Corn Silage Neutral Detergent Fiber: Refining a Mathematical Approach for In Vitro Rates of Digestion

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INTRODUCTION

The digestibility of corn silage is affected by many factors such as hybrid, season, and processing. Explaining this variation is usually the job of the nutritionist with tools that are appropriate and cost-effective. Tools designed to help account for some of this variation are nutritional models such as the Cornell Net Carbohydrate and Protein System (CNCPS) and the Cornell-Penn-Miner (CPM) Dairy program. Inherent to the proper use of the CNCPS and CPM, the digestion rate for the carbohydrate and protein pools must be inputted and should be specific to the feeds that are being used.

At this meeting in 2000, Van Soest et al., discussed a new mathematical approach for the determination of rates of neutral detergent fiber (NDF) digestion. Since that time, we have conducted more long-term fermentations with emphasis on corn silages and continued to develop the mathematical approach for determining rates of NDF digestion. The objective of this paper is to describe the refined mathematical approach for determining rates of digestion and the application of this approach to in vitro corn silage NDF digestion data. A further objective was to develop a system that allowed for the determination of rates with a minimum number of time points.

The Need for Refinement

Since the inception of the CNCPS rumen submodel there has been discordance in the use of Merten's NDF rate data (Mertens, 1973) combined with the use of the 2.4 x lignin ($U_{2.4}$) to calculate the available NDF pool (Chandler et al, 1980). Inherent to the calculation of the rate of digestion is the available pool of fermentable substrate, which Mertens predicted mathematically from 96 hr in vitro data. Chandler's $U_{2.4}$ which is based on 90 to 120 day fermentations in a methane digester came after Merten's calculations of the unavailable fiber pool (U_m). However, the two approaches were utilized within the framework of the CNCPS rumen submodel and potentially resulted in both over and under predictions of ruminal digestion for poor and high quality forages, respectively.

Several factors relating to digestion rate and pool size were discussed in Van Soest et al., 2000 and will be further dealt with here. An observation from the previous work was that the rate of degradation of all the evaluated feedstuffs deviated from first order behavior and this was demonstrated by the observation of "retarding" rates of digestion with changes in ΔU. This has since been more fully understood and is actually due to the presence of two distinctly different pools of fermentable NDF with very different rates of degradation. Further the calculation of U was also discussed and it was suggested that the value of 2.4 as a constant did not appear to be feasible. This has not been completely resolved, however, data generated in our laboratory on 240 hr fermentations suggests the value is, on average, more robust than previously thought. Subsequently, for the purpose of calculating the available ND pool, the 2.4 x lignin value has been adopted for these derivations.

MATERIALS AND METHODS

To refine the calculations for determining rate of fermentation, long-term fermentations were necessary in order to more accurately evaluate the Chandler $U_{2.4}$ value. Additionally we wanted to start building a corn silage data set. Subsequently, 11 forages were chosen for the initial development set and included the first six corn silages in Table 1. Forages were dried in a forced air oven (60° C) and ground through a 1 mm screen in a Wiley Mill. Fermentations up to 96 hr were carried out in 125 ml Erlenmeyer flasks in a 39 °C water bath under constant CO_2 in Goering and Van Soest buffer (1970) and inoculated with rumen fluid from a cow fed hay and grain. Rumen inoculum was not blended and was strained through four layers of cheesecloth and filtered through glass wool to aid in removal of very small particles that would affect the recoveries of indigestible material. Blank samples were run for all time points and used to correct any contamination from the rumen fluid inoculation.

The same cow has been used for all fermentations. Fermentations from 96 to 240 hr were conducted in 250 ml centrifuge bottles using the procedure above except, every 72 hr the samples were centrifuged, the supernatant was poured off, and the sample was re-inoculated. Overall, fermentations were carried out for 6, 12, 24, 30, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hr. Neutral detergent fiber analysis was conducted with amylase, but without the use of sodium sulfite (Van Soest et al., 1991). In addition, the ND residues were filtered in crucibles that contained approximately 4-5 grams of sea sand in order to provide a filtering aid and prevent the loss of very small particles. Lignin was determined from AD residues hydrolyzed in 72% sulfuric acid for 3 hrs (Van Soest et al., 1991).

