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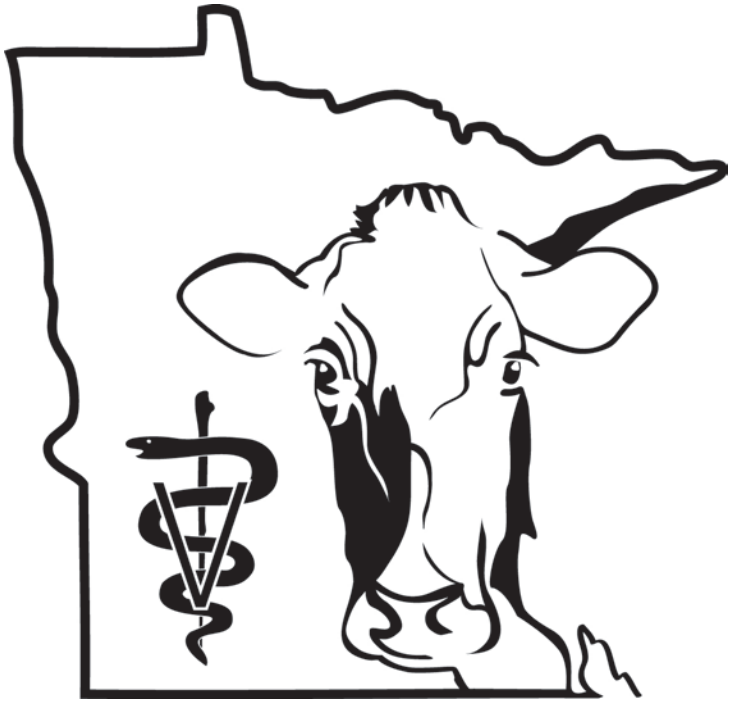

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New Developments in Analytical Evaluation of Forages and Total Mixed Rations

Patrick C. Hoffman
Associate Professor
Department of Dairy Science
University of Wisconsin-Madison

Introduction

There are many new advancements in the analytical evaluation of forages and total mixed rations (**TMRs**). Despite new advancements, the world of "forage testing", as we commonly refer to it, has probably never been in a greater state of confusion. A plethora of concerns and myths exist. This paper will attempt to address new advancements in forage testing and ration evaluation as well as address concerns and myths with analytical procedures and utility of forage evaluation systems.

Summative Energy Equations

The amount of energy a forage contributes to a ruminant diet is arguably the single most important factor in predicting animal performance. It is the author's impression that nutrition consultants and dairy producers have lost confidence in the ability of forage testing systems to predict energy content of a forage. The author commonly hears "I ignore the predicted energy values" when asking nutrition consultants their perspectives on the issue. In the past this perspective was somewhat valid. Empirical equations (Rohweder et al., 1978) were used for many years to predict forage energy content from a single analyte such as acid detergent fiber (ADF). Empirical equations to predict forage energy content by and large were accurate but imprecise. The aforementioned statement simply means that when examining a large data base of forage energy contents predicted by an empirical equation, the empirical equation accurately predicts the average of the data base but cannot precisely predict the energy content of any single forage in the data base. To be of real value, forage testing systems must be able to precisely predict the energy content of any single forage.

Weiss, 1996 proposed using a summative approach to predict energy content of forages. The concept of a summative approach is simple: measure the principal components in the forage that contribute energy, give each component a digestion coefficient, multiply each component by its respective digestion coefficient, and add the products together. The beauty of a summative approach is that it can be used on any forage, grain, commodity, or even total mixed rations. The major drawback of the summative approach is extensive laboratory measurements are needed. Four principal components need to be accurately and precisely measured in the laboratory: crude protein (CP), neutral detergent fiber (NDF), fat, and non-fiber carbohydrate (NFC), which additionally require the measurement of ash and neutral detergent fiber crude protein to facilitate the final determination of NFC. The digestion coefficients assigned to CP, fat, and NFC are well defined by research (Weiss, 1993); however, the digestion coefficient for NDF (NDFD, % of NDF) is not well defined by research and thus requires, if possible, measurement in the laboratory.

An exhaustive discussion of summative energy equations is available (Weiss, 1996; NRC, 2001) and is beyond the scope of this paper. An example of a summative energy equation adopted by the NRC, 2001 to predict the energy content of a legume grass silage is presented in Table 1. The reader should be aware the summative equation concept presented in Table 1 has been modified for corn silage (Schwab and Shaver, 2001) with a detailed discussion of those modifications available in these proceedings.

