based on susceptibility. Since alfalfa DC silage is more digestible than wheat DC silage, it is more susceptible. Thus, less time was alloted for digestion of the wheat straw.

From these data it would appear that ensiling wheat or alfalfa forage as direct cut silage at 39 and 34 percent dry matter, respectively, will result in a silage that is more digestible than when the forage is wilted prior to ensiling. The addition of wheat straw to wheat forage prior to ensiling increased the DMD of the wheat straw by 15 percent, but a decrease of 23 percent was noted when alfalfa forage was used. If silage storage space does not limit how much wheat forage is harvested as silage, then it would be advantageous to add wheat straw at the time of ensiling to increase the DMD of the wheat straw. If storage space is limited, more digestible dry matter would be realized if the straw is mixed with wheat silage at the time of feeding.

Literature Cited

Edwards, R. A. and P. McDonald. 1978. National Feed Ing. Assoc.

Calibration of a Near Infrared Reflectance Spectrometer for Prediction of Forage Quality

S. W. Coleman, F. E. Barton, II and R. D. Meyer

Story in Brief

In order for near infrared (NIR) reflectance spectroscopy to be used to estimate forage quality, the instrument must first be calibrated using a set of samples of known chemical composition. Regression analysis is used to develop a relationship (calibration equations) between one or more of the wavelengths and the chemical or quality component in question. Seventy-six "Old World" Bluestem samples, collected in 1974-75, were used to calibrate the instrument. Chemical data determined near the time the samples were collected could not be used for calibration purposes after the samples had aged either because of changes in composition or erroneous laboratory determinations. Coefficients of determination were increased when the samples were reanalyzed chemically within a few weeks of the time the spectral data were collected. Those with significant differences between "actual" determination and that "predicted" by NIR were reanalyzed a third time and the new data entered into the calibration file. This procedure

improved the agreement between NIR spectra and chemical analysis. Another study indicated that the width of the derivative segment of the spectral data may influence calibration for some constituents. Further, 40 samples selected at random do not appear to be enough to calibrate the instrument. Calibration equations derived from small sample sets tend to be dependent on the sample set and do not have general applicability.

Introduction

Near infrared (NIR) reflectance spectroscopy has been suggested as a method that will decrease the time for laboratory analyses of chemical components of forages used as quality indices (Barton and Coleman, 1981). Ultimately the technique may be capable of predicting the animal performance potential of forages. Previous research suggests that calibration data must be obtained from forages similar in taxonomy, age, location grown, environment, etc., to those being predicted, rather than using data from one kind of forage to develop a general prediction equation which would work for all kinds of feeds and forages. Several variables appear to influence the suitability of calibration data and the resulting equations used to predict quality. Some of these are age of samples, number of samples, accuracy of chemical analysis, derivative segment width of the NIR spectral data and the number of wavelengths (λ) chosen. The purpose of these experiments was to characterize the effects of certain variables on the calibration of NIR spectrometers.

Experimental Procedures

Samples of 5 "Old World" Bluestem (OWBS) varieties (Plains, Caucasian, B,L, and T blends) were collected over two growing seasons (1974 and 75). The samples were oven dried and ground in a Wiley Mill[1] to pass through a 1 mm screen. Between the years 1976-78 the samples were analyzed for dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), permanganate lignin (PML), and in vitro dry matter disappearance (IVDMD) by a modified Tilly and Terry procedure.

In 1981, a random subset of the samples was reground with a Udy[1] cyclone grinder fitted with a 1 mm screen and packed into sample cups approximately 5 cm in diameter by 1 cm thick and sealed with foam-filled poster board backing. Seventy-six of the samples were scanned with a NIR spectrometer through a range of wavelengths from 1100-2500 nm. Reflectance spectra containing 700 data points were smoothed and derivatized (2nd derivative). Chemical analyses, conducted from 1976-78, were regressed on to the spectral data using stepwise linear multiple regression procedures. Statistical comparisons between actual and predicted values were conducted.

