Forage Quality Analysis Using Near Infrared Reflectance Spectroscopy (NIRS) Technology in North Dakota

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Forage breeding programs require a practical method of measuring quality traits on large numbers of plant selections to enhance development of superior cultivars. Standard chemical procedures (reference method analyses) are suitable. But because of the time required for each measurement, these are costly when analyses of a large number of samples are needed.

The technology of near infrared reflectance spectroscopy (NIRS) has evolved within the last decade. The technology is considered to be a fast and accurate method of forage quality analysis, and reduces the need for conventional wet chemistry procedures.

Near infrared reflectance spectroscopy is used by the extension services in Minnesota (Ellingboe et al., 1986), Illinois (Mees et al., 1987), and South Dakota (Twidwell et al., 1989, 1989) for on-site testing of forages on individual farms and ranches, at hay auctions, agricultural field days, annual meetings and state fairs. Forage samples generally are analyzed for quality components such as digestibility and crude protein, cell wall constituents, and specific minerals.

An NIRS laboratory has been established at the Northern Great Plains Research Laboratory at Mandan as a cooperative effort between the USDA-Agricultural Research Service and the North Dakota Agricultural Experiment Station. The laboratory is conducting research on NIRS procedures which evaluate forage quality having application to grass breeding and animal nutrition research programs in North Dakota. The laboratory also will develop calibration equations suitable for regional use.

In this article we provide a report on the NIRS laboratory instrumentation and methodology for NIRS calibration development using data from intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkw. and Dewey] leaf and stem samples collected from nurseries at Mandan.

MATERIALS AND METHODS

NIRS Instrument

The instrument is a model 6250 forage scanning monochromator (see photo) (NIRSystem, Inc., Silver Spring, MD[1]), with a lead sulfide detector and a ceramic standard. The scanning monochromator is coupled to an IBM PS/2, model 50Z computer (International Business Machines Corporation, Boca Raton, Florida). Both the instrument and computer are housed in a temperature and humidity controlled room. Spectra from the monochromator are matched with a master instrument maintained by NIRSystem, Inc. for the purpose of nationwide standardization and to facilitate calibration transfer between laboratories. The instrument's diagnostic performance is routinely checked by scanning ceramic and polystyrene standards, and a sealed soybean-meal check sample. Instrument response also is checked for stray light which would adversely affect results.

Calibration

Calibration is a process of developing mathematical relationships between NIR spectra and concentration of a quality component determined by reference method. The essential point about calibration sample selection is that the sample chosen should represent the range of characteristics (chemical, physical, botanical, etc.) present in the population of interest. Several techniques are used to select samples for calibration. Structured sampling is based on some prior knowledge about the plants sampled, such as difference in maturity class of the plants. Selecting a proportionate number of samples from each maturity class ensures equal representation in the calibration set. Random sampling techniques also are used at times, but these may cause the calibration to be biased (Abrams, 1989).

Samples suitable for use in calibration also may be selected on the basis of spectral characteristics. We used CENTER and SUBSET software programs, developed by the NIRS forage research project network (Windham et al., 1989), to evaluate the spectral characteristics of intermediate wheatgrass leaf and stem samples. The CENTER program calculates a global 'H' statistic (Mahalanobis, 1936) that was used to determine if the spectral characteristics of any samples are significantly different from the majority of forage grass samples collected in previous years. Samples with global H values above 3.0 are considered outliers within a population for a specific product. The SUBSET