Data and Mathematical Methods

To estimate the rate of digestion the data must be in the form of declining values of available substrate A with time. This satisfies the requirements of chemical kinetics where the rates apply to the amount of A_s at any time. The choice of time points coincides with the mean rumen retention times associated with normal production and intake levels (6-36 hr). Further, to obtain the available NDF substrate (A), the lignin x

2.4 ($U_{2.4}$) was subtracted from the ND residues at each time point.

Table 1. Composition of forages set used for long-term fermentations, mathematical development and evaluation.

Forages	NDF	ADF	Lignin	Ash
	------------------ % of Dry Matter -----------------------			
Wheat straw	81.1	55.9	10.61	3.6
Alfalfa Ramon	51.4	40.7	7.72	8.4
Alfalfa Pell	31.3	24.3	5.31	7.8
Timothy '68	81.6	49.7	7.88	4.8
Timothy '93	63.7	37.1	4.62	5.3
Corn silages				
1 (BMR)	48.7	26.7	1.94	3.1
2	43.4	24.1	2.72	4.0
3 (BMR)	39.4	21.4	1.50	3.1
4	40.3	22.1	2.71	3.3
5 (BMR)	49.6	28.5	3.49	2.8
6	46.2	26.4	2.67	3.8
7	41.4	23.6	2.51	2.8
8	47.8	28.3	2.89	4.1
9	50.1	29.6	3.99	3.7
10	39.8	23.8	2.51	3.5
11	46.9	28.8	3.79	5.5
12	43.5	26.5	3.55	5.3
13	46.6	27.7	3.08	3.0
14	52.3	30.7	3.73	2.6
15	48.0	27.6	3.06	3.0
16	46.2	26.1	2.77	3.6
17	51.5	30.2	3.86	2.9
18	45.6	26.8	2.92	4.0
19	39.9	22.6	2.61	3.0
20	38.7	22.3	2.61	3.1
21	47.3	28.2	2.90	2.8

Rates (k_d) obtained from the 6-36 hr range are composites of the respective pools and are in the form currently used in the CNCPS/CPM models. In the work conducted in our lab, the earliest useful time point for NDF digestion has been the 6 hr value and that is used in the lag calculation. Earlier times < 3 hr were not useful because the fermentation had not stabilized.

The first-order differential equation states that the decrease in available substrate per time (t) is governed by a rate constant (k) times the available substrate A:

Equation 1: $$\frac{-dA}{dt} = kA$$

Integration of equation 1 gives

Equation 2: $$\int_0^1 \ln A_0 - \ln A = k\,(t - T_L)$$

where A_o is initial substrate, t is time of fermentation and T_L is lag.

A_o is set to unity (A_o = 1) and values of A are calculated by subtraction of $U_{2.4}$ = 2.4 x lignin contents of NDF from substrate S and divided by initial $S_o - U_{2.4}$.

Equation 3: $$A = \frac{S - U_{2.4}}{S_o - U_{2.4}}$$

Then the value of $\ln A_o$ in equation 2 becomes zero and falls out of the equation.

Equation 4: $$- \ln A = k\,(t - T_L)$$

Equation 4 is converted to logarithms:

Equation 5: $$\ln(- \ln A) = \ln k + \ln(t - T_L)$$

Since lnA is a negative number -lnA becomes positive and its logarithm can be taken. The requisite equations for lag and for rate k are derived from equation 5.

