What Do Today's Forage Analyses Tell Us?

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Two great truths:

- We need forage analyses to formulate rations and to assess feed quality for use and sale.
- Forage analyses have changed a lot over time, and seem to be changing more rapidly now.

Much of the change has been driven by advances in dairy cattle nutrition that drive the need to get a better handle on feed characteristics we think are important to meeting the cow's nutrient requirements. If/When an assay is decided to be nutritionally relevant, some ration formulation program may adopt the assay. Different programs can call for different versions of analyses, so you need to make sure to pick the right one. All well and good. So, how do we go about making sense of the analyses and what they tell us?

Carbohydrates

Some big changes have shown up in carbohydrate analyses (Fig. 1). We used to have acid detergent fiber (ADF), neutral detergent fiber (NDF), and nonfiber carbohydrates (NFC). Now we have different measures of NDF, and NFC has been split into different fractions.

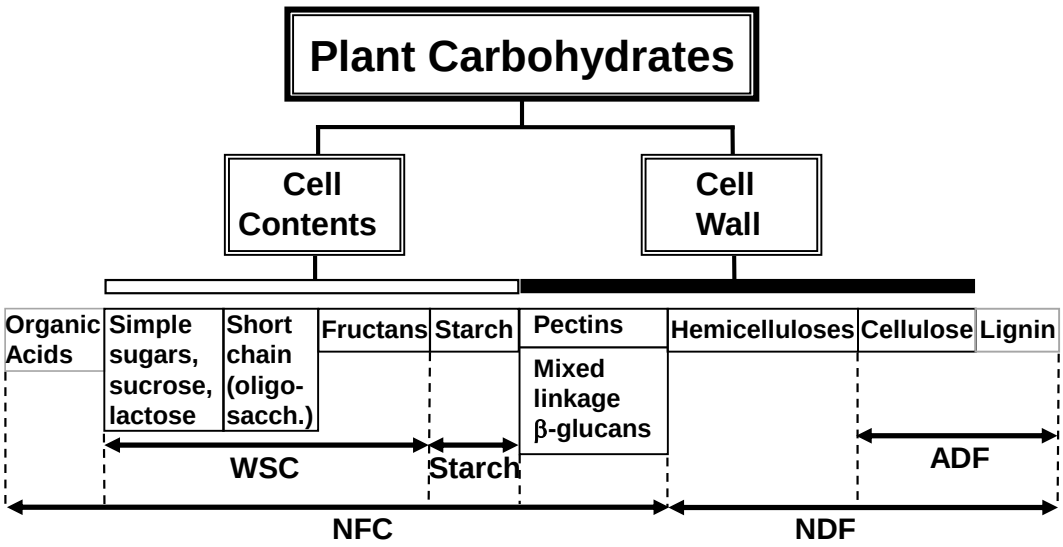

Figure 1. Carbohydrate analyses for ration formulation. ADF = acid detergent fiber, NFC = nonfiber carbohydrates, NDF = neutral detergent fiber, and WSC = water-soluble carbohydrates. Lignin and organic acids are not carbohydrates, but are grouped with them.
Presently, we don't have commercially available, affordable assays that specifically measure fructans and pectins in animal feeds.

NDF tells us about a slowly fermented fiber that is important for meeting the cow's nutrient needs and for keeping the rumen functioning well. It represents the plant cell wall that contains carbohydrates (cellulose, hemicellulose), some protein (neutral detergent insoluble protein = NDICP or NDFCP), and lignin. Cows can't digest fiber, but NDF is fermented by rumen microbes. Forage NDF is used as an indicator of the physically effective form of the feed that enhances rumination and rumen function.

