667

Analysis of Trace Elements in Forages by Near Infrared Reflectance Spectroscopy

D. H. Clark, E. E. Cary, and H. F. Mayland

Analysis of Trace Elements in Forages by Near Infrared Reflectance Spectroscopy

D. H. Clark,* E. E. Cary, and H. F. Mayland

ABSTRACT

Use of near infrared reflectance spectroscopy (NIRS) for elemental analysis has been limited to a few elements including Ca, P, K, and Mg. However, other elements are of interest in the agricultural industry. Therefore, NIRS spectra were collected on forage samples consisting of tall fescue (*Festuca arundinacea* Schreb.), crested wheatgrass (*Agropyron cristatum* and *A. desertorum*), and alfalfa (*Medicago sativa* L.). Elemental concentrations of Ba, Li, Mo, Ni, Pb, V, Al, S, and Si were determined by ICP (Inductively Coupled Argon Plasma) analysis while selenium (Se) was determined by fluorometry. The elemental analyses were regressed against NIRS apparent absorption from 1100 to 2500 nm at 2-nm increments. Coefficients of variation |CV = (standard error of performance / the mean from the chemical procedure) × 100] ranged from a high of 211% for Li in crested wheatgrass to 11% for S in alfalfa. Determination of Ba, Li, Mo, Ni, Pb, and V exhibited enough inconsistency in CVs among the three forages to preclude their determination with NIRS. Aluminum and S appear to be present in an organic form that NIRS is able to detect (CV = 22 and 15, 21 and 12, and 28 and 11%, for tall fescue, crested wheatgrass, and alfalfa, respectively). Silica exhibited slightly more variation than S or Al, with alfalfa having the highest CV (49%). Selenium was only determined on a tall fescue population with a CV = 27%. Using the statistical values as parameters indicative of NIRS utility, it appears that Al and S are the only elements in this group of minerals that can be determined with NIRS for these forage types.

U SE OF NEAR INFRARED reflectance spectroscopy (NIRS) for the analysis of elements in forages was first documented by Shenk et al. (5,6). From this work, commercial laboratories with NIRS instruments began routine elemental analyses on agricultural feedstuffs. The primary elements currently being analyzed with NIRS include Ca, K, P, and Mg. These elements are likely to be associated with certain organic acids (1) which are in the forages, and it is the organic acid salts of these elements that provide the hydrogen bonding detected by NIRS.

Recently we reported the use of NIRS for elemental determinations in three different forage types (1). Inconsistent responses were noted for determination of Ca, P, K, and Mg across the three forages. This precluded the use of NIRS mineral analyses for balancing mineral levels in feed rations. However, the values could be used as general guidelines for ration balancing, the way current feed ingredient tables are currently used. Early researchers (2) reported that S could be detected with NIRS because of the rotational or vibrational energies associated with hydrogen bonds or sulfur molecules. However, those authors used sulfur compounds dissolved in carbon tetrachloride. Whetsel (8) listed the wavelengths found with near infrared for the determination of rare earth metals and

inorganic metal complexes in simple solutions, but the state in which these metals were studied is not likely to be found in forages.

Elements in forages probably exist in both inorganic and organic complexes rather than in simple elemental forms. However, these complexes are not static, and this could contribute to the variability in NIRS results noted earlier (1). In addition, organic acid salt forms may differ between or within genera of forages. Such variability would limit the utility of NIRS for estimating elemental concentrations in feedstuffs as noted earlier (1). The same forages utilized in the previous mineral study (1) were used in this study to examine the accuracy and precision of NIRS in analyzing Ba, Li, Mo, Ni, Pb, V, Al, S, and Si. A different tall fescue population was used to measure Se.

