

## Measurements of Forage Quality

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### Summary

The important take-home messages from this review are:

- Conditions for routine determination of dry matter (**DM**) by oven-drying need to be standardized to improve reproducibility among laboratories. Reference methods need to be adopted that measure DM more accurately than “loss of weight by oven drying” because volatile matter other than water creates errors in DM determination.
- Ash needs to be a routine measurement of quality, and the organic matter (**OM**) in total DM and in forage components should be reported because OM more accurately measures the nutrients and energy value associated with forage quality.
- True protein or amino acids more accurately measure the protein value of forages better than crude protein (**CP**) equivalent. To most accurately account for the mass in feeds, specific conversion factors, other than the average of value of 6.25, need to be used to describe the nitrogen components in forages.
- The ether extract of forages contains many lipids that are not fats, and the measurement of fatty acids provides more nutritionally accurate measurements of forage quality.
- Dietary fiber is a nutritional entity that is different from cell walls in plants. The most accurate measure of total fiber in forages is amylase-treated neutral detergent fiber (**NDF**) OM. Lignin affects fiber digestibility, probably through its effect on the indigestible fiber in forages, and acid detergent lignin is as useful as any of the lignin methods for detecting the nutritional impact of lignin.
- Non-fibrous carbohydrates (**NFC**), calculated by difference, is a crude estimate of the rapidly digestible carbohydrates in feeds that has limited nutritional value only as a diagnostic tool. More specific assays are needed to measure the major nutritional components of NFC (starch, pectins, simple sugars, and organic acids) so that the fraction that is calculated by difference is the smallest possible portion of OM.
- Physical attributes, such as particle size and shape, buoyancy and specific gravity, fragility and ease of disintegration, and spectral properties, need to be included in measurements of forage quality to more fully describe nutritional impacts and utilization.
- Methods of measuring forage quality are evolving to obtain more information about the digestibility of feed components and the digestion kinetics of fractions that are important in ruminal and total tract availability. In vitro systems are inherently variable due to the

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biological variability in ruminal inocula, but methods of minimizing variability by standardizing conditions and using internal references will be developed to make measurements less relative and more useful in modeling intake and digestion processes associated with forage quality.

Current analytical methods provide a solid basis for measuring forage quality. Future enhancements will strive to add more details on the physical attribute of forages that are nutritionally important and increase measurements of digestibility and digestion kinetics.

## Introduction

Current measurements of forage quality are based on a long tradition of determining the chemical composition of feeds that affect animal nutrition. Most of what we accept as routine feed analysis is based on the measurement of protein, fat, ash, and fiber. Although the methods have changed, the basic concept that the chemical composition of feed is important nutritionally was developed more than 150 years ago. The development of the detergent system for measuring fiber in the 1960s was a significant improvement in the nutritional description of feeds (Mertens, 1993) that introduced the idea that dietary fiber and its digestibility were the crucial factors in determining the total digestibility of feeds. More recently, we have focused on the availability (digestibility) of nutrients, as well as their concentration. We are also beginning to understand the importance of time in the digestion process and the interactions among nutrients that affect the digestion of the entire diet (Mertens, 1994). With this new understanding comes the realization that physical and biological availability are as important as composition in determining the animal's response to forage. If we define forage quality as the response of the animal to the forage, then we must increase analytical measurements to more fully explain the intake, digestion, and performance of animals that

are fed forages. The objectives of this review are to: (1) discuss the background and rationale for the current measurements of forage quality and (2) describe the measurements of forage quality that will be needed to more completely characterize nutritional value and address the nutritional challenges facing high producing animals.

## Dry Matter

The DM concentration of forages can range from <15% in fresh forages to >90% of as-is or as-fed hays. The major nutritional consideration related to DM is that it affects the as-is density of nutrients. Although water is a nutrient, it contains no other nutrients and can be considered a diluent because all of the other nutrients are in the DM. It is often observed that forages, especially silage, with low DM result in reduced intakes. It is unlikely that this is a direct effect of water because ruminal contents are typically 12 to 16% DM, which is even lower than DM in immature fresh forages. When eating low-DM forages, ruminants would be expected to simply drink less water to maintain the DM consistency in the rumen. Thus, the effect of low DM in forages is probably an indirect effect of high moisture (< 28% DM) on the fermentation products generated during silage fermentation or of high moisture (< 20% DM) in fresh forages on the rate of passage and ruminal environment of grazing animals.

