INTERPRETATION OF FORAGE ANALYSIS REPORTS

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ABSTRACT

Forage analyses are a valuable tool for determining the economic value of forages and for formulating efficient and profitable rations. To be useful, forage analyses must be determined accurately and interpreted correctly. The sample submitted must truly represent the feed being analyzed and the forage analyses must provide information that is relevant. There are reliable and repeatable methods for measuring moisture, protein, fiber, total ash, fat, and mineral concentrations in feeds. However, forage quality depends upon more than the concentration of nutrients. The true nutritive value of a forage is related to the performance of the animal that consumes it. Thus, forage quality is determined not only by the amount of nutrients it contains, but also by feed-animal interactions associated with intake, digestibility, and metabolic efficiency. Intake, digestion and utilization by the animal cannot be measured routinely; therefore, laboratories use equations that are based on chemical composition to estimate these important attributes of the feed. *In situ* and *in vitro* methods can be used to estimate digestibility, but results are often variable. Research is needed to obtain *in vivo* intake and digestibility observations using lactating dairy cows that can be used to calibrate and evaluate equations and methods for estimating the digestibility and intake of feeds.

Key Words: alfalfa, hay quality, forage testing, feeding, nutritive value, NDF, ADF

INTRODUCTION

Feed analysis can be an extremely helpful tool to both the forage producer and the forage user. For the forage producer, forage analysis can be used to assess management practices and provide a quantitative measure of nutritive value. For the forage user, feed analysis provides many of the inputs needed to formulate rations so that the forage is used effectively. For both, feed analysis provides information useful in determining the economic value of the forage. When properly done and correctly used, forage analysis is a cost effective way for improving decisions about purchasing and using forages. It is difficult to understand why individuals are unwilling to spend $10-30 to obtain information that can improve the utilization of a lot of forage that may be worth 100 to 1000 times as much. If feed analysis information is inaccurate or not used, then the cost of analysis is truly wasted. However with accurate results and proper interpretation, feed analysis information can be a effective tool for improving forage evaluation and utilization.

The nutritive value of a feed represents the productive response of the animal to which it is fed. In reality, the productive worth of a feed cannot be expressed by any single laboratory measurement. Chemical analysis of a feed can provide information about its energy and nutrient content. However, the animal's productive response is not only a function of the amount of

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nutrients and energy in the feed, but also the animal's intake, digestibility and metabolic efficiency.

The ultimate evaluation of a forage is the performance of the animal that consumes it. Unfortunately, animals cannot be used to evaluate feeds routinely. Therefore, laboratories have to use chemical, physical, and biological measurements to estimate the productive response by animals. Feeds can be analyzed for a wide variety of nutrients, chemical components, biological fractions, and physical properties. Before addressing the question of how to interpret forage testing results, one has to address the question of which analyses should be requested.

Although no single feed analysis can indicate the total nutritive value of feeds, the components of feeds can be ranked in order of their nutritional and economic importance. Historically, forages have been the major and most economical source of energy in ruminant diets. Thus, the amount of available energy in forages has been, and probably still is, the most important nutritional information expected from forage evaluation. With current prices of forages, grains, and byproduct feeds, forages may not be the most economical source of energy. Under these circumstances forages play a critical role in supplying the minimum fiber required in dairy rations. Thus, fiber analysis provides useful information in determining the value of forages in dairy rations. Forages are often a major source of protein in animal diets; therefore, protein content is an important component in feed evaluation. Current concern about the excretion of excess nitrogen and minerals by animals indicates that mineral analyses may become an important component of feed evaluation. In unique situations, additional analyses can play crucial roles in defining the nutritive value of feeds.

FACTORS AFFECTING NUTRITIVE VALUE

The animal's productive response to a feed is a function of the feed's nutrient and energy content and feed-animal interactions associated with intake, digestibility and metabolic efficiency. Chemical and physical analyses can accurately measure nutrient and energy contents, but there are no laboratory methods for directly measuring the potential intake, digestibility (bioavailability), and metabolic efficiency of the nutrients in a feed. Thus, important nutritional information like intake potential, digestibility and net energy must be estimated based on chemical and physical analyses. These biological attributes of the feed are estimated most accurately when there are causative relationships between them and the chemical and physical characteristics that are analyzed.

It has been estimated that about 50-75% of the differences in productive response of forages is related to intake, 25-50% is related to digestibility, and 5-15% is related to metabolic efficiency. Thus, intake potential of a forage is its most important biological attribute, but it is also the attribute that is most difficult to measure because intake results from a complex interaction between the animal and feed. When high-energy diets or feeds are fed (that are adequate in protein and minerals), the animal eats to meet its requirement for energy and feed intake potential is limited by the animal's energy demand. To determine the intake potential of high quality forages, the energy demand of the animal must be very high so that the animal is not limiting intake potential. Alternatively, the differences in the intake potential among animals can

be determined using a reference feed and used to adjust observed forage intakes (Osbourne et al., 1974). On the other extreme, when low energy diets or feeds are fed (that are adequate in protein and minerals), the animal eats until it meets its gut capacity to hold or process the feed, and intake potential is limited by characteristics of the feed itself.