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[1] Mention of trade name does not imply North Dakota State University or USDA-ARS endorsement.
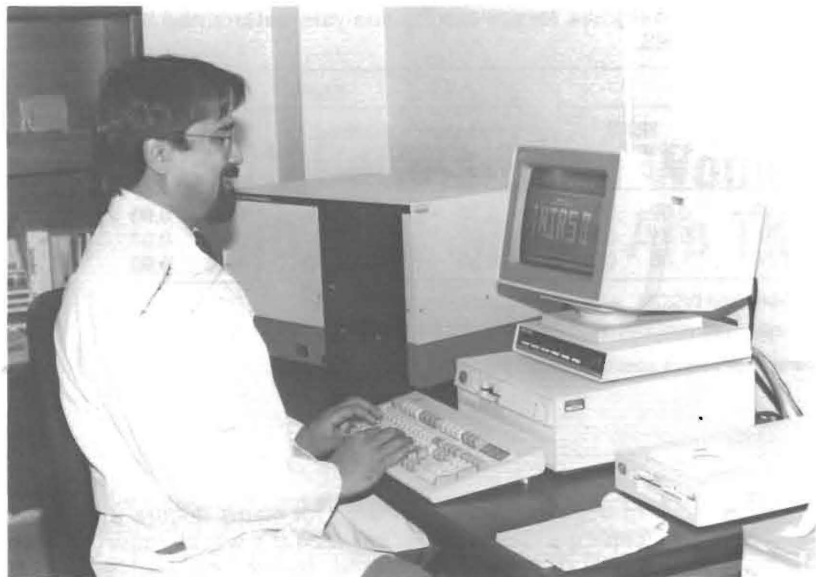
Use of 6250 (Forage) near infrared reflectance spectroscope, a monochromator to collect spectral data is an essential step for forage quality analysis. Syed Dara, Land Reclamation Research Center, North Dakota Agricultural Experiment Station, Mandan, is shown developing NIRS calibration that will reduce conventional wet chemistry analyses.

software calculates an H statistic which is a measurement of the relative spectral proximity of samples to each other and is used to eliminate samples that have identical spectral characteristics. An H value of 0.6, while subsetting for calibration set, is considered appropriate to select an adequate number of samples for wet chemistry reference method analyses.

The intermediate wheatgrass samples taken in North Dakota were collected from the field at mid-dough seed development stage. A sample consisted of 50 plant stems with leaves. Leaf blades and stems were separated and then dried for three days at 60 degrees Celsius. Samples were ground in a cyclone mill and stored in air-tight bottles at room temperature. A portion of each unknown sample was packed in a sample cup and scanned 64 times at 2 nanometer intervals between 1100 and 2500 nanometers. This provides 700 wavelength points of reflectance data.

Using SUBSET procedures, 50 out of 124 leaf and 49 out of 123 stem samples of intermediate wheatgrass were selected for conventional wet chemistry reference method analyses. This represented the entire population of 247 samples. As a wet chemistry reference method, the selected samples were analyzed for *in vitro* digestible organic matter (IVDOM) (Moore and Mott, 1974), neutral detergent fiber (NDF) (Goering and Van Soest, 1970), and Kjeldahl nitrogen (N). *In vitro* digestible organic matter is a laboratory estimate of the portion of forage that can be digested by cattle or sheep. Neutral detergent fiber is a measure of forage fiber, which is the least utilized portion of the plants. Nitrogen multiplied by 6.25 represents the crude protein concentration.

Equation development

The goal of equation development is to select the best predictive equation using the least number of reference method samples for each forage quality component. There are two commonly used regression techniques for equation selection. These are stepwise multiple regression and modi-

fied partial least squares (MPLS) analysis. Unlike stepwise multiple regression, MPLS uses all wavelength data (Wold, 1981, 1983).

We used the MPLS technique in combination with several other mathematical treatments of the spectra data and wet chemistry reference method data to develop calibration (prediction) equations for the leaf and stem samples. A critical 'T' statistic of 2.5 was used to eliminate wet chemistry reference method outliers just as global H values of 3.0 were used to eliminate spectral outliers. The standard error of calibration (SEC) and the standard error of prediction (SEP) were used as guides in selecting the best predictive equations for each quality component. The SEC is an estimate of the error in the calibration model, and the SEP is an estimate of the error between the wet chemistry reference method and NIRS predicted values. Acceptable values for each of these error terms will vary with the forage quality component being predicted.

RESULTS AND DISCUSSION

The distributions of global H values ranged from 0.4 to 3.7 for the 124 leaf samples and from 0.4 to 2.9 for the 123 stem samples (Figure 1). Only one sample had a global H value greater than 2. The stem sample that had a global H value of 3.7 was not eliminated after the wet chemistry reference method values and its contribution to the calibration model was determined. The limited variability in global H values indicated that both the leaf and stem samples were from a homogeneous population.

Calibration statistics for wet chemistry reference method analysis data used in calibration development show that leaf and stem nitrogen had SEC's of 0.09 to 0.04, respectively (Table 1). These SEC values are smaller and compare favorably with published values for nitrogen of 0.66 (Windham et al., 1989). Regression analysis between the wet chemistry reference method and NIRS method predicted that N content of leaves and stems was linear with R^2 value

Table 1. Statistical data for intermediate wheatgrass forage quality analysis determined by the wet chemistry reference method and NIRS.