Chemical components of the samples were routinely determined a second time, and the new chemical data were subjected to statistical analysis with the original spectra. Chemical components were "predicted" using the spectral data, and samples with "predicted" values deviating by more than two standard deviations from chemical determination were reanalyzed a third time. A third data set, identical to the second data set except for replacement of aberrant chemical values by the new chemical values, was reanalyzed statistically.

[1]Mention of trade name, proprietary product of specific equipment does not imply its approval to the exclusion of other products that may also be suitable.

The 76-sample subset plus 24 other samples from the same large data set were randomly divided into two subsets of 40 and 60 samples each. Prediction equations were derived from each set, and the reciprocal set was used as a "blind" comparison to test effect of samples' set size and the derivative segment width on calibration equations derived from regression analysis and their relative usefulness for predicting the chemical components in question.

Results and Discussion

The NIR spectrum as log(1/reflected energy) for two diverse samples is presented in Figure 1. Note obvious differences in the absorbance of the two samples from 1100-1300 nm (lignin), 1500 nm (fiber). 1940 nm (water), 2150 nm (protein), 2200 nm (fiber) and 2350 nm (carbohydrates). However, several physical factors, such as fineness of grind may shift the entire spectrum vertically, thus precluding its usefulness as a predictor. To overcome these nonchemical problems, the spectrum may be normalized by derivative spectroscopy. The first derivative depicts the slope of the various peaks, and the second derivative (Figure 2) depicts the rate of change of the slope of the peak. Compound peaks

