which they were taken.

2. Linear regression; a quick review/overview

Simple linear regression is a statistical method for calculating parameters for the model:

$$\hat{Y} = b_0 + b_1 X \tag{1}$$

Graphically, the model represents a straight line that intercepts the Y axis at b_0 and which has a slope equal to b_1. As X increases, Y either increases or decreases proportionally depending on whether the slope is positive or negative, respectively. The model parameters, b_0 and b_1, can be estimated using least squares regression. This approach is based on an algebraic solution of normal equations and produces parameters that minimize the sum of the squared deviations

of observed values from those predicted by Eq. (1). The regression line intersects the point that represents the means of X and Y unless it has been forced through the origin ($X = 0$, $Y = 0$) and the sum of the deviations from the regression line is zero.

The equation for estimating the slope (b_1) is as follows:

$$b_1 = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sum(X_i - \bar{X})^2} \tag{2}$$

Once b_1 is known, then b_0 can be estimated using the equation:

$$b_0 = \bar{Y} - b_1\bar{X} \tag{3}$$

The assumptions for linear regression are the following: (1) the independent variable X is measured without error; (2) the relationship between X and Y is linear; (3) deviations from the regression are independent; (4) the variance in Y is homogenous or constant across the range in X; and (5) the residuals or deviations from the regression are distributed normally. There are ways to assess whether most of these assumptions are valid or not, and they will be described where appropriate in the examples that follow.

Some straightforward statistics for assessing the fit of a regression equation are the coefficient of determination (r^2) and the standard error of the estimate ($S_{Y \cdot X}$). The coefficient of determination is calculated as follows:

$$r^2 = \frac{\sum\left(\hat{Y}_i - \bar{Y}\right)^2}{\sum(Y_i - \bar{Y})^2} \tag{4}$$

It represents the proportion of total variation in Y explained by the regression model and varies between 0 and 1. A value approaching 1 indicates that the regression equation explains most of the variation in Y and, therefore, does a good job explaining the relationship between Y and X. The coefficient of determination is the square of the simple correlation coefficient (r) that is interpreted as the degree to which X and Y vary together. The correlation coefficient is used to describe the relationship and varies from −1 to +1 indicating whether Y decreases or increases with respect to X, respectively. Values close to −1 or +1 indicate a high degree of association between Y and X. The simple correlation coefficient can be calculated as the covariance between X and Y divided by the square root of the product of the standard deviations in X and Y and thus can be thought of as a standardized covariance.

The standard error of the estimate is calculated from the equation:

$$S_{Y \cdot X} = \sqrt{\frac{\sum\left(Y - \hat{Y}\right)^2}{n-2}} \tag{5}$$

It is the square root of the residual variance of the regression. It describes how well the regression line fits the data with smaller values indicating a better fit. Smaller values indicate less departure of the actual observations from the regression line.

These five equations are all that is needed to fit and assess models that are either linear or intrinsically linear. However, there are other methods and statistics that are useful for this purpose and some of them will be described as the examples that follow are developed.

3. Intrinsically linear models

Nonlinear models that can be linearized by transforming either Y or X are considered to be intrinsically linear and can be fit using simple linear regression [3]. The most common of these involves logarithmic transformations of X or Y to yield a linear model with two parameters: (1) exponential (log-linear), (2) logarithmic (linear-log), and power (log-log) functions.

The exponential model has a number of important uses. With a positive slope (b_1), it can be used to describe exponential growth that is unbounded. With a negative slope, it can be used to describe exponential decay. In this form, it is useful in isotope studies to describe radioactive decay and is used in marker dilution studies in a similar manner. It can be also used to describe first-order kinetics for chemical reactions. We will use it in this latter context in an example on modeling herbage digestion that follows.

The logarithmic and power models are useful for describing responses where the rate of change gradually decreases with respect to increasing X. Many chemical and biological processes are limited and show an asymptotic response. These types of responses are generally better described by an intrinsically nonlinear model that contains an asymptote as a parameter. The Gompertz [4] and Mitscherlich [5] equations are two good examples of such models commonly used to describe biological processes. However, these models require a different approach to estimating their parameters than simple linear regression.

If a nonlinear model cannot be expressed in the form of a simple linear equation through transformation, then it is considered to be intrinsically nonlinear. There is a host of such models, and many of them can be used to describe functional responses relevant to herbivory (see Archontoulis and Miguez, 2013, for a review of 77 nonlinear models). However, fitting these models is somewhat more complicated and requires using a numerical approach that adjusts parameter values iteratively until a solution based on certain criteria is achieved. The criterion typically used is the combination of parameter estimates that results in the minimum residual sum of squares, which is why such algorithms are sometimes referred to as a nonlinear least squares approach [6]. Convergence is then based on identifying the combination of parameter estimates that result in the lowest sum of squared deviations from the value estimated by the regression. This chapter focuses on models for which the parameters can be estimated by simple linear regression all of which are therefore intrinsically linear.

4. Herbage nutritional entities

The forgoing concepts and equations can be used to fit and assess simple linear regression equations. However, before applying them to experimental data in the following examples, a quick overview of some nutritional concepts related to herbage utilization is in order.

The nutritive entities of herbages are broadly grouped into uniform and nonuniform fractions based on the Lucas Test [7]. Uniform fractions are those that have similar nutritional characteristics or true digestibility regardless of the feedstuff [8, 9]. These include most

nutrients contained in the cytoplasm of plant cells including proteins and other nitrogenous compounds and nonstructural carbohydrates. Nonuniform fractions vary in true digestibility among different feedstuffs and even within a single feedstuff. Plant fiber is considered a nonuniform fraction. Its digestibility varies greatly among different feedstuffs and is affected by a number of genetic and environmental factors [10].

The Lucas test itself involves a simple linear regression model. It is performed by regressing the amount of a nutrient that is digestible against its intake. Fractions for which true digestibility is constant over a range of herbages are considered to be nutritionally uniform or ideal [10]. The Lucas Test provided the foundation on which Van Soest [8] developed the detergent system for analyzing feeds. In this system, herbage or feedstuff dry matter is partitioned into cell solubles and neutral detergent fiber by refluxing a sample of the feed in a neutral detergent solution and recovering the residue by filtration. The residue remaining is fiber. The compounds removed with the filtrate are collectively referred to as cell solubles. Cell solubles have a uniformly high true digestibility regardless of the feedstuff they are contained within. They are very nearly completely available when subjected to digestion in ruminants with a true digestion coefficient of 0.98. The residue remaining after treatment with neutral detergent is the fibrous fraction and varies significantly in digestibility among feedstuffs. Both fiber and cell solubles are heterogeneous in composition and can be further partitioned into chemical constituents. Neutral detergent fiber, while structurally complex, is composed of relatively few polymers and consists almost entirely of cellulose, hemicelluloses, and lignin. True, the hemicelluloses represent a fairly diverse set of compounds, but still this is a relatively small number compared with the myriad of compounds found in plant cells. Some complex carbohydrates such as pectins and beta glucans are not recovered in neutral detergent fiber. However, these compounds are easily digested by ruminants and are considered to be part of the cell soluble fraction [11].

A particularly important fraction of the cell soluble fraction is protein. Proteins and other nitrogenous compounds in herbage can be converted to amino acids by rumen microorganisms that incorporate them into proteins. These proteins are eventually passed from the rumen to the lower digestive tract where they are hydrolyzed to amino acids, which are largely absorbed within the small intestine [12]. The example that follows involves using a modification of the Lucas Test to test the hypothesis that rumen degradability of protein is proportional to the concentration of protein in the herbage.