Quantitative vs Qualitative Summative Equations

Simply using a summative equation to predict forage energy content in no way guarantees precision of the forage energy prediction. The old axiom of garbage in equals garbage out still applies. To generate quantitative summative equations that most precisely predict forage energy content requires the laboratory to accurately and precisely measure all of the subcomponents (CP, Fat, NDF, Ash, NDFCP and NDFD). The CP, NDF, and fat content of a forage can be accurately and precisely measured using a near infrared reflectance spectrophotometer (NIRS) (Hoffman et al., 1999; AACC, 1987). Measuring NDFCP is more problematic for NIRS (Hoffman et al., 1999), but the overall influence of NDFCP on summative energy equations is small, therefore a qualitative assessment of NDFCP using NIRS is acceptable and will not greatly compromise the accuracy and precision for the summative energy prediction of the forage. Perhaps most critically the laboratory must measure NDFD (% of NDF) and ash with the highest degree of accuracy and precision possible because they both have a major influence on forage energy content.

Measuring Ash

The measure of ash content of a forage is difficult with NIRS because NIRS is physically unable to detect inorganics (Shenk, 1994). NIRS may be able to associate organic compounds in a forage as related to species or maturity and make an indirect association to endogenous ash content, but data are limited. Even more problematic is exogenous ash in forages. Exogenous ash is dirt, mud, soil, sand, bedding, etc., which somehow, but frequently, get into the forages. Exogenous ash contents can be high in forages, especially forages stored on earth, gravel or in bag and bunker silos. At the Marshfield Soil and Forage Analysis Laboratory, 100 legume/grass silage and hay samples averaged 8.4 % ash with a minimum, maximum, and standard deviation of 5.5, 22.1, and 2.7 % ash, respectively. Because ash has no energy, whatever the measurement error of ash is results in exactly the same prediction error in the forage energy content. Simply stated, if the forage ash content is missed by 5.0 percentage units of DM, the energy prediction is missed by 5.0 TDN units, which is unacceptable precision in predicting forage energy content. Fortunately, endogenous and exogenous ash content of a forage can be economically, accurately, and precisely measured in a laboratory by combustion in a 500° C oven for 2 to 3 h. Utilizing combustion ash determinations ultimately results in a more accurate and precise determination of NFC in the forage, of which NFC is one of the principal components in the summative energy equation.

Measuring NDF Digestibility

Similarly, accurately and precisely predicting the NDFD content of the forage NDF is extremely important in generating a quantitative summative forage energy prediction. Unfortunately NDFD is one of the more difficult assays to conduct in the laboratory. Most laboratories cannot conduct the assay because an in vitro NDFD laboratory procedure requires rumen fluid from a live cannulated cow.

Forage NDFD can be measured in one of two ways. First, forages can be placed in small dacron bags and inserted into the rumen of a cow via a ruminal cannula. The amount of NDF prior to ruminal incubation is compared to the amount of NDF remaining after ruminal incubation and NDFD is calculated. This is called an in situ method. The in situ method is a very viable method to estimate NDFD of forage NDF and is often used in research and other forage evaluation programs. Because of the lack of a large uniform database, the 2001 NRC, however, does not recommend the in situ method as its basis for NDF of forages.

The 2001 NRC uses lignin and NDF concentrations to predict potential NDF digestibility or advises the use of an in vitro system as the basis for direct determination of forage NDFD. Again, advised use of an in vitro system was not made based on analytical superiority over the in situ system, rather the in vitro NDF digestibility data base was larger and more uniform, making interpretation easier. An in vitro NDF digestibility determination (Goering and Van Soest, 1970) is conducted as follows: 1) feed is weighed into a glass flask, 2) buffers, macro- and micro-minerals are added along with rumen fluid extracted from a cow fit with a ruminal cannula, 3) the forage, buffers, and rumen fluid are incubated in a water bath in an anaerobic environment (carbon dioxide) at a cow's body temperature (102° F) for 48 hours, 4) the flask containing the forage, buffers, and rumen fluid is removed from water bath and the remaining solution is refluxed in NDF solution for 1 hour, 5) after refluxing in NDF solution for 1 hour the remaining solution is filtered and the NDF that resisted digestion by rumen bacteria is retained on the filter, and 6) digestible NDF is calculated by difference.

