RUMEN BALANCE AND RATES OF FIBER DIGESTION

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INTRODUCTION

A core concept in the Cornell Net Carbohydrate and Protein System (CNCPS) (1) is the competition between rates of digestion and passage to determine rumen balance. The current status of both CNCPS 4.0 and CPM Dairy feed libraries only provide book values for rates of digestion that are treated as constants. Many of the book values of digestion rates are likely too low for high quality forages. The consequence of underestimating the rate of fiber digestion in the model is the incorporation of more concentrate into the ration leading to potential rumen imbalances. Fiber and lignin increase while digestion rates decline with forage maturity. Relatively, digestion rates are higher for alfalfa followed by timothy and orchard grass (2). Data presented in the current CNCPS do not reflect these differences. Advisors in the field are now attempting to estimate rates from laboratory data with varied results. This paper presents a mechanistic approach to the problem of rate estimation of NDF digestion along with suggestions for required laboratory data.

Most studies of rates of digestion apply first-order kinetics in which a constant rate of digestion is a function of the declining amount of residual substrate. The rate constant is obtained by regressing the natural logarithm of residual substrate upon time in h[*]. In the case of the plant cell wall, an unavailable lignified fraction complicates this application, because direct regression of undigested substrate upon time is nonlinear. Waldo et al. (4) subtracted a residual amount to obtain linearity, but as shown by Mertens (2) this subtracted amount includes a digestible fraction. The subtraction of an amount of residue needed to produce a straight regression between logarithm of substrate and time (4) was nevertheless applied by Mertens (2) to obtain kinetic rates. The practical problem is that rates of fiber digestion are not constants, but decline with time.

Conservatively interpreted, the rates in the Mertens thesis are the basis for current digestion rates in the CNCPS (5), which are treated unrealistically as constant. Unfortunately only these rates are presently available, and they probably undervalue higher quality forages.

The procedure of Mertens and Loften (6) insures that the regression line fits early times of digestion values, and therefore, can be considered maximum initial rates, which decline with time. This decline in rate will affect estimates of rumen fill, since a larger portion of slower digesting residues will remain than what would be expected from first order kinetics.

Any attempt to account for nonlinearity will result in declining k values with time, and will result in a major adjustment in the CNCPS. Undervaluing the rate constant in high quality forages leads to increased feeding of concentrate. The nonlinearity could be resolved by curve-peeling to discern faster and slower pools (attempted by Mertens) (2) or by applying higher order kinetics (7). The difficulty with curve-peeling to discern two or more first-order pools is that the choice of time for inflection points seems purely arbitrary, and the observed fermentation curves of digestion seem continuous. Higher order kinetic systems would avoid this problem.

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[*]A definition of first order kinetics assumes that there is a single factor regulating the rate. Second order systems assume that there are two independent factors affecting rate. These details are aspects of chemical kinetics (3). The value of the order is expressed as n, which is the exponential function in Equations 2 and 3.

This exposition re-examines higher order systems applied to the experimental data of Mertens. It also makes proposals for standard analyses needed to apply these developments in the CNCPS.

Fadel (7) and Robinson et al. (8) have presented two model equations, one surface limited and the other second order, for accounting for nonlinearity (these in addition to a two-pool first order system). The equation dealing with a surface-limited equation, in which the rate is limited by the 2/3 power, has been passed over because, as shown in the following development, the deviations from linearity do not relate to the cube root (as expected from Fadel's first equation) but instead to variable powers exceeding unity. For strategic reasons and in consideration of the nature of the CNCPS, only the second-order equation is considered. Robinson et al. (8) compared first and second order models with rye grass, beet pulp, brewers dried grains, and babassu meal as substrates. It appeared that a simple first order model was superior to the second order one. However, all of these feeds are of low lignification, and there is only one forage in the set.

Mathematical terms used in this paper are defined in Table 1.

Table 1. List of mathematical terms.

A	Available substrate, equals S - U at time t
A_0	Initial available substrate at time zero (T_0)
A_x	Available substrate estimated by subtraction of $U_{2.4}$
A_w	Available substrate estimated by subtraction of U_w
k_w	Rate of digestion reported by Mertens using model of Waldo et al. (4)
k_f	Functional rate of digestion variable with time
S	Total substrate inclusive of undegradable components
S_0	Initial amount of substrate at time zero
T_L	Lag time (delay): subscript L for lag[†]
T_W	Theoretical time required for maximal digestive extent in the Waldo model
T_r	Retardation time = (ΔlnA)/k. (An example is the difference between 30 and 68 h in Figure 1)
T_x	Functional time required for digestion exclusive of lag T_L
t	Time as a variable
U	Generally the unavailable fraction as a proportion of S
$U_{2.4}$	U estimated by Chandler et al. (9) (2.4×Lignin)/NDF
U_M	Mertens' estimate of U
U_T	Traxler's estimate of U
U_w	Estimate of U using model of Waldo et al. (4)
ΔlnA	Differences between -kt and lnA_x (See Figure 1)
ΔU	Value of A_x - A_W, also the difference U_M - $U_{2.4}$

MATHEMATICAL DEVELOPMENT

The second-order equation of Fadel (7), number 22.36 in Nutritional Ecology of the Ruminant page 369 (10) states:

$$1.\quad \frac{-dA}{dt} = k\frac{A^2}{(A+U)}$$

This equation assumes that two factors influence the rate of digestion: the quantity of available substrate (A) and the residual unavailable fraction (U).

[†] For an accounting of lag, see Figures 4 and 5 and their accompanying commentaries.

Equation 1 can be formulated more generally for any order of kinetic expression:

$$2.\quad \frac{-dA}{dt} = k\frac{A^{(n+1)}}{(A^n + U)}$$

where U is equal to or less than unity. This modification is important, since it appears that the value of the exponent n is variable.

As Mertens (2) pointed out, the apparent order of cell wall digestion may be variable where observed n > 1 and the variability of n is open to experimental examination. These equations are biologically realistic because in the case of an unlignified substrate where U equals 0, the equation collapses into a simple first order one. This is a behavior expected of biochemically uniform substrates and seen in very immature unlignified forages. Mertens (2) indicated that degree of nonlinearity seemed to be related to forage maturity.

The mechanistic logic of this equation considers that as digestion of available cell wall carbohydrate proceeds, the concentration of unavailable lignified matter rises, occupying more of the surface exposed to digestion, and reducing accessibility to the remaining available substrate. This slows the kinetic rate of digestion as a function of the ratio of the undigested substrate to the available. The biological effect is possibly surface related, but an inhibitory effect of lignin concentration cannot be excluded. The calibration of this function to the rate are experimentally developed here, was found to be related to the reciprocal of the available substrate raised to a variable power.