In general, there is a positive relationship between forage intake and digestibility. Forages with high digestibility typically have high intake potentials. Although feed intake and digestibility are interrelated, the mechanisms by which feed characteristics affect each biological response is quite different. The primary factor affecting the intake of low quality forages is their cell wall content. In routine feed analysis, plant cell walls are measured as neutral detergent fiber (NDF). Cell walls or NDF determines the volume of the feed and it capacity to fill the rumen. In addition, fiber is selectively retained in the rumen and must be extensively ruminated before it can pass out of it. Mertens (1992) observed that the maximum amount of NDF that dairy cows can consume and process through the digestive tract and obtain maximal production is about 1.2% of their body weight (BW) per day. Thus, the potential intake of a forage (that also maximizes animal performance) can be determined by dividing the animal's NDF intake capacity (BW X .012) by the fraction of NDF in forage dry matter. This calculation represents the forage's intake potential, but the animal's energy demand and physiological status can cause actual intake to deviate significantly from the intake potential. The difficulty of separating a feed's intake potential from the actual intake that is obtained by animals with a specific (but unknown) energy demand is one of the reasons that intake has been neglected as factor in forage quality even though it is the most important factor affecting animal performance.

Much of the focus in feed evaluation has been on the digestibility (degradability, availability) of feeds, especially as it relates to energy. Digestibility can be measured in animals much more accurately than can intake potential. However, characteristics of the animals and the methodology of digestion trials can have a significant impact on the actual measurement of *in vivo* (in the animal) digestibility. Although there are differences among animals and species of animals, the level of intake allowed during the digestion trial is the major factor affecting the measurement of digestibility (Tyrrell and Moe, 1975). As the level of intake increases, the time feed remains in the digestive tract decreases and digestibility declines. Although much debate is generated about the use of sheep to measure digestibility for cows, in fact, a young sheep eating at the level of 3-4% of its body weight per day may have a digestibility closer to that of a lactating cow, than a mature cow or steer that is eating at the level of 1% of body weight per day.

Another major factor in measuring digestibility is the degree of selection of the feed that is allowed during the digestion trial (Zemmelink, 1980). If forages are fed in excess to allow refusals, animals will tend to select the feed that is offered and eat a fraction that typically is higher in protein and lower in fiber than the original feed. This can cause problems when analyses of the feed offered is related to the digestibility of the feed actually consumed. The *in vivo* measurement of dry matter (DMD) or organic matter (OMD) digestibility that is most repeatable occurs when mature animals are fed at a maintenance level of intake. Digestibility measured at 1X maintenance intake is the starting basis used by the NRC (1989) to estimate energy availability in terms of total digestible nutrients (TDN) and net energy for lactation (NEL). The actual measurement of NEL is even more complex than measuring digestibility

because all of the energy consumed, deposited, and excreted (urine, feces, milk, body heat) must be measured.

It is obvious that digestibility, TDN, or NEL cannot be routinely measured during forage analysis. How are the TDN and NEL results reported by forage testing laboratories actually generated? These values are estimated or calculated using equations that relate chemical composition to DMD, TDN or NEL. The equations were developed using statistical regression techniques to relate digestibility measured during research trials to chemical analyses. This approach provides relevant information about nutritive value that can and has been used to evaluate feeds and formulate diets more accurately. However, like any technique that is based on prediction, using regression equations has pitfalls and inadequacies that can affect reliability and applicability. The errors associated with predicted digestibility fall into three broad categories: how well animal digestibility was measured; how well the set of samples with digestibility data represent the population of all forage samples; and how well digestibility is related to laboratory analysis?

The species of animal, production status, level of intake and degree of selection can affect digestion. In addition, the techniques used during the animal trial to actually measure digestibility can have an impact. Total collection and sampling of feces is more accurate than using internal or external markers to measure digestibility. The number of animals used and length of time during which digestion is measured can also affect the accuracy of the average digestibility that is observed. Digestibility is not an inherent characteristic of feeds like protein or fiber content. It is a dynamic response of the animal to the feed that can vary depending on the circumstances under which it is measured.