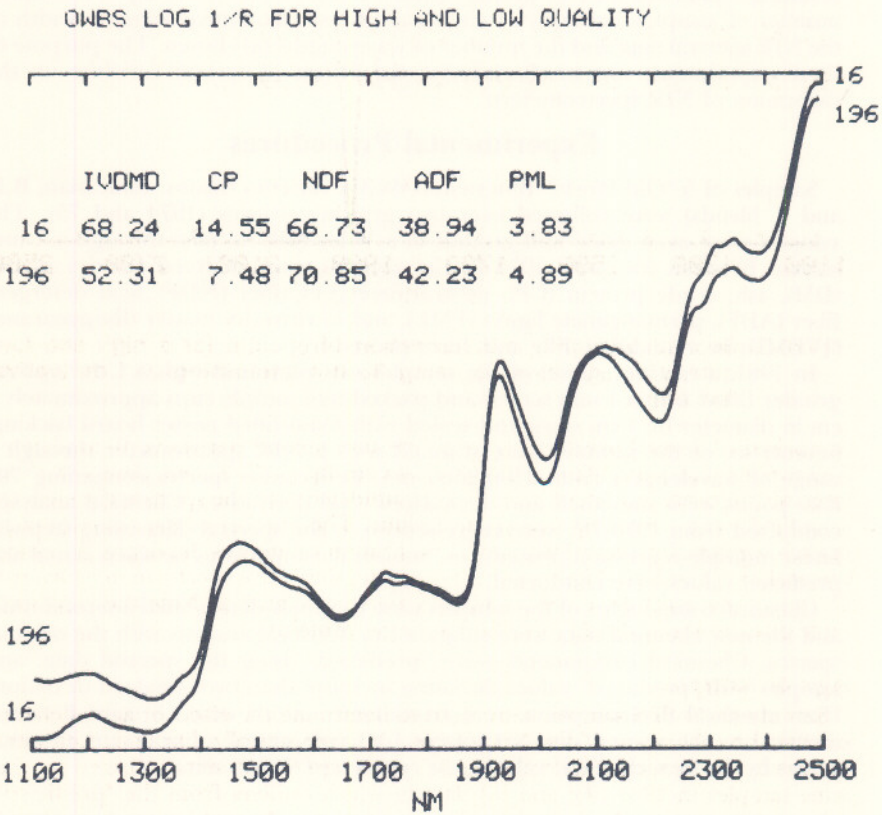

Figure 1. Log (1/Reflectance) spectra for a high and low quanity forage sample

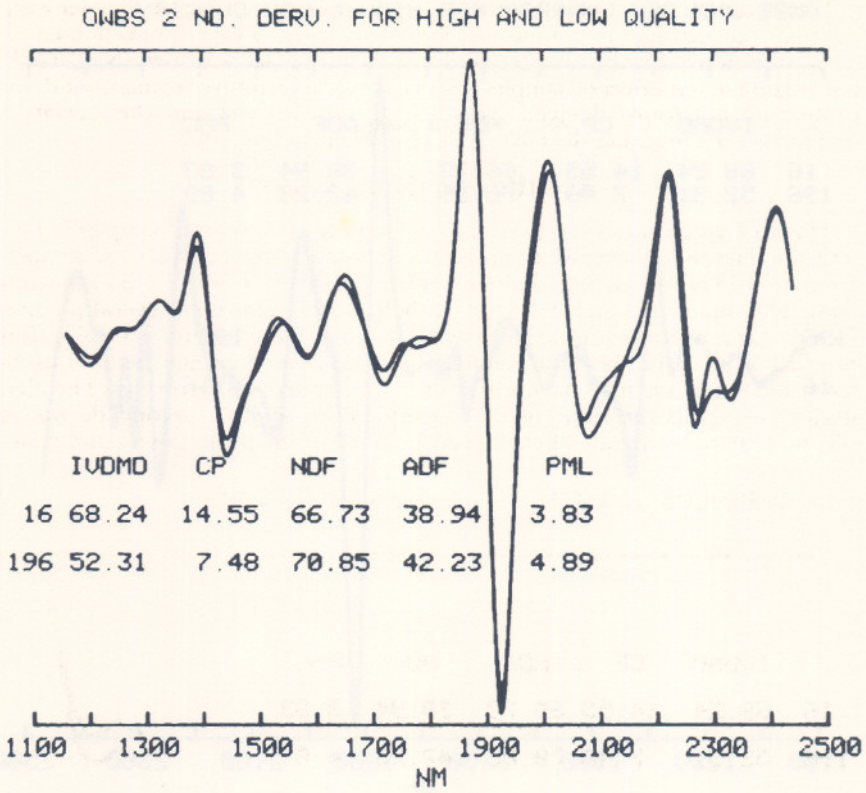

Figure 2. Second derivative transformation of spectra for a high and low quality forage sample using 36 nm smoothing and derivative function

comprised of several overlapping peaks require second derivative treatment to deconvolute the peak. The spectrum is made up of 700 data points at 2nm intervals. To overcome "noise" which is accumulated with the spectra, the curve is smoothed using a running average technique. The number of data points taken in each segment for smoothing and derivatization influences the degree to which the peaks are resolved. Figure 3 shows a second derivative spectrum in which the derivative segments were half as wide as those in Figure 2. Note the increased number of peaks in the 2100-2200 nm range (protein).

Age of sample

Seventy-six OWBS samples were used to determine the effect of age of chemical data on calibration of the NIR (Table 1.) Three sets of calibration data were obtained: (1) chemical data collected 3-5 years ago (I); (2) data collected on the same samples in 1981 (R); and (3) data in which outliers from the "predicted" value were two standard deviation units from the "actual," were reanalyzed chemically and were then added to the calibration data file (C). Only the "actual" means of crude protein and ADF were not changed appreciably by one or more