Given that DM is needed to convert the dry weights of nutrients and feed intake into the as-fed amounts of feeds that must be provided and mixed, it is an extremely crucial measurement of forage quality, especially for silages. It is a common misconception that DM is easily and accurately measured. First, there is no "official method" for measuring DM in wet feeds (< 85% DM) and second, the methods that are used routinely do not measure moisture or water concentration (true DM = 100 – moisture). The "DM" in forages is generally measured by convection or microwave drying.

However, these methods depend on evaporation of water due to heating. Unfortunately, oven drying volatilizes more than water, including volatile fatty acids, alcohols, sugars, urea, and bicarbonate. Thus, what we call moisture (measured by oven drying) should be called loss of weight by oven drying. It is interesting the Europeans (probably because they feed more low DM silages than we do in the U.S.) typically name what is measured as oven dry matter (**ODM**). To complicate things further, the temperature (or microwave wattage) and time of drying can affect ODM results dramatically (as can the depth of material in the drying vessel, the humidity of ambient air, the total load of moisture in the oven, etc.), so an “official method” is needed to improve repeatability among laboratories. True moisture can be measured by the Karl Fisher method, which is based on a chemical reaction with water molecules, or by toluene distillation, which measures the water distilled from the forage, after correction for alcohols and volatile fatty acids in the liquid collected.

We typically observe that Karl Fisher or toluene distillation measures 2 to 15% less moisture (greater DM) than ODM. This difference is not trivial when you are buying DM, mixing rations, or assessing efficiency. For example, early research comparing hay to silage indicated that silages resulted in more milk per pound of DM. However, it was eventually discovered that the DM in silages was underestimated by 5 to 10%, which created an artificial improvement in efficiency. Our interest in more accurately measuring forage quality will result in the use of Karl Fisher as the reference method for measuring moisture and DM in wet feeds. Like its effect on feed economic value and efficiency, changing DM values to truly reflect the moisture in samples will have a significant effect on how dairy cows are fed. All intake measurements and nutrient concentrations that we currently use for dairy cow feeding recommendations are based on a biased underestimate of DM. Changing to true DM will not be a trivial matter, but it is one that needs to be done to more fully measure the volatile components

in forage that affect their nutritional value. An additional impact of measuring true DM will be discussed further in the section on the NFC residue that is calculated by difference.

## Ash

Although specific macro and micro minerals are required by animals, they are typically only a small fraction of ash. The predominant effect of the ash content is to reduce the energy value of forages, and this is why ash concentration should be a routine analysis for forages. Organic matter is the non-ash portion of forages, and it contains all of the major nutrients and the components yielding energy that are absorbed from feeds. Legume forages typically contain more ash (8 to 12% of DM) than grasses (4 to 8% of DM), and corn silage typically contains the lowest concentration of ash (2 to 6% of DM). When estimating net energy or digestible organic matter (**dOM**) as a percentage of DM, the difference in ash content can be one indicator of the legume:grass ratio in mixed forages because higher concentrations of ash indicate a greater proportion of legumes. However, ash also can be an indicator of soil contamination. This contamination can come from soil splashed on plants during rainfall, soil raked into windows during harvest, or soil mixed into silages during feed-out from stone or soil surfaces. Ash in forages is a negative indicator of forage quality because it results in less energy available to the animal. When comparing regression equations for predicting net energy or dOM, it is typically observed that the intercepts for legume are lower than those for grasses, which reflects the higher ash content of legumes.