5. Rumen degradable protein

In this application, a modified form of the Lucas Test is used to evaluate the degradability of herbage in the rumen using an *in situ* technique. The data are from an experiment designed to assess ruminal degradation of smooth bromegrass (*Bromus inermis* Leyss.) and switchgrass (*Panicum virgatum* L.) using an *in situ* analytical technique [13].

The degradability of protein in the rumen varies greatly between cool and warm-season grasses, and this may be one explanation for the observation that animals consuming warm-season grasses perform better than would be expected based on their chemical composition.

The theory is that some plant proteins localized within bundle sheath cells in warm-season grasses are physically protected from degradation by the structure of the cells. These proteins bypass the rumen intact and progress to the lower intestinal tract where they are digested and absorbed as amino acids. Nitrogen in proteins degraded in the rumen is often in excess of microbial needs and that which is not needed is lost as ammonia. Thus, protecting some of the protein from ruminal degradation improves the efficiency of protein utilization [12].

One of the objectives of the experiment was to quantify the relationship between ruminal protein degradation and protein concentration for both species. Linear regression of rumen degradable protein (RDP) on crude protein (CP) concentration was done for several samples of both species with varying CP concentration. The linear equation for this analysis was:

$$\text{RDP} = d_0 + d_1 \text{CP} \tag{6}$$

where d_0 represents the endogenous contribution to RDP and d_1 is the true digestion coefficient for ruminal degradability.

The grasses used in this study were harvested at different stages of maturity and separated into leaves and stems to obtain a range of CP concentrations and were analyzed for RDP using an *in situ* bag technique. Samples of each grass were enclosed in Dacron bags and incubated in the rumen of a live animal for 12 h. The loss of protein from the bag was determined by difference using residual protein remaining after digestion, and RDP was calculated based on protein disappearance from the bag. The endogenous contribution (d_0) in this system represents microbial contributions of protein to the residue remaining after incubation by rumen microbes.

Model parameters for each of the two grass species were estimated using the REG procedure in SAS (Appendix A1). The linear model described the relationship between RDP and CP very well for both species (**Figure 1**) based on the r^2 and standard error of the estimate (RMSE in SAS output). The calculated coefficient for ruminal degradability of CP was 74% for smooth bromegrass and 57% for switchgrass. The null hypothesis that these two slopes are the same can be tested with a *t*-test:

$$t = \frac{b_1 - b_2}{S_{b_1 - b_2}} = \frac{0.739 - 0.572}{0.058} = 2.88 > 2.09 \quad t_{0.05,19df} \tag{7}$$

where

$$S_{b_1 - b_2} = \sqrt{\frac{\left(S^2_{Y \cdot X}\right)_p}{\sum (X - \bar{X})^2_1} + \frac{\left(S^2_{Y \cdot X}\right)_p}{\sum (X - \bar{X})^2_2}} = \sqrt{\frac{38.115}{32499.27} + \frac{38.115}{17466.65}} = 0.058 \tag{8}$$

$$\left(S^2_{Y \cdot X}\right)_p = \frac{(\text{residual SS})_1 + (\text{residual SS})_2}{(\text{residual DF})_1 + (\text{residual DF})_2} = \frac{484.279 + 239.903}{9 + 10} = 38.115 \tag{9}$$

Based on this comparison, it is reasonable to conclude that the two species have different rumen protein degradability and that this difference is constant and persists across a range of maturities and morphological components. These results are consistent with the observation that protein in warm-season grasses seems to be used more efficiently than that in cool-season grasses. However, the mechanism for why this is so is not clear from this study. Based on the protection theory, one might expect protein degradability to vary across tissues within a species and that does not appear to be the case. So maybe there is another explanation that would better describe what was observed in this study.

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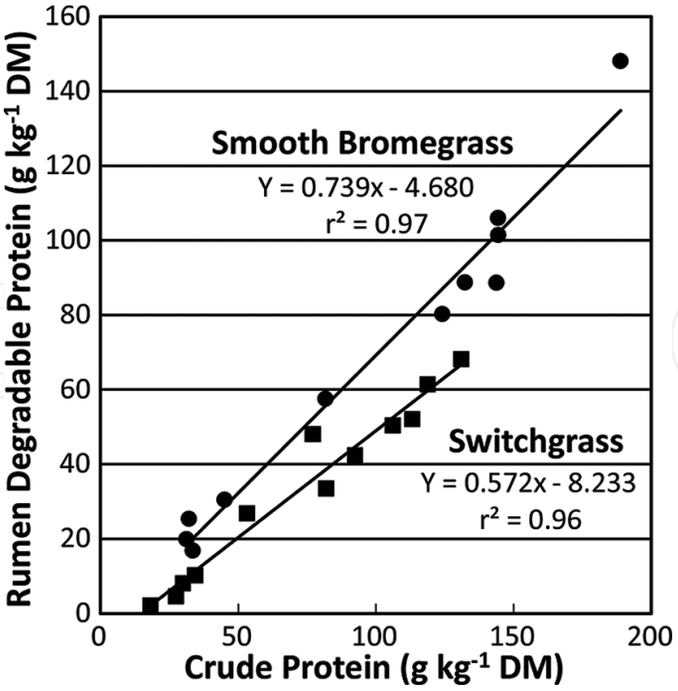

Figure 1. Relationship between rumen degradable protein and protein concentration in smooth bromegrass (●) and switchgrass (■). Individual data points represent leaf and stem samples collected at different stages of maturity. The slope of the equation represents the proportion of the dry matter that is degraded in the rumen and the intercept can be interpreted as the microbial contribution. Data from Ref. [13].

One further conclusion that can be inferred from fitting these equations is that the contribution of microbial CP to residual CP was negligible for smooth bromegrass and small (<1%), but significant for switchgrass. This is based on the test of parameter estimates included in the SAS output. The *t*-test for the intercept associated with the smooth bromegrass model was not significantly different from zero ($P > 0.05$), while that for the switchgrass model was ($P < 0.05$).

6. Fiber digestion kinetics

In this application, we will compare the digestion of alfalfa (*Medicago sativa* L.) and tall fescue (*Festuca arundinacea* Schreb.) by fitting a log-linear model to calculate the rate constant and lag time (Appendix A2). The data are from an experiment that was designed to compare different approaches for estimating the parameters of a first-order digestion model [14]. In this case, we will be using the data to explore some unique aspects of such data sets and how they require some rearrangement and culling of data in order to fit a first-order model to them. Once this is done, we will use simple linear regression to calculate the rate of digestion and then estimate a lag period based on the intercept of the equation.

To comprehend and be able to interpret the parameters of the model, an understanding of plant fiber and first-order kinetics is necessary. The next two sections provide an overview of each of these topics following that we will pick up the example in more detail.

