FUTURE TRENDS IN FORAGE ANALYSIS

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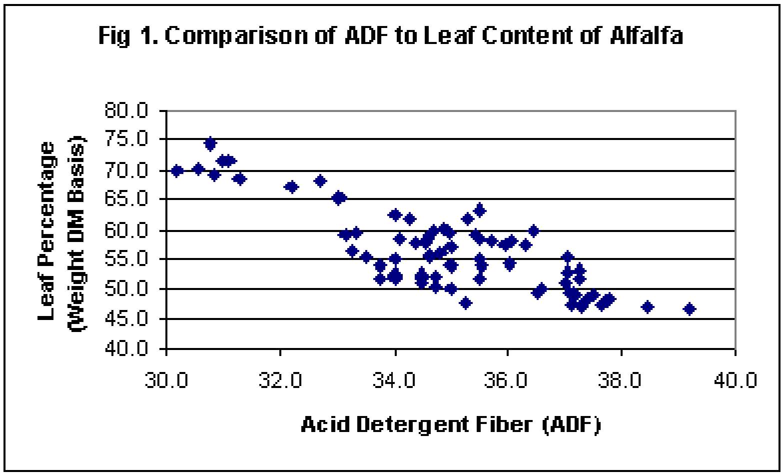
Introduction

Forage analysis is critical to balancing rations for desired level of milk or meat production on a least cost basis. It simply is not possible to judge forage quality by visual appearance. Many individuals are fooled by appearances, thinking if a forage appears similar to something fed previously, the quality is similar. They are often disappointed when animal performance is not as expected. Some of our data in figure 1 indicates how deceptive this can be, as acid detergent fiber content varied but up to 4 units at similar leaf contents. In addition to composition changes, moisture can vary which affects the purchase price and proportions of ration ingredients used in a TMR.



At the same time, nutritionists have become concerned about the accuracy of current forage analyses. They are having considerable difficulty balancing rations and having the herd respond as predicted. This problem has become more acute as higher herd production has increased the need for greater precision in testing ration components.

The lack of animal response to forage analysis relates to the chemical estimates of energy and protein availability that we have used for the last 30 years. While these tests have served us well at lower levels of animal production, it is important to recognize their limitations. The limitations consist of poor relationship between fiber or protein estimate and digestibility, lack of uniform chemical tests, and variation in energy prediction equations from fiber content.

We have become so accustomed to predicting energy from acid detergent fiber that we seldom go back and look at the original relationship to consider its accuracy. The digestibility and TDN relationships were developed by simply developing empirical regression equations of fiber vs digestibility as shown in figure 2. **The original relationships explained about 70% of the variation in forage digestibility** (note that 30% of the variation was not explained). However, when we went to laboratories and collected legume/grass samples that farmers had submitted, we

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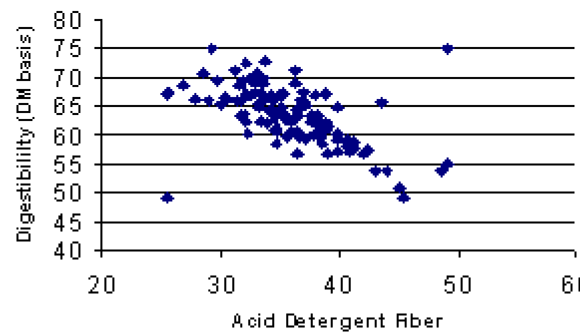
found that ADF explained only 55% of the variation in digestibility. The much lower relationship between ADF and digestibility is likely due to the fact that the original data was based largely on research samples from University Experiment Stations that did not contain the full range of effects seen on farms. I think all will agree that this relationship is too poor to use in today's high producing herds.

Fig 2. Comparison of ADF to in vitro Digestibility of Alfalfa



A second consideration is that such regression data is really only appropriate to apply to the same kinds of samples as were used to develop the relationship. The equation most commonly used to predict TDN was based heavily alfalfa with some alfalfa/grass samples included. Thus it is not appropriate to use the ADF to TDN or digestibility relationship from other regions, elevations, or, especially, for other forages. However, this is commonly done.