Integration of Equation 2 yields:

$$3.\quad \int_0^1 -kdt = C - kt = \ln(A) - \frac{U}{(n+1)A^n}$$

The integration is divided into a logarithmic factor (lnA) and an arithmetic factor of U/A. This partition is discussed below and described in Figure 1. Further, these components are identified as described below.

The value of the integration constant C is complex involving both logarithmic and exponential functions. These are not easily applied. Fadel (7) resolved the solution of Equation 2 by computer iteration. Another approach is taken in this presentation. The value of the integration constant C, which occurs at time zero, is resolved by regression analyses.

We have assumed that the logarithmic factor, Mertens (k_W) value determined by the procedure of Waldo et al. (4) represents a maximum rate dominant at early times of fermentation. Introduction of the expected values of -kt from the respective regressions leads to sets of increasing positive deviations from regression as logarithmic values (ΔlnA) per times of digestion as shown in Figure 1.

We have further assumed that these deviations (ΔlnA) are functions of U/A where U is a fixed value and A declines with time of digestion. This retarding effect is assumed to be the consequence of the rising proportion of unavailable substrate in the digesting matrix as available substrate is removed. The quantitative association between ΔlnA and U/A is estimated by regression analysis. Note that the ΔlnA values are the difference between that of the Waldo regression and the logarithm of the calculated residual A_x. These values of ΔlnA increase with time of digestion and are not linear with U/A although very highly correlated. The nonlinearity results from the rising value of the exponent n with time of fermentation.

Any of the functions of -kt are convertible into an equivalent time value by division by $-k_w$ as indicated in Figure 1, and can be regarded as a time of retardation. It is not a lag function.

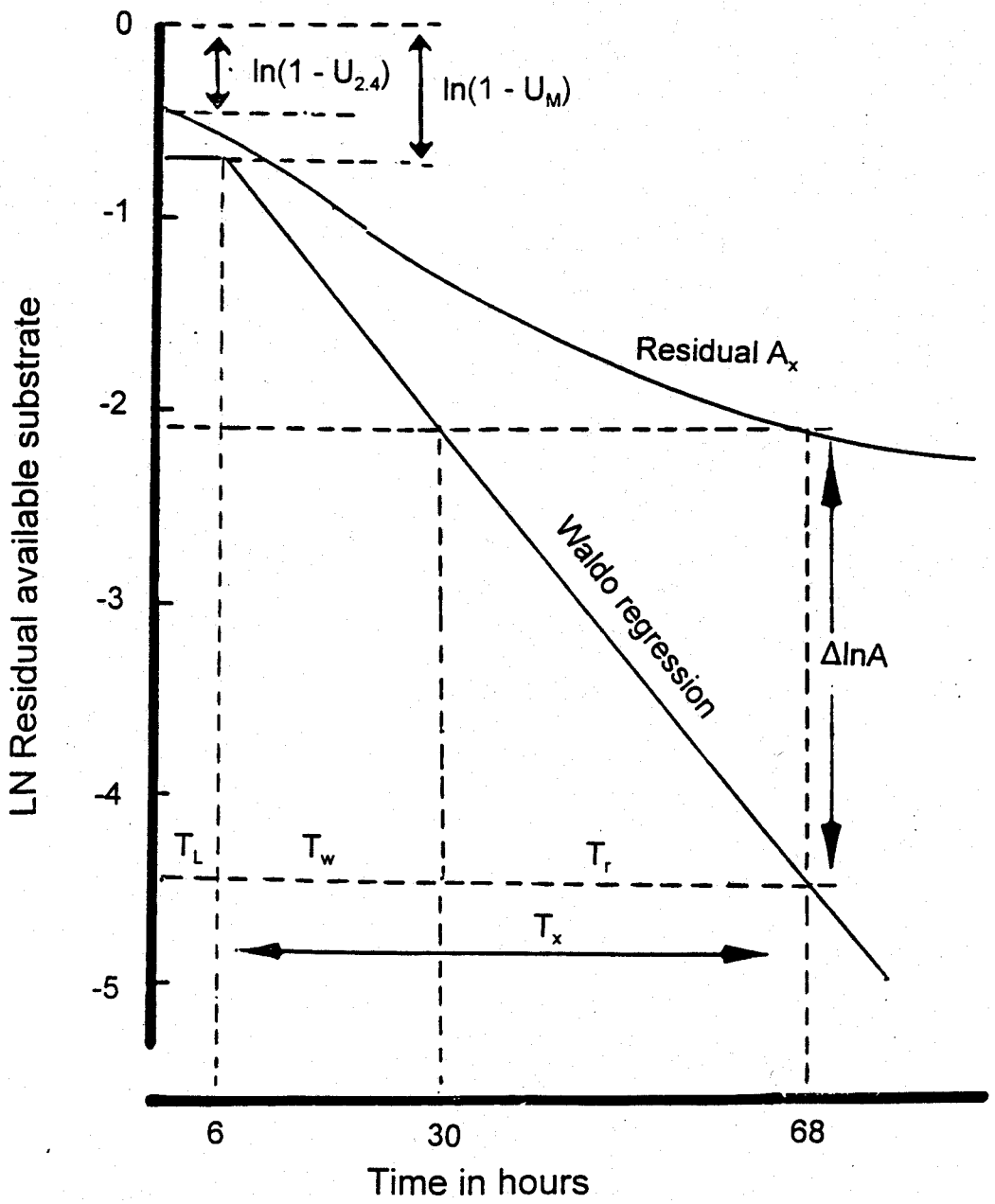

Figure 1. Graphical diagram showing the application of the Fadel second-order equation to an alfalfa with a k rate of -0.06 and a ΔU of 9%. The observed extent of available substrate A_x at time T_x, where U is 2.4 times lignin content of NDF. The total time needed for extent of digestion T_x can be partitioned into lag (T_L) that expected for the Waldo model (T_w) and the retardation (T_r). Correspondingly the substrate is denoted by the initial amount A_0 and A_w the amount expected from the Waldo regression. The figure shows the expected digestion of A at 30 h actually took 68 h with a T_r of 38 h. Definitions of terms are in Table 1.

ANALYSES OF SELECTED DATA FROM MERTENS

The data of Mertens (2) consists of the in vitro cell wall degradations at 0, 3, 6, 9, 12, 18, 24, 36, 48, 72 and 96 h for 260 forages. Lignin and NDF contents are reported along with the k_w values using the model of Waldo et al. (4) as programmed by Mertens and Loften (6). This application of Equation 3 to the Mertens data set utilizes a subtraction of U, which has been calculated as lignin $\times$ 2.4 according to Chandler et al, (9). Reasons for this choice are given in the following section on the evaluation of U.