NDF analyses come with a variety of options: with and without sulfite (Na2SO3), and on a with or without ash / ash-free basis. Almost all NDF analyses are already run using a starch degrading enzyme (amylase) to keep this fiber fraction from being contaminated with starch. The analysis option you choose depends on what you want to do with the number (see NDF table). The "no sulfite" option is used when you need an NDICP value for ration formulation. Sodium sulfite removes protein from the NDF and reduces the NDICP value. Using sulfite may give an NDF value that more correctly describes the carbohydrate available for microbes to ferment. Regarding NDF analysis with or without ash: "ash" is the same as "mineral". Ash is not fiber, but some mineral, like that in soil, is counted as fiber in the NDF analysis. For speed of turnaround, and because the amount of mineral may usually not be large enough to be a concern, most commercial labs have historically analyzed for NDF "with ash". The use of "with ash" or "ash-free" may depend on what the ration formulation program calls for, and how contaminated the forage is with soil (a.k.a., how many mounds of dirt near woodchuck holes were harvested). Ash-free analysis gives a more reliably accurate fiber value because mineral won't be counted as fiber. For chemical analyses, it may take an extra day to get the ash-free results because the lab has to incinerate the sample to calculate the ash-free NDF. The NDF analyses you get with sulfite added or on an ash-free basis likely will have at least slightly lower values than without sulfite or with ash, respectively. There's an effort underway to have NDF abbreviations that tell how the analysis was run. "aNDF" means that amylase was used in the assay. "aNDFom" means that amylase was used and the sample is on an ash-free basis.

NDF Analysis Options	What is it used for?
With heat-stable alpha-amylase	Commonly used NDF assay. Removes starch so that starch is not counted as fiber. Abbreviated "aNDF".
Without sulfite (w/o Na2SO3)	Used for analyses for neutral detergent insoluble protein (NDICP, NDFCP).
With sulfite (w/ Na2SO3)	Removes most/all of the NDICP and gives a better idea of the carbohydrates+lignin, only.
With ash	There's mineral in the fiber!!! Soil or other mineral contamination analyzes as NDF. Most common NDF.
Without ash / ash-free	The NDF value is corrected for any ash contamination = the more accurate fiber value. Abbreviated "NDFom".

NFC, Sugars, and Starch tell us about very digestible carbohydrates that can be an excellent energy source for the cow, or support production of microbial protein if fermented by microbes in the rumen. The nonfiber carbohydrates (NFC) are calculated as 100 – crude protein – NDF – ash – ether extract, sometimes with a correction for NDICP to avoid double subtraction for the NDICP that is already counted in crude protein; if sulfites are used, the adjustment for NDICP is small or not needed. For years we assumed that all of the carbohydrates in NFC were used similarly by the cows and microbes. But, that's not true. Now, we are measuring fractions in NFC that may behave differently and seem to matter nutritionally to the cow and her microbes (See Fig. 1 and NFC table).

The soluble carbohydrates that we've been calling "sugars" are more than just "sugar", but rumen microbes use them relatively similarly. This group of carbohydrates includes simple sugars (glucose, fructose), sucrose, lactose, short chain carbohydrates ("oligosaccharides" like stachyose and raffinose which are in soybeans, and some short chain fructans which are found primarily in cool season grasses), and long chain fructans (also from cool season grasses). These carbohydrates are fermented more rapidly than NDF and may, but don't necessarily, produce lactic acid. Also, rumen microbes can turn them into glycogen, a carbohydrate with the same basic structure as starch that they store inside their cells to ferment later. The rates of fermentation seem to vary mostly by source (example: glucose faster than fructans). The two most popular assays that have been used to describe "sugars" are 80% ethanol-soluble carbohydrates (ESC) or water-soluble carbohydrates (WSC). The WSC include all of the "sugar" carbohydrates, whereas ESC does not include the long chain fructans or lactose. At the end of the day, WSC appeared to be a better assay to use than ESC because it gives a more complete value for this group of carbohydrates. We originally used ESC because we thought it would let us analyze for "sugars" (glucose, fructose, sucrose), but it turned out to measure more than that. So, no more "sugars", and let's just call it WSC: that's what it is, and it's more than just sugars.

Starch, like cellulose, is made up entirely from glucose, but the way the glucose molecules are linked in starch allows both the cow and rumen microbes to digest it. Starch can be a great source of energy to support performance, or can cause digestive upset if fed in excess, so it is important to measure it

and formulate with it properly. We have reliable assays that labs are using to analyze for starch content of feeds.