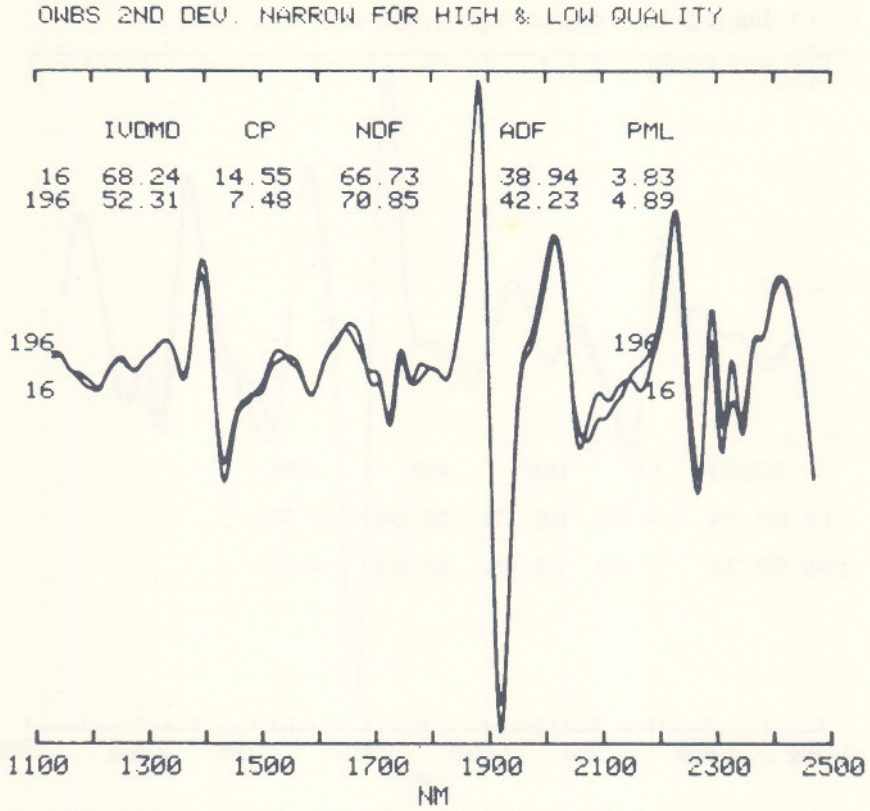

Figure 3. Second derivative transformation of spectra for a high and low quality forage sample using narrow (18 nm) smoothing and derivative function

of the reanalyses. In all cases except IVDMD, R^2 was higher and standard error of calibration lower in data sets R and C compared to 1. It appears from these data that some chemical constituents change with time, but location and reanalysis of probable "error" samples resulted in more consistent improvement in calibration. Further, NIR may be used to detect inaccurate chemical data in a sample set. In every case where predicted vs actual values did not agree, the reanalysis proved the NIR to be more correct. The final equations (C) appear to be satisfactory to predict quality components of OWBS. However, they must be tested against a "blind" set to see if the equation will predict the chemical constituents of samples not included in the calibration data set.

Effect of sample set size and derivative segment width

Results for calibrating the NIR for dry matter (100-moisture) produced mixed results (Table 2). Unpublished results from our lab showed that variations in moisture of 1-2 percentage units occurred due to variations in time the samples spent in the dessicator after oven drying. In our estimate, oven drying samples is

Table 1. Effect of lab data on calibration

Analysis	Run	Mean + SD[a]	#λ's[b]	R^{2c}	SEC[d]	Repeat[e]
Dry matter	I[f]	93.3 ± 1.82	3	.63	1.11	.03
	R[g]	95.3 ± 1.16		.73	.61	.06
	C[h]	94.7 ± 1.06		.84	.43	.02
Protein	I	12.2 ± 1.96	3	.84	.80	.03
	R	12.4 ± 2.00		.87	.71	.04
	C	12.3 ± 2.01		.94	.49	.05
Neutral detergent fiber	I	67.7 ± 3.12	5	.65	1.86	.16
	R	68.2 ± 2.91		.73	1.52	.08
	C	67.6 ± 2.71		.82	1.15	.27
Acid detergent fiber	I	38.8 ± 2.55	3	.63	1.55	.10
	R	38.6 ± 2.95		.81	1.27	.25
	C	38.8 ± 2.90		.87	1.04	.09
Permanganate lignin	I	4.9 ± 1.14	3	.38	.90	.05
	R	3.7 ± 1.23		.66	.71	.01
	C	3.7 ± .87		.61	.54	.01
IVDMD	I	60.4 ± 4.10	3	.68	2.33	.21
	R	60.2 ± 5.78		.65	3.40	.33
	C	62.5 ± 3.34		.83	1.36	.11