A second method is estimate energy of forages is to use summative equations proposed originally by Goering and Van Soest (1970) and, more recently, by Conrad (1984) or Weiss et al. (1992). These do a much better job of predicting energy from multiple chemical analysis rather than analysis of a single component. However these have never been well accepted because of the cost and time involved in doing the multitude of analyses required for such equations. Beware of laboratories claiming to use these equations and then estimating (or using book values) for components other than protein and fiber. When estimates rather than actual analysis are used, the output of the equation will be no better than from using a single component as above.

A third method of analysis is to estimate energy by measuring digestibility. In vitro or in situ digestibility are the methods of forage analysis most closely related to animal performance. While these methods are good research tools, they are not to use for routine forage analysis. These methods are costly and time consuming. Further there is significant run to run and laboratory to laboratory variation. This means the technique is useful for comparing samples run within a batch but less so for results compared over time.

We have also completed analysis of forage samples determining the protein digestion in the rumen vs by-pass protein (rumen undegraded protein). None of the chemical tests currently in use have correlations of higher than 0.50 with in situ degradation of protein.

Another issue with chemical analysis is the lack of uniform analysis procedures. When I came to Wisconsin and surveyed nine laboratories that did forage analysis for farmers of the state, each used a different procedure for dry matter determinations. While things have improved in Wisconsin, the same situation exists nationally. There is no way that similar results can be achieved with all the different procedures. If dry matter is wrong all the rest of the analysis is wrong. Similar differences exist among labs for ADF analysis procedures, especially NDF analysis procedures, and crude protein analysis. The National Forage Testing Association is a national organization dedicated to improving forage quality testing. Table 1 reports laboratory performance data from 1995 (most recent year of such data).

RMA is reference analysis, the procedure exactly as described in AOAC or the best recommendation based on research. These laboratories and have close agreement of analysis among laboratories. Note however that of 86 laboratories participating nationally only 8 to 10 were running the reference method. All others had some procedural deviation from the recommended method. These laboratories had different mean values for analysis and significantly higher stand deviations. Near infrared reflectance (NIR) predictions had similar means and standard deviations as the general wet chemistry because most laboratories have developed their own NIR prediction or biased existing equations to match their wet chemistry.

Table 1. Comparison of Chemistry and NIR Check Sample Results in National Forage Testing Association (NFTA) 1995 Certification.

Statistic	Dry Matter	Crude Protein	ADF	NDF
Alfalfa Hay				
NFTA RMA[1]	92.87	17.82	33.90	39.45
NFTA RMA Std[2]	0.45	0.26	0.46	0.45
Chemistry Average, 86 laboratories	90.99	17.40	33.69	41.16
Chemistry Std	13.14	0.94	1.22	1.86
NIR Average, 44 laboratories	92.90	17.71	32.96	40.66
NIR Std	0.99	0.64	1.51	1.82
Corn Silage				
NFTA RMA	91.66	10.64	29.14	43.17
NFTA RMA Std	0.52	0.34	0.21	0.91
Chemistry Average, 86 laboratories	90.06	10.35	28.93	44.15
Chemistry Std	12.80	0.62	1.49	3.02
NIR Average, 45 laboratories	89.93	10.69	28.55	45.90
NIR Std	12.11	0.70	1.74	3.72

[1] Reference Method Average of 8 to 10 laboratories performing standardized analysis
[2] Std refers to standard deviation of values within the data set.

Note that the NIR mean and standard error for alfalfa dry matter are very close to those used by reference method laboratories. This occurred because a standard equation was released for dry matter determination and illustrates the possibility for NIR. NIR can measure water directly and very accurately while chemistry procedures generally measure water by weight loss on drying. The weight loss can have high variability and other components can be lost, especially from silages.

The third problem with the current chemical analysis system is that different laboratories and nutritionists use different equations to predict TDN or NE_L. This means that the same forage fiber analysis can be sent to different forage testing laboratories or nutritionists and predicted to have different energy values. Further, all equations are based on prediction of energy at maintenance with standard adjustments made for level of feeding. The one thing that we are sure of is that forages do digest at different rates and any constant adjustment from maintenance to a higher feeding level (rate of passage) will be wrong in many cases. Such differences are causing great confusion among individuals involved in the forage testing industry.

