

# Interpreting Forage Analysis

Eric Vanzant  
Department of Animal Sciences  
University of Kentucky

Many factors influence forage quality. Factors such as forage type (e.g. legume vs. grass, type of grass, etc.), plant maturity at harvest, level of fertilization, weather conditions around harvest time, and others, interact to result in tremendous variation in the quality of forages as feed for animals. For this reason, it is impossible to provide standard lists of the nutrient profiles of forages that are useful for formulating rations. To know how much of a given forage to offer, and what other nutrients must be provided in the diet, it is critical for each lot of forage to be analyzed. Over the last century or so, we have discovered several components of forage that are meaningful from a nutritional standpoint. Some of the items on our forage analysis list have been used by livestock producers for over 100 years, whereas some have only been available during the last couple of years. The purpose of this paper is to provide some understanding of the various components that might appear on a forage analysis printout, so that these values will be of use to you in preparing diets for ruminant animals.

Before putting any emphasis on the results of a forage analysis, it is critical to understand the limits of forage analysis, particularly as influenced by factors within your control. Most of the actual analyses conducted in the laboratory are done on ground samples that typically weigh around 1 gram. Considering that we may

be applying the results to a lot of hay that may weigh several tons, it's pretty astounding that this approach works at all. For instance, using a 1 g sample to represent a lot of 100 tons of hay could be equated to asking one person's vote to represent a group of 90,718,474 people. Though this would certainly eliminate the wait at the election booth, no one would expect such a tiny sampling to come close to representing the 'whole'. Yet we routinely expect that the analytical results that we get back from the laboratory will tell us exactly what is in every mouthful of hay. The most important job in trying to maintain any degree of accuracy in the analytical results is actually in your hands, not in the lab. Laboratories, by and large, actually do a pretty good job of getting representative samples of the pound or so of forage that they receive. Their job is made easier by grinding the samples into very small particles, so that they can be accurately mixed and sampled. The tougher job is getting the right stuff into the bag in the first place. With forages, the first job is to determine what a 'lot' is. Generally, any factor that you know could affect forage quality can be used to separate forages into lots. Certainly, hay from different cuttings should be separately sampled and stored. Hay that was put up before and after a rain shower should probably be handled as two lots. Grass samples from pastures on different sites will need to be managed as

separate lots. The definition of 'lots' of forage is only useful insofar as you will be able to separate the lots and manage them individually in a feeding program. If you've got 400 bales of pretty uniform alfalfa hay, and you know you've got a handful from the edge of the field that have some grass in them, it's probably not useful to consider these as two 'lots'. Once you've identified your sampling 'lots', you need to decide how many individual samples to include in your 'representative sample'. Typical recommendations suggest that you obtain 12-20 'grab samples' in the sample you mail off to the lab. More is always better, and it's not a simple task to do a good job with each sample. Always keep in mind that you're trying to obtain a sample that will represent the whole. For hay bales, this means you have to use a forage probe to be able to get samples all the way to the center of the bale. With pasture samples, this means that you want to do your best to mimic the locations in the pasture and the parts of the plant that the cows might actually use. Silage samples should come from freshly unloaded material only and should come from several spots throughout the pile. Close attention to representative sampling is so important that some nutritionists suggest that you're better off to throw the sample away and simply use a book value if you're not going to take the time and effort for a good sample.

## **Components of the forage analysis report:**

**1. Dry Matter (DM)** Forages can vary tremendously in the amount of water (or

moisture) in the sample. For this reason, the most fundamental determination on any sample is the dry matter content. If you're looking at buying 100 T of hay that has 87% DM vs. 100 T of hay that has 88% DM, you'll buy a full ton more water (and less hay) with the 87% hay - and remember, you're paying hay prices for the water that comes along for the ride. You'll also notice that this measure forms the basis for two ways of expressing all of the other components in a forage analysis. Typically, you'll have one column for DM-basis, and one column for as-fed or as-received basis. The best rule of thumb is to ignore the results under the 'as-fed' column and focus your attention on the DM-basis column. This eliminates differences in components that are just due the dilution effect of the water present.

**2. Protein fractions** Chemically, one of the things that makes proteins what they are is that they contain the element nitrogen (N). Unfortunately for our categorization schemes (or fortunately, considering the biochemical implications) there are also a lot of other things that have nitrogen in them. This is unfortunate because it's considerably easier to measure N than it is to actually measure protein. As a result, over 100 years ago, researchers coined the term 'crude protein' (CP) which is actually a measure of the total amount of N in a sample (multiplied by 6.25 to account for the weight of the other elements in the average amino acid), not just the protein. Since the majority of the nitrogen in most feed samples is, in fact, protein, this system has worked pretty well for us over the years. However, we

