Forage and feed proteins are complex

URING nutrition workshops with students and industry professionals, I generally break nutrition analysis training down into sections corresponding to nutrients or nutrient digestion measures. Within the nutrient section, the classifications we discuss include protein, carbohydrates, fat, ash and minerals, and fermentation compounds. Protein and nitrogenous compounds tend to be the first nutrient class we delve into only because these compounds are listed first on most forage and feed analysis reports.

Crude protein is the anchor

Forages tend to be between 5% and 30% crude protein on a dry matter basis. Explained in pounds-per-ton terms, this equates to between 100 and 600 pounds of crude protein per dry ton. With expensive soybean meal and protein prices, this makes protein an incredibly valuable component of forage; however, crude protein is just what the name implies — a crude measure of the actual amino acid and protein content in the forage.

In the feed analysis laboratory, crude protein is determined by measuring the total nitrogen (N) and then multiplying that value by 6.25. Crude protein doesn't necessarily equate to usable true protein and amino acids in fermented forages, hence a better understanding of nonprotein or bound protein measures in forage crops becomes important.

Nonprotein nitrogen differs

Fresh forages and hay crops tend to have very small amounts of nonprotein-N (NPN), except in cases where nitrate-N is taken up by the plant in considerable quantities, and the living forage is unable to convert this nitrate-N into amino acid and protein fast enough. This situation tends to be associated with extreme drought stress followed by a rain event and harvest, or soon after a killing frost. In both cases, there can be a considerable amount of nitrate-N included in the crude protein fraction; however, this NPN is not true protein and is toxic to ruminants in



Measured crude protein isn't always usable true protein. It's important to understand nonprotein and bound protein measures.

high amounts, acting like cyanide.

Fermented forages contain a different NPN form from fresh or hay crops. This NPN is measured as ammonia-N and represents broken down protein and amino acid, resulting from fermentation bacteria and microbial protein degradation. Ammonia-N can also result from proteolysis following extensive enzymatic activity.

We can use ammonia-N measures in a couple of practical ways — either to identify an inefficient forage preservation or to assess the extent that corn grain and silage has fermented.

A 10% ammonia-N rule of thumb applies to haylage crops, whereas the goal is to have 10% or less of the total crude protein measured as ammonia-N. Alternatively, this means that 90% of the crude protein has been conserved in protein or amino acid form. When ammonia-N is greater than 10% of the total crude protein, the forage has likely fermented inefficiently and the true protein value of the feed isn't optimized.

For corn grain and silage, the protein in the feed is of lesser interest relative to the starch and energy component. Silage and high-moisture corn offer exceptional energy per pound due to excellent starch digestibility following ensiling. With these feeds, 10% or greater ammonia-N indicates adequately fermented silage or high-moisture corn, and this implies that the silage or grain is feeding to its full potential.

Bound protein of little value

The final protein fraction laboratory measure is undigestible protein, which is found in the acid detergent fiberbound fraction. Some protein is inevitably locked within forage fiber. Of particular interest is the protein that is bound so tightly to fiber that even a strong acid detergent can't break the protein loose. This is defined as acid detergent insoluble crude protein, or ADICP.

With an efficient fermentation or hay preservation and no heat damage, the ADICP should be less than 1% of the total forage. If this value is greater than 1%, there is reason to believe that substantial heating in the ensiling or curing process took place and bound too much protein into an indigestible form.

Hay that heats because it was harvested at too high of a moisture content will often have a high ADICP value, especially if it "caramelizes." This undigestible protein passes through the animal without providing any value and should be subtracted from the total crude protein when balancing diets.

Looking toward the future of animal nutrition, we'll likely progress toward amino acid measurements as a more accurate depiction of the true protein content in feeds. However, for the immediate future, the crude protein, nonprotein nitrogen, and bound protein measures remain the bedrock for dairy or beef nutritionists to formulate diets.

Next time you review your forage analyses, identify and interpret the protein measures discussed here. In following "Feed Analysis" columns, we'll cover the other sections with a goal to advance your feed analysis report interpretive skills. •

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Carbs are foundational to forage quality

I N THE August/September "Feed Analysis" column, we delved into forage protein and its associated complex feed analysis measures. The nitrogen and amino acids in forage are valuable, with protein supplement costs near record highs. However, a majority of your forage quality is better defined by a different feed fraction, namely carbohydrates.