In many experiments, the set of samples with measured digestibility is narrow and reflects only the effect of date of cutting and maturity of the first growth as the main sources of variation in forages that were evaluated. Variation associated with forage species, differences among first and regrowth cuttings, and environmental effects of climate, soil, and growing season, differences in fertilization and fields often are not included. These problems can be overcome by combining sets of samples from various experiments, regions of the country, species of forage, and dates and sequences of cuttings that reflect current management practices. Most of the equations used by forage testing laboratories were derived from relatively diverse sets of digestibility experiments, but the data is relatively old. These equations need to be tested for accuracy using observations that are obtained from digestion and performance trials with dairy cows that reflect current and future levels of production.

Perhaps the greatest source of error in estimating digestibility from laboratory analysis is the statistical relationships between digestibility and chemical components in the feed. It appears that the major biochemical and physiological factors limiting digestion are associated with the plant cell wall or NDF in the feed. Research shows that most of the indigestible or slowly digesting components of a feed are contained in cell walls or fiber. The variable digestibility of fiber is related to factors such as lignin, silica, intrinsic chemical characteristics of cellulose and hemicellulose, three-dimensional structure and crosslinking of cell wall components, and rates of fermentative digestion, particle size reduction and passage. Because the factors that limit digestion reside in fiber, it is logical that fiber fractions are most highly correlated with

digestibility measurements. This explains why digestibility and net energy are predicted from acid detergent fiber (ADF) or NDF concentrations in feeds.

Plant cell walls (as measured by NDF) are the most important single feed characteristic determining nutritive value because of its impact on intake, digestibility, and metabolic efficiency. However NDF often obtains a poorer relationship with DMD than ADF presumably because ADF contains a higher concentration of the indigestible components in cell walls or fiber. Poor relationships between NDF and DMD are due to the highly variable digestibility of NDF. Plant cellular contents or neutral detergent solubles (NDS) include proteins, fats, starches, soluble carbohydrates and organic acids all of which are essentially completely digestible (Van Soest, 1994). It follows that the problem in predicting DMD is that of estimating NDF digestibility. Goering and Van Soest (1970) proposed the use of a summative equation to predict DMD that is based on the biological principle that NDS and NDF have distinctly different digestibilities:

$$\%DMD = -12.9 + 0.98*\%NDS + Dc*\%NDF;$$

where –12.9 is the endogenous loss of DM during digestion, 0.98 is the relatively constant true digestibility of NDS and Dc is the variable digestibility of NDF. Conrad et al. (1984) and Weiss (1993) expanded the summative equation by separating the NDS fraction into various components and providing functions or constants to describe the digestibility of each. They estimate the variable digestibility of NDF using a complex function of lignin and NDF:

$$\%TDN = -7.0 + 0.93*CP + 0.98*NFC + 2.25*(EE-1) + .75*(NDF_{CP} - L)(1 - (L/NDF_{CP})^{2/3});$$

where CP = crude protein, NFC = 100 - NDF_{CP} - CP - EE - Ash); EE = ether extract; NDF_{CP} = NDF corrected for crude protein; and L = lignin.

The use of chemical analyses to estimate digestibility or energy availability has clear limitations. First, only known relationship between chemical components and digestibility are used. Secondly, when fiber is the chemical component used to measure digestibility there is an implicit assumption that all fiber has the same digestion coefficient. This can be demonstrated by solving the Goering and Van Soest (1970) summative equation for NDF:

Given: %NDS = 100 – %NDF,

Then: $\%DMD = -12.9 + 0.98*(100 - \%NDF) + Dc* \%NDF,$

$\%DMD = -12.9 + 98 - 0.98*NDF + Dc *\%NDF$

$\%DMD = 87.1 - (0.98 - Dc)*NDF.$

The coefficient for NDF (0.98 – Dc) in the solution indicates that the regression coefficient for NDF (or ADF) in regression equations for predicting DMD is actually the difference between the true digestibility of NDS (or ADS) and a constant average digestion coefficient (Dc) that is implicitly assumed for fiber.

Ruminal microorganisms are affected by all of the factors (known and unknown) that limit digestibility of fiber. Differences in fiber digestibility are the primary variable that determine differences in DMD, and most fiber digestion (70-100%) occurs in the rumen. Consequently, it should be evident that ruminal digestion can measure digestibilities with greater accuracy than any chemical component(s). There are two major ruminal techniques that can be used to determine ruminal digestibility or DMD directly. In *in situ* systems (sometimes called *in sacco* or nylon bag techniques), feeds are suspended in the rumen of fistulated cows inside indigestible

nylon or Dacron bags. In *in vitro* systems (sometimes called artificial rumen systems) feeds are fermented in tubes or flasks using microbial inoculum that is obtained from fistulated cows.