We're working with evolving recommendations for starch in dairy cattle rations that balance against fiber and rate of starch fermentation that support good performance but help to avoid acidosis. Rate of starch fermentation is mentioned here because it is important to consider at least relative rates of fermentation and not just starch amount in ration formulation to keep cows healthy and productive; brief discussion on starch fermentation assays is included later. Rates of starch fermentation in the rumen can vary greatly, but these are not reflected by the starch composition assay. Differences in how rapidly starch ferments are affected by the crystal structure of the starch (think dry ground vs. steam flaked corn), the protein matrix around the starch granules that limit access of microbes and enzymes to the starch (think "hard"/flinty corn vs high moisture corn that's been ensiled for months), how finely ground the corn is (more finely ground ferments faster than coarsely ground), and source of the starch (wheat and oats is faster than corn or sorghum).

NFC Analyses	What's in it? What does it tell you?
"Sugars"	These are glucose, fructose, sucrose, and lactose and other simple sugars. The "sugars" on feed analysis sheets are usually ESC or WSC results, but those assays include more carbohydrates than just "sugars" proper. Need to find out how the lab defines its "sugar" assay.
ESC (80% ethanol-soluble carbohydrate)	Simple sugars (glucose, fructose), sucrose, short chain carbohydrates (stachyose, raffinose, short chain fructans).
WSC (water-soluble carbohydrate)	Everything that is in ESC plus lactose and long chain fructans. Readily fermented carbohydrates excluding pectins.
Starch	Contains only starch.
Calculated NFC	All non-NDF carbohydrates, and the mistakes we made in all the analyses we used to calculate it.

Fat

Fat is an energy rich portion of the diet that can be used by the cow, but not by the rumen microbes, though biohydrogenation of fatty acids in the rumen may affect milk fat test.

Fat Analyses	What is it?
Crude fat	Fats, waxes, cutin, pigments, and other ether-soluble things, whether they are digestible or not. Also called Ether Extract.
Total fatty acids	This is the portion of the crude fat that is digestible by the cow.
Individual fatty acids	The individual types of fatty acids that make up the total fatty acids. Different fatty acids can have different effects on cow performance (repro, butterfat test).

Energy

Net energy of lactation (NEL) values estimate the energy available to the cow during lactation to support maintenance, milk, reproduction, growth, and putting on body condition. In feed analysis reports, the energy values are calculated based on the composition of the feed, and the energy equations are based on extensive research with cows, much of it done at USDA, to measure how cows converted feed to energy. The 2001 Dairy NRC used the OARDC (Ohio Agricultural Research and Development Center) equation developed by Dr. Bill Weiss (of THE Ohio State University). It estimated digestible nutrients that contributed to energy by adding together NFC assumed to be 98% digestible, CP adjusted for ADICP, fat as ether extract percentage minus 1 (to adjust for indigestible ether extract) or using the measured value for total fatty acids, and NDF carbohydrate adjusted for lignin effects or using a 48 hour in vitro NDF digestibility value determined in the lab by the Goering and Van Soest method. Energy is only counted as coming from portions of the diet that are predicted to be digested. This is why, after estimating what portions of the feed are digestible for an animal at maintenance, the total digestible nutrient values are discounted for the intake of the animal – the more a given animal eats, the more rapidly feed passes through her system, and the digestibility decreases.

The number of variations in NEL values has expanded beyond the National Research Council (NRC) equation as variations that incorporate digestibility assays have been developed. To calculate NEL values, commercial labs are using equations that are based on 1) the Dairy NRC - OARDC equation or 2) ADF, or may include 3) NDF digestibility based on in vitro fermentation or, 4) predicted starch digestibility. And there may be more variations out there. The NEL values for a feed can differ among the equations. The laboratory measures of digestibility can differ between labs (see digestibility assay section below). So, which analysis should you use? There's no absolute answer to that. If cow performance agrees with the Dairy NRC NEL value, and no other obvious ration or environment issues appear to be holding the cows back, that value may be "right" and good for use. If cow performance disagrees with the feed analysis, it is not the cows that are wrong. As you are investigating and addressing other potential contributors (feeding management, cow comfort, ventilation, fresh water supply, spoilage in feeds, etc.), getting further information on NDF and starch digestibilities that can be integrated into the NEL values could be useful for sorting out what is affecting the herd in order to come up with an energy value that is closer to the truth. Remember, all energy values are calculated estimates. Different ration software programs adjust them differently – you need to verify which values are needed for the program used to run your herd's rations.