Quality parameters	Leaf				Stem			
	Reference method		NIRS		Reference method		NIRS	
	Mean	Range	SEC	R^2	Mean	Range	SEC	R^2
N, % of DM	2.61	2.04- 3.42	0.09	0.92	1.33	1.08- 1.59	0.04	0.90
NDF, % of DM	53.16	47.96-60.04	0.81	0.92	64.82	60.70-70.41	0.47	0.97
IVDOM	67.24	63.75-70.94	1.06	0.72	58.90	52.26-66.24	1.08	0.90

N = nitrogen, NDF = neutral detergent fiber, and IVDOM = *in vitro* digestible organic matter.
DM = dry matter.
SEC = standard error of calibration.
R^2 = Regression coefficient of quality parameter versus spectra wavelength.
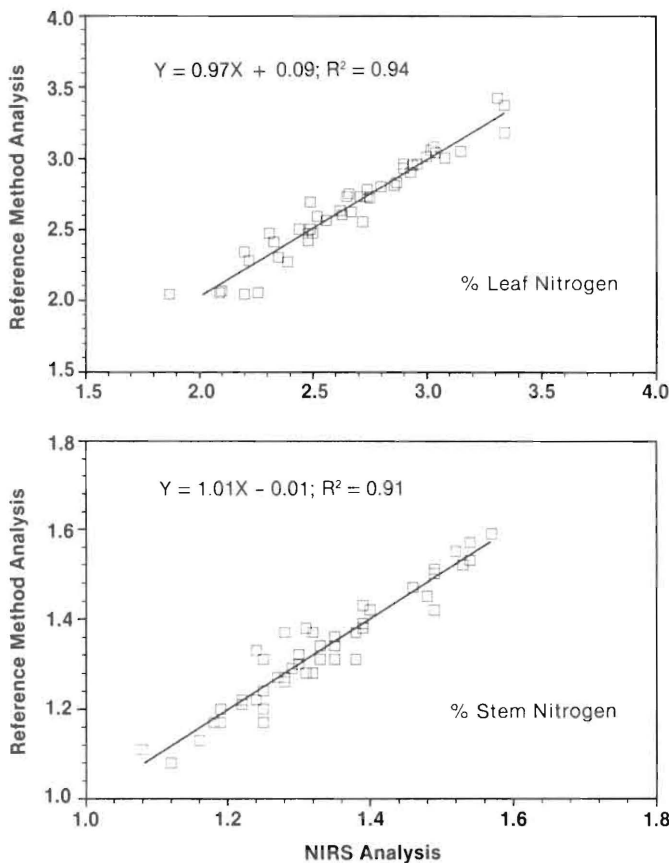

Figure 1. Distribution of mathematically defined spectral boundaries for 124 leaf and 123 stem samples are displayed according to their distance from the spectral mean.

of 0.94 for leaves and 0.91 for stems (Figure 2). This indicates good agreement between the wet chemistry reference method and NIRS method. Regression analysis indicated that both procedures will provide similar results.

The calibration R^2 value for IVDOM in leaf samples was lower (0.72) than for stem IVDOM (0.90) (Table 1). The lower calibration R^2 value for leaf samples can be attributed to a much narrower range in variability in the calibration subset for digestibility in leaf samples than stem samples.