European systems of forage evaluation are based on the measurement or prediction of “d” or “D” value, which is the starting point for their system of energy utilization [which is similar to using total digestible nutrients (**TDN**) at maintenance as the starting point for estimating net energy in U.S. feeding systems]. The “d” or “D” value is the dOM in the

DM of the feed. It is either measured using ruminal or cellulase enzyme in vitro systems or predicted by nutrient concentrations or near infrared reflectance spectroscopy (NIRS). Because energy can only be obtained from OM, basing metabolizable or net energy on dOM (or TDN, which also excludes ash) rather than digestible DM has a more sound biological and chemical foundation. Measurement of forage quality in the U.S. probably should transition to dOM rather than digestible DM or DM digestibility.

## Protein

Almost everyone interested in measuring forage quality are familiar with the concepts of CP, soluble CP (**solCP**), peptides, non-protein nitrogen (**NPN**), acid detergent insoluble CP (**ADICP**), neutral detergent insoluble CP (**NDICP**), and ammonia (**NH<sub>3</sub>**). Instead of NPN, I prefer non-amino acid nitrogen (**NAAN**) because the ultimate use of the nitrogen is based on amino acid structure and not protein, which is defined by amino acid chain length. Differences in methods, difficulties in measuring, and nutritional implications of solCP, peptides, NAAN, ADICP, NDICP, and NH<sub>3</sub> are issues that need to be resolved to make these components more accurate indicators of forage quality. However, I will not discuss these issues because they would require an entire paper to characterize and interpret in detail. Instead, I will focus on an issue which is often overlooked and affects our ability to allocate mass correctly so that the weight of all nutrients will sum to the total mass of OM.

We all accept the fact that we actually measure nitrogen (**N**) in the laboratory and convert this number to CP ( $CP = N \times 6.25$ ). Because the measurement of N is relatively accurate, we assume that the measurement of protein is also accurate. However, this assumption disregards the fact that true protein is not CP and that CP depends on a conversion factor that is not as constant as we too

often assume. The multiplication factor for CP (6.25) is based on the fact that “average” true proteins contain an average of 16% N ( $6.25 = 1/0.16$ ). The conversion factor of 6.25 certainly is not valid for NAAN but also is not valid for specific proteins (Mossé, 1990). Individual amino acids vary in N concentration (tyrosine contains 7.7% N and arginine contains 32.2% N); therefore, true proteins with different amino acid concentrations will also vary in N concentration from 13 to 19% (conversion factors ranging from 7.69 to 5.26).

The concentration of N in nitrates, urea, and NH<sub>3</sub> is 22.6, 46.7, and 82.4% N, respectively, which creates an even bigger problem for accurate mass calculation based on CP equivalent. For example, if a silage contains 18% of DM as true protein containing 17% N and 2% of DM as NH<sub>3</sub> containing 82.4% N, the N concentration in DM would be 4.7% ( $= 18 \times 17/100 + 2 \times 82.4/100$ ), which converts to 29.4% CP equivalent. Thus, instead of equaling the actual 20% of the mass of the feed, the CP conversion indicates that it is over 29.4% of DM. You can begin to see that this is potentially a greater error in calculation of non-fibrous residue than any correction of fiber for protein content. If ADICP is the result of heat-damaged protein, the Maillard products created are an amino acid linked to a sugar, and will typically have one half of the N concentration of true protein, or 8%, (a conversion factor of 12.5 is needed to convert its N content to mass correctly).

Finally, I would like to emphasize that it is not the digestibility of protein in forage, or feeds in general, that is of concern, rather it is the conversion of amino acids and ammonia into microbial protein and the amino acid balance of the microbial and ruminal escape protein that is absorbed in the intestines of ruminants. All animals have evolved a very efficient system for digesting protein to insure that any protein consumed is absorbed and utilized. With the exception of heated and tannin-containing forages, the true digestibility of protein is greater than 90% regardless of the source.