MATERIALS AND METHODS

Tall fescue, crested wheatgrass, and alfalfa samples were used to quantify the relationship between traditional chemical and NIRS determinations of elemental concentrations. The history of the forage samples used in this study is documented elsewhere (1). The ground samples (1 mm stainless steel screen) were dried at 70 °C for 24 h before subsamples were weighed (ca. 0.2 g) into quartz digestion tubes containing 5 mL concentrated HNO_3 and 0.3 mL 70% $HCLO_4$ acid (17:1). Perchloric acid digestion was performed according to Schilt (7). Samples were allowed to oxidize overnight at room temperature. They were then heated to 160 °C for 2 h and then to 185 °C until the tube was dry. The tubes were then cooled to room temperature and 5 ml of 3 M HNO_3 was added. The tube and contents were heated for a short time over a flame to promote dissolution. A cap was placed on the tube and the contents were cooled to room temperature. Multi-elemental analysis was performed using a simultaneous vacuum ICP spectrometer (Applied Research Laboratory[1] Model 34000, Sunland, CA).

The tall fescue population used for Se analysis consisted of spring and fall harvested plants collected in each of 2 yr from an experimental nursery separate from the other tall fescue population grown at Columbia, MO. The samples were collected and processed as described in the earlier study (1). Selenium was determined fluorometrically with the method used by Olsen (3).

After subsampling for chemical analysis, the balance of the samples were ground to pass a 1-mm screen, using a UDY[1] cyclone mill (UDY Corp., Fort Collins, CO), and a portion (ca. 2 g) was packed into cups for NIRS scanning. Apparent absorption values (Log 1/R, where R = reflectance) were collected for all samples from 1100 to 2500 nm at 2-nm increments using a Pacific Scientific Model 6350 scanning monochromator (Pacific Scientific Corp., Gardner/ Neotec Instrument Div., Silver Spring, MD) coupled to a DEC PDP 11/23 computer (Digital Equipment Corp., Nashua, NH). The NIR data for the two tall fescue populations, crested wheatgrass, and alfalfa samples were each stored in separate files. Elemental data from ICP and fluorometric analysis of each sample were entered into the computer. For calibration, 102 tall fescue samples (251 tall fescue samples for Se), 100 crested wheatgrass samples, and 44 alfalfa sam-

[1] Mention of a trade name does not imply an endorsement or recommendation by the USDA or Utah State Univ. over similar companies or products not mentioned.

ples were randomly selected. The absorption data and elemental analyses for these samples were used in developing multiple regression equations for each element.

Software used to collect reflectance spectra (absorption data) and to develop and test the equations are part of the USDA National NIRS Forage Network software (4). Equation development was performed using the BEST program (4). Samples used for calibration were further split (generally 3:1; starting with sample no. 3) during the calibration process to produce a "validation" set as an aid in choosing the optimum equation. Equation selection, by the operator, was based on a combination of statistics from calibration development (each wavelength in the equation having a partial $F > 8.5$, low standard error of calibration (SEC) and standard error of performance (SEP), and the high R^2 and r^2). Therefore, the best equations within each mineral and forage type were chosen based on both calibration and validation statistics. The remaining samples, 101 tall fescue (251 tall fescue for Se), 100 crested wheatgrass, and 15 alfalfa, were used as the final validation of the chosen equations.

The concentration of the analyzed element was regressed on the 700 data points as follows: Amount Analyzed Component = $B_0 + B_1X_1 + B_2X_2 + B_3X_3 \ldots$ where X_1, X_2, X_3 are reflectance (absorption) measurements or derivatives of these, at wavelengths λ_1, λ_2, λ_3. The regression constant B_0 and B_1, B_2, B_3 are partial regression coefficients. Standard error of performance, bias, and r^2 for each element within each forage type were used to measure the amount of error associated with the determination of each element.

Coefficients of variation (CV) were calculated for every element, except Se, as a means of comparing the same element across the three forage types. Standard error of calibration (SEC) and SEP were calculated as follows:

$$SEC = (\text{mean square error})^{0.5} ;$$

$$SEP = \{[(X - x)^2]/n\}^{0.5} - (bias/n) ;$$

where X = NIRS values, x = chemical values, n = number of samples, and $bias$ = NIRS mean minus the chemical mean.