There is debate about which ruminal digestion system is better. Each has advantages and disadvantages. It is argued that the *in situ* system is more similar to actual digestion determined in the animal because the ruminal contents are not placed in an artificial environment. But the *in situ* system is somewhat artificial in that the diet of the fistulated cow does not represent the feeds that are suspended in the bags, and the bags are an artificial environment themselves. The advantage of the *in situ* system is that the ruminal microorganisms remain in their natural environment. Natural salivary buffers are secreted continuously by the cow and the end products of digestion (volatile fatty acids, methane, and carbon dioxide) are continuously removed. A disadvantage of the *in situ* system is that undigested feed can escape through the pores of the bag and appear to be digested. Conversely, feed particles and microorganisms from the rumen of the cow can pass into the bag and be measured as undigested feed. Washing the bags to remove ruminal contamination and microorganisms is a crucial step in the *in situ* procedure. An additional disadvantage of the system is that the ruminal environment is variable throughout the day depending on the feed and feeding management provided to the fistulated cow and it may not represent what would happen if the test feed in the bags were actually fed. Finally, it should be remembered that *in situ* methods only measure ruminal digestion and that digestion in remainder of the gastrointestinal tract is not measured.

In vitro systems have the advantage that feed particles cannot be lost from the fermentation vessel nor can material from ruminal contents enter the vessel (except that contained in the inoculum which can be measured using a blank). In addition, conditions during fermentation are more stable than in the rumen and can be controlled more easily. The major disadvantages of *in vitro* systems are that volatile fatty acid end products are not removed during fermentation (which could result in a inhibition of long-term fermentations), ruminal microorganisms can be lost or damaged during the preparation of the inoculum, and buffer and dry matter concentrations in *in vitro* systems differ from those in the rumen.

The most commonly used *in vitro* method and the one demonstrated to be superior to other *in vitro* methods is the Tilley and Terry (1963) procedure. This method involves a 48-h fermentation with ruminal microorganisms followed by a 48-h acid pepsin digestion. This two-step procedure is intended to mimic DMD in the total digestive tract. Numerous studies have shown that it is highly correlated with DMD measured in animals and has a low standard error in predicting DMD. In general, it tends to overestimate animal digestibility slightly.

Van Soest et al. (1966) developed the *in vitro* true digestibility method by replacing the second stage of the Tilley and Terry method (acid pepsin digestion) with an extraction using boiling neutral detergent solution. This procedure is two days shorter than the Tilley and Terry method and typically has a lower standard error of prediction and smaller bias. In addition, this method can be used to determine the digestion coefficient for NDF that is useful in summative equations. True digestibilities measured using this system differ from the apparent digestibilities obtained by animals and the original Tilley and Terry system by the amount of endogenous loss excreted in the feces (which averages about 12.9% of dry matter intake).

In vitro and *in situ* digestibilities are measured after a specific time of fermentation. In general, *in vitro* digestibilities measured at 48 h match animal digestibility at maintenance levels of intake. This raises the question of the length of *in vitro* or *in situ* fermentation needed to represent digestibility in dairy cows at 3-4 times maintenance levels of intake. It has been suggested that 30 h of fermentation may be more relevant to dairy cow digestion, but most of the *in situ* or *in vitro* digestibilities of fiber or dry matter have been measured at 48 h.

Digestion is a dynamic process that results from the competition between rates of digestion and rates of passage. Thus, static measurements of digestibility that are measured at specific times or levels of intake have limited utility. We know that digestibility measured in animals at maintenance levels of intake will be higher than those measured in animals at 3-4 times maintenance levels of intake. Furthermore, the digestibility of a forage may be different depending on interactions with other feed ingredients in the total ration. In general, when rapidly digesting feeds are added to a ration, ruminal pH decreases and sometimes fiber digestion is adversely affected. To estimate digestibility at various levels of production and intake and in various types of rations, it is most useful to know the rate at which a forage is digested (the dynamic changes during digestion are often called digestion kinetics). Using rates of digestion and passage, digestibility of a feed can be estimated for any level of intake that is expected. Both *in situ* and *in vitro* systems can be adapted to measure rates of digestion. Digestion is measured at several times of fermentation (e.g., 0 3, 6, 9 12, 18, 24, 36, 48, 72 and 96 h) and the curve of fiber, protein, or dry matter digestion versus time is used to determine the rate and potential extent of digestion (plateau of the curve).

A large amount of time and effort is needed to measure rate of digestion. Multiple samples must be obtained over time and analyzed to obtain this information. This has stimulated research to develop methods that are more rapid and easy. Gas is produced by ruminal microorganisms during fermentation and it is produced in proportion to the amount of feed that is digested. By fermenting the feed in sealed vessels and measuring the gas pressure produced during fermentation, a digestion curve can be developed from a single sample of the feed. Furthermore, the system can be automated so that a computer collects the gas production information over the entire fermentation. If validated, *in vitro* gas production could be a valuable technique for measuring digestion kinetics.