Few changes have been made to the in vitro NDFD assay over the years, but some researchers and laboratories have reduced the incubation times from 48 hr to 30 or 24 hr, claiming that shorter incubation times better describe the digestion potential of NDF in high producing lactating dairy cows. Reducing the incubation time of the in vitro NDFD assay to 30 or 24 hr is logical because feed is not retained in the rumen of a high producing dairy cow for 48 hr. In the larger sense, however, this issue is somewhat irrelevant because changing the incubation time of the assay reduces the amount of NDF digested; therefore, NDF digestibility values obtained from 30 or 24 hr digestions cannot easily be compared to NDF digestibility values listed in the NRC, 2001. The recommendation of a 48 hr NDFD value by the NRC, 2001, is to facilitate calculating TDN content of forages at maintenance intakes (which is TDN). The most important issue with NDF digestibility at this time is for laboratories to report forage NDF digestibilities that have a common scale and reference. Because the NRC, 2001 advises the use of a 48 hr in vitro NDF digestibility procedure to calculate TDN contents of forages at maintenance intakes, it is most logical to identify with the 48 h NDFD reference and scale.

The NDFD values in the NRC, 2001 however, were not measured values per se; they are calculated using lignin and NDF content of forage as primary independent variables and there is controversy whether NDFD values calculated from lignin, 48 h NDFD or 30 h NDFD values are the best values to use to calculate forage TDN content. At present it is critical for any user of NDFD values to thoroughly understand the universe of which they were created. Listed in Table 2 are typical NDFD (% of NDF) values as measured in a 48 h in vitro incubation. Substituting wet chemistry in vitro 48 h NDFD values into summative energy equations can increase the accuracy and precision of forage energy estimates but may slightly overestimate the contribution of digestible NDF because of the long incubation time. Using 30 h incubations to estimate the energy contribution of NDF may better represent the true digestibility of whole forage NDF at maintenance but the error rate of the NDFD assay increases with shorter incubation times and NDFD values can become despairingly low if the analytical system for NDFD is not perfect.

The NDFD content of a forage can be predicted using NIRS, but generally there is some loss of precision. Combs (1998) has used NIRS to predict in vitro 48 h NDFD contents of legume grass forages with success. The NIRS NDFD equations developed by Combs (1998) are commercially available and are currently being used in commercial forage testing laboratories. Development of accurate and precise NIRS equations for the NDFD content of corn silage has proven more problematic because of the narrow range of NDFD in corn silage and the heterogeneous nature of corn silage (Lisa Baumann, 2000-2002, personal communication).

Ultimately, prediction of NDFD in forages by NIRS would be preferred because laboratories using NIRS prediction systems can be easily standardized. It is likely that large data bases of forage NDFD contents will be required to facilitate accurate and precise measures of forage NDFD by NIRS. Such projects are in progress and therefore it is likely that prediction of NFDF in forages using NIRS will improve in the future.

Typical NDFD, 48 h values for forages evaluated at the Marshfield Soil and Forage Analysis Laboratory are presented in Figure 2. In general, legumes have less total NDF and lower NDFD values due to greater lignification as compared to grasses. Grass silage and hays have a very wide range of NDFD values because grass species are so diverse and are utilized at extreme ranges in maturity (e.g., grazing vegetative grass versus feeding straw). Corn silage can have a wide range of NDFD but it is uncommon to see the extremes because corn silage is harvested and stored at a relatively narrow range of maturity. Extremes in corn silage NDFD can occur when corn silage is harvested late at an over-mature stage (NDFD = low) or in well managed brown midrib mutant varieties (NDFD = high).

Summative Energy Equations for Forages
Qualitative or Quantitative?

Nutrition consultants can chose between qualitative and quantitative summative energy predictions in forages. In general, rapid (1 day), low cost evaluation of forages using only NIRS will result in a qualitative prediction of forage energy content because some precision is lacking in predicting the NDFD and ash content of the forage, resulting in more variance occurring in the energy prediction. Examples of laboratory procedures yielding qualitative (summative) energy predictions for alfalfa silage and corn silage are presented in Tables 3 and 5. Qualitative energy

predictions are adequate for routine screening of forages, but when special situations occur, such as problematic diets, feeding new season forages, animal health problems, switching dietary forage sources, etc., a quantitative assessment of forage energy content should be made. In this case (presently), the NDFD and ash content of the forage should be evaluated wet chemistry to increase the accuracy and precision of the energy prediction. The author's choice of methods to facilitate a quantitative assessment of alfalfa silage and corn silage energy content is presented in Tables 4 and 6.

