Potential of Near Infrared Reflectance Spectroscopy for Measuring Forage Quality

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Story in Brief

Research on the application and potential uses of near infrared (NIR) reflectance spectroscopy has been initiated at Ft. Reno. The instrumentation has been set up and preliminary studies begun to evaluate the instrument and develop the appropriate calibration data to predict chemical and nutritive composition of forages. Emphasis will be placed on forages native to or grown in the southwestern U.S. Results have been obtained for *in vitro* dry matter digestibility (IVDMD) and crude protein (CP) of "Old World" bluestem. All laboratories participating in the NIR network project have obtained good calibrations for IVDMD, CP and fiber from a set of thirty samples which have been analyzed by all locations. The precision of the instrument allows researchers to obtain more accurate data than is possible using conventional wet chemistry techniques, i.e., Wende, Van Soest, etc. The results of the analyses indicate that we can predict forage quality accurately and rapidly with NIR. Potential uses of NIR spectroscopy include general forage analysis and better screening methods for plant breeders, resulting in more reliable, less costly means of balancing rations.

Introductions

Over a century ago the Wende proximate analysis system was developed. This system consisted of five measurements: dry matter (DM), by oven drying; fat, by ether extraction; ash, by incineration; crude protein (CP), by Kjeldahl analysis for nitrogen; and crude fiber (CF), by successive extraction with dilute alkali and acid. These procedures are still in use today for the analysis of forages and other fibrous materials. In 1963 Tilley and Terry published their two-state *in vitro* dry matter digestibility (IVDMD) procedure. This technique has been adapted worldwide as the best laboratory measurement of feed digestibility. During the mid 1960's Van Soest introduced the detergent fiber analysis which included acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin. These analyses more closely define the components of plant structural carbohydrates than the Wende System and provide a means of predicting animal performance from analysis of the feed the animal consumes.

All of the standard laboratory wet chemistry procedures for forage analysis have two things in common. They are time consuming and labor intensive. When a sample is analyzed by standard wet chemical methods for CP, NDF, ADF, lignin, and IVDMD, the time required per sample will be two to three weeks before all the analyses are complete. During this time the samples are weighed and dried and transferred numerous times with the ever-increasing chance for human error. Even so, these methods are far more time and labor efficient than using tons of forage over several months to obtain animal feeding data, the only way to obtain forage nutritive value. The time to know the nutritive value of a forage is not, however, several weeks after the last bale has been fed.

Considerable progress has been made in the laboratory by constructing facilities to do multiple repetitive analyses. Laboratories can usually process 500 tube IVDMD

runs which will give the results of 250 samples/week. This analysis, however, is the only one that a group of 2-4 technicians can handle. To conduct all of the analyses mentioned would require 10-12 people and at least 3 weeks for 250 samples.

Near-infrared (NIR) reflectance spectroscopy has been used to predict the quality of forages and their chemical composition. The NIR method is fast and requires much less labor. On a properly calibrated instrument, an operator and one person to prepare samples are all the labor needed to analyze a sample in 40 sec. and predict 10 compositional or animal performance factors. If automated, the instrument could analyze several hundred samples a day, and its initial cost ($50,000 to $100,000) could quickly be regained. The NIR method has been accepted by Canada and the U.S. as the method of choice for measuring protein content of grains.

Currently, the Southwestern Livestock and Forage Research Station is participating in a National NIR Network system utilizing a monochromator and a computer to collect, process and analyze the spectral data. As part of the network, researchers at the Ft. Reno station and at five other locations will be evaluating identical new instruments. Further, we will evaluate laboratory procedures which are used to obtain calibration data. Results of the studies conducted at Ft. Reno can be compared with those from the other five locations. These will make possible the standardization of all six instruments and the rapid exchange of data among locations.

Experimental Procedures

Sample preparation

Thirty samples of forages were assembled form three locations, University Park, Pennsylvania; Athens, Georgia; and El Reno, Oklahoma. The samples included alfalfa (*Medicago sativa*), orchardgrass (*Dactylis glomerota L.*), Clair Timothy (*Phleum pratense L.*), tall fescue (*Festuca arundinacea* Schreb.) and bermudagrass (Cynodon dactylon(L.) Pers.). Fresh frozen, freeze-dried samples and sun-cured hay samples of various qualities were included. Each was analyzed for crude protein (CP), ash, dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), and permanganate lignin (PML). *In vitro* dry matter disappearance was determined by a modification of Tilley and Terry procedures. A second set of 130 samples of "Old World" bluestems (*Botriochloa*) was analyzed by the same procedures.

The 30 samples, referred to as Near Infrared Network (NINW) samples, were ground through a 1mm screen in a Wiley Mill prior to being packed in the cups while the "Old World" bluestem samples (OWBS) were ground a second time through a 1mm screen in a Udy cyclone grinder.All samples were packed into sample cups approximately 5 cm in diameter and 1cm thick. A foam-filled poster board backing was used to seal the cups.

Instrument description and operation

The instrument system is comprised of two parts—the spectrometer and the computer. The spectrometer is a Neotec model 6100 monochromator. It produces monochromatic light in the near infrared region from 1100 nm to 2500 nm with a holographically ruled grating and uses lead sulfide detectors. The spectrometer is unique because of its high energy throughput and rapid scan time as compared with other monochromators such as the Cary 14 or J-Y HRS-2. Sixty-four scans of each sample are taken and averaged. Seven hundred data points at 2.0 nm intervals are recorded.