We have non-randomly selected 15 forages as examples shown in Table 2. Criteria for selection were range, relevant forage species, and maturity.

The Mertens' data reports a value U_M determined to give maximum linearity of the regressions of ln substrate (A_w) upon time. These values are higher than those ($U_{2.4}$) estimated by (2.4×lignin)/NDF. The differences between these estimates of U are termed ΔU where $\Delta U = U_m - U_{2.4}$. Note that these values are arithmetic and are relatively constant with time as opposed to the ΔlnA values that increase with time of digestion.

Generally ΔU increases with estimated U, although large differences between grasses and legumes exist. The value of ΔU increases generally with plant maturity, as would be expected from the model that would allow for retardation of digestion with larger indigestible residues. The value of ΔU is inversely related to the kinetic rate. The highest values of ΔU coming from some tropical grasses and very mature temperate ones. The lowest values generally come from immature forages.

Order of reaction and retardation of rates

The kinetic rate of digestion deviates from first order as the digestion is retarded. This results in an increase of the power function (n) in Equation 2. Estimation of the values of n were performed on 15 selected forages described in Table 2. The value of n was determined by regressing the logarithm of the logarithm (absolute value)[‡] of the available substrate $\ln|\ln(A_x)|$ using unitized values of A_x = (S – (2.4×Lignin)/NDF)/A_{xo}, upon the logarithm of time. This is an adaptation of general procedures for estimating the order of physicochemical reaction (3). The values of n were evaluated over 3 time points beginning at 6 h. The earliest time point at 3 h is confounded by lag and is omitted. In some cases estimates from the Merten's regression were interpolated to provide consistency.

The regressions of $\ln|\ln(A)|$ upon the logarithm of time become increasingly nonlinear with increase in time of digestion (Table 2). Curvilinearity is driven by ΔU. When a relatively unlignified forage such as Timothy in the vegetative stages (9-14-1) is evaluated, n is near unity which is consistent with a first order reaction. Values tend to rise with increasing time and plant maturity. For most forages, values of n are near unity at early times and tend to rise particularly after 24 h. The rise in the value of the exponent n in Equation 3 indicates a slowing down of rate of digestion. Values of n near unity at early stages of fermentation indicates a portion of the cell wall is relatively unaffected by lignin, even in very lignified forages. At later stages of degradative fermentation, the non-homogenous distribution of the lignified matrix becomes manifest. This kinetic analysis indicates that forage cell walls are non-homogeneous and are mixed matrices of lesser and more lignified parts.

The mechanism of the nonlinear association between ΔlnA on U/A may be surface related. A model can be constructed where unavailable lignified residue is embedded in a sponge-like association with available substrate. Removal of available substrate reduces available surface while that of the unavailable surface increases out of proportion to its quantity. The exponential values of n obtained by such a model between unavailable and available mass can easily reach and exceed power 2 (personal calculations). The increasing value of n indicates nonuniformity of the substrate, which cannot be easily peeled into subpools. This situation is analogous to a coal miner who as he removes good coal exposes ever more useless rock, which comes to dominate the scene and slows down production.

[‡] Bars indicate absolute values because one cannot take the logarithm of a negative number.

Table 2. Composition, rate of digestion, value of ΔU and order of reaction (n).

Forage	Code	Stage[1]	Source	NDF	L/NDF	ΔU	k_w	Value of n at time of fermentation[4,5], h				
				%	%	%	%	6-18	12-24	18-36	24-48	36-72
Alfalfa	12-1-1	Veg	NY	31.5	15.9	2.8	12.5	1.0	1.2	1.8	2.6	3.0
	5-1-6202	EBL	MI	42.9	16.8	15.4	8.8	1.1	1.1	2.2	2.2	3.7
	8-1-6204	FBL	UT	51.4	16.1	19.7	6.5	1.0	1.1	2.8	2.1	2.1
	5-1-6402	2nd	MI	52.9	17.8	18.5	5.9	1.0	1.0	1.8	1.4	2.3
Orchardgrass	9-10-1	Veg	WV	50.5	4.0	8.6	9.9	1.0	1.4	1.6	1.8	4.3
	9-10-4	EBL	WV	67.0	9.4	11.5	5.6	1.0	1.1	1.2	1.4	3.0
	9-10-8	Seed	WV	73.3	8.9	29.1	4.1	1.1	1.0	1.4	1.7	2.1
	9-10-209	2nd	WV	56.2	6.2	6.6	9.2	0.9	1.3	1.6	1.7	2.4
Timothy	9-14-1	Veg	WV	47.6	5.0	0.7	12.0	1.0	1.0	1.2	1.1	1.1
	7-14-1001	BL	PA	62.6	4.0	7.3	7.1	1.1	1.0	1.3	1.4	2.6
	7-14-1004	Seed	PA	75.8	8.3	16.2	3.4	0.9	0.9	1.0	1.2	1.2
	9-14-210	2nd	WV	60.8	7.5	10.1	9.2	1.1	1.1	1.5	2.5	3.2
C. Bermuda	3-6-40	---	GA	67.8	6.0	7.4	7.5	1.0	1.0	1.9	1.9	3.1
Guinea	12-17-1	---	PR[2]	71.6	7.8	17.2	5.9	1.0	1.1	1.4	1.8	2.4
Napier	10-16-404	---	PP[3]	66.8	7.7	10.6	5.7	0.9	0.9	0.9	1.3	1.8

[1] Stages: Veg = vegetative, EBL = early bloom, FBL = full bloom, 2nd = 2nd cut.

[2] Puerto Rico

[3] Philippines

[4] Three and 96 h fermentation values have been excluded from these estimates.

[5] Values of n are the power slope of the regression of ln(ln(residue)) versus ln(time) conducted within each time period using 3 points. Values are calculated on an overlapping basis. R^2 of these regressions are 0.98 or more.

EVALUATION OF THE INDIGESTIBLE FRACTION (U)

Evaluation of the proportion of U as a part of the substrate is required for solution of Equation 3. The value of U must be subtracted from the residual substrate to obtain an estimate of residual unfermented available substrate A. In the second term of Equation 3, U is divided by a power function of A. These operations set limits upon U: it cannot exceed the fermented residue at long times, as this would result in an available substrate of less than zero and also the division by zero in the second term. Division by zero results in a mathematical explosion and failure of the model. This problem occurred in some immature forages when Traxler's estimate at 96 h was used. Therefore, a longer time estimate of indigestibility is needed.