Protein

Protein tells us about the nitrogen and amino acid containing compounds in a feed that can support rumen microbe and cow needs, and may be used to describe digestibility of starch.

Protein Analyses	What is it? What does it tell you?
Crude protein (CP)	Nitrogen measured in the feed x 6.25 is a gross measure of "protein" in a feed. Useful with ruminants because rumen microbes can convert nitrogen to amino acids that the cow can use. Does not describe digestibility.
Soluble protein	CP soluble in buffer. Used to describe readily rumen available CP, including nonprotein nitrogen and true protein. In fermented high moisture shell corn, has been related to increased starch digestibility.
Ammonia (NH_3)	In fermented feeds represents protein that was broken down by microbes; high levels may be found in butyric acid fermentations. In high moisture shell corn, together with particle size, it has been used as an indicator of starch fermentation rate (Hoffman et al., 2012).
Neutral detergent insoluble protein (NDICP, NDFCP)	The CP associated with NDF. Used in some ration programs to describe a slowly degrading CP fraction. Also used to correct for CP in NDF when sulfite is not used for NDF analysis to give an NDF value that more accurately reflects NDF carbohydrate content.
Acid detergent insoluble protein (ADICP, ADFCP)	The CP associated with ADF. High values may indicate heat damage of feeds. Used in some ration programs to describe an indigestible or very slowly degrading CP fraction.
Prolamins	A type of slowly degrading protein in corn grain. In dry ground corn, together with particle size, it has been used as an indicator of starch fermentation rate (Hoffman et al., 2012).
Amino acids	The building blocks of true protein & what the cow uses to meet her nutritional requirements. Amino acid analysis does not indicate whether they are ruminally degradable or undegradable, so this analysis may be less useful for cattle diets than for swine or poultry.
Nonprotein nitrogen (NPN)	Includes nitrogen containing compounds not found in true protein, including ammonia, urea, free amino acids not in protein molecules, short chains of amino acids (peptides), nucleic acids (DNA, RNA), etc. These can be used by rumen microbes. Except for free amino acids and peptides, cannot be used by cows to meet protein needs.

Fiber Digestibility

Fiber digestibility measured in the lab gives a way to estimate the potential for fiber to be converted to nutrients that the cow can use. In vitro NDF digestibility (NDFD or IVNDFD) is measured by using rumen microbes to ferment a ground feed sample in the lab, and measuring how much NDF remains after a certain number of hours of fermentation. The curves in Figure 1 show patterns of

how feed NDF disappears over the course of a fermentation. Initially, there's a lag time where not much fermentation occurs, then the microbes go into full swing, fermenting