[a]Standard deviation.
[b]Number of wavelengths used in equation.
[c]R^2 = Coefficient of determination.
[d]SEC = Standard error of calibration.
[e]Repeat = Repeatability error.
[f]I = File data several years old.
[g]R = Samples were all reanalyzed routinely.
[h]C = Samples from R which were statistical outliers were reanalyzed and the new data were incorporated into the file.

not sufficiently accurate to calibrate the NIR for dry matter. For this reason, the Karl Fisher method is being investigated by the national USDA-NIR project.

Calibration and prediction data for crude protein is presented in Table 3. The best prediction equation was obtained with the 60 samples and 36 nm (wide) derivative segment. A slight bias (.18 percent) did occur when the predicted values of the 40 samples were compared to the actual wet chemistry values. It might be noted that agreement between actual and predicted values was better for crude protein than the fiber components or IVDMD. The reason is that crude protein measures a distinct chemical entity whereas fiber and IVDMD are not chemically defined. The lowest bias was found with the narrow (18 nm) derivative segment though the standard error of prediction (.65) was higher, and the coefficient of determination (R^2 = .88) was lower than that for the wide derivative segment. The standard errors are similar to those for multiple runs of the same samples in the laboratory.

Table 2. Effect of sample number and derivative width on calibration and prediction of dry matter

File	Function	#λ's[a]	Bias[b]	SE[c]	R^{2d}	Repeat[e]
OWBS60W[f]	Calibrate	3	—	.61	.74	.04
OWBS40	Predict		.79	1.05	.84	—
OWBS60N[g]	Calibrate	3	—	.58	.76	.14
OWBS40	Predict		.15	.70	.81	—
OWBS40W	Calibrate	2	—	.45	.88	.01
OWBS60	Predict		−.58	1.16	.32	—
OWBS40N	Calibrate	2	—	.44	.89	.05
OWBS60	Predict		−.38	1.18	.24	—
OWBS100W	Calibrate	3	—	.64	.74	.04
OWBS100N	Calibrate	2	—	.70	.69	.10

[a]Number of wavelengths used in the equation.
[b]Difference in mean of "actual" and "predicted" values.
[c]SE = Standard error of calibration for calibrate; standard error of the difference of actual vs predicted for predict.
[d]R^2 = Coefficient of determination.
[e]Repeat = Repeatability error.
[f]W = Wide derivative segment — 36 nm.
[g]N = Narrow derivative segment — 18 nm.

Table 3. Effect of sample number and derivative width on calibration and prediction of crude protein

File	Function	#λ's[a]	Bias[b]	SE[c]	R^{2d}	Repeat[e]
OWBS60W[f]	Calibrate	3	—	.46	.97	.04
OWBS40	Predict		.18	.57	.96	—
OWBS60N[g]	Calibrate	2	—	.52	.96	.10
OWBS40	Predict		−.05	.65	.88	—
OWBS40W	Calibrate	3	—	.53	.93	.04
OWBS60	Predict		.33	.71	.94	—
OWBS40N	Calibrate	3	—	.51	.92	.09
OWBS60	Predict		−.09	.62	.95	—
OWBS100W	Calibrate	3	—	.49	.96	.04
OWBS100N	Calibrate	2	—	.57	.95	.10

[a]Number of wavelengths used in the equation.
[b]Difference in mean of "actual" and "predicted" values.
[c]SE = Standard error of calibration for calibrate; standard error of the difference of actual vs predicted for predict.
[d]R^2 = Coefficient of determination.
[e]Repeat = Repeatability error.
[f]W = Wide derivative segment — 36 nm.
[g]N = Narrow derivative segment — 18 nm.