At least 50% of the forage energy is derived from carbohydrates, so estimating quality based on carbohydrates is reasonable. The carbohydrate nutrient class is the second we'll cover in our dedicated column series discussing feed analysis interpretation.

Forage fiber, starch, and sugar levels prove pivotal in defining forage quality because these nutrients relate to both energy and feed intake. From an energy perspective, these three different carbohydrates each contain approximately 4 calories per gram in potential energy. Yet, forage fiber contributes only half the digestible energy value per gram relative to starch and sugar due to limited fiber digestion in dairy and beef cattle. Here, we'll focus our attention on improving your understanding of the different carbohydrate fractions reported on the feed analysis.

A detergent rinse

Fiber relates to both energy and feed intake. Cornell University's Peter Van Soest developed a detergent system for fiber analysis in ruminant nutrition. His research laid the foundation for our commercial feed analysis, where forage testing laboratories use different detergents to rinse away nonfiber feed fractions and then measure the insoluble remaining fraction.

Through Van Soest's work, the neutral detergent fiber and acid detergent fiber measures were born and are reported on your forage analysis as aNDF and ADF. In reality, the aNDF and ADF describe neutral and acid detergent insoluble fiber, respectively.

The aNDF and ADF laboratory measures are like a laundry machine cycle. The laundry detergent washes away the dirt and grime, and you're left with clean clothing. At the forage



Beef and dairy cattle derive energy from forage fiber, starch, and sugars.

lab, technicians use detergents to wash away starch, sugar, protein, and other nonfiber compounds, and then fiber is left. Lignin is also measured in a similar fashion to ADF and aNDF, but with a concentrated sulfuric acid. Lignin values reflect a completely indigestible component in fiber and forage.

The "a" in the aNDF acronym describes amylase, which is important alongside the neutral detergent to extract starch in starch-rich feeds such as corn silage. There is also a bit of soil and ash that's retained in the fiber measures; aNDFom accounts for this by subtracting the ash that's in the insoluble NDF. Think of this like sand in your pants pockets after the laundry rinse.

The ADF and aNDF measures have become the bedrock for forage quality measures such as relative feed value (RFV). For more discussion around RFV and other hay evaluation index measures, reference the three articles from 2020 that Dave Mertens and I wrote in *Hay & Forage Grower*.

Generally speaking, aNDF is a focal point on the forage analysis, with higher quality dairy forage being 40% to 45% aNDF or less. In forages, less fiber means more energy because the calories in fiber are partly locked in lignin and are less digestible relative to starch, sugar, and other feed components. Hence, more fiber dilutes the other energy-rich components in fiber.

Additional carbs

Mary Beth Hall, a dairy scientist with the U.S. Dairy Forage Research Center in Madison, Wis., standardized the starch analysis over the past decade. Starch is a chain of glucose molecules, and laboratories measure starch by breaking it down into glucose. Then the technicians measure the resulting glucose with a hospital grade glucose analyzer. Following Hall's work, starch content in forage and grains is now one of the most accurate measures on your report.

While starch is reliable and quantifies a known compound, crude sugar measures are a bit different. Sugar is like crude protein, with crude sugar measured using either ethanol or water as solvents to extract sugar-like compounds. The water-soluble carbohydrate (WSC) or ethanol-soluble carbohydrate (ESC) measures represent an estimate of true plant sugar. Water extracts more sugar-like compounds than ethanol, which is why WSC is typically greater than ESC. In the future, measuring known forage sugars such as fructose, sucrose, and glucose will replace the WSC and ESC measures on your forage analysis.

For current dairy and beef nutrition purposes, focus your attention on the WSC measure to assess sugar levels. Hay and baleage will contain more sugar than silage due to sugar being used by fermenting bacteria in the ensiling process. Typical WSC measures in forage range from near zero to 10% or more.

Bringing this article full circle, remember that carbohydrates define forage quality. With an improved understanding of the fiber, starch, and sugar measures on your forage analysis report, rank your forages based upon these carbohydrate measures, with lower fiber and more starch or sugar being the goal. Fiber and starch digestibility are also important for ranking forages, and interpreting this aspect of your forage analysis report will be covered in a future column.