One of the issues in measuring digestibility or digestion kinetics is how to prepare the sample for measurement. For most analyses, the sample needs to be finely ground so that it can be uniformly mixed before subsampling and be adequately extracted during chemical analysis. To finely grind the sample requires that it be dried. However, drying and grinding the sample may destroy some of the properties of the feed that are important to its digestion and intake by the cow. If we want to investigate the intrinsic limitations to digestion at the molecular level then studying the digestion of finely ground samples is appropriate. It might even be appropriate for estimating extent of digestion in animals because the particles in the feces are relatively fine indicating that they are extensively chewed during digestion and passage. However, feeds typically do not enter the rumen as finely ground particles. This has led to the suggestion that digestion should be measured on whole or very coarsely chopped material. Although it is logical to assume that using finely ground samples in *in vitro* and *in situ* systems may over estimate animal or *in vivo* digestion or digestion kinetics, it is just as likely that whole or coarsely

chopped samples may underestimate them. The best particle size for measuring digestion *in vitro* or *in situ* or the optimal way of adjusting data to reflect *in vivo* performance has not been defined.

Routinely measuring digestibility or digestion kinetics using *in situ* or *in vitro* methods is difficult because it is time consuming, fistulated animals are needed, standard methods are not clearly defined, the relationship between *in vitro* or *in situ* results and actual *in vivo* measurements is unknown, and repeatability among laboratories is probably much greater than for chemical methods. These biological methods of analysis are inherently more variable because of the variability among diets and individual animals used as the fermentation chambers or inoculum sources. One alternative is to use a single facility to generate the *in situ* or *in vitro* data and then use this information to develop a calibration for near infrared spectroscopy (NIRS) that would be used universally (Hoffman et al., 1999). Another alternative is to develop *in vitro* methods that use commercially available and standardized mixtures of enzymes to simulate ruminal digestion. No matter whether *in vitro* or *in situ* results are generated directly, predicted using NIRS, or simulated using enzymatic methods, there is a clear and definite need for *in vivo* measurements of digestibility that can be used as the gold standard in calibrating and evaluating methods for estimating digestibility.

FEED ANALYSES AND THEIR IMPORTANCE

Feed typically represents more than 50% of the cost of producing meat and milk. Accurate formulation of rations is not possible using average values from tables because the chemical composition of forages is quite variable. Typically fiber values can vary by a factor of 3, protein by a factor of 4, and minerals by a factor of 10 or more. Thus, it is important to know the exact composition of a forage as the first step in determining its value and its potential contribution to an animal's diet. Although feed analysis is relatively inexpensive in relation to the value of the feed itself, it is not without cost and it is pertinent to ask what information is needed, which analyses provide the needed information, and how should the results be interpreted. In the remaining discussion, a distinction will be made between those results that are analyzed directly and those that are calculated.

Moisture or Dry Matter. Moisture is determined analytically by drying in an oven at a specified temperature for a specified time (typically 135 °C for 2 h). Oven drying is not as simple or as accurate as it might seem. In silages, significant volatiles that are created during silage fermentation are evaporated by oven drying and are analyzed as water. Karl Fisher (KF) methods for measuring water using a chemical reaction are more accurate as long as all of the water is extracted into the KF reagent. The water peak for NIRS is relatively unambiguous and calibrations of NIRS using KF data are quite accurate.

Moisture in feed has no special properties and typically it is an expensive way to obtain water – it is much more economical to provide drinking water. Correct measurement of water is crucial for the determination of the value of a feed because it is the dry matter in the feed that contains the nutrients. Feeds should be priced and compared for nutritive value on a constant dry matter (DM) basis (typically 100% DM, but sometimes hays are compared on a air-dry or 90% DM basis).

Feed moisture or dry matter content can provide information about the storage conditions and stability of feeds. Silages with <30% dry matter may have abnormal fermentations that can affect intake. Silages with dry matters >60% may have significant heating and heat-damaged protein. Hays that have <85% moisture may mold and heat, thereby causing significant nutritional and storage problems.

Water in feed is simply a diluent of the nutrients and energy in feeds and correct moisture determination of silages is key to formulating accurate rations. This is especially true when minimum forage rations are fed. For example, if a farmer is mixing 2000 lbs of silage that is 35% DM with 1000 lbs of concentrates that are 90% DM the forage to concentrate ratio in the ration is 700 lbs of silage DM to 900 lbs of concentrate DM or 44:56. However, if it rains on the bunker silo and the 2000 lbs of silage contains 200 lbs of rain water or the silage changes to 31.5% DM, the ration now contains only 630 lbs of silage DM and 900 lbs of concentrate DM for a forage to concentrate ratio of 41:59. If the fiber concentration in the ration was low, this change in the F:C ratio can have serious consequences.