Neutral detergent fiber (NDF) (Table 4) is one of the more difficult parameters for which to calibrate the NIR because it is not a single chemical entity but represents both the hydrolyzable and non-hydrolyzable cell wall components. As such, the NIR attempts to predict total cell wall components including carbohydrate (pentose and hexose chains), lignin and residual protein, most of which we cannot measure chemically with any degree of accuracy. Bias was approximately half as large using 60 samples to calibrate and 40 to predict than when the reverse was used. Both 18 and 36 nm derivative width gave similar results. Five wavelengths significantly contributed to the calibration equation for the 60-sample set, but may result in "overfitting" the data.

Table 4. Effect of sample number derivative width on calibration and prediction of neutral detergent fiber

File	Function	#λ's[a]	Bias[b]	SE[c]	R^{2}[d]	Repeat[e]
OWBS60W[f]	Calibrate	5	—	1.06	.89	.19
OWBS40	Predict		.57	1.67	.60	—
OWBS60N[g]	Calibrate	5	—	1.10	.88	.26
OWBS40	Predict		.65	1.44	.67	—
OWBS40W	Calibrate	3	—	1.13	.75	.16
OWBS60	Predict		−1.04	2.21	.64	—
OWBS40N	Calibrate	3	—	1.09	.77	.19
OWBS60	Predict		−1.47	2.44	.63	—
OWBS100W	Calibrate	3	—	1.38	.77	.22
OWBS100N	Calibrate	4	—	1.19	.83	.24

[a]Number of wavelengths used in the equation.
[b]Difference in mean of "actual" and "predicted" values.
[c]SE = Standard error of calibration for calibrate; standard error of the difference of actual vs predicted for predict.
[d]R^2 = Coefficient of determination.
[e]Repeat = Repeatability error.
[f]W = Wide derivative segment — 36 nm.
[g]N = Narrow derivative segment — 18 nm.

The best calibration equation to predict ADF (Table 5) was from 40 samples using the wide derivative segment. ADF appears easier to calibrate for than NDF. Further, the range of ADF in the 40 samples may be more representative of the 100-sample population than the 60 samples. No differences were observed in calibration errors with the wide and narrow derivative segments, but predictions were better when the wide derivative segment was used. This would suggest that broad, smooth peaks, including some combinations, may be best for parameters which are not well defined chemically.

Permanganate lignin (Table 6) contained little variation from which to predict; therefore, R^2 was quite low. Bias and standard errors were also low. However, general observation of individual predicted vs actual values would suggest the prediction may be about as good as the chemical determination.

Although IVDMD (Table 7) is not a chemical component, several chemical components have a bearing on its magnitude. Therefore, with a combination of wavelengths, it is possible to predict IVDMD. The R^2 value was not as high as with other constituents, and it appears that the narrow derivative segment might be more beneficial than the wide. Bias was high, but the IVDMD procedure itself

Table 5. Effect of sample number and derivative width on calibration and prediction of acid detergent fiber

File	Function	#λ's[a]	Bias[b]	SE[c]	R^{2d}	Repeat[e]
OWBS60W[f]	Calibrate	3	—	.94	.90	.06
OWBS40	Predict		1.00	2.00	.72	—
OWBS60N[g]	Calibrate	3	—	.96	.90	.14
OWBS40	Predict		.37	1.64	.59	—
OWBS40W	Calibrate	4	—	.73	.91	.07
OWBS60	Predict		.04	1.32	.81	—
OWBS40N	Calibrate	4	—	.74	.91	.15
OWBS60	Predict		−1.15	2.07	.77	—
OWBS100W	Calibrate	5	—	.87	.91	.09
OWBS100N	Calibrate	4	—	.98	.88	.15

[a]Number of wavelengths used in the equation.
[b]Difference in mean of "actual" and "predicted" values.
[c]SE = Standard error of calibration for calibrate; standard error of the difference of actual vs predicted for predict.
[d]R^2 = Coefficient of determination.
[e]Repeat = Repeatability error.
[f]W = Wide derivative segment — 36 nm.
[g]N = Narrow derivative segment — 18 nm.