RESULTS AND DISCUSSION

Data associated with equation development for tall fescue, crested wheatgrass, and alfalfa are shown in Tables 1, 2, and 3, respectively. Means and standard deviations are listed to show the concentration level and variation among forages. The SEC and R^2 values for quality parameters (e.g. protein, fat, oil, moisture, etc.) will generally indicate how well the equations will perform within the same population. However, with elements, the SEC and especially the R^2 are not good indicators, because the instrument is not directly measuring the element. The R^2 and r^2 values for elemental determinations are governed more by the amount of variability (range in concentration) present than by direct relationship between concentration change and apparent adsorption. For most elements, the SEC values are lower than the SD, indicating that NIRS can be used to determine concentration change. Some elements have SEC values close to the SD. Indicating that, although NIRS can make the determination, the results are not exact.

Selenium results are hard to interpret because of the small range in concentration. The R^2 is low ($R^2 = 0.24$) and suggests that NIRS cannot determine Se concentration. Selenium was not determined on the crested wheatgrass or alfalfa, so no comparisons for wavelength selection can be made.

Wavelengths are listed in order of decreasing (highest to lowest F) contribution to the overall equation (Tables 1, 3, and 5). Wavelength comparison is a weak method for determining the relationship between reflected energy and concentration change. If the instrument can make the determination for an element, then common wavelengths may appear in the equations for the different forage types. However, for common wavelengths to be used, the elements must be associated with the same organic molecules in each forage type. The fact that the elements are found in different complexes and the complexes appear to be different within or among forages, will contribute to the differences in wavelengths used for the equations (1). Multiterm (wavelengths) equations are used frequently by NIRS, and some interdependency will occur among wavelengths. Also, because of the use of derivatives and different segment lengths that are subtracted for derivative calculations, the wavelengths will not be the same for a given element across the different forage types.

The only common wavelengths found for Ba were for tall fescue (1958 nm) and crested wheatgrass (1982 nm). Some wavelength areas were common for Li determinations between two forage types (1398, and 1402; 1898, and 1868 for tall fescue and alfalfa, respectively; and 1842 and 1868 for crested wheatgrass and alfalfa, respectively), but only 1808 to 1842 and 1398 to 1418 nm areas were used in all three forages. Molybdenum determinations found one common wavelength area for tall fescue and alfalfa (2118–2192 nm). However, 1132 and 1182 nm were common for crested wheatgrass and alfalfa, respectively; 2298 and 2292 for tall fescue and crested wheatgrass, respectively. Only tall fescue and alfalfa used 1712 to 1732 and 1832 to 1852 nm areas for NI determinations.

All three forages had the 1398 to 1458 nm region in common for Pb, while tall fescue and crested wheatgrass also had the 1752 to 1778 and 1892–1898 nm regions in common. Vanadium responded to two wavelength regions in common for tall fescue and alfalfa (1398–1402 and 1822–1838 nm) with crested wheatgrass and alfalfa also sharing the 2110 to 2138 nm region. No wavelengths were common for all forage types for the determination of Al, however many wavelengths were shared by at least two of the forage types.

Spectra of cystine, cysteine, or methionine show strong absorption in the areas of 1700 and 2300 nm (Karl Norris, unpublished data), possibly attributable to a S-H stretch. Only crested wheatgrass responded in this area (1732 nm). None of the S equations had wavelengths common for all three forages. Many wavelength areas were common between two of the forages. No wavelengths were common for Si for all three forage types. Two areas were common for crested wheatgrass and alfalfa (1152 and 1312–1352 nm), while tall fescue and crested wheatgrass shared one area (2290–2312 nm).