Crude Protein. Crude protein is measured analytically as nitrogen multiplied by 6.25%. This assumes that all nitrogen in the feed comes from protein and that protein is 16% nitrogen. Nitrogen is measured using combustion or acid hydrolysis (Kjeldahl method). Measuring and using actual feed crude protein to formulate rations helps to ensure that adequate protein is fed and also to ensure that excess protein is not fed which is detrimental both economically and environmentally.

Some of the protein in heated feeds can be bound in complexes that are not utilized by the animal. Heating can occur naturally as in the case of moist hays or high DM silages (spontaneous heating typically occurs when DM is between 60 and 80%). Heat also can be supplied as a part of the cooking or drying process of some concentrate and byproduct feeds. Feeds that are dark tan to brown and have a toasted or sweet caramel aroma should be checked for heat-damaged protein. This can be determined analytically as **acid detergent insoluble nitrogen** or protein (sometimes called ADF-N). Most of the heat-damaged protein is unusable by the animal and should be discounted when formulating rations.

Fiber. Fiber is unusual in that it is measured analytically using chemical methods, but the nutritional concept of fiber is based on biological attributes. Traditionally, fiber was defined as the undigested ballast or bulk in a feed. More recently it has been defined for ruminants as the indigestible or slowly digesting fraction of the feed that occupies space in the gastrointestinal tract. Historically, fiber was measured as crude fiber. However, crude fiber does not contain the slowly digesting hemicellulose in plant cell walls and contains variable amounts of the indigestible lignin. Currently fiber is measured routinely as either ADF or NDF.

The ADF method was developed as a preparatory step for the determination of indigestible lignin. It was never intended to be a measure of total fiber because the ADF procedure dissolves hemicellulose and recovers only cellulose and lignin (also small amounts of cutin, silica, and heat-damaged protein when it occurs in the feed). Because it contains a higher proportion of indigestible material than NDF it is often more highly correlated with DMD. Although ADF can

indicate relative differences in fiber within a feed or forage type, it does not discriminate in fiber value among feed types and is not the best measure of fiber in a feed. For example, samples of alfalfa hay, corn silage, and bermudagrass hay can all have similar ADF values of 30-31%, but their NDF values will be 41, 58 and 66%, respectively, and probably represents their feeding value more accurately.

The NDF method is the best routine method available for measuring the total fiber in a feed. It is related to forage intake potential and separates feeds into a soluble fraction that is essentially completely digestible (NDS) and a NDF fraction that contains all of the indigestible and slowly digestion components in feeds. Unfortunately, the NDF method has undergone several modifications in procedure and has developed a reputation for being more variable and difficult to analyze. The original NDF method described by Georing and Van Soest (1970) used sulfite to remove protein from the fiber residue, but did not adequately remove starch from concentrates and corn silage. Robertson and Van Soest (1980) developed the neutral detergent residue (NDR) method that uses a heat-stable amylase to remove starch, but they eliminated sulfite from the method because they were concerned that it might remove some of the phenolic compounds like lignin. We developed the amylase-treated NDF (aNDF) method which uses both sulfite and heat-stable amylase (Hintz et al., 1996). The three modifications of the NDF method obtain slightly different values for forages, but the discrepancies are quite large for feeds that have been heated such as brewer's and distiller's grains.

Total Ash. Total ash is determined analytically by ashing the feed at 500-600 °C for 3-5 h. It is a measure of the total mineral matter in feeds and can have a significant impact on the estimation of available energy because ash contributes nothing to the energy value of feeds. Legumes and protein supplements tend to have higher ash values than grasses and grains. Sometimes the ash value can provide an indication of contamination of feeds with supplemental minerals or soil. Energy values can be estimated most accurately when ash is measured and taken into account.

Minerals. Rations are routinely balanced for some minerals, especially calcium and phosphorus. Environmental concerns about the excretion of excess phosphorus in manure is stimulating increased interest in analytically measuring the phosphorus in feeds and adjusting rations accordingly. In addition, the calcium to phosphorus ratio in forages can provide an indication of the relative proportions of legumes and grasses in forage mixtures. Legumes will have calcium to phosphorus ratios of about 5.5:1, whereas grasses will have a ratio of about 1.3:1.

Ether Extract. Lipids are extracted analytically using ether. Lipids contain compounds other than true fats (or triglycerides) such as waxes, cutins, essential oils, and other compounds. Because lipids contain compounds other than fat, it is best to measure total fatty acids in feeds. Fatty acids are important for the estimation of available or net energy in feeds because they contain 2.25 times as much energy as carbohydrates. In general, forages contain very little ether extract or fat, and tabular values can be used as reasonable estimates. However, some concentrate feed sources, especially oil seeds and grain byproducts in which fat has not been extracted, can have significant fat that has a large impact on the energy value of these feeds.