6.1. Plant fiber

Fiber is a nutritional concept that refers to the less degradable and more variable constituents of an herbage or feedstuff. Chemically, it is comprised of plant cell walls the composition of which varies greatly among and within herbage species. Even within a single plant, the organs, cells, and tissues vary remarkably in fiber composition and digestibility [15]. The primary chemical constituents of plant fiber are cellulose, hemicellulose, and lignin, although there are others that comprise a much smaller fraction. These constituents are aggregated and arrayed in three-dimensional space in various ways creating a network of nonliving tissues that play an important structural role in the architecture of their plant [11]. Having a rigid cell wall is one of the defining characteristics of higher plants. Cell walls and thus fiber evolved to fulfill specific roles in plants, which do not include being a source of energy for herbivores. Their structure and function in a plant are in many ways counter to their use as a nutrient source. Even though fiber is composed of plant cell walls, it is functionally different. It is defined by its properties when subjected to digestion by an animal and has attributes that are only relevant in this context. The two terms are thus not really interchangeable [11].

Not all the fiber in a plant is degradable by ruminants. The degradation of plant fiber involves the hydrolysis of the principal polysaccharides by enzymes secreted by rumen bacteria. Because of the close physical and chemical interactions among plant cell wall constituents, some of the glycosidic linkages are not accessible to the hydrolases that would otherwise cleave them and render them digestible. The fraction of fiber that cannot be digested because of these interactions is indigestible and cannot be degraded within the digestive system. When determining the kinetics of fiber digestion, the indigestible (C_I) portion must be considered separately and removed from that which is potentially digestible (C_D) [16, 17].

The indigestible fraction is usually considered to be that which remains after being subjected to *in vitro* or *in situ* digestion for a period of time. This is usually between 48 and 96 hours and well past the expected residence time that it would be exposed to digestion in the rumen of an animal. The potentially digestible fraction is the difference between total fiber (C_0) and indigestible fiber ($[C_D]_t = [C_D]_0 - [C_I]$), and its concentration decreases exponentially during digestion asymptotically approaching zero according to the first-order rate law. The key to defining the indigestible fraction is to subject the herbage to digestion long enough to approach an asymptote after which time no further digestion occurs (**Figure 1**). Once this is achieved, there is very little change in the concentration digested. Indigestible fiber is usually calculated as the concentration of fiber remaining after incubation for 72–96 h *in vitro*. The period needed to reach this point varies with substrate and periods as short as 24–36 h for rapidly degraded herbages are not uncommon.

When calculating first-order digestion parameters, it is important only to include fiber concentrations at time points where digestion is actively occurring ($[C_D]_t \neq [C_D]_0$ and $[C_D]_t \neq [C_I]$; $[C_D]_0 > [C_D]_t > [C_I]$). Including time intervals in the calculation where no change in fiber concentration has occurred biases the estimates of the parameters. Most importantly, time intervals where the fiber concentration is not different from either the initial or final concentration should be excluded from the calculations. Moore and Cherney [13] suggested a simple method for selecting time intervals for rate calculations. Since replicate samples are usually collected at each time point during a digestion study, it is possible to compare the mean con-

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centration between pairs of time points using a t-test. Time intervals within the lag period can be identified as those for which the fiber concentration is not significantly different than the initial concentration. Time intervals occurring after digestion has ceased will have concentrations that are not different than the longest time point, which is usually used to determine the indigestible fiber fraction. It is entirely possible for digestion to occur throughout the sampled period, but it is more often the case that some time points will need to be excluded.

The data in Appendix 2 were collected by incubating an herbage sample in buffered rumen fluid for 0, 3, 6, 9, 12, 16, 24, 36, 48, 60, 72, and 96 h [14]. Samples were refluxed in a neutral detergent solution following fermentation to extract undegraded fiber using the procedures described by Cherney et al. [18]. The concentration of fiber remaining after each time interval was calculated on the basis of initial dry matter subjected to fermentation (**Figure 2**).

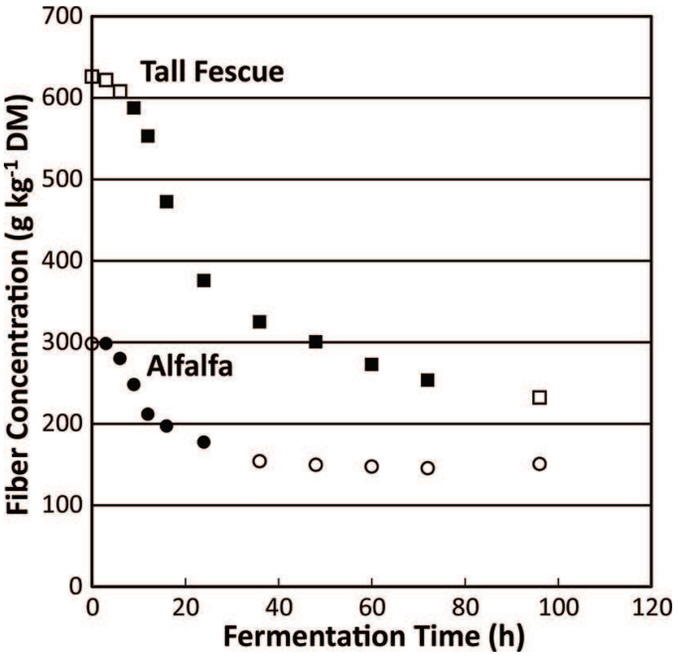

Figure 2. Concentration of fiber remaining during fermentation of alfalfa (●) and tall fescue (■) samples incubated in buffered rumen fluid. Symbols represent means of four subsamples and those that are closed were determined to be different to adjacent means using a least significant difference at alpha 0.05. Data from Ref. [14].

In order to calculate an unbiased estimate of digestion rate (k), time intervals where no change in concentration occurred must be excluded from the dataset. This was accomplished using the method referenced above using the SAS code presented in Appendix A2.1. This involved conducting a one-way analysis of variance followed by a post-hoc mean comparison using a least significant difference (LSD). Because the measurement represented a wide range in concentrations and the concentrations were quite small after longer time intervals, a test of homogeneity was performed to validate the use of a pooled error for calculating the LSD. Abridged versions of the results that include output from the homogeneity test and LSD are presented in Appendix A2.1.

Based on the Levene test for homogeneity, the variances observed in neutral detergent fiber (NDF) concentration were homogeneous across each time interval and this was true for both

species. This simply confirms that using the LSD procedure for comparing the mean NDF concentration remaining at each time interval was appropriate. Had they been found to be heterogeneous, it would not have been possible to use a pooled estimate of the variance for comparing means and either a data transformation to stabilize the variance or the use of different variances for each comparison would be needed. There are other tests of homogeneity that can be used to assess whether treatment variances can be considered equal. The Levene test is the default method when using GLM in SAS because it is widely used and accepted, but others are available and can be specified if desired.

The mean concentration associated with each time interval is reported in the output along with a capital letter denoting which grouping it belongs to (Appendix A2.1). Means associated with the same letter are not different from each other at alpha 0.05. By comparing the groupings, it is possible to infer the intervals during which digestion began and when it ceased. Digestion of alfalfa fiber began after the first interval so the concentration at 3 h would be included in the calculation of k (**Figure 2**). However, for tall fescue digestion did not begin until sometime between 6 and 9 h so the concentrations at 3 and 6 h would be excluded (**Figure 2**). Digestion of alfalfa fiber ceased after 24 h as there was no difference between the concentrations at this time and the next one at 36 h. For tall fescue, fiber digestion continued throughout the remainder of the incubation period once it began.