The value of U in the present version of the CNCPS is (2.4×lignin)/NDF, a value obtained from the study of Chandler et al. (9) where diverse substrates were fermented for 90 or 120 days (Table 3).

Table 3. Comparison of the experimental values of Chandler et al. (9) with those predicted by Traxler[1] et al. (11) for ratios of NDF to lignin at final digestion.

	Initial Lignin Content of NDF (%)	Days Fermented	Final ratio NDF/Lignin	
			Digestion	Power Equation 0.76 (U_T)
Newsprint	23.6	90	2.9	2.7
Treated kelp	18.1	120	2.7	2.9
Water hyacinth	14.5	120	2.3	3.0
Cow manure				
R_3	16.1	120	2.1	3.0
R_2	14.9	120	2.2	3.0
R_1	14.1	120	2.0	3.1
Elephant manure	13.5	120	2.5	3.1
Cattails	13.4	120	2.7	3.1
Wheat straw	11.6	120	2.0	3.2
Corn meal	9.1	90	1.5	3.4
Corn stalks	7.8	120	2.7	3.5
Chicken manure	7.5	120	2.5	3.5
Corn leaves	6.5	120	2.0	3.7
Pig manure	5.4	120	2.9	3.8
Mean			2.4	3.2

[1]Equation for power 0.76 is (lignin/NDF in g/kg)$^{0.76}$ divided by initial lignin content of NDF (11).

The object in the Chandler study was the estimation of methane potential where the value of 2.4 has been treated as a constant. However, the more recent development of surface models to account for the effects of lignification (11, 12, 13) indicate that 2.4 cannot be constant. Analyses of the data of Mertens (2) indicate that a low lignin content in immature forage has a larger impact on the value of U than in more mature ones (11, 14). However, these observations are based on 96 h extents; which are incompletely digested. The mean ratio of indigestible NDF to lignin using the power 0.76 gives higher values than those observed by Chandler (Table 3). This indicates that the 96 h residues likely contain significant remaining unfermented available substrate relative to the longer fermentation times of Chandler et al. (9).

However, a consistent relationship of the final NDF to lignin ratio with initial lignin content has no significant association in the Chandler data. Possibly this is due to a lack of samples with a range in forage maturities. The extent of digestion can be increased (and

the residual U reduced) by decreasing the power of the Traxler equation. A power slope of 0.69 – 0.70 will reduce the predicted residue to give (NDF/Lignin) ratios on the order of 2.4. However, a resolution to this problem will require more long time (> 96 h) fermentations, which are not available at the present time. As a result we have deferred the use of the Traxler power function system and retained the use of the Chandler calculation, even though the database is limited.

DATABASE SELECTION AND PREDICTION OF RATES AND ΔU

The object of this investigation is to generate a system by which values for rate of digestion and its retardation can be predicted and used in the field. At the present time there is no system for deriving digestion rates apart from those in the feed dictionary. Analytical values available in the field, include NDF, ADF, lignin, crude protein (CP) and sometimes in vitro digestibility at 24 or 30 h. Rates of digestion and ΔU will need to be predicted from these observations. From Table 2 it is seen that there is very large variability in the values of K_w and of ΔU, which is the factor retarding retention time.

Forages were selected randomly from the database of Mertens, (2) and used to predict equations for ΔU and k using feed concentrations of neutral and acid detergent fibers, sulfuric lignin, CP, *in vitro* digestibility of dry matter (30 h), and their second order interactions. Selected forages were divided into two categories (grass and legumes plus mixes) and 60% of each category was randomly selected to derive the equations whereas the remaining 40% were used to validate them. Species known to contain secondary factors such as tannins or alkaloids have been excluded. These species include vetches, fescues and Reed canary grasses. The Mertens data set contains no corn silages. Data for corn silages need to be found or generated and further evaluated.

Mertens' dataset does not provide 30 h digestions. Values here are estimated by averaging 24 and 36 h values according to Equation 4. Equation 5 was used to estimate ΔU.

4. $IVTDM = (1 - [(Residue_{24} + Residue_{36})/2]/Residue_0) \times NDF + (100 - NDF)$
5. $\Delta U = (U_M/Residue_0 - (2.4 \times Lignin)/NDF) \times 100$

Feeds having a negative ΔU were removed from the database and the lignin value was calculated from permanganate lignin (0.81 x sulfuric lignin - 0.1) for feeds missing sulfuric lignin (15).

Statistical analysis

Regardless the phase of analysis (derivation or validation), outliers were identified using the plot of studentized residue against the predicted (Y-variate) and Cook's D influence statistic (SAS Inst. Inc., Cary, NC). Feeds with a studentized statistical residue outside the range -2 and 2 were considered as outliers and removed from the database.

The procedure PROC REG (SAS Inst. Inc., Cary, NC) was used to obtain the parameter estimates of the regressions. In multiple regressions, the stepwise selection method was used to select the best set of variables and finally the sequential sum of squares was used to select the appropriate variables (16).

Bias (%) was calculated by dividing the mean of the Y-variate (observed) minus the mean of the X-variate (predicted) by the mean of the X-variate. A positive bias means that the observed values had greater values than the predicted ones. The correlation matrix is shown in Table 4. In vitro digestibility has the highest correlations with ΔU and rate.

Table 4. Correlations between forage composition and digestibility with ΔU and rate of digestion.

	Development dataset N = 102		Validation dataset N = 76	
	ΔU	ln(k)	ΔU	ln(k)
NDF	0.07	-0.50	0.16	-0.52
ADF	0.43	-0.61	0.56	-0.48
Lignin	0.46	-0.26	0.61	-0.32
Crude protein	-0.31	0.61	-0.39	0.47
Digestibility[1]	-0.73	0.81	-0.71	0.60

[1] In vitro true digestibility at 30h. All values correlated are on a dry matter basis, except for rate which is %/h.

Prediction equations using in vitro digestion at 30 h, ADF and CP are shown in Table 5, for rate of digestion k_w and ΔU. These equations can be used to predict ΔU and rate of digestion from 30 h in vitro digestion, NDF, ADF and lignin measurements.

The last column of Table 5 shows the mean squares from the calibration sets. Comparison of the predicted values and the observed ones for the validation set are shown in Table 6. Figures 2 and 3 show the relationship between observed and predicted values of ΔU and k_w, respectively.

The statistics from the validation set (Table 4) indicate that in vitro rate of digestion at 30 h is the most highly correlated for both rate and ΔU. The ADF and CP values are less satisfactory. From the pattern of association between rate of digestion k_w and ΔU both are affected by plant maturity but with different quantitative effects. The ΔU and rate have considerable independence.