Calculation of Discrete Lag Time

Lag time (T_L) is the amount that needs to be subtracted from fermentation time (t) to produce equality in equation 6. The requisite equation 6 is derived by subtracting equation 5 from itself as the difference between two fermentation times. The logarithm of the six hour time and its value of available substrate $\ln(-\ln A_6)$ are subtracted from later times $\ln t_n$ and $\ln(-\ln A_n)$. The six-hour value used in the above calculations must be in equilibrium with the kinetic rate and, therefore, later than the discrete lag time. The ln k is eliminated in the subtraction.

Equation 6: $$\ln(t_n - T_L) - \ln(6 - T_L) = \ln(- \ln A_n) - \ln(-\ln A_6)$$

Equation 6 is solved for T_L: There are at least three different algebraic solutions to equation 6. All give the same numerical values for T_L. However, the one given here avoids negative numbers, and is easiest to use and is more user friendly, although it is not the simplest algebraic form. The critical steps that distinguish this solution are as follows:

Antilog transformation:

Equation 7: $$\frac{t_n - T_L}{6 - T_L} = \frac{\ln A_n}{\ln A_6}$$

Substitute δ for $\frac{\ln A_n}{\ln A_6}$

Equation 8: $$\delta = \frac{t_n - T_L}{6 - T_L}$$

Multiply by ($6-T_L$).

Equation 9: $$6\delta - T_L\delta = t_n - T_L$$

Rearrange: subtract 6δ and T_L; multiply through by –1.

Equation 10 $$T_L\delta - T_L = 6\delta - t_n$$

Factor out T_L and divide by (δ -1).

Equation 11 $$T_L = \frac{6\delta - t_n}{\delta - 1} \quad \text{where} \quad \delta = \frac{\ln A_n}{\ln A_6}$$

Calculation of rate (k_d)

Rearrangement of equation 5 gives:

Equation 12 $$\ln k = \ln(-\ln A) - \ln(t - T_L)$$

The logarithm of rate k is the difference between ln (-lnA) and ln ($t-T_L$) for any time point. Thus the rate k can be estimated from a single fermentation value. The rate of digestion (k_d) is calculated by substituting the determined value for lag from equation 11 into equation 12. The k_d is taken as the antilog.

Application

A sample calculation for T_L is given for the corn silage 1 (Table 2). The 6 hr value for A is 0.905 and was used as the six-hour base and δ was calculated in Table 2 according to equation 11. The four values of T_L were averaged to give a mean value of 4.21 hours and used in the 6-hour time point to calculate a k_d. Values of time minus the respective lag and the residual value of A were substituted into equation 12 to calculate ln k_d. The value of k_d is taken as the antilog.

Table 2. Time point fermentation data and calculations from corn silage 1 based on 6 to 36 hr fermentations.

Time, h	S[a]	A[b]	δ	Lag, h	kd, %/h
6	0.895	0.884	---	(4.21)	6.91
12	0.594	0.551	4.82	4.43	7.87
24	0.322	0.250	11.17	4.23	7.02
30	0.260	0.182	13.77	4.12	6.59
36	0.215	0.132	16.38	4.05	6.34
			Means:	4.21	6.95

[a]Neutral detergent residues as a proportion of the initial NDF
[b]Unitized values of S calculated according to equation 3. $U_{2.4}$ = 0.956

Various Time Point Calculations

The time-point calculation allows the calculation of k_d at a single time observation, except that lag calculation will require two-time point values. However, if an average lag value is used the single point calculation can be made. An evaluation of the overall time point calculations, and three different approaches are found in Tables 3 and 4. For the single point 24-hour calculation and the two-point (12 and 24 hr) calculation, a constant 3 hr lag was used. The use of a 6 hr digestion value allows for the calculation of lag, which is then used to calculate a 24 hr k_d in the third column. All time points (6-36 hr) are used to calculate the k_d values in the last column. The correlation between the use of the complete data (6 to 36 hr) with the alternative calculations was r = 0.97 with a coefficient of variation of 7%. The 24 hr time is advantageous because it lies in the middle of the first pool and is a time that field laboratories will find convenient.