This analysis (Appendix A2.1) can also be used to determine the concentration of indigestible fiber that must be subtracted from the concentration ($[C]_t$) remaining after each time interval (**Figure 2**). The longest time period is often selected based on the assumption that no further digestion will occur beyond it and the concentration remaining at that time is considered to be indigestible and is used to define $[C_I]$. In Example 2.1, $[C]_{96}$ might be selected to define this fraction. However, instead we will use the mean of the concentrations that were determined to be not different after the end of the period of active digestion. For alfalfa, this will be the concentrations for intervals 48–96 h and $[C]_I$ is then 148.2 g kg^{-1} DM. The 36-h interval is excluded because the t-test was ambiguous about which group it belonged to. Since digestion continued through 96 h for tall fescue, the concentration at that time, 232.3 g kg^{-1} DM, was used to define $[C_I]$.

The concentration of digestible fiber is the initial fiber concentration minus the indigestible fraction ($[C]_0 - [C_I]$) which in this study was 153.1 g kg^{-1} DM for alfalfa and 393.8 g kg^{-1} DM for tall fescue (**Table 1**). This is the fraction or pool to which first-order kinetics applies. To calculate k, the rate of fiber digestion, C_I must be subtracted from each observed value of C that occurred during the incubation. Perhaps a better way of describing this is to say that indigestible portion of the residue remaining after each time interval is constant and must be subtracted from the total amount remaining for first-order kinetics to apply.

6.2. First-order model

The rate of fiber digestion in the rumen is dependent on the concentration present and, therefore, follows first-order kinetics:

$$\frac{d[C_D]}{dt} = k[C_D] \tag{10}$$

where $[C_D]$ is the digestible fiber concentration remaining at time t and k is the first-order rate constant.

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Parameter/quantity	Alfalfa	Tall fescue
Fiber $[C]_0$, g kg^{-1} DM	298.3	626.0
Indigestible fiber $[C_I]$, g kg^{-1} DM	148.2	232.3
Digestible fiber $[C_D]$, g kg^{-1} DM	150.1	393.8
Cell solubles $[C_S]$, g kg^{-1} DM	701.7	374.0
Rate of fiber digestion k, h^{-1}	−0.111	−0.044
Lag time, h	1.7	5.3
C_D/C_0	0.503	0.629
True digestibility, g kg^{-1} DM	851.8	767.8

Table 1. Model parameters calculated for fiber digestion and true digestion of alfalfa and tall fescue (Example 2).

The absolute rate of change in concentration per segment of time depends on the concentration present during that segment. Higher rates of digestion for a given herbage substrate correspond to higher concentrations since the relative rate (k) is constant. So even though the absolute rate changes during fermentation, the proportional rate at which C is degraded is constant throughout. The units of the rate constant are in reciprocal time, which for plant fiber is generally measured in hours (h^{-1}). Because k is a proportional constant, it can be expressed as a percentage as well as a fraction and is sometimes given in percentage units.

Eq. (10) can be written in differential form and divided by C_D to give the equation:

$$\frac{d[C_D]}{[C_D]} = -kdt \tag{11}$$

Integrating both sides gives:

$$\ln[C_D] = -kt + C \tag{12}$$

where C is the constant of integration.

This is a convenient form of the equation because the parameters can be calculated by simple linear regression of the logarithm of concentration remaining over time. This is the exponential decay model referred to in the discussion of intrinsically linear models above. Concentrations in Eq. (12) are in log units. C is the constant of integration and in this context is the logarithm of digestible fiber concentration (C_D) in the absence of a lag period. It is the intercept where the regression line intersects the ordinate at $t = 0$. Because a lag period is observed for most *in vitro* incubations, the value given by C is rarely equal to the logarithm of initial digestible fiber concentration. Whenever a lag period occurs, the concentration at $t = 0$ will be greater than the known concentration in the substrate.

The rate constant (k) and lag time (L) can be calculated using linear regression on data collected from *in vitro* or *in situ* incubations. Samples of herbage are incubated for several time intervals, and the concentration of fiber remaining is determined for each one. Since kinetics apply only to the fraction of fiber that is potentially digestible, the fraction that is indigestible must be determined and subtracted from that remaining at each time interval. In Example 2, the indigestible fiber concentration was calculated as the mean concentration remaining after digestion had ceased and was determined by using a mean comparison test to determine the time interval

after which no further decreases in fiber concentration occurred (Appendix A2.1). The natural logarithm of the fiber concentration remaining ($[C]_t$) minus the indigestible fiber concentration ($[C_I]$) during this period of active digestion is shown for both species in **Figure 3**. The symbols in the figure represent the mean of the four replicate measurements of fiber concentration that were made at each time point. Regression of $\ln([C]_t - [C_I])$ on time using individual data points (i.e., replicate data) or mean at each X (i.e., time) results in the same estimates of the parameters for the linear model (Eq. (12)), although the fit statistics will vary. However, since there are multiple values of Y for each X, it is possible to partition the residual sum of squares (SS) into lack of fit and pure error [19]. Theoretically, if the model fits, then squared deviations of each observation from the regression and squared deviations from the mean of each of X value should be the same. The difference between these two values is called lack of fit and its significance can be assessed using an F-test. If the lack of fit variance is determined to not be different from that for pure error, then it can be concluded that the correct model was used to describe the relationship between Y and X. For both alfalfa and tall fescue, there was no lack of fit of the linear model and we can be confident in the parameters that were estimated by the regression (Appendix A2.2).

There are also qualitative methods for assessing the appropriateness of a model for a set of data. A residual is defined as the difference between the observed value and that estimated by the regression. Examination of residuals is a fast and easy way to visualize the fit of a model and determine if it is biased for some values of X. Ideally, the residuals will appear to be randomly distributed around the regression line with no obvious clustering above or below along any segment of X. This can be observed for the example in **Figure 3** that displays the mean digestible fiber concentration at each time point and regression lines for both species. The residual at each X is the distance between the symbol and the line. A plot of actual residuals is a common output of statistical analysis programs, but the patterns are easily visualized when the regression line is plotted with the means as shown in **Figure 3**. In this case, no patterns are discernible in the residuals for either species, and it is readily evident that the linear model fits the data.

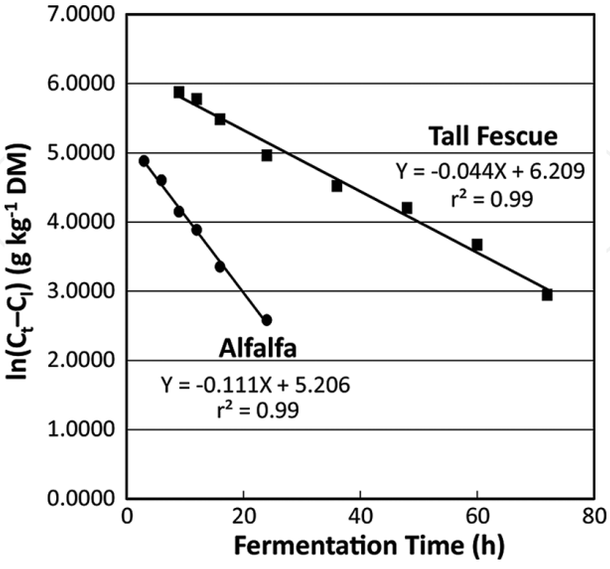

Figure 3. Regression of the natural logarithm of digestible fiber on time of fermentation for alfalfa (●) and tall fescue (■) samples. Only data from within the period of active digestion were included in the calculation. The slope of the line represents the first-order rate of digestion. Data from [14].