After equations are developed, they are tested on a different set of samples and the statistical values from this test are used to measure accuracy. Most researchers use the SEP, bias, and r^2 values to evaluate equation performance. Using r^2 values to evaluate equa-

Table 1. Equation calibration statistics for element concentrations (mg kg⁻¹) in 102 tall fescue samples.

| Element | Wet chemistry | | SEC† | R^2 | TRT‡ | Wavelengths§ |
	Mean	SD				
Ba	35.2	12.9	10.6	0.19	2	2058 2298 1958
Li	0.6	0.3	0.3	0.89	1	1808 1418 1898 1398 2398 1298
Mo	1.8	0.4	0.3	0.52	1	1178 2118 2298 1738
Ni	2.0	0.5	0.3	0.51	1	1832 1712
Pb	3.2	0.9	0.5	0.66	2	1432 1752 2292 1892 1822
V	2.3	1.3	0.3	0.87	1	1422 1402 2462 1882 1902 1822
Al	797	639	161	0.86	1	1428 2168 1388 1868 1908
S	2578	343	208	0.66	2	1992 2152 2252 1412 1252
Si	220	87	58	0.16	0	2290 1970
Se¶	0.1	0.01	0.3	0.24	2	1408 1888 2028 1668

† SEC = standard error of calibration (mg kg⁻¹).
‡ Math treatments: 1 = first derivative; 2 = second derivative; 0 = log 1/Apparent absorption.
§ Wavelengths (nm) used in the equation in order of decreasing contribution.
¶ Se data are for 251 tall fescue samples which are not a part of the 101 tall fescue population in the first section of this study.

Table 2. Equation calibration statistics for element concentrations (mg kg⁻¹) in 100 crested wheatgrass samples.

| Element | Wet chemistry | | SEC† | R^2 | TRT‡ | Wavelengths§ |
	Mean	SD				
Ba	22.1	5.5	2.2	0.43	2	1282 1902 1482 1982 1542
Li	0.8	2.3	1.2	0.12	2	1842 1402 1942 1462
Mo	0.7	0.4	0.2	0.63	2	2292 1852 1232 1292 1132 2192
Ni	0.5	0.2	0.2	0.22	1	1152
Pb	1.6	0.7	0.5	0.35	2	2418 1778 1898 1458 1578
V	0.7	0.3	0.2	0.66	0	1670 2110
Al	330	166	59	0.82	1	1412 1892 2032 2272 2312
S	1414	279	155	0.65	1	1232 2112 1732 1812
Si	165	53	38	0.45	2	1352 1152 2152 2312 2052

† SEC = standard error of calibration (mg kg⁻¹).
‡ Math treatments: 1 = first derivative; 2 = second derivative; 0 = log 1/Apparent absorption.
§ Wavelengths (nm) used in the equation in order of decreasing contribution.

Table 3. Equation calibration statistics for element concentrations (mg kg⁻¹) in 44 alfalfa samples.

| Element | Wet chemistry | | SEC† | R^2 | TRT‡ | Wavelengths§ |
	Mean	SD				
Ba	20.5	9.6	5.8	0.32	1	1742 1602 1642 1822
Li	1.1	0.6	0.4	0.49	1	1418 1868 1818 1378
Mo	1.7	0.8	0.6	0.42	2	1942 1362 1182 2122 2382
Ni	0.9	0.3	0.2	0.51	1	1632 1852 1732 1972
Pb	1.5	0.8	0.7	0.03	1	1472
V	0.6	0.3	0.2	0.72	2	1398 2098 1838 2138
Al	169	142	74	0.73	2	1332 2452 2172 2012
S	2237	492	172	0.88	2	2062 1982 2222 2002
Si	71	79	35	0.78	1	1792 1152 1312 1832

† SEC = standard error of calibration (mg kg⁻¹).
‡ Math treatments: 1 = first derivative; 2 = second derivative.
§ Wavelenghts (nm) used in the equation in order of decreasing contribution.

tions can be misleading, because the amount of variation in chemical data will affect the values. The SEP and bias values can be affected by one or two samples that are not part of the sample set (abnormal chemical values or spectral properties). This laboratory proposed using CV values (1) as an estimate of equation performance, especially when comparing different concentrations for the same component or different types of material. Because CV values are affected by the mean from the chemical procedure and SEP, researchers should be able to use these values as a tool in evaluating equation performance across several elemental parameters.