Digestible Dry Matter, Total Digestible Nutrients and Net Energy. These estimates of available energy in feed are all calculated primarily using regression equations (Undersander et

al., 1993). In most, if not all instances, these energy values are estimated based on ADF. Different equations are used by forage testing laboratories which explains why two labs that reported the same ADF value for a feed might provide different estimates of TDN or NEL. The problems and errors associated with these equations have been discussed earlier.

Some laboratories also use the Conrad et al. (1984) or Weiss (1993) summative equations (also called the Ohio State equations) to calculate TDN and NEL. These equations requires that NDF, lignin, crude protein, acid detergent insoluble protein, ash and fatty acids be measured or estimated from tabular values to calculate energy values. Although this equation appears to predict the energy value of concentrates much more accurately than can be done based solely on fiber content, it is unclear whether they provide a more accurate estimate of energy value for forages. Fats do not make a significant contribution to energy value in forages and the theoretical function of lignin and NDF used to calculate the digestibility of NDF has not been extensively evaluated. The use of *in vivo*, *in situ*, or *in vitro* methods to determine NDF digestibility may improve the accuracy and utility of these equations for forage evaluation.

Nonfibrous Carbohydrates. There is increasing interest in the rapidly fermenting carbohydrates in feeds that are not fiber. The easiest way to estimate these carbohydrates is to calculate them as: NFC = 100 – NDF – CP – EE – Ash. Sometimes this fraction is also called nonstructural carbohydrates (NSC) or total nonstructural carbohydrates (TNC). However, these last two terms also refer to the measurement of starches + sugars in feeds using analytical methods. The calculated and measured rapidly fermenting carbohydrates provide different results and it is important that the terminology be standardized and used consistently among laboratories. If you are uncertain which result is being reported, contact the laboratory to determine if they calculate or measure the result. It is suggested that the calculated value be called nonfibrous (NFC) or neutral detergent soluble carbohydrate (NDSC) to indicate it origin as being calculated based on fiber analysis and that the measured result be called NSC or TNC in agreement with the original nomenclature of these methods.

Relative Feed Value. Relative feed value is a relative indicator of the digestible dry matter intake potential of a forage. It is calculated as the product of potential intake based on NDF content and dry matter digestibility based on ADF. It was derived as a single number index of potential feed value that can be used to rank forages of differing NDF and ADF concentrations using a biologically relevant scale.

FACTORS AFFECTING FORAGE TESTING RESULTS

Sampling. A representative sample is crucial to the value of feed analysis. Testing results are no better than the sample that was submitted. Each lot of feed or forage should be sampled for an analysis separately, and additional samples should be taken when a change in the forage is observed or suspected. A lot of forage is defined as one cutting harvested from the same field at the same stage of maturity with the same type of weathering and storage. Obtaining a sample that can represent several tons of forage is a challenge. Collecting a truly representative sample requires that multiple samples be taken and combined or composited. At least 12 randomly selected hay bales should be sampled using a core sampler. Samples should be taken from the

ends of square bales and the sides of round bales. Mix the sample thoroughly and pour into a plastic bag. Press air out of the bag and seal with a twist tie or cord.

Silage is more difficult to sample because only a small portion of the silage is accessible at any one time. For bunker silos, 12 samples should be obtained from the entire face of the silage. A double handful should be collected from each site. When mechanical unloaders are used, use a container of 3 to 4-quart capacity and collect at least 5 samples during a single feeding. Combine samples in a clean bucket and mix thoroughly. Place a two-quart subsample in a plastic bag, remove the air from the bag, and seal it air-tight with a rubber band or twist tie. If the sample has to be shipped to the laboratory, it is best to freeze it overnight before shipping to minimize spoilage during transit.

It is often very difficult, if not impossible, to sample hays and silages after they are harvested and stored. It may be easier to get a representative sample during harvest by collecting a sample from each load or fixed number of loads as the forage is harvested. This material can then be mixed and a subsample submitted for analysis. It may be more informative to composite the samples by field or day to get an indication of the variability that can be expected in the forage that was harvested. By sampling during harvest, feed analysis information will be available before the feed is fed. However, it has the disadvantage that changes can occur during ensiling and storage that are not measured. Thus, spot sampling at feeding should be used to determine these changes.

Laboratory Proficiency. The vast majority of forage testing laboratories attempt to provide reliable and repeatable results to their clients. They recognize that their reputation and success is dependent upon providing results that are accurate. In many cases, the price or acceptance specification of a feed is based on laboratory results, and it is not uncommon for the forage to be tested by both the buyer and the seller. No one is benefited by discrepancies among laboratories. Even in situations when the forage or feed is grown and fed on the same farm, the accuracy of laboratory results is crucial to accurate ration formulation. When laboratory results are inaccurate serious nutritional and financial consequences can occur.