Table 6. Effect of sample number derivative width on calibration and prediction of permanganate lignin

File	Function	#λ's[a]	Bias[b]	SE[c]	R^{2d}	Repeat[e]
OWBS60W[f]	Calibrate	3	—	.49	.73	.03
OWBS40	Predict		.04	.67	.63	—
OWBS60N[g]	Calibrate	3	—	.46	.75	.08
OWBS40	Predict		−.06	.67	.38	—
OWBS40W	Calibrate	4	—	.49	.68	.06
OWBS60	Predict		.15	.70	.56	—
OWBS40N	Calibrate	4	—	.49	.68	.14
OWBS60	Predict		.51	.97	.42	—
OWBS100W	Calibrate	3	—	.55	.66	.04
OWBS100N	Calibrate	5	—	.51	.71	.09

[a]Number of wavelengths used in the equation.
[b]Difference in mean of "actual" and "predicted" values.
[c]SE = Standard error of calibration for calibrate; standard error of the difference of actual vs predicted for predict.
[d]R^2 = Coefficient of determination.
[e]Repeat = Repeatability error.
[f]W = Wide derivative segment — 36 nm.
[g]N = Narrow derivative segment — 18 nm.

Table 7. Effect of sample number and derivative width on calibration and prediction of in vitro dry matter disappearance

File	Function	#λ's[a]	Bias[b]	SE[c]	R^{2d}	Repeat[e]
OWBS60W[f]	Calibrate	3	—	1.53	.86	.07
OWBS40	Predict		−1.94	2.75	.76	—
OWBS60N[g]	Calibrate	3	—	1.73	.82	.16
OWBS40	Predict		−.80	1.58	.78	—
OWBS40W	Calibrate	3	—	1.02	.88	.16
OWBS60	Predict		.63	2.32	.73	—
OWBS40N	Calibrate	4	—	1.08	.86	.29
OWBS60	Predict		−1.81	2.98	.67	—
OWBS100W	Calibrate	3	—	1.45	.85	.20
OWBS100N	Calibrate	5	—	1.38	.87	.43

[a]Number of wavelengths used in the equation.
[b]Difference in mean of "actual" and "predicted" values.
[c]SE = Standard error of calibration for calibrate; standard error of the difference of actual vs predicted for predict.
[d]R^2 = Coefficient of determination.
[e]Repeat = Repeatability error.
[f]W = Wide derivative segment — 36 nm.
[g]N = Narrow derivative segment — 18 nm.

can only be used for ranking taxonomically similar plant samples and not for widespread comparisons across sample types and plant ecotypes.

These data would suggest that different smoothing and derivative segment widths are desirable for predicting different plant constituents. The classical method for selecting derivative width is to take the interval width of a peak at half its height. Computer programs for instruments in the national NIR project are presently being written to arrive at the proper width for each constituent.

The number of samples required for calibration appears to be greater than 40 for most chemical constituents. Even when the set of 40 gave the best equation, the equation may be sample set dependent and may not be of general value for predicting other OWBS data sets. Samples used for calibration should be scanned with the NIR instrument as soon as possible after the chemical laboratory analysis is obtained. These data indicate that changes in the samples may take place over a period of time which affect the relationship between NIR spectra and laboratory chemical data.

Literature Cited

Barton, F. E., II and S. W. Coleman. 1981. Potential of near infrared reflectance spectroscopy for measuring forage quality. Okla. Agr. Exp. Sta. Res. Rep. MP-108:73.