Barium analysis produced varying CV values with low r^2 and high SEP values for the three forage types (Tables 4, 5, and 6). Because of this variation (CVs ranging from 25.4 to 44%) it does not appear that NIRS consistently relates spectral information (apparent ab-

sorption) to changes in Ba concentration. Results from Li determinations exhibited more variation in CVs than results from Ba. Molybdenum, Ni, Pb, and V all exhibited inconsistent results. This inconsistency across forage type would preclude the routine use of NIRS for these elements. The CV values for Al were more consistent (ca. 20%) across forages, suggesting a relationship between spectral changes and Al concentration. However, because no common wavelengths were found among the forage types, chemical components with which Al is associated probably differ.

Sulfur is associated with many different organic components in forages. This may explain the low statistical values (SEP and r^2) shown in the tables. However, the CV values were consistently low for each forage, suggesting a strong relationship between S concentration and apparent absorption. Silicon in alfalfa had the highest CV, followed by tall fescue and crested

Table 4. Statistical data for element concentrations (mg kg⁻¹) determined by wet chemistry and NIRS for 101 tall fescue samples.

Element	Wet chemistry		CV†	SEP‡	r^2	Bias	Standard deviation	
	Mean	Range					Chemistry	NIRS
Ba	36.9	13.9–86.0	34.3	12.6	0.07	2.2	12.8	6.2
Li	0.6	0.3–2.5	15.9	0.6	0.80	0.0	0.2	0.2
Mo	1.7	0.8–2.9	20.1	0.4	0.30	0.0	0.4	0.2
Ni	2.0	1.2–3.6	16.2	0.3	0.35	0.0	0.4	0.3
Pb	3.2	1.6–7.0	24.6	0.8	0.21	0.0	0.9	0.6
V	2.4	1.0–10	16.1	0.4	0.81	0.0	0.9	0.8
Al	827	241–4861	22	180	0.78	24	384	365
S	2660	1350–4149	15	403	0.62	127	416	251
Si	215	85–754	33	70	0.05	−8	71	28
Se§	0.1	0.05–0.2	27.3	0.03	0.24	0.0	0.03	0.02

† CV = coefficient of variation = ((SEP/mean of chemical procedure) × 100).
‡ SEP = Standard error of performance (mg kg⁻¹).
§ Se data are for 251 tall fescue samples which are not a part of the 102 tall fescue population in the first section of this study.

Table 5. Statistical data for element concentrations (mg kg⁻¹) determined by wet chemistry and NIRS for 100 crested wheatgrass samples.

Element	Wet chemistry		CV†	SEP‡	r^2	Bias	Standard deviation	
	Mean	Range					Chemistry	NIRS
Ba	22.0	8.1–34.0	25.4	5.6	0.06	−1.2	5.3	3.5
Li	0.8	0.1–13.0	211.5	1.6	0.03	0.0	1.7	0.5
Mo	0.7	0–1.6	43.5	0.3	0.52	0.0	0.4	0.4
Ni	0.4	0–1.0	44.4	0.2	0.19	0.0	0.2	0.1
Pb	1.6	0–9.1	46.2	0.7	0.17	0.0	0.8	0.4
V	0.7	0.4–2.6	39.1	0.3	0.36	0.0	0.3	0.3
Al	330	155–1150	21	71	0.72	−18	127	127
S	1364	896–2419	12	159	0.61	−25	254	200
Si	165	86–340	28	46	0.26	−6	53	32

† CV = coefficient of variation = ((SEP/mean of chemical procedure) × 100).
‡ SEP = standard error of performance (mg kg⁻¹).