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The constant for rate of fiber digestion was 0.111 h^{-1} for alfalfa and 0.044 h^{-1} for tall fescue. These two slopes can be compared using a *t*-test similar to that used in the first example:

$$t = \frac{b_1 - b_2}{S_{b_1 - b_2}} = \frac{0.111 - 0.044}{0.0156} = 4.30 > 2.01\ t_{0.05,52df} \tag{13}$$

Based on this comparison, it is clear that the rate of fiber digestion is quite different between the two species with the digestion of alfalfa fiber occurring at over 2.5 times the rate as that of tall fescue. For reasons we will see, interpreting the rate of fiber digestion independently of concentration and degradability can be misleading. However, at this point, for whatever reasons, we conclude that in this study the rate at which fiber was digested in alfalfa was much faster than in tall fescue.

It is possible to test multisample hypotheses about slopes when more than two equations are being compared; for example, if an additional species were being considered in this example. This can be accomplished using analysis of covariance [20], but the test is not easily implemented using statistical software. The calculations involved are laborious enough to consider avoiding making them altogether and instead making pairwise comparisons between slopes using the *t*-test described above. In many cases, as in this example, the slopes are clearly different, and it would be reasonable to proceed with interpreting what the differences mean under that assumption. There are other ways to convince yourself and others that this is appropriate. For example, a significant interaction between species and time in an analysis of variance of digestible fiber concentration remaining over time indicates that the slope of the response is different between species. Even when the slopes are the same, it does not mean that the equations are the same. The intercept can differ between two simple linear equations with the same slope. There are again statistical tests that may be used to compare the intercepts of linear equations [20], but in this case, it is far easier to conduct an analysis of variance or *t*-test on the initial digestible fiber concentrations (**Table 1**).

It is common for there to be a lag period before fiber digestion begins in *in vitro* digestion systems [21]. This is usually attributed to the time required by the rumen bacteria to colonize the sample and begin growing in number. There is often no measurable lag time in *in situ* digestions systems [22]. Sometimes, a negative lag time is observed *in situ* that is attributed to washout of particles from the bag containing the sample and is an artifact of the method. Whether or not there is an actual lag time before digestion begins in the rumen is a subject of debate. It is most likely a function of the substrate and depends on the chemical and physical attributes of the plant material. Longer lag times have been observed for more mature herbage, suggesting that lignification may play a role in delaying active digestion [14]. It takes time for fibrolytic bacteria to colonize herbage particles and for hydrolytic enzymes to access their target polysaccharides. It is reasonable to assume that these processes occur faster for herbage consumed naturally. Regardless, when a lag period occurs during an incubation in which the rate of digestion is to be determined, it must be accounted for in the calculations.

The lag time (*L*) can be calculated by determining the time when *C* is equal to the initial concentration of digestible fiber:

$$L = \frac{\left(\ln\left[C_D\right] - C\right)}{k} \tag{14}$$

That is L is equal to time required to digest the difference between the actual digestible fiber concentration and that predicted by the regression. The lag period will be equal to the time required to reach the initial concentration. Lag time is a constant and for this reason is considered discrete. It can be calculated by first solving for k and C in Eq. (12) by linear regression and using these values to solve for L using Eq. (14).

The foregoing discussion raises the question of how many time points are needed to accurately estimate kinetic parameters. Theoretically, the answer is only two as long as the substrate concentrations are measured accurately, concentrations at the two selected time points are different than the initial concentration and final concentration, and the first-order model can be assumed. With only two time points, the calculation of k is simplified:

$$k = \frac{\ln\left(C_{t_1} - C_I\right) - \ln\left(C_{t_2} - C_I\right)}{t_2 - t_1} \tag{15}$$

As long as fiber digestion is occurring throughout the interval defined by the two time points, then k should be constant throughout the entire time period during which digestion occurred. This may be the most practical method for estimating k when the fiber digestion of a large number of treatments is being evaluated as it greatly reduces the number of subsamples that must be incubated.

There are advantages to using more than two points. Each additional time point decreases the leverage of the others. Since it is not possible to measure the fiber concentration with absolute accuracy, the slope of a line computed with only two points may be subject to higher error than one calculated with more points. Because a line is defined by two points, it not possible to assess if the linear model describes the relationship so it is necessary to assume that it does. However, one might argue that it is better to have more replications to estimate a mean concentration for a few values of X rather than several unreplicated values at many values of X. As long as the assumptions for fitting the model hold, it probably does not much matter, although there may be other nonstatistical reasons for using one approach or the other.

7. Fiber digestion model

The parameters k, C_D, and L together describe the digestion kinetics of herbage fiber and can be used to predict the expected concentration of digestible fiber remaining after an interval of digestion:

$$\left[C_D\right]_t = \left[C_D\right]_0 e^{-k(t-L)} \tag{16}$$

This equation is the nonlinear form of Eq. (12) that has been adjusted for L and uses actual units of concentration rather than log units. The parameters of this equation can be calculated using nonlinear regression. However, using that approach can lead to biased estimates of k and L for herbage with long lag periods, so the log-linear approach is preferred [14].

This equation indicates that the digestible fiber concentration at any given time (t) is a function of the initial concentration ($[C_D]$) multiplied by a proportion equal to $e^{-k(t-L)}$. This proportion can

be thought of as the inverse of the digestion coefficient for $[C_D]$ at t. By subtracting the concentration of digestible fiber remaining $[C_D]$ predicted by the model from the amount present at time zero $[C_D]_0$, it is possible to calculate the amount digested at any point in time during the incubation:

$$[D]_t = [C_D]_0 - [C_D]_0 e^{-k(t-L)} \quad (17)$$

which factors to:

$$[D]_t = [C_D]_0 (1 - e^{-k(t-L)}) \quad (18)$$

The units of $[D]_t$ are in concentration per unit of dry matter (e.g., g NDF kg^{-1} DM). Fiber digestibility can be calculated by dividing $[D]_t$ by the concentration of fiber initially present (**Table 1**).

Eq. (18) is one form of a common nonlinear model that is used to describe asymptotic increase. It goes by many names including the monomolecular equation that is used to describe chemical reactions involving a single molecule and the Mitscherlich equation that is used to describe crop yield responses to fertilizer. Archontoulis and Miguez [1] simply refer to it as "Exponential gives rise to maximum." It is useful for characterizing a host of biological relationships that exhibit asymptotic behavior. In its simplest form, it is a two-parameter equation, but a third parameter is sometimes used as a scaling factor to reduce the pool size [23] affected by the proportion defined by $e^{-k(t-L)}$, essentially creating a nonzero intercept on the *Y* axis. Perhaps more to the point, the monomolecular equation has been used to describe the digestion of herbage [24, 25], although it has been used mostly for characterizing protein degradation which also follows first-order kinetics.

As presented in Eq. (18), the monomolecular equation cannot be linearized in a manner that lends itself to an algebraic solution for all parameters. Estimates of all parameters, however, can be obtained simultaneously using nonlinear regression. In the case of fiber digestion, the parameters are well defined chemically and biologically, and it is more straightforward and, therefore, advantageous to determine their values directly and sequentially as demonstrated in Examples 2.1 and 2.2 (**Table 1**).

