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Accurate analysis: NIRS versus wet chemistry

WITH today's high grain prices and tight margins, dairy producers need to better understand and control nutrient variation. Accurate and timely forage analysis, therefore, becomes even more important for reducing feed costs and maximizing production. It may also be important to look closely at the nutrient variation of commodity feeds and place a higher value on consistency.

Accuracy versus precision

There are two primary approaches to testing forages and feeds: wet chemistry and NIR or NIRS (Near Infrared Reflectance Spectroscopy). Understanding the differences and when to use each is important for obtaining the best information for the testing dollar spent.

Wet chemistry methods are the most accurate at analyzing feeds and forages for nutrient content. Nutrients are isolated using chemicals and heat to break down the forage. For example, NDF (neutral detergent fiber) is the percentage of fiber in a forage sample that is not solubilized after boiling the sample in neutral detergent solution. Samples are precisely weighed before and after a chemical or heat process with the difference calculated as the amount of the nutrient. Unfortunately, wet chemistry analysis is time-consuming, costly and requires skilled technicians.

With NIRS, a spectrophotometer is used to analyze the light spectrum reflected off of a sample when it is exposed to infrared light. Each nutrient has unique reflection characteristics based on its molecular structure (carbon, nitrogen and hydrogen bonds). The reflectance of test samples is compared with that of a set of similar samples (calibration set) that have been analyzed by wet chemistry. NIRS as an analytical technique is faster than wet chemistry methods and requires less labor. However, NIRS requires sophisticated calibration development, instrument standardization and constant quality control to ensure good results.

NIRS is a secondary method based on reference evaluation of nutrients and by definition will never be more accurate than the methods on which it is based. However, as an analytical tool, NIRS is often more precise, or repeatable, than wet chemistry analysis and can be used to reduce analytical variation (Table 1).

Minerals do not reflect infrared light. So, NIRS mineral analysis is an indirect estimate based on the typical relationship of minerals with other nutrients in the forage. Many nutritionists do use NIRS mineral analyses, but one should keep possible issues with NIRS mineral analysis in mind. If there are potential mineral issues with the cows, minerals should be analyzed by wet chemistry.

Table 1. NIRS reduces variation

Nutrient (% DM)	Average	SD*, wet chemistry	SD*, NIRS
CP	15.8	0.33	0.05
ADF	31.4	0.78	0.32
NDF	41.9	0.70	0.28
Ash	11.3	0.20	0.07
Fat	3.44	0.12	0.02

*Standard deviation

Developing NIRS calibrations

The NIRS system used by the lab must be calibrated with feeds and forages analyzed by wet chemistry methods. NIRS is fairly accurate if the test sample is in the range of the calibration sample set in terms of forage type, geographic region and growing conditions. It is important that the sample be accurately identified so that the most appropriate NIRS equation can be used.

The quality of an NIRS calibration for a specific nutrient depends on several things. There needs to be a spectral relationship between the near infrared reflectance and the nutrient in question. For water and most organic constituents, NIRS will "see" the nutrient quite well. In order to develop a good prediction, there needs to be significant range in the nutrient and good repeatability of the wet chemistry assay that produces the data for calibration. The rule of thumb is that the range of the nutrient should be 10 times the error of the assay.

Protein in most plant species has significant range and the analytical method is generally precise, providing opportunity to develop excellent calibrations. By contrast, the starch prediction in alfalfa is not nearly as good because there is little range, and the starch assay is less precise.

Is the calibration good?

There can be significant differences in the quality of nutrient calibrations used by laboratories. NIR nutrient calibrations are described by certain statistics that are used to assess calibration quality. Table 2 shows statistics for certain NIRS nutrient calibrations of Cumberland Valley Analytical Services.

For each feed, the table shows the number of samples in the calibration, the average nutrient value and the standard error of prediction. The R-square is the amount of variation explained by the calibration. We want low standard errors of prediction and a high R-square value. A good R-square value for an NIRS nutrient prediction will be over 0.95; below 0.80 is considered to be a questionable prediction.

Protein is a nutrient with high repeatability by wet chemistry and with significant analytical range in most feeds. For the hay protein calibration there is a large number of samples, a low standard error and a very high R-square. We would expect this calibration to predict well.

While the bakery waste starch equation has many fewer samples, starch is a relatively uniform chemical entity and should predict well. The standard error of prediction is low, and the R-square is quite high.

On the other hand, the standard error of prediction for vomitoxin in distillers is high compared to the average vomitoxin value, and the R-square value is much lower than for traditional nutrients. Both the vomitoxin and nitrate predication are considered to be of lower predictive quality.

Nutrient and fiber analysis

With the development of excellent calibration sets and mathematical techniques, NIRS has become a well-respected method for nutrient analysis. The NIRS Forage and Feed Testing Consortium has found that, when the analyzed protein value of a feed is different when run by wet chemistry and by NIRS, the rerun wet chemistry result turns out to be the same as the NIRS result 80 percent of the time. For ADF and NDF, the wet chemistry reruns turn out to be the same as the initial NIRS results about half the time.

Estimates of NDF digestibility (NDFD) are obtained by in vitro laboratory procedures using microbes taken from the rumen of a fistulated cow. NIRS can be used for routine characterization of NDFD. The R-square of the NDFD 30-hour digestibility prediction for corn silage is good, and the standard error of prediction is reasonable. It is probably still wise, however, to check NDFD using in vitro laboratory procedures when benchmarking quality or troubleshooting problems.

Table 2. Nutrient prediction better with high R-square values

Feed	Nutrient	Number of samples	Average	Standard error	R-square
Hay	Protein	4,747	15.7	0.56	0.99
Corn silage	NDF	1,915	43.5	0.76	0.97
Corn silage	NDFD (30 hr.)	3,237	61.0	1.14	0.96
Bakery waste	Starch	359	9.1	0.46	0.97
Hay	Nitrate	1,000	0.3	0.08	0.90
Distillers	Vomitoxin	338	4.9	0.79	0.84

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