In cooperation with the American Forage and Grasslands Council and the National Hay Association, forage testing laboratories formed the National Forage Testing Association (NFTA) in 1976 to improve the accuracy of forage testing among laboratories. Each year the NFTA sends the 150 participating laboratories six samples of forage. The labs analyze these samples for DM, crude protein, ADF, and NDF and submit their results to the association. The results of all labs are compared to the average of labs that are using reference methods and each lab is given a grade for each analysis for each sample. This information can be used by the laboratory to identify when and were they may be having problems with their analyses. Reference analytical methods for each analysis were published in a Forage Analyses Procedures Manual by the NFTA (Undersander, et al., 1993). Based on the cumulative grade for all samples and methods, labs with satisfactory results are awarded a Proficiency Certification for the following year. A list of proficient labs is published on the NFTA website at "www.foragetesting.org/prolabs.html".

It is recommended that you use forage testing laboratories that participate in proficiency testing programs and are certified as proficient. This will help to ensure accuracy and reduce discrepancies when samples of the same forage are submitted to different labs. However, it is

still possible for two NFTA certified laboratories to obtain different results. Sometimes the problem is related to the difficulty of accurately splitting a forage sample. It is nearly impossible to adequately split a forage sample without grinding and mixing it first. One way to alleviate this problem is to ask the laboratories to exchange their ground samples. When results are different among labs there are several factors to consider. Which of the variations in analytical methods do they use? Are they reporting the results on the same DM basis? Do their DM analyses agree? Sometimes the discrepancy in fiber results can be due to adjusting fiber analyses to a 100% DM basis using different DM results. Do they have a quality assurance program and do they use internal standards and reference materials to monitor their analytical results? How often do they calibrate and update their equipment and equations?

CONCLUSIONS

Laboratory analyses are available that can characterize the nutritive value of feeds. The contents of dry matter, crude protein, acid detergent fiber, neutral detergent fiber, total ash, and fats can be determined with the accuracy that is necessary to make sound nutritional and economic decisions. However, methods for estimating the digestibility and energy value of feeds are less accurate because these results are typically calculated from chemical composition. Research is needed to develop new methods for measuring or estimating digestibility and to obtain animal intake and digestibility data that can be used to calibrate and evaluate laboratory methods for estimating digestibility.

REFERENCES

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.

Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analyses. USDA Handbook No. 379. US Gov. Printing Office, Washington, DC. 20 pp.

Hintz, R.W., D.R. Mertens, and K.A. Albrecht. 1996. Effects of sodium sulfite on recovery and composition of detergent fiber and lignin. J.A.O.A.C. Inter. 79:16.

Hoffman, P.C., N.M. Brehm, J.J. Hasler, L.M. Bauman, J.B. Peters, D.K. Combs, R.D. Shaver, and D.J. Undersander. 1999. Development of a novel system to estimate protein degradability in legume and grass silage. J. Dairy Sci. 82:771.

Mertens, D.R. 1992. Nonstructural and structural carbohydrates. p 219. In Large Dairy Herd Management, H.H. Van Horn and C.J. Wilcox, eds. Am. Dairy Sci. Assoc. Champaign, IL.

NRC (National Research Council). 1989. Nutrient requirements of dairy cattle. National Academy Press. Washington, DC. 85 pp.

Osbourne, D.F., R.A Terry, G.E. Outen, and S.B. Cammell. 1974. The significance of a determination of cell walls as the rational basis for the nutritional evaluation of forages. p. 374. In Vol.3 Proc. XII Inter. Grassland Congr., Moscow.

Robertson, J.B., and P.J. Van Soest. 1980. The detergent system of analysis and its application to human foods. p. 123. In The Analysis of Dietary Fiber in Food. W.P.T. James and O. Theander (eds.). Marcel Dekker, Inc., NY.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassland Soc. 18:104.

Tyrrell, H.F., and P.W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151-1163.

Undersander, D., D.R. Mertens, and N. Thiex. 1993. Forage Analyses Procedures. National Forage Testing Assoc., Omaha, NE. 154 pp.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd edition. Cornell University Press. Ithaca, NY. 476 pp.

Van Soest, P.J., R.H. Wine, and L.A. Moore. 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. p. 438. In Proc. Xth Inter. Grassland Cong.

Weiss, W.P. 1993. Predicting energy values of feeds. J. Dairy Sci. 76:1802.

Zemmelink, G. 1980. Effect of selective consumption on voluntary intake and digestibility of tropical forages. Agric. Res. Rep. (Verl. Landouwk. Onderz) 896. Wageningen.