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Fat and fatty acid analysis is evolving

R EED and forage analysis interpretation in most cases begins and ends with a focus on protein and carbohydrate measures. The prior two articles in this series centered upon improving your ability to understand these two nutrient content sections on the feed analysis report.

In each of these previous two categories, there are historic crude measures and also more current detailed and accurate measures. For example, amino acid measures are more accurate and precise than crude protein measures. Or, in the carbohydrate category, sucrose and fructose measures are more definitive than the crude water-soluble carbohydrate sugar measure.

Animal nutrition is advancing with more accurate laboratory measures in support of more precise and efficient nutrition programs on dairy or beef farms. Over the past five to 10 years, the fat analysis section on your feed analysis report has evolved in a similar fashion. This energy dense section of the feed analysis reports crude fat measures as well as more precise and accurate total and individual fatty acid amounts.

Prior to describing the fat measures, we need to differentiate fat's nutritional impact from protein or carbohydrates to better interpret the fat feed analysis report section. Protein provides usable amino acids for growth and development. Carbohydrates provide digestible energy, which kick off a nutrient digestion and metabolism cascade in the rumen. Protein and carbohydrates are somewhat co-dependent with one another, but fat is different.

Packed with energy value

Fat is energy dense, containing roughly twice the caloric value of starch and sugars and substantially more energy than protein. Traditional nutrition programs have balanced fat in diets to ensure adequate calories and energy are available, with an emphasis on maintaining body condition or supporting high dairy cow performance. Crude fat, also known as ether extract, has done a great job



In the future, forage varieties and hybrids will be selected for unique and specific fatty acid characteristics.

of accounting for fat's caloric value on your feed analysis report.

In the laboratory, this fat extraction technique uses an ether reagent to extract fat and fatty acid like compounds, and then reports total fat content by difference. For example, if a laboratory technician starts with 100 grams, and extracts 3 grams with the ether extraction technique, the fat by ether extract value is reported as 3% of dry matter.

As dairy nutrition and research has evolved, more accurate and precise measures have led the way. Similar to the other feed analysis sections, more current and precise measures for fat are now reported. Individual and total fatty acid measures have become common on your feed analysis report. These measures have become a focal point in nutrition, recognizing that fat in dairy and beef diets has brought more than just energy to the diet.

The specific fatty acid effects can be positive or negative. For example, a mere 2 grams of trans-10, cis-12 C18:2 fatty acid bypassing the rumen will crash milkfat in dairy cows. This fatty acid is derived from unsaturated fatty acids in the diet, and corn oil is rich in unsaturated fatty acids. Do not worry about the biochemistry involved here, but recognize that nutritionists are beginning to account for specific fatty acids in diet formulation while also balancing total fat. Hence, feed analysis reports today list myristic, palmitic, stearic, oleic, linoleic, and linolenic fatty acids.

These fatty acids are 14 to 18 carbon chains long. Palmitic and stearic acids

are predominant fatty acids in animal fat, or lard. These are saturated fatty acids and are fairly inert within the rumen. The oleic, linoleic, and linolenic fatty acid concentrations are unsaturated fats. These unsaturated fatty acids are known to have different effects in animal nutrition.

Not all are equal

Nutritionists will feed fatty acid supplements that are rich in these different fatty acids to achieve different goals. They will also sum up the oleic, linoleic, and linolenic acid values to define rumen unsaturated fatty acid load, or RUFAL. Nutritionists monitor the total diet RUFAL amount as a risk factor with milkfat depression. Oleic acid is a focal point with dietary fatty acid digestibility and is also a predominant fatty acid in Plenish soybeans. The RUFAL focus or Plenish soybeans represent two examples where specific fatty acid measures are relevant.

There are not well-recognized benchmarks for forages to consider at this point. In the future, we will continue to explore varieties or hybrids with unique, specific fatty acid characteristics. We will also further move away from crude fat measures as we balance dietary fatty acids much like we do when balancing for amino acids.

The fat section on the feed analysis report is relatively brief compared to protein or carbohydrate categories. However, this energy dense section of the report has evolved in complexity much like protein and carbohydrate measures have become more specific. Future animal nutrition efforts will continue to emphasize both the total caloric value from fat as well as targeting specific fatty acids to improve animal health and performance.