Similar standard deviations were found for the wet chemistry reference method and the NIRS method for all three
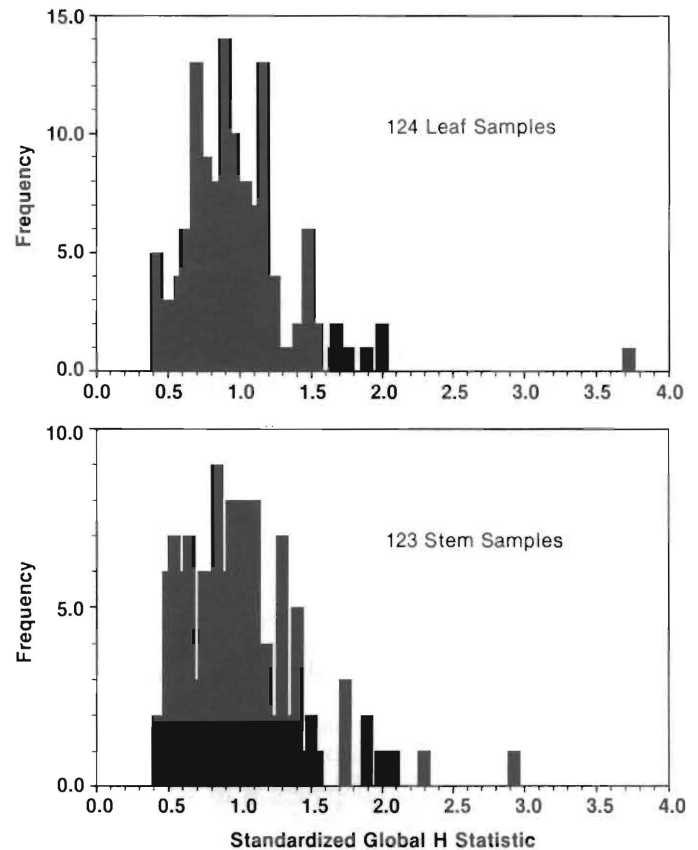

Figure 2. Relationship between NIRS and the wet chemistry reference method analysis for leaf and stem nitrogen.

quality components from both leaves and stems (Table 2). There was excellent agreement between wet chemistry reference methods and NIRS measurements for all three quality components. Prediction biases for NDF and IDVOM ranged from none to very low, indicating excellent agreement between wet chemistry and NIRS predictions (Table 2). The R^2 values for all three quality components ranged from 0.88 to 0.98, indicating an excellent prediction accuracy.

SUMMARY

The homogenous nature of intermediate wheatgrass leaf and stem samples along with consistent sample preparation techniques provided calibration equations for N, NDF, and IVDOM with supporting statistics equal to or better than previously reported. Only one sample of leaf tissue was eliminated from the final calibration. Selection of calibration samples based on spectral characteristics and principal components rather than on random sample selection can be advantageous when resources are limited for analyzing a large number of wet chemistry reference method samples.

The calibration equations developed in this study are useful for leaf and stem samples with similar chemical and physical properties. However, with minor recalibration and inclusion of new additional samples, these equations may have expanded use. In vitro digestible organic matter in leaf samples with a narrow range in variation had a lower coefficient of determination than stem samples, which had a wide range of IVDOM.

The modified partial least squares (MPLS) technique is a new approach in NIRS calibration. Future applications of the NIRS method for measuring forage quality traits likely will make use of the MPLS technique.

The cost of analyzing large numbers of forage quality samples can be reduced with use of NIRS technology. Flexibility and ruggedness of the newer instruments and new technique of spectra handling have made the technology more versatile than earlier models. The NIRS technology can be used for forage quality analysis to determine the value of hay, help formulate dairy feeding rations, analyze consumer dairy products, for small grain quality analysis, and even to determine crude oil grades. The present NIRS research and development program at the Northern Great Plains Research Laboratory includes mineral and animal nutrition studies and development of improved quality in grass cultivars of western wheatgrass, crested wheatgrass, Russian wildrye, Altai wildrye, little and big bluestem and other native and introduced species. Cooperative research is in progress with researchers of various North Dakota Agricultural Experiment Stations and USDA-SCS Plan Materials Center at Bismarck.

The NIRS technology can complement other Agricultural Experiment Station and Extension Service activities that serve North Dakota farmers and ranchers in the future.

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Table 2. Statistical data for forage quality analysis of intermediate wheatgrass determined by the wet chemistry reference method and NIRS.

Quality parameters	Standard deviation		SEP	Bias	R^2
	Ref. method	NIRS			
LEAF					
N, % of DM	0.35	0.35	0.09	0.00	0.94
NDF, % of DM	3.15	2.98	0.74	0.00	0.94
IVDOM	1.95	1.84	0.66	-0.07	0.88
STEM					
N, % of DM	0.12	0.12	0.04	0.00	0.91
NDF, % of DM	2.55	2.51	0.39	-0.03	0.98
IVDOM	3.52	3.41	0.75	-0.02	0.95

N = nitrogen, NDF = neutral detergent fiber, and IVDOM = in vitro digestible organic matter.
DM = dry matter.
SEP = standard error of prediction.
Bias = observed value – predicted value.
R^2 = Regression coefficient of wet chemistry reference method versus NIRS method.

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