The computer is a Digital Equipment Corporation PDP/11-03, 16-bit word, 32K mini-computer. In its current configuration it has one RL01 hard 5.2M byte disk, dual 512K byte floppy disks and Decwriter III and Decwriter II hard-copy terminals. The

operating softwear was developed at Pennsylvania State Univesity and consists of eight programs to scan the samples, create files of the data, add calibration data, perform mathematical transformations and stepwise linear multiple regression analyses, make statistical comparison of actual vs. predicted data, and measure instrument noise and wavelength accuracy.

Results and Discussion

Table 1 contains data obtained from 30 samples which were circulated to six NIR laboratories for wet chemical analysis as an inter-laboratory check on accuracy of results and precision of laboratory procedures. The standard error of calibration (SEC) is quite low for such a diverse sample population, and the coefficient of determination R^2 values are good, particularly for protein and IVDMD. Repeatability is an estimate of similarity of multiple readings by the NIR instrument for each sample and is a measure of what the error limits (precision) for the instrument would be for any one sample. The last two columns contain data on 10 samples which came from Ft. Reno, Oklahoma. These were five samples, three bermudagrass and two fescue hays, submitted as duplicates. The SDP is the average standard deviation between duplicates for predicted values, and SDA is the average standard deviation between duplicates for the laboratory or "actual" analysis for IVDMD, CP, ADF and ADL. Only in the case of CP was the laboratory analysis more precise than NIR, reflecting the precision of analysis for nitrogen by the Kjeldahl method.

The most critical factor in NIR analysis is the quality of the laboratory analysis used for calibration, The study with the NIR network sample described above has shown that NIR can be used to find erroneous results. In fact, the SDA for IVDMD in Table 1 is large primarily for a difference of 10 percentage units between one set of duplicates. Statistical analysis of the residuals from "actual" analytical values of Location 1 versus predicted values using equations generated from Location 2 calibration data, and vice versa, have shown biases and errors which otherwise would have been ignored. Such things as differences in the diet of rumen fluid donor animals will bias IVDMD. Consistantly high NDF values are usually obtained because samples were not filtered while still boiling. Titrating to a different end point will bias crude protein, especially between operators. These biases affect the calibration value but really do not affect the prediction capability as much as would be expected since the prediction equation line is "averaged" through all calibration samples.

Table 2 contains data obtained from 100 "Old World" bluestem hay samples. In this case IVDMD was rerun several times on those samples with prediction values that differed by more than 25 percent from the laboratory value. In all cases the laboratory value was found to be wrong. When a set of good (consistent in three runs) IVDMD

Table 1. Calibration of NIR instrument for a broad spectrum of samples

Analysis	N[2]	SEC[3]	R^2	Repeatability	SDP[4]	SDA[5]
IVDMD	3	1.74	.92	0.16	0.37	1.72
CP	2	0.72	.98	0.02	0.22	0.09
ADF	3	1.6	.88	0.08	0.24	0.28
ADL	3	0.78	.87	0.06	0.12	0.29

[1]Set of 30 samples included 13 bermudagrass, 5 alfalfa, 4 orchardgrass and 1 timothy grass. Twenty were hays and 10 were fresh-frozen and freeze-dried.
[2]Number of wavelengths.
[3]SEC is standard error of calibration.
[4]SDP is the average standard deviation of the predicted values of the 10 El Reno samples which were duplicated, i.e. 5 different samples.
[5]SDA is the average standard deviation of the actual laboratory values for the 10 Ft. Reno samples which were duplicated.

Table 2. Standard errors of calibration and prediction for IVDMD of Old World Bluestem[1]

	Number of 's in equation		
Error	2	3	4
Calibration	1.65	1.52	1.50
Prediction	1.81	1.98	2.00
R^2	0.84	0.86	0.86
Repeatability	0.06	0.07	0.08

[1]Calibration was made on 60 Old World Bluestem samples and the resulting equations were used to predict IVDMD of a different set of 40 samples.

data for 60 samples was used for calibration, the results were those obtained in Table 2. These calibration equations were applied to the spectra of a different set of 40 "Old World" bluestem samples. The standard error of prediction, i.e. how far these samples deviate from the calibration regression line, is 1.81-2.00 (Table 2) equations using 2-4 wavelengths. These results are about as good as we can hope to do in the laboratory.

Conclusions

The near infrared method has potential in several areas. First, as a means of rapid laboratory analysis it is extremely valuable. It takes 5 minutes or less to run a sample and get 10 different analytical results. With automation, several hundred per day could be run. These analyses would be conducted with prediction equations generated from broad sample sets so that many species could be analyzed with the same set of equations. Alternatively, the computer, through the use of discriminant functions, could be programmed to choose the appropriate equation for a given sample's spectra.

Second, plant breeders could use the NIR technology as a rapid means of screening many samples. Here, specific calibrations would be required for each line of germ plasm. In many instances the extreme values would be the ones of most interest to the breeder, and the statistical routines could be modified to provide that information.

Third, the NIR spectra could be interpreted to the extent that wavelengths could be physically related to particular chemical entities in the plant giving an absolute analysis. This, in turn, could be related to an animal performance factor. This aspect will be an undertaking of a least one of the laboratories participating in the NIR network.

Finally, the concept of chemametric analysis, analysis by prediction, is just now becoming an accepted analytical procedure. There exists an unlimited potential for using the computer and regression analysis with a host of non-destructive, rapid spectroscopic methods which will provide detailed information more rapidly in the future.