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Quantifying nutrient digestibility starts with fiber

VE recently opted for a diesel truck, primarily to step up the range between fuel stops. It's got a 33-gallon fuel tank, but the range equation is more than just a larger fuel tank. Both tank size and fuel conversion efficiency are needed to project distance to empty. Sorting out energy value in your forage analysis is similar.

Nutrient content and digestibility measures are needed to project the energy value of forage. Nutrient amount is like the fuel tank size, then measuring nutrient digestibility is like the miles per gallon in the energy value equation.

Measuring and reporting forage nutrient digestibility began with neutral detergent fiber (aNDF) decades ago. Fiber, sugar, and starch each contain the same calorie potential, but the energy released from fiber by dairy or beef cattle is roughly half that of starch or sugar due to limited digestibility. Fiber digestibility is also related to how much forage dairy or beef cattle can consume. Hence, growers, feeders, and nutritionists have focused on fiber content and digestibility initially to better quantify forage energy value.

Forage testing laboratories use in vitro rumen techniques to assess fiber digestibility. These biological assays are complex. The short method explanation is that living rumen microorganisms are collected from cannulated donor cows via rumen fluid, and the fluid is used to inoculate and digest a feed sample on a lab bench. The term for this approach is in vitro, meaning simulated rumen.

Expanded measures

The undigested fiber amount (uNDF) is measured after a designated time period. Laboratories began with a 48-hour in vitro rumen incubation and have expanded to report many more digestion time periods. The common fiber digestibility measure (NDFD) is actually a calculated value. Laboratory technicians measure total fiber and undigested fiber and then calculate NDFD. This has been a confusing topic, with forage reports listing both uNDF and NDFD on forage reports. For clarification, the math is as follows:

- uNDFX, % DM = undigested fiber after X time period, as a % of feed dry matter
- NDFDX, % aNDF = (aNDF uNDFX) / aNDF x 100

Initially, a NDFD48 was a good and simple start, but the aNDF digestibility section within the feed analysis report has grown to be exceedingly complex over the past decade. The following NDFD time points are now reported for different feeds: 12, 24, 30, 48, 72, 96, 120, and 240 hours. Further, Cornell University researchers noted that fiber contains a bit of ash contamination. Thus, fiber digestibility measures are now reported on an organic matter basis as well. The math is as follows:

 uNDFXom, %dry matter = uNDFX minus residual ash, as a % of feed dry matter

Less repeatable

Consider the following points to aid in fiber digestibility data interpretation. Laboratory NDFD is a biological measure, originating from living cattle. We readily understand that cattle and farms are quite different from one another; hence, a lab assay originating from an animal's rumen should be recognized as less repeatable relative to lab measures like crude protein or starch, which rely upon standard chemical reagents.

Further, forage testing laboratories also utilize multiple different wet chemistry fiber digestion methods such as a rumen fluid standardization protocol (standardized) or a traditional rumen fluid protocol (traditional). In general, fiber digestion results should not be compared between different laboratories or methods.

Beyond NDFD48, NDFD30 has become more popular with nutritionists and incorporated into the relative forage quality (RFQ) index. The RFQ is a more robust forage quality ranking than the relative feed value (RFV) calculation, partly because it accounts for NDFD. These feed index calculations are covered in more depth by Dave Mertens and me in previous columns originally published in the April/May and August 2020 issues.

In addition to NDFD30 or 48, the 12to 72-hour measures have been brought online to estimate fiber digestion over time and calculate digestion rate in nutrition models. Think of this like how your truck measures fuel consumption per mile over a trip to calculate fuel conversion efficiency.

Similar to rebar

Then the 120- and 240-hour measures have been brought onto the report to quantify the lignified and indigestible fiber. Think of this fraction as equivalent to the rebar in concrete. The uNDF240 measure has become useful to benchmark different crops and make forage to concentrate adjustments within farms. In general, compare your forage uNDF or NDFD results to laboratory and method benchmarks and within time point and laboratory measures.

Lastly, the 24-, 30-, 48-, and 240hour in vitro rumen measures have been integrated into a total tract NDF digestibility (TTNDFD) measure, which the University of Wisconsin's Dave Combs spent roughly a decade investigating and verifying. The TTNDFD equation is similar to that used by advanced nutrition models, accounting for both lignified fiber and digestion rate. It is comparable between forages, and, in general, the TTNDFD goal is 45% or greater.