Estimates of the digestion model parameters (Eq. (18)) are presented in **Table 1**. We have already concluded that the rate of digestion was over two times as fast for alfalfa as for tall fescue. However, this rate only applies to the digestible portion of fiber, which was much greater in tall fescue. The absolute rate of fiber digestion (Eq. (10)) at the onset of digestion (L) was 16.7 g kg^{-1} DM/h for alfalfa and 17.3 g kg^{-1} DM/h for tall fescue reflecting the much higher concentration of digestible fiber in the latter, which was over two times greater than that for alfalfa (**Figure 4**). Evaluating k only without considering the size of C_D would be very misleading when evaluating the contribution of fiber to the digestible energy available from a particular herbage.

In the next section, we will combine the information we developed in Example 2 with a theory developed by Van Soest and colleagues [8] to estimate the true dry matter digestibility of herbage.

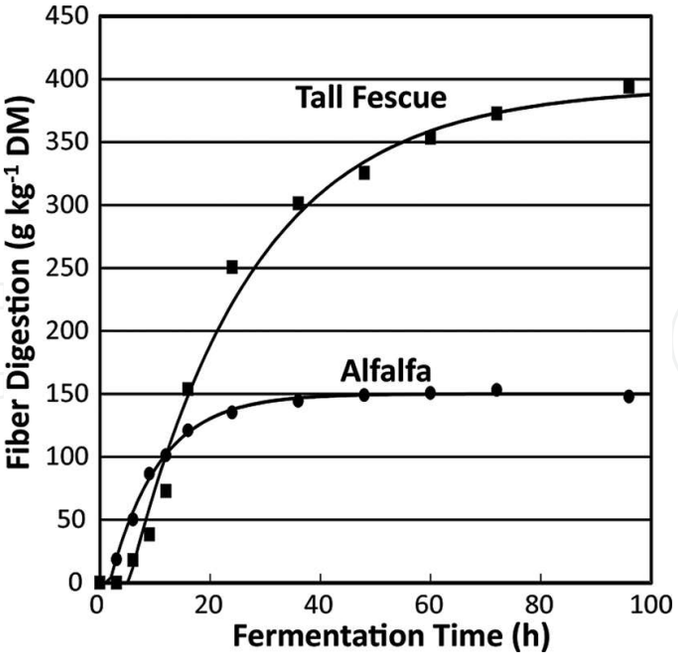

Figure 4. Amount of fiber digested during fermentation for alfalfa (●) and tall fescue (■). The values estimated by the line were calculated using Eq. (18). The first-order rate constant (k) was calculated using the subset of data points that were determined to occur during the period of active digestion and the lag time (L) before digestion began was calculated using Eq. (14). Data from Ref. [14].

8. True digestion model

True digestion of herbage dry matter is distinguished from apparent digestion by the contribution of the animal to fecal dry matter [10]. That is, some of the dry matter contained within the feces arises from the animal and microbes inhabiting its digestive system. This includes microbial cells and material sloughed from the walls of the digestive tract [8]. The animal, however, does not contribute fiber to the feces. All the fibers contained within the feces originate from the plant matter consumed by the animal. Given that all dietary constituents other than fiber are virtually digested completely, the true digestibility of the diet can be calculated by excluding nonfiber components from the fecal dry matter. Thus, the true digestibility coefficient of an herbage or diet can be calculated as:

$$TD = \frac{DMI - C_U}{DMI} \tag{19}$$

where DMI is the dry matter intake; the amount of DM consumed and C_U is the undigested fiber; the amount of undigested fiber excreted in the feces.

Depending on the composition of the diet and its physical form, there may be a difference between undigested fiber and that which is truly indigestible [22]. In diets with high passage rates, some of the fiber that may have been digested if exposed to the rumen environment for a longer time escapes undigested and is recovered in the feces. However, for diets that consist entirely or mostly of herbage, it is reasonable to assume that the indigestible fiber fraction reasonably reflects that portion of the diet that is undigested. In *in vitro* and *in situ* assessments of

herbage digestibility, there is no influence of rate of passage and recognizing that dietary and other factors may influence *in vivo* measurements, the true digestibility values obtained from these assessments may be thought of as representing potential values and may not be reached *in vivo* under certain circumstances.

The comprehensive system for feed analysis developed by Van Soest [8] partitions herbage dry matter into two primary fractions based on studies of nutritive uniformity using the Lucas test: cellular contents and neutral detergent fiber. Cellular contents are nutritionally uniform in that they have the same true digestibility across a range of herbages and other feedstuffs. They are virtually completely digestible and according to Van Soest have a true digestion coefficient of 0.98. Digestibility of fiber varies greatly among herbages as we have seen.

Van Soest [8] used these relationships to develop a summative equation for predicting true and apparent digestibility. Accordingly, true digestibility is the sum of cell contents (×0.98) and digestible fiber (C_D). Digestible fiber was estimated either from *in vitro* analyses or a calculation using a simple linear relationship between lignin concentration and fiber digestibility. Apparent digestibility is true digestibility minus 129 g kg^{-1} DM, the latter quantity representing endogenous contributions of nonfiber material to fecal dry matter. Based on these principles, it is possible to estimate true digestibility by summing the output from the fiber digestion model with the contribution from cell solubles:

$$TD = [C_S] + [C_D]_0(1 - e^{-k(t-L)}) \tag{20}$$

where $[C_S]$ is the concentration of cell solubles (1000—$[C]_0$) [26]. This varies slightly from Van Soest's equation by assuming that cell solubles are completely digestible; i.e., have a digestion coefficient of 1.0 rather than 0.98. For the purposes for which this equation can reasonably be used this variance matters little, but it can be easily corrected by applying Van Soest's digestion coefficient to the cell soluble fraction.

Adding the $[C_S]$ term to the equation essentially changes the *Y* intercept. Since the concentration of cell solubles is completely digestible, at $t < L$, the true digestibility is equal to $[C_S]$ at the beginning of fermentation. Once *t* exceeds *L*, then the proportion of [CD] defined by $(1 - e^{-k(t-L)})$ is added to this amount and true digestibility continues to increase until all of the digestible fiber fraction is digested (**Figure 5**).

Having a model that includes the principal parameters affecting herbage digestion allows assessment of how each entity and the parameters acting upon it influence herbage digestibility and by extension energy availability. Two herbages with similar true digestibility may differ greatly in how that value is achieved. They may have different concentrations of cell solubles and digestible fiber and rate of fiber digestion. Based on the model (Eq. (20)), strategies for increasing the true digestibility of herbage could include simply increasing the cell soluble concentration, increasing the concentration of digestible fiber, and/or increasing the rate at which the latter is digested. Focusing on improving any one of these parameters in isolation of the others would not necessarily lead to an improvement in true digestibility because whatever gains achieved in one could be lost from negative changes in the others. Using any or all of these strategies, the end result is a decrease in the concentration of indigestible fiber

(C_l), which in the end makes sense because it is the mathematical inverse of true digestibility (i.e., TD = 1000 − C_l). As it turns out, there is an additional benefit to decreasing indigestible fiber that can be described with a simple mathematical model.