Table 6. Statistical data for element concentrations (mg kg⁻¹) determined by wet chemistry and NIRS for 15 alfalfa hay samples.

Element	Wet chemistry		CV†	SEP‡	r^2	Bias	Standard deviation	
	Mean	Range					Chemistry	NIRS
Ba	17.9	12.7–29.0	44.0	7.9	0.55	−3.0	4.4	4.0
Li	2.2	0.3–10.3	148.6	3.3	0.01	1.4	0.6	0.6
Mo	1.2	0.5–1.7	51.3	0.6	0.06	−1.4	0.4	0.6
Ni	0.5	0.4–0.9	33.3	0.2	0.13	−0.6	0.1	0.2
Pb	1.9	1.5–3.5	34.4	0.7	0.01	0.5	0.6	0.2
V	0.5	0.2–1.0	58.3	0.3	0.02	0.3	0.2	0.2
Al	223	84–402	28	62	0.69	40	111	92
S	2330	1692–3711	11	256	0.68	1	370	352
Si	105	32–203	49	52	0.34	64	57	56

† CV = coefficient of variation = ((SEP/mean of chemical procedure) × 100).
‡ SEP = standard error of performance (mg kg⁻¹).

wheatgrass (49, 38, and 28%, respectively). Legumes generally have lower Si amounts than grasses, and the chemical components that Si are associated with would be expected to vary between legumes and grasses as well as among grasses. Selenium results from tall fescue show a high CV, but without results from the other forage types no definite conclusions can be drawn.

CONCLUSIONS

Use of common statistical values to determine accuracy for NIRS use in elemental analysis is limited because NIRS measures elements indirectly. Because we do not see the high r^2 values common for NIRS measurements of protein, fiber, oil, etc., it does not follow that NIRS cannot measure trace elements. From the statistical values, it appears that NIRS is somewhat sensitive to the presence of these elements within a forage type. However, the results were variable among forage types for all elements except Al and S. Elements are undoubtedly found in different forms in different forage types, which contributes to the inconsistent NIRS results and differential availability to animals. The mineral values generated from NIRS are more accurate than values from feed tables, which have a high degree of variation associated with them. If exact mineral results are required, then NIRS is not the method to use; however, in many instances the mineral values obtained from NIRS are an acceptable first approximation.

REFERENCES

1. Clark, D.H., H.F. Mayland, and R.C. Lamb. 1987. Mineral analysis of forages with near infrared reflectance spectroscopy. Agron. J. 79:485–490.
2. Kay, W. 1954. Near infrared spectroscopy. I. Spectral identification and analytical application. Spectrochemica Acta 6:257–287.

3. Olsen, O.E., I.S. Palmer, and E.E. Cary. 1975. Modification of the official fluorometric method for selenium in plants. J. Assoc. Off. Chem. 58:117–121.

4. Shenk, J.S. 1985. Public software. p. 18–21. In G.C. Marten, J.S. Shenk, and F.E.Barton, II (ed.) Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality. ARS Agric. Handb. 643, U.S. Gov. Print. Office, Washington, DC.

5. ———, I. Landa, M.R. Hoover, and M.O. Westerhaus. 1981.

Description and evaluation of a near infrared reflectance spectro-computer for forage and grain analysis. Crop Sci. 21:355–358.

6. ———, M.O. Westerhaus, and M.R. Hoover. 1979. Analysis of forages by infrared reflectance. J. Dairy Sci. 62:807–812.

7. Schilt, A.A. 1979. Perchloric acid and perchlorates. G. Fredrick Smith Chemical Co., Columbus, OH.

8. Whetsel, K.B. 1968. Near infrared spectrophotometry. Applied Spectroscopy Rev. 2:1–67.