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The next frontier: protein and starch digestion

ORAGE nutrient digestion and energy yield is a function of the feed's nutrient content and subsequent digestion by the animal. Fiber content and digestion has been a major focus in ruminant nutrition, laboratory measures, and forage quality for decades. Fiber quality is important and has been discussed in recent Feed Analysis interpretation columns. However, dairy and beef nutrition programs will be expanding focus over next decade to include a couple different nutritional analysis metrics in feed evaluation - protein and starch digestibility.

Protein and starch are highly valuable nutrients within forages. The protein contributed by high-quality grass or legumes provides an offset for expensive purchased feeds like soybean meal or canola meal. The protein digestibility is generally high for lush forages, though, in some cases, heat damage through inefficient fermentation or poor hay curing robs the animal from capturing the protein.

Intestinal digestibility analyses can help nutritionists account for lost protein. Several different laboratory measures capture and express protein intestinal digestibility in two different ways: protein digested in the intestine after bypassing the rumen (% of rumen undegraded protein) and the percentage of total feed protein that is undigestible in the intestines. The latter is a fairly straightforward way to understand the damaged protein in feed. The goal is less than 10% undigestible crude protein, meaning that 90% or more of the protein is used by the animal. Consult with your nutritionists for more insight here.

Corn and sorghum differ

Starch is a bit more like fiber and focuses on the rumen. Starch-rich feeds such as corn silage or sorghum silage contribute substantial pounds of starch to dairy and beef diets. Starch in these two forages is valuable and can offset purchased corn grain when the feed's starch is highly digestible. However, nutritionists treat corn and sorghum silage starch very differently due to differing starch digestibility between the two. For example, nutritionists may discount the total starch amount in sorghum silage because sorghum berries are less digestible than corn kernels.

There is potentially valuable starch in sorghum berries much like corn kernels, but sometimes cattle are less able to access the starch in berries due to the hard grain. That's not to say that sorghum silage isn't a valuable alternative forage. Sorghum silage will outperform corn in energy yield under extreme drought conditions. This is an unfortunate reality for many; hence, there will likely be more sorghum in dairy and beef diets in the future, and starch digestibility needs to be accounted for. Thankfully, today's feed analysis report accounts for rumen starch digestibility.

In the rumen

Beyond sorghum silage, we are increasingly focusing on rumen starch digestibility to separate high-quality feeds from poor ones. Starch digested in the rumen yields energy for gain and milk production and provides energy and substrate to grow valuable microbial protein. The new crop slump associated with corn silage is mostly due to limited starch digestibility.

Fermentation enhances starch digestibility, which impacts how well the silage feeds. Over the past 10 years, feed-testing laboratories have brought feed rumen starch digestibility measures onto feed analysis reports, and we can accurately account for these factors.

As with fiber, laboratories measure rumen starch digestibility at different rumen incubation time points and express starch digestibility as a percent of total starch. These measures help rank feeds and tighten up diet formulation. With corn silage, the 7-hour starch digestibility goal is greater than 85% to 90%. The range in rumen starch digestibility is as low as 40% to 50% of total starch and as high as 95% for extremely well-processed and fermented silages.

It's a balancing act

Within diet formulation software, rumen starch digestibility measures are combined with total feed starch to calculate digestible starch. Nutritionists now formulate for both total dietary starch and digestible starch. The latter stands to be more predictive for milk production potential or milk fat depression. Too little digestible starch hampers performance, but slightly too much rumen digestible starch can be costly in milkfat depression in dairy cows.

For dairy and beef cattle, starch is digested both in the rumen and lower digestive tract. Combine the two and we end up with total tract starch digestibility (TTSD). TTSD is accurately predicted with a manure starch analysis. The starch content in manure is known to negatively correlate to TTSD for dairy and beef cattle and even growing calves. The goal for TTSD in dairy and beef cattle is greater than 98% of total starch, understanding that the best farms can achieve these levels. There is little tolerance for valuable corn grain passing through in the manure.

Whether analyzing protein rich forage, starch-filled silage, or manure, the protein and starch digestibility metrics reported on nutrition analyses are worth considering when evaluating feeds or formulating diets. Using these protein and starch digestibility metrics can help your farm optimize your feeds' potential and limit costly protein or energy supplements.

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