Fitting the Fermentation Curve

One test for the precision of the predicted values of rate and ΔU is to calculate the value of residual substrate (S) relative to fermentation times. Table 7 shows calculations for an average alfalfa using the predicted rate k_w and ΔU from 30 h in vitro digestibility. Values have been calculated using no lag and for an arbitrary discrete lag of 4 h, which is about the average in Merten's data.

Without lag the calculation overestimates digestion at early times, particularly at 6 h. Inclusion of lag reduces this error considerably. Whether or not lag should be used is open to discussion, because in vitro systems have longer lags than in vivo (17), the difference in time being at least 2 h. In vitro systems are dilute, require oxygen scavenging and microbial attachment, due to handling and preparation, whereas the in vivo rumen is an actively functioning system at the time of feeding. Much of the effect of lag will be compensated when sequential meals are integrated (see section on retarded rates).

The data in Figure 4 shows that as time progresses the difference between calculated and observed tend to converge on the error of prediction of ΔU. In this case the value from Mertens data is ΔU = 14.6 as opposed to a predicted value of 15.1 leading to a positive difference at long times. Introduction of a 4 h lag greatly reduces the deviation at early times. The large deviation at 18 h probably indicates an erratic fermentation in Mertens' data set. The CNCPS does not employ lag. However, lags also occur in in vitro data and may be in part an artifact of the procedure. We feel that lag should be employed in calculating rates from in vitro data even if they are not used in the model application. Figure 4 is a fitting of Mertens' data which would include laboratory induced lag in the in vitro system.

Table 5. Prediction equations for estimating rate (k_w) and ΔU[1]

Forage type and equations	N	R^2	MSE
Grasses			
$\Delta U = 95.0076 – 0.8765 \times IVDDM - 0.1445 \times NDF - 0.0361 \times NDF \times Lig$	75	0.775	4.62
$k_w = \exp(-1.935 + 0.04834 \times IVDDM - 0.1267 \times Lig + 0.00308 \times NDF \times Lig)$	72	0.857	0.011
Legumes and Mixes			
$\Delta U = 143.49 - 1.652 \times IVDDM - 0.1923 \times NDF + 1.911 \times ADF - 4.807 \times Lig - 0.02095 \times NDF \times ADF$	27	0.894	4.63
$k_w = \exp(1.935 - 0.0055 \times IVDDM + 0.0320 \times NDF - 0.1046 \times ADF - 0.0006595 \times NDF \times IVDDM + 0.00195 \times ADF \times IVDDM)$	26	0.716	0.011

[1] N = number of samples, MSE = mean squared error.

Table 6. Analysis of the regression validation[1].

Equations	N	r^2	MSE	Bias[2], %
ΔU: $Y = -0.77 + 0.996 \times X$	77	0.781	6.56	-6.6*
k_w: $Y = 0.068 + 0.974 \times X$	78	0.793	1.16	-1.7

[1] Y = observed and X = predicted, N = number of samples, MSE = mean squared error.

[2] A negative bias means that observed values were lower then predicted ones (overprediction). Asterisk means different from zero at $P < 0.05$. Intercept was not different from zero in both regressions.

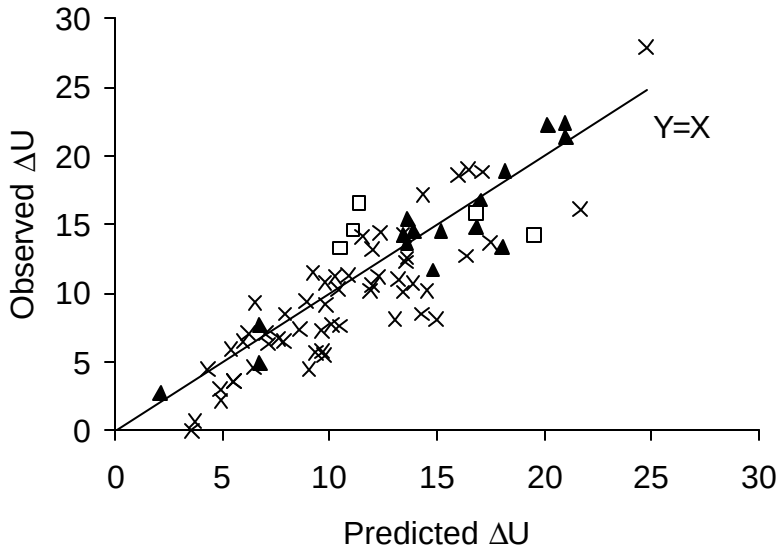

Figure 2. Relationship between observed and predicted values of ΔU for grasses (×), legumes ( ), and mixed ( ). The line represents the unity slope. See Tables 5 and 6 for statistics.

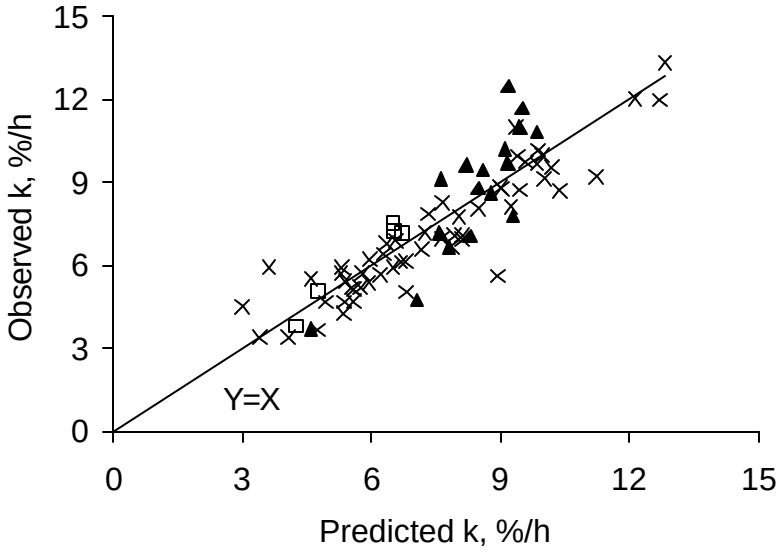

Figure 3. Relationship between observed and predicted values of rate k_w for grasses, legumes, and mixed. Symbols as in Figure 2.