## Fat

Fat is a minor element of quality in forages because true fat (triglycerides) or fatty acids are a small fraction (< 4%) of DM. The typical method of measuring lipids in feeds is by hexane or ether extraction (**EE**). Lipids contain more than triglycerides and fatty acids, and this is a particular problem in most forages, which contain waxes, cutins, and other ether-soluble materials that have limited digestibility and do not provide the 2.25 times as much energy as carbohydrates if digested. The trend toward fatty acid analysis instead of EE (Palmquist and Jenkins, 2003) is beneficial in the measurement of forage quality. However, like using CP equivalent, measuring only fatty acids results in additional matter being included in the non-fiber residue that is not carbohydrate. In forages, about 1 to 2% of DM is ether or hexane extractable but is not fatty acids.

## Fiber

The history of fiber methods as measures of forage quality has been extensively reviewed many times and only a few salient issues will be presented because it is more important to discuss current understanding and the future development of fiber characteristics as measures of forage quality. Crude fiber was successfully used to define nutritional value within feed types because it is highly correlated with total dietary fiber within feed types. However, it failed to explain differences in quality between forages and concentrate feeds. The reason for this is that the alkali extraction that is a part of the crude fiber system dissolves lignin and therefore puts lignin in the supposedly easy-to-digest nitrogen-free extract. Acid detergent fiber (**ADF**) was developed (Van Soest, 1963) as a preparatory step in the isolation of acid detergent lignin (**ADL**). Detergents were used to remove protein, which often contaminates lignin isolated by non-detergent methods. Although ADF is highly correlated with digestibility, it does not measure all of the fiber in

feeds because the strong acid used dissolves most of the hemicelluloses in forages. Neutral detergent fiber was developed (Van Soest and Wine, 1967) to measure the total fiber in feeds and to separate it from the almost completely digestible neutral detergent solubles (**NDS**).

## NDF

The measurement of NDF has evolved over the years and continues to evolve. The initial NDF method used sodium sulfite to improve the extraction of protein from fiber residues. However, it became evident that the original NDF method did not adequately remove starch from concentrate feeds and corn silage. The neutral detergent residue (**NDR**) method was developed (Van Soest et al., 1991) that included amylase treatment to assist in the removal of starch but removed sodium sulfite because some research indicated that it extracted phenolic complexes that might be limiting digestion. Most recently the amylase-treated NDF (**aNDF**) method was developed (Mertens, 2002) that includes the use of amylase and sodium sulfite. The aNDF method was evaluated in a collaborative study and received Official Method designation by the AOAC, International. Although these 3 methods of measuring total fiber (NDF, NDR and aNDF) are highly correlated, they result in consistently different values for feeds. Therefore, it is important for laboratories to indicate which of the methods they use, but unfortunately, most laboratories are still referring to their results as NDF, regardless of the method actually used.

During the development of aNDF, it was observed that protein could not be extracted from heated or cooked feeds and animal products without the use of sodium sulfite (Hintz, et al., 1996). It was also observed that the NDICP of aNDF was less than that of NDR. This creates a problem for the estimation of the slowly digesting protein pool in the Cornell Net Carbohydrate and Protein Model (Cornell University, Ithaca, NY), which uses NDR



without sulfite to estimate NDICP and requires that 2 neutral detergent extractions be done, one with sulfite and one without. The use of sulfite also creates a problem in the “correction” of aNDF for NDICP contamination. When sulfite is not used (NDR), the NDICP is large and correction of NDR for CP contamination is needed, especially for cooked or heated feeds. However, the NDICP of aNDF is much lower, and given the errors in the NFC calculation, it is relatively unimportant (see explanation in the last section of this paper). The worse case is to use the NDICP measured without sulfite to “correct” aNDF measured with sulfite because it can result in an overcorrection. Correction of aNDF for ash contamination is much easier to address. The official method for aNDF includes the measurement of aNDFom, which is the aNDF residue corrected for ash. The measurement of aNDFom is the preferred method for routinely measuring total fiber in feeds with a minimum of contamination.