Listed below is a set of guidelines to follow if a quantitative assessment of forage energy content is desired.

1) Use NIRS to predict CP, NDF, Fat, and NDFCP. Using NIRS for these nutrients is accurate and precise enough and keeps the overall cost of the evaluation down.
2) Evaluate the ash content of the forage using combustion techniques. This assures ash is predicted precisely and/or exogenous ash is not missed in the evaluation. The result is a more precise prediction of NFC.
3) Evaluate the NDFD (% of NDF) content of the forage using a 30 or 48 h in vitro assay at a laboratory with proven records that NDFD values are reasonably relevant to the NDFD values listed in the NRC, 2001. Not all in vitro NDFD analyses are suitable for summative equations.
4) Laboratories conducting in vitro NDFD assays should report a minimum of 2 NDFD standards with each in vitro NDFD report. The reporting of NDFD standards helps the user gain confidence in the NDFD values of unknown forages submitted for analysis. The 48 h in vitro NDFD report of the Marshfield Soil and Forage Analysis Laboratory is presented in Table 7. Note that BMR corn silage, bud alfalfa, and full bloom alfalfa standards are run and reported with each unknown sample. When such standards are reported it is easy for the user to compare the unknown NDFD content of their forage to other recognizable forages.
5) Do not request or expect fast turnaround times from your laboratory. Quantitative summative energy predictions are special tests that can take 3 to 4 days to conduct. Be willing to wait longer and pay for quantitative summative forage energy predictions.
6) Make sure the laboratory can accommodate the mixture of analysis into their reporting system.
7) Work openly with your laboratory and ask a lot of questions.

Evaluation of Total Mixed Rations

In the author's opinion, summative energy prediction systems have the greatest utility in the evaluation of total mixed rations. The author realizes that sampling and laboratory analysis of TMRs is controversial. One of the greatest concerns with laboratory evaluation of TMRs is sampling error. Recently, Hutjens, 2002 warned against TMR sampling error and suggested evaluating TMRs via wet chemistry for DM, CP, and ADF to determine accuracy of mixing. The recommendation of Hutjens, 2002, is logically conservative but overlooks the great potential to use a precision summative technology to estimate of TMR energy content as compared to relying on commonly fabricated computer generated ration energy contents. The Marshfield Soil and Forage Analysis Laboratory now offers the first precision summative estimate of TMR energy contents. The system is relatively simple with CP, NDF, Ash, Fat, NDFCP and 48 h in

vitro NDF digestibility of the TMR evaluated in duplicate via wet chemistry procedures, thus minimizing potential lab error. The energy content of the TMR is then estimated using NRC, 2001 summative models and precision estimates are achieved because lab error is tightly controlled. An example report of the Marshfield Soil and Forage Analysis Laboratory TMR-Quality Control Report is presented in Table 8. As previously stated, criticism of laboratory evaluation of TMRs is speculation that sampling error is high, although no data are available to substantiate this speculation. To assess TMR sample error, the author extracted random raw data of laboratory analyses conducted on a static research trial TMR over a 7 day period (Hoffman and Esser, 2001). The author selected these data because the diet was static (no feed changes), was sampled by the sample technician, and laboratory analysis was likewise conducted by the same technician. Thus the variation observed in Table 9 is mostly sampling error. While empirical, the standard deviation (**SD**) of nutrients in the TMR is relatively small and appears to be of minor concern (Table 9). Likewise, the normal variation of nutrients in a field sampled TMR which was sampled in triplicate is presented in Table 10. Again, the variance of nutrients due to sampling error appears small and workable under most field nutrition settings. The data in Tables 9 and 10 can be compared to data in Table 11 which contains nutrient profiles of 20 TMRs from individual dairy herds evaluated using the TMR-Quality Control procedures at the Marshfield Soil and Forage Analysis Laboratory. The TMRs in Table 11 were extracted from the TMR data base at the Marshfield Soil and Forage Analysis Laboratory and TMRs containing 27.0 to 27.9% NDF were purposely selected. As selected, all TMRs in Table 11 contain approximately 27.0% NDF, but other nutrients vary widely including energy content (NE_L), which ranges from .71 to .79 Mcals/lb. The reader is reminded that there is little chance of laboratory error, due to the use of precision wet chemistry laboratory methods. The variation of TMR nutrients in Table 11 far exceeds the TMR sampling variation observed in Tables 9 and 10; therefore numerous TMR diets in Table 11 are likely incorrectly formulated or fed. In addition, it should be noted that high group lactating cow diets containing a common 27.0 to 28.0% NDF can vary dramatically in dietary energy content. More research is needed on the normal relative sampling errors associated with TMRs. For the first time, however, the dietary energy content of a TMR can be systematically predicted if proper laboratory procedures are used. The precision summative TMR evaluations are, however, slow (1 week minimum) and expensive to conduct (≅$50.00). Dairy producers and nutrition consultants should not confuse precision summative TMR analysis systems with other common TMR testing systems. Evaluation of TMRs using NIRS or with TMR energy estimates made using empirical equations or book values are of limited value. Listed in Figures 1 to 4 are the ranges of NE_L, NDF digestibility, phosphorus, and ash observed in high group TMR evaluated using precision summative technology.