have developed more sophisticated techniques that have allowed us not only to separate out the 'true protein' from the non-protein nitrogen (NPN), but also to categorize the true protein into fractions that have nutritional relevance for ruminant animals. The most well-known members of the NPN group are urea and ammonia. One of the unique things about ruminant animals is that they can (through the actions of the microorganisms in their gut) actually convert NPN into protein that their bodies can use. However, there are limits to the amount of NPN that the rumen microorganisms can use and, importantly, levels can become toxic to the animal. Therefore, it is important to separately categorize the amount of NPN in diets, particularly if outside sources of NPN have been added. But, it's not enough to separate N fractions into NPN and protein alone. For years, nutritionists have recognized that all protein is not created equal. Certainly, one of the most important differences among proteins is variety in the amino acid composition. However, for ruminants, most (usually) of the dietary amino acids are broken down by microbial action in the rumen, and converted into new amino acids. The amounts of the various amino acids that are 'created' by the microorganisms are very different than the amounts in the diet. Therefore, measuring amino acids in the diet doesn't tell us much about the amino acids that are actually absorbed by the animal. Because absorption of protein fragments (peptides and amino acids) occurs predominantly in the small intestine, we would like to have a profile of these protein fragments that arrive at the small intestine. There are two primary sources of these fragments. The first,

microbial protein, typically represents the majority of protein flowing to the small intestine. Microbial protein is the net result of the amino acid transformations that occur in the rumen. Protein that is degraded by ruminal microorganisms, and thus made available for resynthesis into microbial protein is termed Rumen Degradable Protein (RDP). The second source is feed protein that escapes degradation in the rumen (Rumen Undegradable Protein, RUP). If we can quantify the amount of dietary protein that ultimately is transformed by microbial activity, and, by difference, the amount of dietary protein that arrives at the small intestine intact, we will have a reasonably good picture of the total protein that is available for absorption at the small intestine. However, we also know that of the protein that escapes ruminal degradation, a portion is indigestible, and thus, cannot be absorbed. This fraction is named from the analytical procedure used to measure it - Acid Detergent Insoluble Nitrogen (ADIN) aka Acid Detergent Insoluble Protein (ADIP). Though nutritionists have been interested in these fractions for years, it was not until fairly recently that laboratory techniques became available that allowed us to define these fractions with any degree of certainty. In fact, these techniques are new enough that only a handful of commercial laboratories are routinely including them in forage analysis packages. To accurately balance dietary protein, however, it is essential to partition protein into RDP and RUP.

**3. Fiber Fractions** Fiber is somewhat unique among the forage components routinely measured by laboratories in that

it is not a clearly defined chemical entity. There are a number of different chemical compounds that are actually included with the various fiber fractions. For this reason, fiber fractions have generally been named for the procedures used to isolate them. Historically, Crude Fiber (CF) was the first standard fiber fraction to be measured in feedstuffs. As its name implies, this is a crude preparation that presumes to measure the fiber component of diets. From a diet formulation standpoint, the chemical constituents that we isolate from feed samples are only important if they help us predict how the feed will be utilized by an animal. Over 50 years ago, nutritionists recognized that CF could lead to some very inaccurate predictions. For that reason, techniques were developed in the 1960s to improve our predictive ability. Both Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) have become commonplace in forage analytical laboratories because they relate more closely to nutritionally important parameters. Although these two components are intimately related (ADF actually represents a subset of the components of NDF), knowing the quantities of both fractions can be useful for diet formulation. The reason these components are useful is that they relate to two of the key parameters that determine how well a forage will support a given level of animal performance, namely, intake and digestibility. Typically, NDF, which represents the entire cell wall of plant materials, is associated with intake, and ADF is associated with digestibility. Importantly, both of these relationships are inverse. That is, as the level of fiber increases, we typically will

see decreases in intake and digestibility. Another fiber component that is inversely related to digestibility is lignin (aka Acid Detergent Lignin or ADL). Lignin is a very rigid structural component of plant cell walls and, as a consequence, is indigestible. Furthermore, lignin can 'protect' other cell wall components from digestion. Thus, as the level of lignin increases, the digestibility of forages decreases.

#### **4. Other Carbohydrate Fractions**

Fiber is part of the larger family of nutrients known as carbohydrates. Other members of this family include sugars, starches, pectin, and some organic acids. From a nutritional standpoint, Non-Fiber Carbohydrates (NFC) serve primarily as a readily-available energy source by ruminal microorganisms. Thus, for diet formulation purposes, we typically group them together under the NFC heading. A related group is the Non-Structural Carbohydrates (NSC), so-named because the members of this family are found primarily outside the cell wall of plant materials. Generally, NSC contains sugars and starches, and thus, comprises the largest subgroup of the NFC.