### *Cell walls*

In early publications about NDF, it was associated with the term “cell walls.” In many instances, it was stated that NDF was an estimate of cell walls, but often the terms were used interchangeably. This is unfortunate because the concepts of “fiber” and “cell walls” are different in origin, intent, and composition. Cell walls refer to a specific anatomical part of plant cells. It consists of the lignin and structural polysaccharides that make up the outer boundary of plant cells and the pectin-containing matrix that exists between plant cells. Thus, cell walls contain lignin, cellulose, hemicelluloses, and pectins. Dietary fiber is defined as the indigestible or slowly digested fractions of feeds that occupy space in the gastrointestinal tract. Easily extractable pectin is completely digested; therefore, it should not be included in “fiber.” The ethylenediaminetetraacetic acid (EDTA) in the neutral detergent solution chelates much of the calcium in the pectin complex, and when combined

with refluxing in a hot solution; it almost completely extracts pectin. Thus, NDF is not cell walls, although it is highly correlated with it within forage types.

Cell walls are measured by a complex system of cell wall isolation, solubilization in 72% sulfuric acid, and determination of neutral sugars and uronic acids. The solubilization of cell walls in 72% sulfuric acid leaves a residue that is defined as Klason lignin. Klason lignin is highly correlated with ADL, but it is much higher in value, especially for grasses. Klason lignin probably isolates phenolic complexes more effectively than detergents, which probably extracts them. This raises the question as to the difference in the definition of lignin between nutritionists and plant physiologists. Should phenolic compounds and small lignin fragments be included in the definition of lignin? Do they have the same impact on digestibility of cell walls or fiber? Is the lignin of concern to nutritionists only the polymeric lignin that binds or encapsulates structural polysaccharides and limits their digestion? Does the detailed analysis of cell wall carbohydrates provide information that is nutritionally important? These questions must be resolved before it is prudent to suggest that the routine measurement of cell walls as opposed to NDF is warranted.

### *Lignin*

There is little disagreement that lignin limits the degradation of cell walls and fiber. However, the large discrepancy between Klason lignin and ADL in forages raises questions about the role of different types of phenolic compounds on fiber digestibility. There is evidence that some phenolic compounds are solubilized by acid detergent extract. There is also evidence that Klason lignin can contain a significant amount of nitrogenous contamination because cell wall isolation does not extract proteins as effectively as detergents. Thus, there is indication that additional work needs to be done to determine how simple phenolic complexes and polymeric lignin affect digestion. Is it possible that the phenolic

complexes extracted by detergents are not digested and result in significant reduction in the digestibility of NDS? The high true digestibility of NDS certainly suggests that the pectins and solubilized phenolics have little effect on digestibility. However, it is possible that the neutral detergent extract of feces also solubilizes the simple phenolic compounds, implying that they are apparently digested. Only an input-output analysis of phenolic complexes as they pass through animals can resolve this issue.

### *Indigestible fiber*

During the anaerobic microbial fermentation, it appears that the fiber of all forages plateaus at some level less than 100%, even after long fermentation times (> 72 hr). This indicates that there is a fraction of fiber that is indigestible (Smith et al., 1972) and will not disappear by anaerobic fermentation, even at infinite time. The existence of indigestible NDF (**iNDF**) has several consequences that make it an important component in the measurement of forage quality. Because iNDF can only disappear from the gastrointestinal tract by passage, it has a significant effect on the filling effect of forages, and because of this, it negatively affects intake. Because iNDF is indigestible, its proportion in DM has a significant negative effect on DM or OM digestibility. The iNDF in forages is highly correlated to ADL concentration (Traxler et al., 1998), and Van Soest et al. (2000) speculated that it is a constant proportion of ADL. However, others have observed that the relationship between ADL and iNDF is variable from one forage to another. Regardless of the quantitative relationship, it is becoming apparent that the ADL exerts its effect primarily by influencing iNDF. In reality, it is iNDF that is more important to measure than lignin, and methods will be developed to measure this fraction as a crucial component of forage quality.