The use of an average 3 hr lag does not appear to introduce any great error. If a forage had a true lag less than 3 hr use of the constant value will slightly enhance the k_d. If the true lag is longer than 3 hr use of the constant lag results in a slightly lower value of k_d. Thus the variation of k_d caused by use of a constant lag is consistent with forage quality. During the course of our evaluations we found feeds that calculated to have negative lags. Obviously those values are not kinetically possible. Under those circumstances we first recommend checking your undigested ND values for errors. If the undigested residues are appropriate, then you must set the lag to zero. Based on the data we have generated to date, a negative lag implies that the sample digested faster in the first 6 hr than at later times. From the single point 24 hr k_d determination of the corn silages, varying the lag from 0 to 5 hr results in deviation of ±12% relative to the use of the 3 hr fixed lag.

Table 3. Rates of fermentation k_d (%/hr) of selected forages based on various time point calculations, 24 hr with fixed lag, 6 and 24 hr with variable lag, 12 and 24 with fixed lag and 6 – 36 hr with variable lag and the calculated lag from 6-36 hr.

Forage	24 hr k_d, fixed lag, (3 hr)	6 and 24 hr k_d variable lag	12 and 24 hr k_d fixed lag (3 hr)	6 – 36 hr k_d, variable lag	Lag, hr
Alfalfa 83	5.48	5.51	5.27	5.02	2.74
Alfalfa 93	7.70	7.19	9.43	8.19	1.64
Timothy 68	2.96	2.82	2.52	2.27	0.57
Timothy 93	6.28	6.28	6.89	6.59	2.87
Orchard grass	3.22	3.31	3.19	3.26	3.45
Wheat straw	1.88	1.72	1.97	1.74	1.20

Table 4. Rates of fermentation k_d (%/hr) of corn silages based on various time point calculations, 24 hr with fixed lag, 6 and 24 hr with variable lag, 12 and 24 with fixed lag and 6 – 36 hr with variable lag and the calculated lag from 6-36 hr. Known BMR varieties are noted.

Corn silages	24 hr k_d, fixed lag (3hr)	6 hr and 24 hr k_d, variable lag	12 and 24 hr k_d fixed lag (3hr)	6 – 36 hr k_d, variable lag	Lag, hr
1 (BMR)	6.61	7.02	6.62	7.10	4.21
2	5.23	5.40	5.47	5.67	3.77
3 (BMR)	6.59	6.85	6.93	7.25	3.87
4	4.81	4.87	5.12	5.02	3.23
5 (BMR)	4.57	4.67	4.26	4.29	3.22
6	6.15	6.45	5.78	6.05	3.82
7	5.09	5.26	4.98	4.99	3.45
8	4.57	4.48	4.48	4.22	2.29
9	4.79	4.85	4.96	4.84	3.13
10	4.56	4.57	4.58	4.37	2.72
11	4.73	4.71	4.06	3.87	2.09
12	4.47	4.50	4.58	4.26	2.83
13	4.35	4.50	3.74	3.66	3.09
14	3.33	3.24	3.48	3.17	2.24
15	3.71	3.22	4.38	3.50	0.06
16	3.20*	2.88	3.91*	3.67	0.00
17	4.02	3.76	4.42	3.83	1.42
18	4.95	4.56	5.43	4.53	1.00
19	3.96	3.62	4.23	3.50	0.72
20	3.96	3.52	4.63	3.69	0.31
21	3.22	2.90	3.90	3.59	1.59

*For these calculations, lag was set to zero.

However, some field labs are doing 30 hr fermentations, which can also be used in the estimation of k_d. Thirty hr is later in the fermentation sequence, and as a result the k_d values for higher quality forages will be underestimated because by this time the very fast k_1 is nearly exhausted and the rate becomes more influenced by the slower k_2. There are 20 comparisons between mean k_d and 30 hr k_d. The regression of mean k_d (Y) on 30 hour k_d (X) is Y = 1.20 x – 0.63. Values in the range of 3-5% are comparable, but above 6% will require adjustments. A 48 hr value cannot be used because it is near the inflection point of the fermentation curve into the slow pool of ND residue. It is very advisable that some standard forages be made available to the various laboratories.