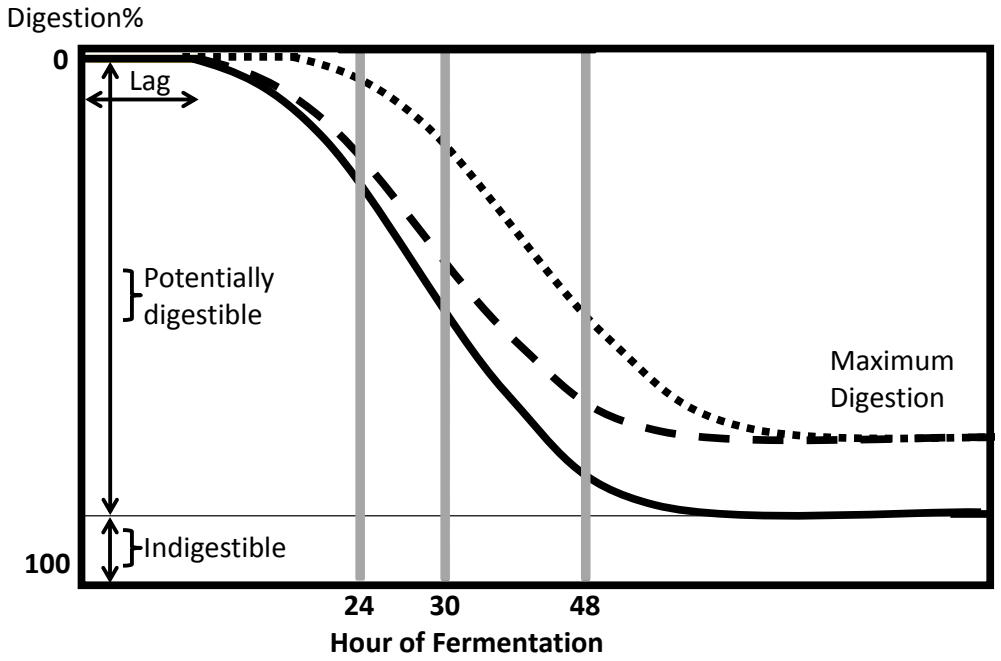

Figure 2. Examples of patterns of NDF digestion over time. "0" = no digestion, "100" = complete digestion, Lag = time before a sample starts fermenting. The dotted line has the longest lag, the solid line has the greatest final NDFD. The dotted and dashed curves have the same fermentation rate, but would differ in NDFD because of differences in lag (Hall, 2014).

the NDF more rapidly, and gradually slowing until they reach the limit of what they can ferment, which is the maximum extent of fermentation. There's debate about what time point to use for NDFD: 24, 30, or 48 hours. The earlier 24 and 30 hour time points may show more differences related to how rapidly the fiber ferments, but are also affected by lag time. They are also sensitive to lab procedures that can create more variability or noise (see Figure 3). As Figure 2 shows, changes in lag time or rate of fermentation translate into differences in NDFD between samples in the earlier hours of fermentation, no matter what their final amount of digestion. The 48 hour value can have less variability and you can detect which forage has a relatively greater extent of digestion than another, but you can't tell the route – lag or rate -- by which it got there.

Labs can differ in the NDFD method that they use, and the methods can give very different results. The 2001 Dairy NRC lists 48 hour NDFD by the Goering and Van Soest method as the one to use for the NEL calculation. More recently, another method that uses different rumen fluid handling procedures has been used (Goeser et al., 2009; Goeser and Combs, 2009), and this method gives lower NDFD values than the Goering and Van Soest method. Since factors that affect the actual NDF digestibility in cows vary by individual cow, and in vitro NDFD is a lab assay, none of the in

vitro NDFD are necessarily more "biologically correct" than the others. So, chose one – whatever assay and time point your ration formulation software uses – and use the same lab.

Some labs and research farms may measure an "in sacco" or "in situ" NDFD. This is different from "in vitro" NDFD in that it involves placing ground feed samples in porous nylon bags and placing them in the rumens of ruminally cannulated cows to ferment for varying periods of time. This has the advantage of fermenting feeds under real rumen conditions. Some downsides include variation among cows, higher costs, and longer times required to get results.

How should we interpret NDFD? Fiber digestibility is useful for comparing relative energy values of forages, but it is not a very precise number. This is not because labs are doing a bad job. *All* feed analysis methods have some variability, so you do not get *precisely* the same number with each and every analysis. The NDFD assay combines multiple steps that make the assay more variable than chemical analyses like crude protein. For example, commercial and research labs running 30 hour NDFD assays on 14 forage samples over multiple fermentations showed that, within a given lab, 95% of the results for a given forage sample fall between ± 4.9% NDFD from the average (Figure 3; Hall and Mertens, 2012). Individual labs can vary somewhat from this, but the variation is similar. If a sample is run in different labs, the results fall into a range that is ± 6.6% NDFD from the average. The labs did a good job of ranking forages in order of NDFD, but statistically, you could not separate samples that were closer than 5% NDFD apart. Take home: 1) if NDFD values are closer than 5% NDFD, they may not really be different, 2) for best consistency stick with one lab for NDFD, and 3) pay attention to how feeds rank or change relative to one another as that can reflect differences in energy content.