### *Digestion kinetics*

The determination of iNDF is also crucial for the measurement of digestion kinetics (rate of digestion). It is a central tenet of kinetic analysis that pools must have homogenous kinetic properties. In effect, iNDF has a rate of digestion (kd) of zero. Given that total NDF contains both iNDF and one or more potentially digestible NDF (pdNDF) pools, it is clear that total NDF is not kinetically homogeneous and its rate of digestion cannot be estimated. Only pdNDF pools have rates of digestion that can be determined. This means that iNDF must be measured and subtracted from total NDF to obtain the potentially digestible pools that have fractional rates. The reason we measure NDF digestion at long fermentation times has nothing to do with the time feed spends in the rumen of a cow, but these long fermentation times are necessary to obtain a reasonable estimate of iNDF so the fractional digestion rate of physically effective NDF (**peNDF**) can be determined.

Extremely long in vitro and in situ fermentations (>120 hours) suggest that peNDF may consist of rapidly and slowly digesting pools. Typically the slowly digesting pool is less < 20% of the NDF and has a rate of <0.01/hour. Although a two-pool model for peNDF may be most accurate for research use, its value in applied models of ruminal fermentation has not been demonstrated and none of the current applied models have 2 digestible pools of NDF.

Regardless of the number of digestible pools, it is clear that measurements of fiber digestion kinetics will be the next step in the measurement of forage quality. Several issues need to be resolved before digestion kinetics become routine. Should fiber digestion kinetics be measured by one or two digestion pools? What method and time of fermentation is needed to determine the iNDF for each model? What is the minimum number of replications and fermentation times needed for

acceptable accuracy in the determination of pools and fractional rates of digestion? There is tremendous interest in measuring the digestion kinetics of fiber and research is in progress to address these and other questions so that fiber digestion kinetics can be added to our methods of measuring forage quality.

### *Physical attributes*

Fiber is somewhat unique in that its physical attributes not only influences its intake and digestion but also influences the ruminal environment and health of dairy cows. Because most of the fiber in dairy rations originates from forages, it is evident that when fiber in the ration is minimized, the physical particle size of the forage is an important factor in measuring forage quality. The rumen in a cow functions most effectively when it is biphasic, with a floating mat of long buoyant particles on a liquid layer with small dense particles. The floating mat stimulates chewing activity. Thus, the peNDF index of chemical fiber and particle size was based on forage measurements that stimulate chewing activity (Mertens, 1997).

There are 2 major problems in the evaluation and application of peNDF as a measurement of forage quality. The first problem is the diverse methods used to measure particle size. In both research and application, numerous methods of measuring the particle size of feeds and setting the threshold for minimum size for peNDF are being used, without research to evaluate them or a valid separation principle to guide them. The only particle size method that has been directly compared to the peNDF values derived from chewing activity is based on vigorous vertical shaking of dried samples. This separation technique maximizes the measurement of small particle, and because it bounces particles on end, it measures the width of particles rather than their length. Using this method, the 1.18-mm sieve was selected as the threshold for peNDF because it not only matched the peNDF values derived from chewing activity but also matched the size of particles found in the feces. Part

of the rationale for using the 1.18-mm sieve with vigorous shaking to define peNDF is that particles found in the feces escape the rumen and therefore do not need or stimulate chewing.

Although the Penn State Particle Separator (**PSPS**; Kononoff et al., 2003) is a practical tool for assessing particle size on the farm that was intended to be rapid, it is a crude method of particle size separation. It is based on an entirely different mechanism of separation compared to vigorous vertical shaking. The PSPS system is based on: (1) horizontal shaking and measurement of particle length, (2) wet particles which tend to clump and stick together, and (3) incomplete separation is defined by the number of shakes. It can be a great diagnostic tool, but it was neither designed nor is it acceptable as an analytical measurement. Combining data across research experiments that use different methods of measuring peNDF is risky unless the effect of experiment is included in the statistical model to account for method differences.