The potential exists to remedy most of these problems by judicious use of near infrared reflectance spectroscopy (NIR). NIR has and advantage of being a wavelength range where it can actually 'see' organic compounds, such as cellulose, starch and protein. Further, it can detect physical differences of the forage where chemical tests cannot. These two factors combine to make NIR a powerful tool for forage analysis. All of the discussion above indicates that we need to go to more direct animal estimates of performance rather than chemical estimates. Due to animal variability across animals and over time, the only economically feasible way we get closer to direct animal estimates is to use NIR to estimate digestion of compounds or fractions within the forage.

Near infrared reflectance spectroscopy was introduced in the early 80's and was vastly oversold in terms of its capabilities. Results were disappointing. The first problem was that each laboratory had to develop its own equations because instruments were not standardized. This meant that all the chemistry problems mentioned above were involved in equation development. Second, the first NIR instruments and associated equation development processes were relatively unsophisticated compared to the instruments and techniques used today.

Changes in Near Infrared Reflectance Instrumentation and Procedures

Major improvements in NIR technology have occurred in the last 5 years. The first improvement has been the standardization of instruments. Previously, if the same sample was read with the same equation on two different NIR instruments, each instrument would predict different output values due to instrument variation. In 1991, Martin *et al*. showed that instruments could be standardized electronically, and the standardization maintained, among several commercial forage testing laboratories. This development means equations could now be developed and distributed to standardized instruments with very repeatable results. Previously, best results came from each laboratory developing it's own equations (meaning that equations reflected the procedural differences and errors from individual laboratory wet chemistry). It also limited equation development for new parameters because there could be no shared effort (and expense).

The second improvement in NIR has been the development of new methods for predicting results. Until a few years ago equations were developed by using stepwise regression to select a few wavelengths with the highest correlation to the factors of concern and using these wavelengths for prediction. The problem with this procedure was that as the breath of sample was increased, accuracy of prediction was lost. New software (ISI Version 3.0, 3.1) by Infrasoft

International use PLS software to develop equations. This equation development software involves more wavelengths than previously and greatly improves predictive characteristics over broader ranges of samples. Equations developed with this software can be much more robust than previously and still maintain the desired accuracy.

The next generation of software (ISI version 4.0) works from a database rather than equations. The procedure is to read a sample, have the computer select the most similar spectra from the database and develop an equation to predict analysis values of the specific sample. A new equation is developed for each sample. Prediction accuracy with this approach is limited only by the range samples in the database.

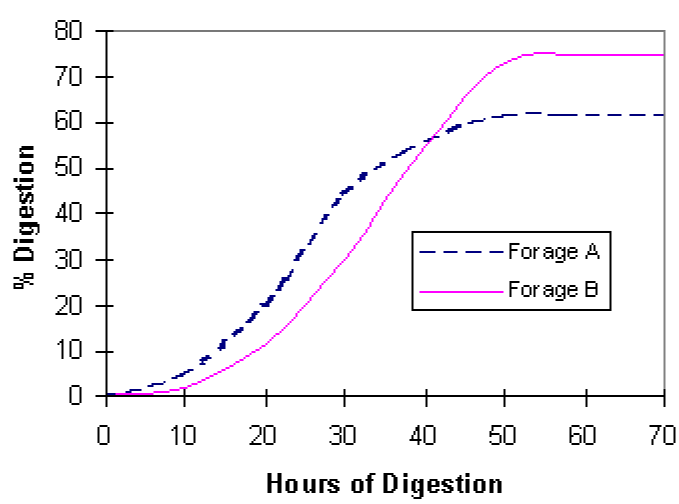
These changes mean that NIR can now predict chemistry of forages better than before and that equations can be developed and shared across laboratories. The latter is critical as the cost of equations for new determinations is great and can only be afforded if several laboratories share the cost of development.