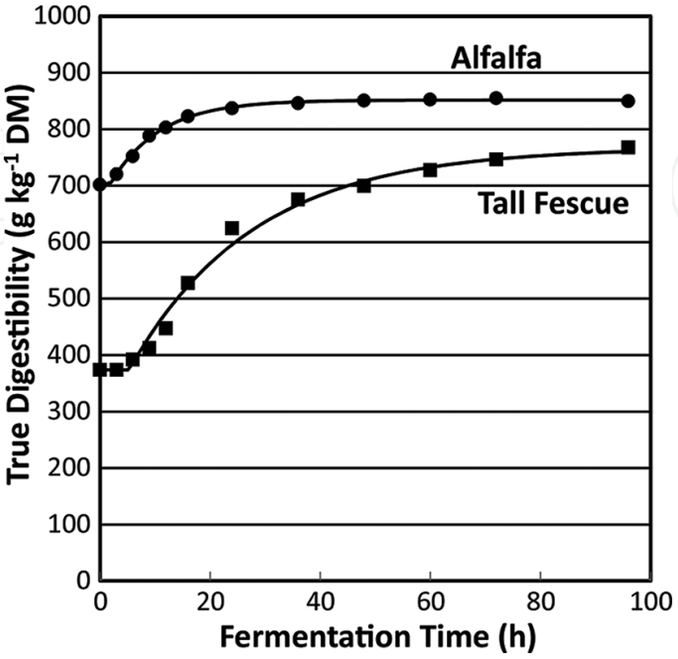

Figure 5. True digestion of forage dry matter during fermentation of alfalfa (●) and tall fescue (■). The values estimated by the line were calculated using Eq. (20). The point where each line intersects the ordinate represents the cell soluble concentration, which in Eq. (20) is considered to be completely digested. The overall picture that emerges is quite different from that where fiber digestion is considered in isolation. Data from Ref. [14].

9. Dry matter intake

The nutritional value of herbage depends largely on the amount of digestible energy that the animal derives from consuming it [27]. In this application, we present a model for predicting true digestibility that relates directly to the energy concentration available to support maintenance and production. How much energy the animal actually ingests, however, also depends on the amount of herbage consumed. Digestible energy intake is the product of dry matter intake and digestibility and is often limited for herbage diets.

There are many factors that influence the amount of herbage that is consumed by an animal. Some of these are related to the animal and its body size, plane of nutrition, and psychogenic factors that influence palatability [28]. There are chemostatic controls that regulate intake and tend to suppress it once the animal's demand for energy has been satisfied [29]. Intake of diets that are predominantly herbage, however, often are regulated by physical distention of the digestive tract. This latter mechanism is generally referred to as fill volume because it represents the quantity of undigested herbage than can be accommodated by the size of the digestive system.

The intake of indigestible fiber is often observed to be relatively constant across similar herbage diets that vary in digestibility suggesting a limit in the amount of indigestible material

that an animal can consume [30]. As the digestibility of the diet increases for animals of similar size and nutritional status, the amount of dry matter that can be consumed also increases because there is less undigested material to retard its passage through the digestive system. Because of this, dry matter intake is often correlated to indigestible fiber concentration and a simple fill model can be used predict it:

$$DMI = \frac{F}{C_I} = \frac{F}{1 - TD} \tag{21}$$

where F is a fill constant and has the same units as DMI. It represents the intake capacity for indigestible fiber. Intake is often expressed as a percent of animal body weight (% BW), so in this case, F would represent the daily intake capacity for indigestible fiber expressed as a percentage of body weight. The concentration of indigestible fiber then should be expressed as a decimal proportion of forage dry matter. It is possible to linearize this equation so that F can be estimated from experimental data but is easier just to calculate the average indigestible fiber intake across a range of forages with varying DMI and indigestible fiber concentrations.

In a brief survey of the literature Moore et al. [31] found that growing beef steers consumed between 0.4 and 0.6% of their body weight of indigestible fiber when fed diets consisting of warm-season grasses. A graph showing predicted intake as a function of C_I using a fill constant of 0.5 is presented in **Figure 6**. Using this relationship, the estimated DMI would be 3.4% BW for alfalfa and 2.2% BW for tall fescue evaluated in Section 7. These are realistic estimates and could be reasonably accurate as long as the fill constant is similar for the class of animal consuming these diets.

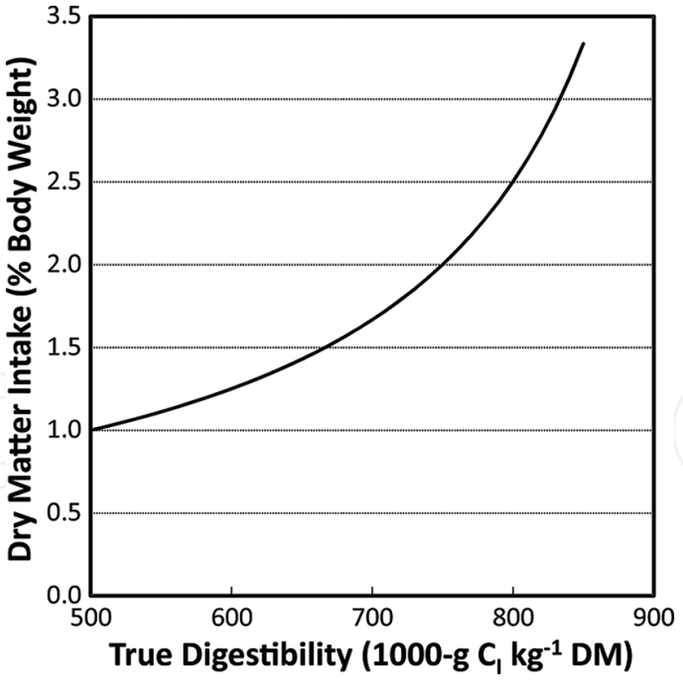

Figure 6. Predicted dry matter intake of warm-season grasses by growing beef steers using a fill constant of 0.5% of animal body weight (Eq. (21)). Adapted from Ref. [31].

It should be obvious that something as complex as DMI cannot be universally predicted using a simple model with one parameter. However, that is beside the point when using the model

to develop strategies for improving forage quality. There should be no disagreement that for a given animal, there is a physical limit on how much indigestible dry matter they can consume. However, assuming that it is the same for all animals or even all animals within a specific class is probably unreasonable. This does not negate the utility of the concept for understanding how indigestible fiber affects DMI and nutritive value of herbage. The model is useful in that it demonstrates why modest improvements in true digestibility usually result in disproportionate increases in digestible energy intake [32].

10. Considerations

The value of using a simple model to describe biological responses is that it enables a better understanding of the response. It is one thing to say that observed values are different, another to say how they are different, and still yet another to say why they are different. Fitting a model to the response creates the possibility of accomplishing all three outcomes. It is important to realize, however, that the parameters of some models that fit a response cannot be easily interpreted. The coefficients from a quadratic equation used to fit the data from Section 5 would be difficult to interpret relative to any biological meaning or significance even though the model fits reasonably well (r^2 = 0.81 and 0.97 for alfalfa and tall fescue, respectively). Knowing that the digestion of fiber follows first-order kinetics is much more informative and the logical conclusions that can be made once this is accepted are quite useful.

The examples presented in this chapter demonstrate the utility of using simple mathematical models to explain nutritional aspects of herbivory. It should be understood that simple models cannot be expected to fully explain complex phenomena. There are too many factors involved in most biological systems to be able to do so. This does not mean that the models are not valid within the constraints they are used, but that they should not be generalized to other situations without validating their predictive performance in those situations.