The second problem in the application of peNDF is related to dietary interactions. The peNDF of forages is only important if the ration is designed to have minimum fiber. In these cases, it is an effective measurement of quality because it is the minimum fiber requirements for effective ruminal function and chewing activity that are the crucial limit in the diet. When there is adequate total fiber in the ration, it is unlikely that peNDF of the forage will have any measurable effect on dairy cow responses. This issue is especially important in the harvesting of corn silage. It must be realized that there is an implicit compromise between chopping length and kernel processing. As the chop length increases, the larger size of cob and stalk particles force the rollers of the kernel processor apart, thereby reducing the efficiency of kernel fragmentation. The rollers will reduce the size of these cobs and stalks, but we are forcing the rollers to reduce particle size, which is very inefficient compared to chopping. With expensive corn and



rations that contain adequate total fiber, one should question long chop length for corn silage. It slows harvest, requires more fuel, and decreases the fragmentation of corn kernels, which are the most valuable part of the crop.

Physical attributes will continue to be an important factor in forage quality. However, with the high cost of grains and adequate total fiber in rations, the use of physical measurements may shift from peNDF and its value in minimum fiber rations to the effects of physical attributes on intake, digestion and passage. Particle size will increase as a quality measurement, especially for forages that contain grains with starch (Mertens, 2005) but the emphasis will be on effectiveness of digestion. In all forages, particle size and shape, buoyancy (density or functional specific gravity), and fragility (ease of particle size reduction) will become useful measurements of quality as we gain knowledge about their impacts on digestion, and passage, and develop nutritional software that can use this information.

### **Non-Fiber Carbohydrates**

For any system of analysis to be complete, its parts must sum to 100% of DM. To accomplish this with the analyses that are routinely measured and already discussed requires a component that is calculated by difference. This fraction was designated NFC because the main constituents of the component are carbohydrates that are not in fiber ( $\text{NFC} = 100 - \text{ash} - \text{CP} - \text{EE} - \text{aNDF}$ ). Because it is calculated by difference, NFC is a crude estimate of the more completely digested carbohydrates. It not only contains all of the errors associated with the measured components used to calculate it, but it also contains all other components in the feed that are not measured. These unmeasured components include: starch, simple sugars, disaccharides, oligosaccharides, pectins, fructans, beta-glucans, organic acids, volatile fatty acids, non-fat lipids, alcohols, aldehydes, and minor organic

compounds. Depending on the forage or feed, the composition of NFC can vary significantly. It is also important to note that NFC calculated by difference is not non-structural carbohydrate (NSC). The term NSC should be used for the analytical measurement of starch plus glucose.

There is debate about “correcting” NDF for protein and ash contamination as a means of improving the accuracy of NFC calculation. However, the errors associated with CP equivalent for NAAN are often a much greater error, and the use of fatty acids instead of total lipid analysis will introduce another significant error. When we measure true DM (including the volatile compounds that are evaporated by oven drying), even more volatile matter will be included in NFC. It should be accepted that NFC is a crude estimate of nutritional value that allows the total mass to sum to 100 (within limitations), but refining its estimation does not appear to be worth the analytical cost and gains little for nutritional evaluation.

Except for coarsely processed corn, the constituents of NFC typically have high digestibility, but they vary greatly in the fermentation end products produced and in effects on the rumen environment. Without knowing the source of NFC, it is difficult to judge its impact on the ration and animal. Generic NFC has little utility in ration formulation. Because it is inversely and highly correlated with NDF, it tells you little about the ration that cannot be deduced from NDF concentration. Because it is calculated by difference, it is actually less accurate than NDF and because its composition is variable, it is less uniform and informative than NDF.

The primary value of NFC is as a check on the concentrations of the components, if you know the feed source. In alfalfa, the primary constituent of NDF is pectin; in whole plant corn and cereals, it is starch; and in grasses, it is fructosans and sugars. In silages, fermentation acids are a significant part of NFC. Thus, if you know the forage source, NFC

can be used to more effectively estimate concentrations of specific components (starch and pectins) to validate measured values or to predict them when they are not measured. For example, if the measured starch concentration exceeds NFC, something must be wrong. In silages, 10 to 15% of the DM will be fermentation products and other non-starch constituents. In grains and grain products, starch will be 90 to 100% of the NFC. Although NFC can be used as a diagnostic tool, it would be more productive to directly measure the major components in NFC (pectin, starch, and sugars) and reduce the magnitude of the undefined residual that is calculated by difference.