Since the Cornell model currently uses $U_{2.4}$, the values determined herein are more consistent with current use. Further, this indicates that current tabular values in the model generally over value the digestibility of most forages and seriously so with lower quality forages.

DISCUSSION

Mathematical equations and procedures are presented that allow direct calculation of lag time and rate of digestion (k_d). For rate determination, a single time point allows calculation of k_d. Lag requires two time points, although an average lag of 3 hours can be used with a single point to calculate k_d. The time-point calculation of k_d represents the slope of the logarithmic disappearance curve at a particular time. These procedures have the advantage of requiring minimum data that may be available from field laboratories. Since the calculations are direct and use no statistical regression procedures, they are simpler to implement because many observations are not required. If a number of time point digestions are available, means and standard deviations of the respective lag and rate values can be calculated and their uniformity examined.

Some comparisons of k_d using this mathematical procedure to the values found in Mertens's thesis have been made, but are not shown here. Rate of digestion values of about 12% in Mertens thesis are about 10% using this new mathematical approach. At the lower ranges, k_d values from Mertens at 6% are about 3% in the determinations. Thus the proportional drop in rates estimation is much greater for low quality forages. The drop occurs because of the change in estimated $U_{2.4}$. The value of U affects the rate number mathematically, the lower the U the lower the k_d estimates. Lower values of U yield lower rates because the fermentable pool is increased: Mertens' values of U are the residual near 96 h and contain considerable fermentable substrate from the second pool (Van Soest et al. 2002). The use of the $U_{2.4}$ decreases the estimate of ultimate extent and is largely responsible for the lower determined values. Further, among the corn silages, the relationship between the k_d and $U_{2.4}$ was r = – 0.66.

The k_d needed for the Cornell model covers the range in time from 6-36 hr, the time period for the main digestion of NDF in the rumen. The k_d is the average of these time points. Since the $U_{2.4}$ is used in the Cornell model these revised values are appropriate. The k_d values over the 6-36 h period are composites of fast and slow NDF pool rates. The new values expand the range in k_d which the book values do not reflect.

CONCLUSIONS

Mathematical procedures are presented for the direct calculation of lag and rate of digestion k_d. Rate of digestion can be calculated from a single digestion value and lag from two values at different times. Calculation of k_d from a single time point (24 hr) using a constant average lag of 3 hours gives a very close estimate of the average k_d, and would allow easy calculation in the field if NDF, lignin and 24 hr in vitro digestion of NDF were available.

Comparison of the directly determined k_d in the data of Mertens (1973) gives lower values for k_d than those of Mertens. Values of k_d are proportionally lower for mature forages. Low quality forage has been overvalued by previous systems. This occurs because of the overestimation of U and the existence of a second pool. These lower rates would place a greater penalty upon mature forages when these rates are used in the Cornell model.

The high correlations between all of the parameters - lag, rate and ultimate extent validate Merten's original conclusions. However, the direct algebraic approach taken in this paper is easily done on a hand calculator, and allows direct calculation from individual fermentations.

Recommendations for Use

1. We recommend that labs using this mathematical approach conduct some 6 hr fermentations in order to determine the lag inherent to their system. This will help establish the appropriate data points required to make accurate calculations of k_d. Further, we have not evaluated the effect of grind size on these calculations and data exists that demonstrate grind size will affect rate of digestion. Our work is based on 1 mm grinding in a Wiley Mill.

2. To date none of the data used in our k_d calculations have come from an ANKOM Daisy System, thus the influence of the system on lag has not been evaluated.

3. If you are conducting multiple time point fermentations and calculate a negative lag, first check your values and if they are patent, set the lag to zero.

4. The use of standards or reference samples among runs and between laboratories should be considered.

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