Appendix

A1. Rumen degradable protein

Dataset

Bromegrass		**Switchgrass**	
CP	**RDP**	**CP**	**RDP**
188.9	148.1	131.1	68.1
144.5	106.0	118.9	61.4
144.5	101.4	106.3	50.3
124.2	80.2	113.3	52.1

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Bromegrass		Switchgrass	
CP	**RDP**	**CP**	**RDP**
132.4	88.7	92.5	42.3
143.8	88.6	82.0	33.5
81.8	57.5	77.2	48.0
32.1	25.3	53.3	26.8
45.1	30.4	34.4	10.2
31.3	19.9	30.0	8.0
33.6	16.8	27.5	4.5
		18.3	2.0

CP = crude protein concentration (g kg^{-1} DM); RDP = rumen degradable protein (g kg^{-1} DM).

SAS Code

```
proc reg;
 by species;
 model RDP = CP;
run;
```

SAS Output (abridged)

```
---------------------------------- Species=Brome ----------------------------------

                                Analysis of Variance
                                       Sum of           Mean
      Source                  DF       Squares         Square    F Value    Pr > F
      Model                    1         17755          17755     329.97    <.0001
      Error                    9     484.27891       53.80877
      Corrected Total         10         18239

                  Root MSE              7.33545    R-Square     0.9734
                  Dependent Mean       69.35455    Adj R-Sq     0.9705
                  Coeff Var            10.57673


                                Parameter Estimates
                     Parameter     Standard
      Variable   DF     Estimate        Error  t Value  Pr > |t|    Type I SS   Type II SS
      Intercept   1     -4.70460      4.63830    -1.01    0.3369        52911     55.35812
      CP          1      0.73911      0.04069    18.17    <.0001        17755        17755
---------------------------------- Species=Switch ----------------------------------

                                Analysis of Variance
                                       Sum of           Mean
      Source                  DF       Squares         Square    F Value    Pr > F
      Model                    1     5720.58349     5720.58349     238.45    <.0001
      Error                   10      239.90318       23.99032
      Corrected Total         11     5960.48667

                  Root MSE              4.89799    R-Square     0.9598
                  Dependent Mean       33.93333    Adj R-Sq     0.9557
                  Coeff Var            14.43416


                                Parameter Estimates
                     Parameter     Standard
      Variable   DF     Estimate        Error  t Value  Pr > |t|    Type I SS   Type II SS

      Intercept   1     -8.25067      3.07601    -2.68    0.0230         13818    172.59967
      CP          1      0.57212      0.03705    15.44    <.0001    5720.58349   5720.58349
```

A2. Fiber digestion

Dataset

	Alfalfa				Tall fescue			
h/Rep	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
0	293.2[1]	296.7	297.1	306.2	642.3	617.5	639.4	604.9
3	276.6	291.3	274.4	276.6	637.2	615.1	632.4	603.0
6	237.2	254.9	242.7	257.7	623.3	605.0	608.7	594.9
9	208.1	218.9	205.4	214.3	603.8	593.9	581.3	571.3
12	194.7	195.8	190.6	207.4	555.1	561.3	551.5	544.3
16	177.1	180.2	168.9	182.6	461.2	489.4	471.8	467.1
24	169.9	170.6	155.4	156.8	394.8	366.7	373.0	367.2
36	151.2	146.3	152.7	165.5	307.5	326.1	345.2	320.1
48	146.9	144.6	152.2	154.0	299.9	307.7	316.4	277.9
60	145.3	142.9	147.6	154.4	260.0	285.1	281.3	264.5
72	142.8	141.5	143.3	153.4	241.2	264.6	249.8	257.9
96	148.3	147.8	151.4	154.3	219.0	243.1	238.3	228.6
120	137.4	136.8	141.5	150.2	200.9	236.9	207.7	201.3

[1]Values are neutral detergent fiber (NDF) concentration at h (g kg^{-1} DM).

A2.1. Determining time intervals to include in regression

SAS Code

```
proc glm;
by species;
class h;
model NDF = h / ss3;
means h / lsd hovtest;
run;
```

SAS Output *(abridged)*

http://dx.doi.org/10.5772/67342

---------- species=Alfalfa ----------

Levene's Test for Homogeneity of NDF Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
h	11	15155.3	1377.8	1.10	0.3910
Error	36	45202.8	1255.6		

t Tests (LSD) for NDF

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	44.07785
Critical Value of t	2.02809
Least Significant Difference	9.521

Means with the same letter are not significantly different.

t Grouping		Mean	N	h
	A	298.300	4	0
	B	279.725	4	3
	C	248.125	4	6
	D	211.675	4	9
	E	197.125	4	12
	F	177.200	4	16
	G	163.175	4	24
H	G	153.925	4	36
H		150.450	4	96
H		149.425	4	48
H		147.550	4	60
H		145.250	4	72

---------- species=Fescue ----------

Levene's Test for Homogeneity of NDF Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
h	11	154556	14050.6	0.73	0.6997
Error	36	689475	19152.1		

t Tests (LSD) for NDF

Alpha	0.05
Error Degrees of Freedom	36
Error Mean Square	180.9521
Critical Value of t	2.02809
Least Significant Difference	19.291

Means with the same letter are not significantly different.

t Grouping	Mean	N	h
A	626.025	4	0
A	621.925	4	3
A	607.975	4	6
B	587.575	4	9
C	553.050	4	12
D	472.375	4	16
E	375.425	4	24
F	324.725	4	36
G	300.475	4	48
H	272.725	4	60
I	253.375	4	72
J	232.250	4	96

A2.2. Calculating rate constants using linear regression

SAS Code

Note that this procedure requires that time intervals where no digestion occurred have been deleted from the active data set, the indigestible fiber concentration ($[C_I]$) has been subtracted from the fiber concentration ($[C]_t$) remaining at each time point, and that this difference has been transformed by taking the natural logarithm. This quantity is included in the model statement as lnCD.

```
proc reg;
by species;
model lnCD = h / lackfit;
run;
```

The lackfit option in the model statement requests that the residual variance be partitioned into lack of fit and pure error in order to test if the model describes the response.

SAS Output*(abridged)*

```
---------------------------------- species=Alfalfa ----------------------------------

                              Analysis of Variance
                                     Sum of           Mean
Source                    DF        Squares         Square    F Value    Pr > F

Model                      1       14.17745       14.17745     220.39    <.0001
Error                     22        1.41526        0.06433
  Lack of Fit              4        0.05980        0.01495       0.20    0.9359
  Pure Error              18        1.35546        0.07530
Corrected Total           23       15.59271

                              Parameter Estimates

                             Parameter       Standard
Variable     DF       Estimate          Error    t Value    Pr > |t|

Intercept     1        5.20629        0.10175      51.17      <.0001
h             1       -0.11145        0.00751     -14.85      <.0001

---------------------------------- species=Fescue ----------------------------------

                              Analysis of Variance
                                     Sum of           Mean
Source                    DF        Squares         Square    F Value    Pr > F
Model                      1       30.23112       30.23112     467.38    <.0001
Error                     30        1.94045        0.06468
  Lack of Fit              6        0.35437        0.05906       0.89    0.5152
  Pure Error              24        1.58608        0.06609
Corrected Total           31       32.17158

                              Parameter Estimates
                             Parameter       Standard
Variable     DF       Estimate          Error    t Value    Pr > |t|
Intercept     1        6.20922        0.08401      73.91      <.0001
h             1       -0.04431        0.00205     -21.62      <.0001
```

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