**5. Energy** Energy is not a nutrient per se, but rather a function of several of the classes of nutrients. Dietary energy comes from the breaking of some of the chemical bonds that hold molecules together (oxidation). The amount of energy available from a dietary constituent depends on a number of factors. Most important among these is the digestibility of the dietary constituent. If a component is not digestible, the

energy contained in the chemical bonds will still be present in the feces, and obviously, not available to the animal. We can measure the energy available in chemical bonds by completely oxidizing them and measuring the amount of heat released. However, for this to have meaning from a nutritional standpoint, we have to make this measurement on both the feed and the feces in order to account for the chemical energy that was never made available to the animal. Energy that was made available (didn't show up in the feces) is collectively known as digestible energy (DE). Also, some energy is lost in the urine, some in fermentation gases like methane, and a substantial amount is lost as heat. The amount of energy available to the animal for maintenance and production, after accounting for all of these losses, is known as Net Energy (NE). Because energy availability is more a function of how efficiently it's used rather than how much is present in a feedstuff, we don't typically measure energy in feedstuffs. What we do is measure other things that are related to the available energy. This is true not only for forage analyses from commercial laboratories, but also for most 'book values' of energy concentrations in feeds. For example, it was mentioned above that ADF is negatively correlated with digestibility. Many studies have been conducted that have allowed us to develop regression equations to relate the amount of ADF in a forage with its digestibility (more specifically, with total digestible nutrients, or TDN), or with its NE. A number of different equations exist to do this. One of the more common sets of equations is known as the Penn State Equations. Within this group are

equations that have been developed for specific types of feedstuffs to increase their accuracy. For example, there are separate equations for corn grain, corn silage, and grass hay, etc. Often, laboratories will ask you to categorize a submitted feed sample in order for them to apply the correct set of equations to determine energy values. It is important to recognize that this approach to estimating energy values, which is used by most laboratories, is inherently subject to some error. The energy value is typically predicted from a single chemical constituent, ADF, and thus is subject to errors in prediction, as well as errors in the ADF measurement itself. Thus, these values should be used as approximations, and you need to be prepared to make adjustments to these values if cow performance doesn't match calculations.

Researchers have recognized the deficiencies in this approach to estimating energy values and, over the past several years, have developed an approach to estimating energy based on what is known as summative equations. Theoretically, this approach is preferred to the single component regression approach for a couple of reasons. First, summative equations, as their name implies, work on the basis of summing the contributions to dietary energy from a number of dietary components. Because this approach involves the use of more than one dietary constituent, it inherently deals with more sources of variation to available energy. In other words, we know that factors other than just variation in ADF contribute to variation in available energy. For example, fat has 2.25 times

as much energy as an equivalent weight of carbohydrate. Thus, by accounting for the amount of fat in a feedstuff, we can more accurately predict its energy than just by accounting for ADF. Secondly, this approach is a mechanistic approach. This means that the equation that is used to predict energy is designed around mechanisms that affect digestibility. This is in contrast to the empirical approach used with regression equations. Empirical approaches simply describe a numerical relationship between variables (in this case say, ADF and NE) based on observed measurements from past studies. This approach is limited in that, if data is entered that falls outside of the original data set, the predictive ability of the equation is unknown. For example, an equation used to predict NE from ADF for alfalfa hay samples will not give accurate predictions if you try to use it to predict NE from orchardgrass hay ADF. Alternatively, mechanistic approaches are more robust. Because they are based on known digestibilities of individual forage components and estimate digestibility of others based on known nutritional relationships (or in vitro digestibility), they are less prone to errors from new sample types. Experience to date indicates that the commonly used 'Ohio State' summative equations work well for most common forages. However, with some by-product feedstuffs (especially highly digestible fiber sources) the equations appear to underpredict actual energy values. In such cases, it is advisable to use 'book values' as estimators of the energy content.

**6. Index Values** For years, the Relative

Feed Value (RFV) index has been used as an 'integrated measure' of forage quality. This value is based on both the ADF and NDF values of forages. The idea is that, because NDF is negatively correlated with intake and ADF is negatively correlated with digestibility, an index value can be developed from both components that gives an estimate of the voluntary intake of digestible DM. If accurate, such an estimate would be a very strong indicator of overall forage quality. In practice, the value calculated from ADF and NDF is divided by a value for a theoretical 'standard' alfalfa hay. Thus, the 'standard' value is 100 and hay with estimated digestible DM intake greater than the standard will have RFV values greater than 100. This value has been widely adopted by the industry and has been used as a key value for setting hay prices on the open market. However, as you may have already guessed, such a simplistic approach is bound to be subject to some errors. As with the energy prediction approaches, RFV is based on a very limited set of forage components, namely ADF and NDF. A new index called Relative Forage Quality (RFQ) is rapidly supplanting the use of RFV. This index has been developed using concepts based on the summative equation approach for estimating energy values. Like the RFV, the RFQ is an index value that is based on a standard forage having a value of 100. Generally, RFQ is considered to be a better index of true forage quality than RFV. The developers of the RFQ approach have suggested that it works well for all forages with the notable exception of corn silage (the equation does not account for variation in starch digestibility seen with corn silage).