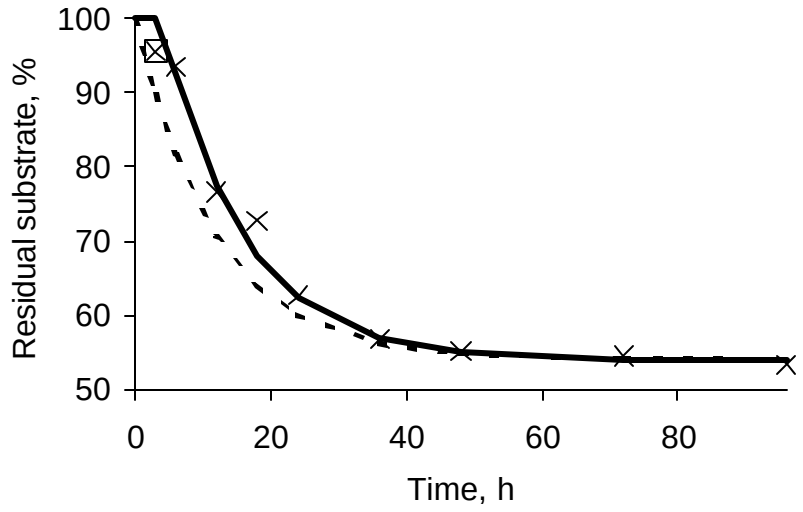

Figure 4. Comparison of observed (×) and predicted residual substrate without (dotted line) and with a 4h lag (solid line) of an Alfalfa (NDF = 48.7, Lignin = 7.9, $U_{2.4}$ = 38.9, predicted ΔU = 15.1, predicted k_w = 8.49, observed ΔU = 14.6, and observed k_w = 7.52). Estimate of $S = (100 - U_{2.4} - \Delta U) \times \exp(-k_w \times t) + U_{2.4} + \Delta U$. The dashed line shows the predictions uncorrected for lag.

Retarding Rates in a Feeding Situation

The rumen fill is a composite of residues from previous meals (Figure 5). The cow eats meals that peak about twice daily. Each meal adds new bulk to the rumen system, for which the digestion and passage rates will slowly dissipate.

Because of the laws of kinetic turnover (14) the rumen is an equilibrated balance of previous meals, their cumulative residues contributing to rumen fill (Figure 5). The oldest residues will exhibit the slowest rates of digestion while the most recent feeding will be the fastest. Each new meal presents faster fermenting substrate that dilutes the older slower fermenting substrates leading to an average rate which is the weighted mean of all previous meal residues (Tables 8 and 9). The rate may indeed undulate with feeding frequency. The net rumen balance of undigested NDF is likely the major factor determining feed intake. The effect of lag and delay in consumption of meals will largely compensate, since time between meals will be unaffected as will net fill.

The estimation of variable and declining digestion rates can be accomplished by estimating decline in k_f as a function of ΔU. Integration between narrow time limits will give k_f values that decline with time using Equation 6, which gives mean values of k_f between times t_1 and t_2.

$$6. \quad k_f = \frac{\ln(A_{wo,t-1}\, e^{(-k_w \times \Delta t)} + \Delta U_{t-1}) - \ln(A_{wo,t}\, e^{(-k_w \times \Delta t)} + \Delta U_t)}{\Delta t}$$

Where: $\Delta U_t = \Delta U \times \exp(-0.005 \times t)$

Table 7 shows the residual cell wall with time with no passage in order to calculate the retarded rate per 12 h time interval (column 4), assuming the animal is fed twice daily. Although cows may eat several meals a day, the rumen is not filled to a constant level, but instead probably undulates diurnally even when dietarily restricted (18). Mertens (2) noted a higher R^2 between estimates of fill and intake when estimates of fill allowed two meals a

day for the integration, as opposed to a constant fill. This was in the case of sheep fed ad libitum.

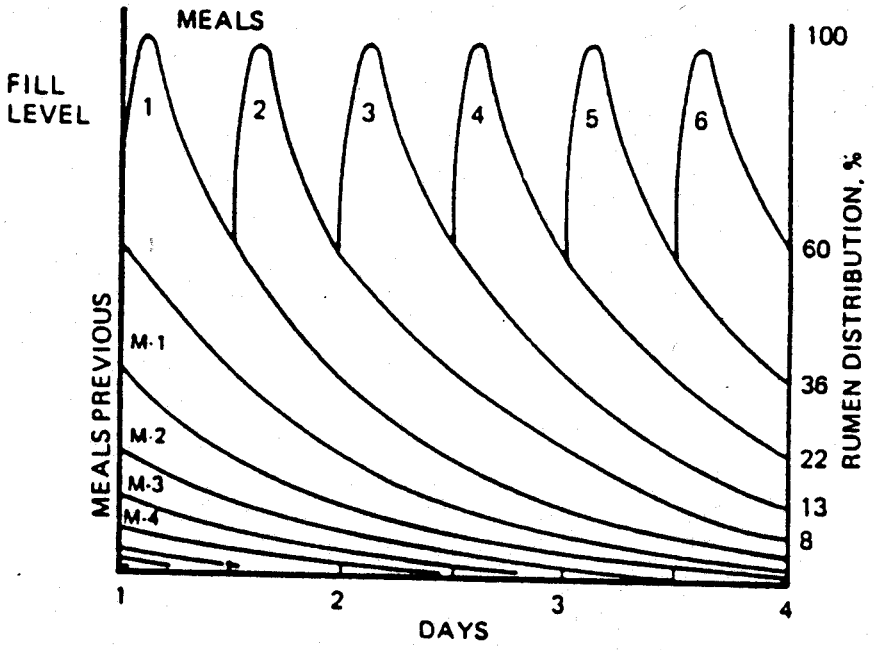

Figure 5. Rumen fill as a composite of undigested previous meals. Each curve represents the disappearance of a meal through digestion and passage. (From Van Soest (10), figure 23.1 page 372.)

Table 7 presents an example as to how declining rate can be fitted into the CNCPS model. The integration of previous meals and the context of a net rumen fill is a realistic feature, which has not been previously considered. It is suggested that this integration be calculated on a net daily basis. The values in Table 7 are unitary, as a percent of net available digestible NDF residue. For practical use the coefficients of net residue will need to be multiplied times net feed intake.

Digestible residues for each 12 h period are subjected to the escape equation: $E = k_p/(k_p + k_f)$ using the respective retarded rate and a rate of passage of 3.33% (30 h retention). The digestible residues are partitioned into digested and escaped fractions for each 12 h interval (columns 9 and 10). Undigested potentially digestible residues, column 6, are added to the residual indigestible fractions ($U_{2.4}$), respectively, (column 7) to obtain net residue per time period (column 8). The net passed (column 11) is the sum of escaped digestible and indigestible NDF. No lag function has been used in this simulation.