Finally, it is the author's opinion that laboratory evaluation of TMRs for energy density using precision summative technology is infinitely superior to ration energy estimation using most current ration balancing techniques.

Other New Developments in Forage Evaluation
Bypass Protein

Recent work from our laboratory (Dorshorst et al., 2000; Hoffman et al., 1999a, b, c) has demonstrated that NIRS can predict (R^2 = .87) bypass protein (3 x maintenance) content of legume grass silages (Hoffman et al., 1999c) and legume grass hays (Dorshorst et al., 2000).

The NIRS system to predict bypass protein of these forages was developed using a calibrated cow in situ technique and was then converted to NIRS techniques. The NIRS evaluation system is commercially available, but has limited use in field applications because the sample cannot be microwave dried because of protein matrix alteration due to overheating. Very good bypass protein numbers can be generated for legume/grass hays or silages if samples are dried at 55° C, then evaluated using bypass protein using NIR systems.

pH

Some laboratories now routinely offer the prediction of pH in ensiled forages using NIRS. Reeves et al., 1989 observed that NIRS could predict silage pH, but prediction was somewhat imprecise. The actual utility of silage pH is somewhat vague, but could be used as a screening tool to conduct further silage fermentation analyses.

Silage Fermentation Analysis

Similar to silage pH, silages can be evaluated for fermentation profiles which generally include pH, acetic, lactic, butyric, propionic (acids) and ammonia (NH_3). Silage fermentation analyses are generally done using high pressure or gas chromatography although some labs use NIRS on undried, unground samples which has been demonstrated to be feasible (Reeves et al., 1989). Silage fermentation analysis can be used to trouble shoot silage fermentation problems, assess potential dry matter intake problems, or evaluate silage inoculant performance.

Conclusions

There have been a number of new advancements in analytical evaluation of forages. Nutrition consultants and dairy producers need to be aware that these analytical advancements often exceed the program capabilities of commercial forage testing laboratories. To take advantage of these new analytical advancements, nutrition consultants and dairy producers should work closely with their laboratory to eliminate false expectations. Listed below are some general guidelines and concepts to keep in mind when working with any forage testing laboratory.

1) The old NIR vs wet chemistry argument is a moot point in modern forage evaluation. NIRS is an excellent tool for many nutrients, but not all nutrients.
2) Expect to pay more and wait longer for quantitative (precise) forage energy predictions.
3) Rapid, low cost forage evaluation systems are routine screening tools. It is difficult for any laboratory to provide accuracy and precision of every nutrient under these conditions.
4) Explain to your laboratory exactly what you are looking for and design a forage evaluation system to meet your needs. Be willing to pay more and wait longer for custom or high precision forage evaluation systems.
5) Do not underestimate the importance of providing a good forage or TMR sample to your laboratory for analysis.
6) Because a laboratory can run an assay does not guarantee the results of the assay have a utility.
7) Evaluate TMRs using precision summative technology.
8) Be aware of forage and TMR testing gimmicks. Ask for research data to support a particular forage testing system.

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