New Developments in Analysis for Ration Balancing

We have known for many years that *in vitro* or *in situ* estimates of forage digestibility relate better to animal performance on a forage than using fiber to estimate animal performance. The problems with using *in vitro* or *in situ* methods to determine digestibility are that the procedures are expensive, slow, and show variation from run to run. The latter is acceptable for research where comparisons can be made within a run but not for analysis of farmer samples were the same result must be obtainable week to week.

Further, it is important to recognize that actual digestibility in the animal relates both to the forage and to rate of passage. Digestion of forage A shown in figure 3 would be about 58% for beef cattle or dairy heifers were rumen retention is about 48 hours but only 40% for a dairy cow where forage stays in the rumen about 30 hours. Forage B is a second alfalfa hay with different digestion kinetics; it digests more slowly but to a greater extent. Forage A is best if fed to dairy cattle and forage B is best if fed to beef cattle or dairy heifers. These differences can be described where rate of digestion is determined.

Fig 3. Digestion of Two Forages Over Time



We at the University of Wisconsin have developed and released to forage testing laboratories, NIR equations for digestibility, involving digestion kinetics. Energy available for ruminant animals from a forage depends largely on the rate rumen microbes digest the forage (as well as rumen retention time). Therefore an estimate of rate of digestion would most accurately predict animal performance across the wide range of animal categories and feeding and performance levels. We believe that the best procedure for the long term is to estimate rate of digestion (k) plus the totally digestibly fraction (A fraction), partially digestible fraction (B fraction), and undigestible fraction (C fraction) (figure 4). These four factors will allow calculation of digestibility for the specific animal type and conditions under the ration is being fed. Development of NIR *in situ* rate of digestion equations is a major undertaking that will require 3 to 4 fistulated animals and one full-time technician working for 2 to 3 years. However this change, when implemented will far better characterize the diversity of forages fed to cattle than current analyses.

Digestibility = 1 - ($B^{-k(t-lag)}$ + C)

where:
B = potentially digestible fiber
C = indigestible fiber
k = rate of digestion
lag = lag time to beginning of digestion
t = time of digestion

NIR equations can also directly predict animal performance. This approach has been used in Northern Ireland where digestion and intake studies were done with sheep and the NIR used to predict farm samples based on calibration to the sheep trials. It would be the best approach worldwide but is extremely expensive.

Rumen undegraded protein (RUP, bypass protein) is an extremely important consideration in both fast-growing beef cattle and high-producing dairy cattle. There is no current widely accepted method of accurately estimating RUP differences in forages. We have developed an NIR equation for estimation of rumen undegraded protein in forage based on *in situ* digestion of protein from forage. These equations have not been available previously because individual laboratories could not afford to maintain the animal and equipment necessary for digestibility determinations.

As the data in figures 4 to 6 show, neither soluble protein nor acid detergent fiber nitrogen have a very high correlation with in situ bypass protein (0.5 and 0.25, respectively). Near infrared reflectance, on the other hand, was able to predict in situ rumen undegraded protein with a correlation of .95.