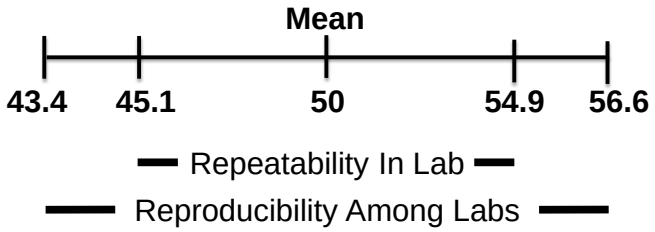

Figure 3. Variation within and among labs in 30 hour NDFD measurements. For a given sample, 95% of the values made in 1 lab will fall within ± 4.9% NDFD of the mean (average); across labs values will fall within ± 6.6%. For example, in a lab, a sample with a 50% NDFD would analyze with real values ranging from 45.1 to 54.9% NDFD.

The NDFD assays may also have other uses: predicting undigested NDF (uNDF). A 240 hour fermentation to give a uNDF 240 is being recommended as a replacement for lignin for predicting how much NDF will be fermented and contribute to NEL (Cotanch et al., 2014). uNDF values are also being suggested as ways to predict how much slowly fermenting, bulky material may limit intake through impact on rumen fill. This is the other side of fiber digestibility: undigested fiber can

fill the rumen and reduce dry matter intake. Work is underway now to evaluate how well intake can be predicted based on uNDF measured at 30 (Jones and Siciliano-Jones, 2014) and 240 (Cotanch et al., 2014) hours of fermentation. There is agreement that even with uNDF, the fineness of chop on the forage as well as its fragility will affect how uNDF relates to intake – finer and more fragile material will likely pass more quickly and have less impact on fill.

Starch Digestibility

As with NDFD, digestibility of starch as measured in the lab is designed to give estimates of how it will digest in the cow. In the Dairy NRC, NFC (which includes starch) were estimated to be 98% digestible. That may be largely true of the water-soluble carbohydrates (sugars, oligosaccharides, fructans), but not necessarily for starch. How finely ground, or fermented, or dry a feed is, or how bound the starch is in a protein matrix will affect starch digestion. Starch degradability assays have not yet been directly linked to in vivo digestibilities, but, they can give an index for how rapidly the starch is fermented for consideration in ration formulation, and the assays have been included in ration formulation programs.

Present starch digestibility assays include a 7 hours in vitro fermentation like that used for NDFD but with slightly more coarsely ground samples (to retain the effect of grain structure on starch degradation). The relative differences in how much starch is fermented by 7 hours gives an indication of how rapidly the starch is fermenting. Another assay relates protein composition (prolamin for dry ground corn, or ammonia in high moisture corn) and particle size as an indicator of starch fermentation rate (Hoffman et al., 2012; used in the University of Wisconsin Feed Grain v2.0 Evaluation system to predict energy content of corn grain). Historically, soluble protein measures in high moisture corn have been used in the beef industry as a proxy for starch digestibility. Prolamin describes the part of the protein matrix that interferes with microbial or enzyme access to starch granules in dry corn. Ammonia or soluble protein in high moisture corn describe how much the protein matrix around the granules has broken down and opened access to the starch granules.

Another approach using gas production measurement from in vitro fermentation of starch containing samples gives rates of fermentation. Gas production does not evaluate “starch”, but gives describes more rapidly or slowly fermented fractions of feeds that may be aligned with the NFC (generally more rapidly fermented) and fiber (more slowly fermented).

Take Home

The rate of development and release of new feed analyses has accelerated, at least in part in response to demands from the field. One of our challenges is that it takes time after a new analysis is released to sort out how to use it to improve ration formulation. The new values need to be put into context with all of the other feed information across a large variety of rations so that it can be made reliably reliable. And the values need to be integrated into ration formulation software so that they

complement the other feed values with which the programs were developed and calibrated. Assays that are newer than the ration program may not "fit" in that program, so be careful how you use them. Ask the labs and your nutritionist how to interpret the new results. Do not just substitute new methods/values for the "old" analyses unless you have verified that it's ok to do so. You can also use the "old" assays, but keep the new results in mind when formulating (not all numbers need to go into a software program).

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