Column 12 indicates the order of previous meals, the residues of which are summed at the bottom of the table. The total of net residues is about 120% whereas 100 was the input, approaching a ratio of 30/24 h. The amount digested was 34.6% and 26.5% potentially digestible NDF was lost through escape. Net passed (65.3%) and net digested (34.6%) account for 99.9% of initial substrate. The calculation has been carried to 180 h. Selective retention has not been taken into account.

A mean value for rate of digestion has been calculated from the weighted average using data from Table 7 and shown in Table 8. The weighted average value is 5.06%. Alternatively, a value can be obtained by integrating between 0 and 30 h (retention time). This gives a lower value of 4.34%. The difference occurs because the faster fermenting pools at 12 and 24 h represent a proportionally larger portion of the rumen pool. Thus it seems necessary to calculate k_f per time period. The results (Table 8) show that retardation significantly affects rumen balance at practical levels of feeding. The calculated value of k_f is less than k_w because of the presence of older pools from previous meals in the rumen.

FIELD APPLICATION AND RECOMMENDATIONS

A possible system for introducing realistic rates of fiber digestion into the CNCPS model is presented. For alfalfa and grasses, rates can be predicted from 30 h in vitro true dry matter digestibility. No calibration set exists at present for corn silages. For these and other fibrous feeds for which rates of digestion data are unavailable, measurements will be needed from the field laboratories.

Measurement of digestion rates by field laboratories present problems. Some labs are currently doing 6, 12, 18, and 24 h sequences of fermentation. Twenty-four h are not long enough in time to ascertain a correct rate and an unavailable fraction U. For this purpose we suggest at least 6, 12, 18, 30, 48, 72 and 96 h measurements of NDF digestion. Longer times are needed for degradation of ΔU.

Although long times are beyond the mean retention time of the cow, measurements at long times of fermentation are needed to anchor the regression line that determines the rate (k_w). There are also significant amounts of digestible residue surviving the rumen after 24 h and integration beyond 100 h will be needed to account for rumen balance (Table 7). This model deals with NDF turnover. Further work is needed to account for non-NDF matter and liquid turnovers. Basic information for non-NDF turnover lies in the thesis of Cannas (19). This needs to be developed.

We are not suggesting the above sequences as a routine, but rather to build a pool of digestion data from which k_w and ΔU can be predicted as in Table 5. Here 30 h measurement will suffice. Thirty h digestion is approximately the retention time of a lactating cow eating at 3M. Note that a 24 h measurement falls short of this, and underestimates digestibility. This leads again to undervaluing the forage and overfeeding of concentrates in the model.

Table 9 shows the set of equations needed to employ the model in the CNCPS. Analytical data for using this model include NDF, ADF, lignin and 30 h in vitro true dry matter digestibility. Laboratories should use standard techniques (20) and use reference samples for interlaboratory comparison.

In vitro rumen digestion should follow the neutral detergent modification of Tilley and Terry using Ankom equipment or the original system of Goering and Van Soest (21). Buffer should be that of Goering and Van Soest (21) and not that from Kansas, which has inferior buffering capacity. If in situ bags are used, standards are mandatory and bags must be boiled in neutral detergent after digestion to remove microbial contamination.

Biological complexity induces complicated models. There is no reason why such complex models may not be employed, for in this day of computers the most complex integrations can be attained. These, however, require valid data which are treated in a mechanistic manner according to biological and physicochemical principles. There must be a warning to those who wish to simplify the system, which may lead to empiricism and a lack of understanding of how the real world works. For these reasons we have provided programmable equations for implementation of these mechanistic principles (Table 9).

As a personal note the senior author through all this effort has never touched a computer. All calculations have been on a pocket calculator. This method has always given him a close feel for numbers and the mathematics.

Table 7. Estimated retarded rates and the cumulated digested, escaped digestible, and undigested passed neutral detergent fiber for previous meals for alfalfa 8-1-6104. All residue values are percentages of initial substrate and rates (%/h).

Time (h)	Residual		Retarded Rate[1] (k_f)	$K_f + k_p$	Digestible Residue[2]	Residual $U_{2.4}$ after Passage	Net Residue	Amount Digested	Potentially Digestible Escaped	Net Passed	Previous Meals M
	A_{wo}	A_o									
0	45.96	61.07	-	-	61.07	38.93	100.00	-	-	-	-
12	16.59	30.82	5.70	9.03	20.66	26.11	46.77	25.50	14.90	27.72	M-1
24	5.99	19.39	3.86	7.19	8.72	17.51	26.23	6.42	5.53	14.13	M-2
36	2.16	14.78	2.26	5.59	4.46	11.74	16.20	1.72	2.54	8.31	M-3
48	0.78	12.67	1.29	4.62	2.56	7.87	10.43	0.53	1.37	5.23	M-4
60	0.28	11.48	0.82	4.15	1.56	5.28	6.84	0.20	0.81	3.40	M-5
72	0.10	10.64	0.63	3.96	0.97	3.54	4.51	0.09	0.50	2.23	M-6
84	0.04	9.96	0.55	3.88	0.61	2.37	2.98	0.05	0.31	1.48	M-7
96	0.01	9.36	0.52	3.85	0.38	1.59	1.97	0.03	0.19	0.98	M-8
108	0.00	8.81	0.51	3.84	0.24	1.07	1.31	0.02	0.12	0.65	M-9
120	0.00	8.29	0.50	3.83	0.15	0.72	0.87	0.01	0.08	0.43	M-10
132	0.00	7.81	0.50	3.83	0.10	0.48	0.58	0.01	0.05	0.28	M-11
144	0.00	7.35	0.50	3.83	0.06	0.32	0.38	0.00	0.03	0.19	M-12
156	0.00	6.93	0.50	3.83	0.04	0.22	0.25	0.00	0.02	0.13	M-13
168	0.00	6.52	0.50	3.83	0.02	0.14	0.17	0.00	0.01	0.08	M-14
180	0.00	6.14	0.50	3.83	0.02	0.10	0.11	0.00	0.01	0.06	M-15
Sum[3]					40.55	79.06	119.60	34.59	26.46	65.30	

[1] Retarded rate calculated according to Equation 4. Predicted value of k_w is 8.49% and observed value is 7.52. This rate includes a constant degradation rate of 0.5% for ΔU.

[2] Undigested available residue at time t calculated as residue from previous time period times $\exp(-(k_f + k_p)\times\Delta t)$.

[3] Excluding the zero time value.

Table 8. Calculation of mean rate of digestion of neutral detergent fiber.