Fig 4. In situ rumen undegraded protein vs phosphate soluble crude protein

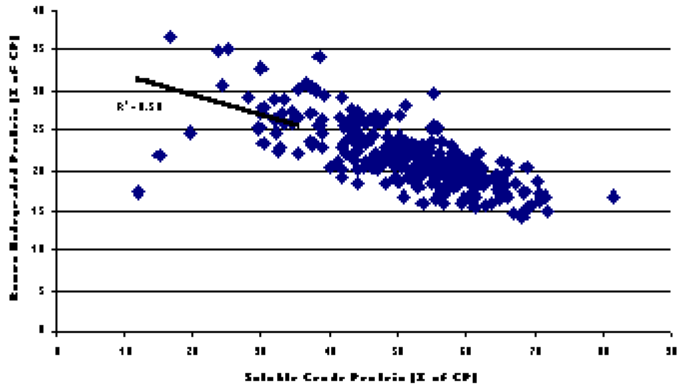


Fig 5. In situ rumen undegraded protein vs acid detergent fiber nitrogen

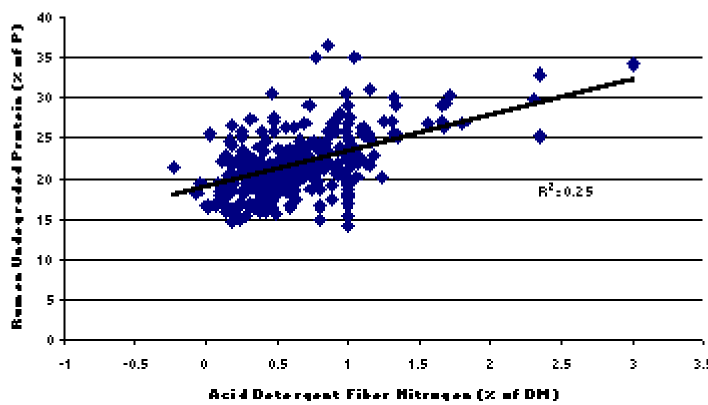
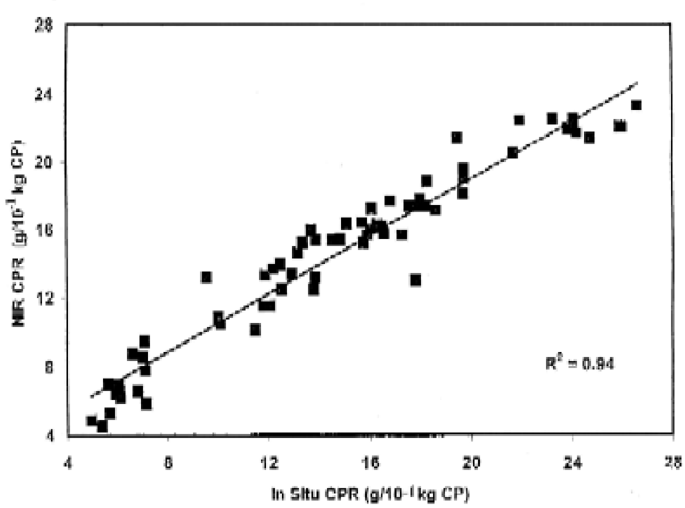


Fig 6. NIR Estimation of In Situ RUP



Future tests will also allow

measurement of individual amino acids, to determine if they are limiting for high-producing cows.

Other tests are being developed to measure additional feed factors important to animal performance. For example, it may be possible to determine particle size of the forage or TMR by NIR. This would reduce the analysis cost to the farmer and increase use of particle size determinations in animal ration balancing.

Another new test we are currently are using for research purposes is to predict the grass percentage in a legume hay or haylage. This test can be a double check to determine if fiber values appropriate. It can also give a nutritionist information about other parameters to modify when balancing a ration. This parameter could also be of use in marketed hay, as the buyer often is interested in knowing percentage of grass in the alfalfa hay being sold. Additionally, the test could also be used to determine whether or not stands are too grassy to keep or in need of herbicide.

The grass percentage determination is also an example of the totally new use of forage analysis to make production management decisions. Alfalfa fields with high grass percentage may justify spraying to remove the grass for future cuttings of dairy feed. It may also be used as an indication of when to plow down alfalfa fields and replant. On the other hand, pasture with low legume percentage would suggest the need to seed legume back in.

The leaf to stem ratio of alfalfa can also be determined by NIR. This can indicate forage quality but also relates well to marketability. NIR is also able to determine color of the hay. This can be useful because color determinations are currently very subjective, depending on the individual, light quality that day, and storage situation.

We have also been able to determine if certain diseases are present in alfalfa. This may be useful to the grower to determine if fields should be replanted due to the yield limiting ability of the disease.

Presence of certain insects can be determined. When determined in the hay, the insect has already done its damage. However, it may be useful to know that the insect has occurred and should be watched more carefully for in the next cutting.

In summary, forage testing is changing dramatically. Not only is near infrared reflectance better able to accurately estimate of standard parameters, such as ADF, NDF and crude protein but it is the only technique that can approximate animal performance on a feedstuff due to its ability to directly measure both organic compounds and physical parameters of the forage. Thus it will help farmers by being able to provide many additional tests results from the forage in a cost effective manner. This additional information will improve ration balancing and add production decision aids.

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