### *Starch*

With the exception of corn and cereal silages, most forages have little starch. However, corn and cereal silages are a major forage source in many, if not most, dairy rations. Starch is unique among the components of NFC because its digestibility can vary greatly, and its fermentation can have direct effects on the ruminal environment. Because of these unique nutritional characteristics, starch should be measured directly in those forages and feeds in which it comprises >5% of the DM. Although there is no official method for starch analysis, most of the values reported appear to be reasonable (in relation to NFC) and relatively reproducible among laboratories. However, there are variations in methods that are important. Some laboratories measure starch directly without a pre-extraction to remove sugars. Because these results will contain glucose from sugars as well as starch, they should be more appropriately reported as NSC and not as starch. Even if sugars are removed by pre-extraction, starch results can vary based on the effectiveness of converting starch to glucose (which is what is actually measured and converted to a starch basis). Fine grinding and adequate gelatinization and extraction of starch are important for obtaining correct results.

### *Starch digestion*

Unlike other components of NDS or NFC, starch digestion can be significantly less than 98%, depending on its particle size and length of time in fermentation storage. The variability in starch digestion, especially in corn and sorghum, has stimulated the direct measurement of starch digestion using ruminal and enzymatic *in vitro* systems, as well as research into the characteristics of starch that affect its digestion (Hoffman and Shaver, 2009). Because starch can affect ruminal fermentation, there is also interest in digestion kinetics of starch in the rumen using *in vitro* starch disappearance or gas production. It appears that starch digestion is much more difficult to measure than fiber digestion because amylolytic activity of ruminal inoculum is more variable. It appears that amylolytic activity in ruminal fluid is inducible and much less stable than fibrolytic activity because amylolytic activity in the rumen is more sensitive to donor animal diet and time of sampling. At present, the repeatability of starch digestion is less reproducible among laboratories and less repeatable within laboratories. As we gain greater understanding of amylolytic activity and starch digestion, *in vitro* methodologies will be improved to reduce variation in laboratory estimates of starch digestion.

There is circumstantial and some direct evidence that starch digestion is a function of particle size, fermentation activity, processing (gelatinization), and intrinsic properties of the starch granules in grains. The predominant effect on starch digestion is particle size, and methods for measuring particle size and extent of kernel fragmentation, such as the corn silage processing score (Mertens, 2005), will become an important benchmark in the evaluation of starch utilization in forages. However, a method is needed to convert laboratory digestion of ground samples to the digestion kinetics and digestibility of the starch in the form in which it is fed. After particle size, the intrinsic nature of the starch granule is probably the next most important factor affecting

starch digestion. Starch granules are surrounded by a protein matrix (such as zien in corn) that serves as a barrier to digestion. These protein matrices are made of prolamines and measurements of prolamines can provide information about the starch digestion. Fermentation and storage time of high moisture corn and corn silage has been observed to increase starch digestion. Ammonia in fermented corn may reflect the breakdown of the protein matrix around starch granules and be an indicator of more rapid starch digestion, along with other indicators of the extent of fermentation during storage. Increased value of grains will generate greater interest in the most efficient use possible of starch in corn and cereal silages.

### *Pectin*

Pectins are the major carbohydrate in legumes and in some non-forage fiber sources, such as beet and citrus pulps. Pectins are unique because they digest rapidly and completely but result in acetic acid production in the rumen instead of propionate from starch. Increasing the pectin content of legumes would appear to be a beneficial goal and pectin analysis may be a worthy addition to measurements of forage quality.

### **Conclusions**

It is unlikely that there will be a revolution in the measurement of forage quality. It is more likely that there will be an evolution of current routine analysis to include new physical attributes and more accurate estimates of digestibility and digestion kinetics. Whenever possible, future methods of analysis should be more specific (amino acids instead of CP; starch instead of NFC) and should provide more information about the digestion kinetics (rates and extents) so that nutritional decisions can be made that improves our measurement of forage quality and the use of forages in dairy rations.

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