Period	Rate[1] (%/h)	Amount Digested[2]	Product	Mean Rate[3]
0 – 12	5.70%	25.5	145.4	
12 – 24	3.86%	6.4	24.8	
24 – 36	2.26%	1.7	3.9	
36 – 48	1.29%	0.5	0.7	
48 – 60	0.82%	0.2	0.2	
60 – 180	0.52%	0.2	0.1	
Sum	---	34.6	175.0	5.06%
0 – 30[4]	---	---	---	4.34%

[1] From Table 7.
[2] Column 8 from Table 7.
[3] Mean rate = 175.0 ÷ 34.6
[4] Calculated from Equation 5.

Table 9. Set of equations to predict retarded degradation rate of neutral detergent fiber.

No. Equations

6. If $t = 0$, then : $A_{w0,\,t}(\%) = 100 - \Delta U - U_{2.4}$
   otherwise : $A_{w0,\,t}(\%) = A_{w0,\,t-1} \times \exp\left(-k_w \times \Delta t/100\right)$
7. If $t = 0$, then : $A_{x,\,t}(\%) = 100 - U_{2.4}$
   otherwise : $A_{x,\,t}(\%) = A_{w0,\,t} + \Delta U \times \exp(-0.005 \times t)$
8. $k_f\,(\%/h) = \dfrac{\ln\left(A_{x,\,t-1}/100\right) - \ln\left(A_{x,\,t}/100\right)}{\Delta t}$
9. If $t = 0$, then : $\text{Digestible Res}_t(\%) = A_x$
   otherwise : $\text{Digestible Res}_t(\%) = \text{Digestible Res}_{t-1} \times \exp\left(-(k_{f,t} + k_p) \times \Delta t\right)$
10. If $t = 0$, then : $\text{Residual U}_{2.4,\,t}(\%) = U_{2.4}$
    otherwise : $\text{Residual U}_{2.4,\,t}(\%) = \text{Residual U}_{2.4,\,t-1} \times \exp\left(-k_p \times \Delta t\right)$
11. If $t = 0$, then : $\text{Net Residue}_t(\%) = 100$
    otherwise : $\text{Net Residue}_t(\%) = \left(\text{Digestible Res}_t + \text{Residual U}_{2.4,\,t}\right)$
12. $\text{Digested}_t(\%) = \left(\text{Digestible Res}_{t-1} - \text{Digestible Res}_t\right) \times \left(\dfrac{k_f}{k_f + k_p}\right)$
13. $\text{Digestible Escaped}_t(\%) = \left(\text{Digestible Res}_{t-1} - \text{Digestible Res}_t\right) - \text{Digested}_t$
14. $\text{Net Escaped}_t(\%) = \text{Digestible Escaped}_t + \left(\text{Residual U}_{2.4,\,t-1} - \text{Residual U}_{2.4,\,t}\right)$
15. $\text{Amount Digested}_t(\%\,\text{DM}) = 100 \times \text{Digested}_t \times k_{f,t}$
16. $\text{Mean Rate of Digestion}\,(K_f, \%/h) = \dfrac{\text{Amount Digested}}{\text{Digested}} \times 100$

REFERENCES

1. Fox, D. G., T. P. Tylutki, M. E. Van Amburgh, L. E. Chase, A. N. Pell, T. R. Overton, L. O. Tedeschi, C. N. Rasmussen, and V. M. Durbal. 2000. The Net Carbohydrate and Protein System for evaluating herd nutrition and nutrient excretion: Model documentation. Mimeo No. 213. Animal Science Dept., Cornell University, Ithaca, NY.

2. Mertens, D. R. 1973. Application of theoretical mathematical models to cell wall digestion and forage intake in ruminants. Ph.D. dissertation. Cornell University, Ithaca.
3. Atkins, P. W. 1990. Physical Chemistry (4th Ed.). W. H. Freeman, New York, NY.
4. Waldo, D. R., L. W. Smith, and E. L. Cox. 1972. Model of cellulose disapperance from the rumen. J. Dairy Sci. 55:125-129.
5. Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70:3562-3577.
6. Mertens, D. R. and J. R. Loften. 1980. The effect of starch on forage fiber digestion kinetics in vitro. J. Dairy Sci. 63:1437-1446.
7. Fadel, J. G. 1984. Development of mathematical techniques to model ruminant fiber digestion systems. PhD Thesis. Cornell University, Ithaca.
8. Robinson, P. H., J. G. Fadel, and S. Tamminga. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim. Feed Sci. Technol. 15:249-271.
9. Chandler, J. A., W. J. Jewell, J. M. Gossett, P. J. Van Soest, and J. B. Robertson. 1980. Predicting methane fermentation biodegradability. In: J. Biotech. and Bioengineering Symp., 10, John Wiley & Sons. pp. 93-107.
10. Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant (2nd Ed.). Comstock Publishing Associates, Ithaca, NY.
11. Traxler, M. J., D. G. Fox, P. J. Van Soest, A. N. Pell, C. E. Lascano, D. P. D. Lanna, J. E. Moore, R. P. Lana, M. Vélez, and A. Flores. 1998. Predicting forage indigestible NDF from lignin concentration. J. Anim. Sci. 76:1469-1480.
12. Conrad, H. R., W. P. Weiss, W. O. Odwongo, and W. L. Shockey. 1984. Estimating net energy lactation from components of cell solubles and cell walls. J. Dairy Sci. 67:427-436.
13. Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39:95-110.
14. Van Soest, P. J., J. France, and R. C. Siddons. 1992. On the steady state turnover of compartments in the ruminant gastro-intestinal tract. Journal of Theoretical Biology. 159:135-145.
15. Van Soest, P. J. and R. H. Wine. 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. J. Assoc. Offic. Anal. Chem. 51(4):780-785.
16. Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. Applied Linear Statistical Models (4th Ed.). McGraw-Hill Publishing Co., Boston.
17. Licitra, G., P. J. Van Soest, I. Schadt, S. Carpino, and C. J. Sniffen. 1999. Influence of the concentration of the protease from *Streptomyces griseus* relative to ruminal protein digestibility. Anim. Feed Sci. Technol. 77:99-113.
18. Gill, M., A. J. Rook, and L. R. S. Thiago. 1988. Factors affecting the voluntary intake of roughages by the dairy cow. In: P. C. Garnsworthy (Ed.) Nutrition and Lactation in the Dairy Cow. Butterworths, London.pp. 262-279.
19. Cannas, A. 2000. Sheep and cattle nutrient requirement systems, ruminal turnover, and adaptation of the Cornell Net Carbohydrate and Protein System to sheep. PhD Thesis. Cornell University, Ithaca.
20. Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.
21. Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis: Apparatus, reagents, procedures, and some applications. Agric. Handbook No. 379. ARS, USDA, Washington, DC.